PROBING INTERACTIONS BETWEEN CELL WALL BINDING PROTEINS AND THE BACILLUS ANTHRACIS SECONDARY CELL WALL POLYSACCHARIDE USING SYNTHETIC COMPOUNDS

by

ROBERT N. CHAPMAN JR.

(Under the Direction of Geert-Jan Boons)

ABSTRACT

The secondary cell wall polysaccharide (SCWP) of *Bacillus anthracis* plays a key role in the organization of the cell envelope. Through non-covalent interactions, it anchors S-layer proteins (SLP) and S-layer associated proteins (BSL) to the cell surface. Proper S-Layer assembly was shown to be important for virulence and various cell maintenance functions. Specific functionalities on the non-reducing SCWP trisaccharide unit including a 4,6-*O*-pyruvyl ketal, a free amine, and O-acetylation are thought to play a role in SLP and BSL binding. Herein, the synthesis of a library of compounds based on the non-reducing unit of *B. anthracis* SCWP is described. The compounds were utilized in microarray and ELISA experiments to probe the importance of various structural features for binding against a panel of mCherry fusion proteins constructed from the S-Layer homology binding domains of various SLP and BSL proteins. A number of synthetic challenges were addressed including the development of synthetic procedures and glycosylation conditions for the unique pyruvylated donors. The SCWP structural features vital for SLP and BSL binding were successfully identified.

PatB1 is a putative O-acetyltransferase believed to play a role in the 3-*O*-acetylation of the penultimate β-GlcNAc on the *B anthracis* SCWP. In order to characterize PatB1 specificity and specific activity in the context of its natural environment, a panel of oligosaccharides based on the trisaccharide repeating unit was synthesized with galactose substitutions at biologically relevant positions. A common

trisaccharide intermediate was synthesized with levulinoyl, fluorenylmethyloxycarbonate, and 2-naphthylmethyl protecting groups installed at key branching points. The orthogonal protecting groups were regioselectively removed allowing for site specific installation of galactose. A trisaccharide based on the non-reducing SCWP unit was also synthesized to determine if the 4,6-*O*-pyruvyl ketal and/or free amine can affect PatB1 activity and specificity. It was found that the presence of galactosylation did not alter specificity, but led to a decrease in specific activity. Furthermore, it was found that the trisaccharide bearing the 4,6-*O*-pyruvyl ketal and free amine showed a significant increase in specific activity demonstrating that these features are likely utilized *in vivo* as PatB1 binding epitopes and help to facilitate 3-*O*-acetylation of the penultimate β-GlcNAc.

INDEX WORDS: Glycosylation, Bacillus anthracis, Bacillus Cereus, Secondary Cell Wall Polysaccharide, S-layer, PatB1, Microarray, ELISA, Oligosaccharide, Sap, EA1, BSL

PROBING INTERACTIONS BETWEEN CELL WALL BINDING PROTEINS AND THE BACILLUS ANTHRACIS SECONDARY CELL WALL POLYSACCHARIDE USING SYNTHETIC COMPOUNDS

by

ROBERT N. CHAPMAN JR.

BSc. Chemistry, University of North Florida, 2006

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2017

PROBING INTERACTIONS BETWEEN CELL WALL BINDING PROTEINS AND THE BACILLUS ANTHRACIS SECONDARY CELL WALL POLYSACCHARIDE USING SYNTHETIC COMPOUNDS

By

ROBERT N. CHAPMAN JR.

Major Professor: Geert-Jan Boons

Committee: Ryan Hili
Vladimir Popik

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2017

DEDICATION

To my very patient wife Catherine

ACKNOWLEDGEMENTS

I would like to acknowledge and thank my mentor and advisor Prof. Dr. Geert-Jan Boons for providing me with the opportunity to pursue my PhD degree in his research group. His guidance not only helped me to become a better Chemist, but a better writer and presenter as well.

I would also like to thank my doctoral advisory committee, Prof. Dr. Vladimir V. Popik and Prof. Dr. Ryan Hili for their helpful suggestions and guidance. They were always willing to rearrange their perfectly staggered schedules to make time for my graduation requirements.

I would like to thank David Sychantha and Anthony Clarke at the Univerity of Guelph for their work on the PatB1 project. Thanks to Olaf Schneewind and his group at the University of Chicago for providing me with a wide array of mCherry fusion proteins. A big thanks to the Boons' group members past and present for making my long hours in the lab a more pleasurable experience (There are way to many of you to name individually). I extend a special thanks to Dr. Lin Liu for being a great friend and labmate and for providing me with great advice for navigating the travails of synthetic chemistry. Next, I would like to thank Dr. Andre Venot for keeping the labs in perfect shape. I will miss the handmade holiday cards and homemade chocolates. I would also like to thanks to Dr. John Glushka for helping me with all things NMR. Thanks to Dr. Margreet Wolfert for training me in various biological and biochemical techniques. I would also like to thank Dr. Anthony Prudden for the great discussions and the occasional dose of much needed levity. Finally and most importantly, I would like to thank my family for their support and encouragement.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SCHEMES

Page

LIST OF ABBREVIATIONS

Ac ..... Acetyl
Ac_2O ..... Acetic Anhydride
AcOH ..... Acetic Acid
Alloc ..... Allyloxycarbonyl
BB ..... Building Block
BF_3-.Et_2O ..... Boron Trifluoride Diethyl Etherate
Boc_2O ..... Di-tert-butyl dicarbonate
Bn ..... Benzyl
BnBr ..... Benzyl Bromide
BSA ..... Bovine Serum Albumin
BSL ..... Bacillus anthracis S-Layer associated protein
Bz ..... Benzoyl
CBD ..... Carbohydrate Binding Domain
CIP ..... Contact Ion Pair
COSY ..... Correlation Spectroscopy
DBU 1,8-Diazabicycloundec-7-ene
DCC ..... N,N'-Dicyclohexylcarbodiimide
DCE 1,2-Dichloroethane
DCM ..... Dichloromethane
DDQ 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEA ..... Diethylamine
DPS ..... Diphenylsulfoxide
DSP ..... Dithiobis(succinimidyl propionate)
DTT ..... Dithiothreitol
DTTSP ..... 3,3'-dithiobis(sulfosuccinimidyl propionate)
DHB ..... 2,5-Dihydroxybenzoic Acid
DIC ..... N,N'-Diisopropylcarbodiimide
DIPEA ..... Diisopropylethylamine
DMAP ..... 4-N,N'-Dimethylaminopyridine
DMF ..... N,N'-Dimetylformamide
DTBMP 2,6-Di-tert-Butyl-4-Methyl-Pyridine
Fmoc 9-Fluorenylmethyloxycarbonyl
FmocCl 9-Fluorenylmethylchloroformate
Gal ..... Galactose

Glc ..... Glucose
HMBC ..... Heteronuclear Multiple Bond Correlation Spectroscopy
Lev ..... Levulinoyl
LG ..... Leaving group
MAB ..... Monoclonal Antibody
MALDI-TOF ..... Matrix Assisted Laser Desorption Ionization Time-Of-Flight
Man ..... Mannose
Me ..... Methyl
M.S. ..... Molecular Sieves
MsCl ..... Methanesulfonyl chloride
Nap 2-Methylnaphthyl
NH-TFA ..... Trifluoroacetamide
NIS ..... N-Iodosuccinimide
NMR ..... Nuclear Magnetic Resonance
OMe ..... Methoxy
PBS ..... Phosphate Buffered Saline
PMB ..... p-methoxybenzyl
Ph ..... Phenyl
S-Layer ..... Surface Layer
Py ..... Pyridine
SLH ..... S-Layer Homology
SLP ..... S-Layer Protein
SSIP ..... Solvent Separated Ion Pair
TBAF ..... Tetrabutyl Ammonium Fluoride
T-BuOH ..... t-Butanol
TDS ..... Dimethylthexylsilyl
TCA ..... Trichloroacetyl
TCEP ..... Tris(2-carboxyethyl)phosphine hydrochloride
TEA ..... Triethylamine
TES ..... Triethylsilane
Tf_2O ..... Trifluoromethanesulfonic Anhydride
TfOH ..... Trifluoromethanesulfonic Acid
TFA ..... Trifluoroacetic Acid
THF ..... Tetrahydrofuran
TLC ..... Thin Layer Chromatography
TMEDA ..... N,N,N',N'-Tetramethylethylenediamine
TMSOTf ..... Trimethylsilyl Trifluoromethanesulfonate
TTBP 2,4,6-Tri-tert-butylpyrimidine
TOCSY ..... Total Correlation Spectroscopy
Troc 2,2,2-Trichloroethyloxycarbonyl
TS ..... Transition State
TsCl ..... p-Toluenesulfonyl chloride

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Carbohydrates in Gram-positive Bacilli cell walls; General Information: In nature, all cells harbor an array of freestanding or covalently linked carbohydrates. These glycans play an important role in the interactions between cells and the surrounding environment. Being a major component of cell surfaces and secreted macromolecules, they are able to serve a variety of functions ranging from providing structural support to mediating cell-cell and host-guest interactions. The outer portion of the bacterial cell wall is known as the cell envelope (Figure 1.1). In Gram-positive bacteria, the envelope includes the cytoplasmic membrane, a thick peptidoglycan (murein) layer with associated secondary cell wall polymers and, in some species, an additional capsule and/or S-layers is present.

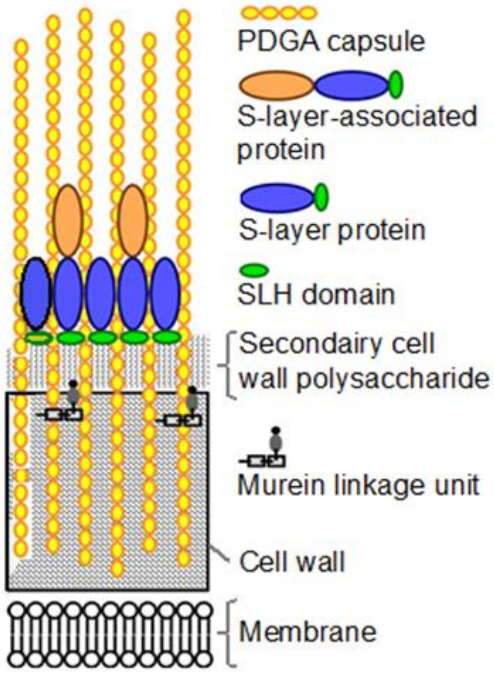


Figure 1.1 The Cell Wall *of B. anthracis.*

The capsule is comprised of a layer of excreted polysaccharides or polypeptides that surrounds the bacterial cell. The capsule can act as protective shield enabling the bacteria to evade the host immune system *via* inhibition of macrophage mediated phagocytosis.[1,2] The cytoplasmic membrane is a semi-permeable membrane that separates the internal components of the cell from the external environment. It serves to retain the cytoplasm of the cell and acts as a barrier against uncontrolled ion exchange. The membrane is made up of a phospholipid bilayer with embedded proteins that can serve as ion transporters, receptors, and enzymes.[3] A thick rigid layer of peptidoglycan (PG) surrounds the cytoplasmic membrane providing structural integrity to the cell. The PG layer prevents lysis of cells through osmotic pressure and confers form and strength to cells by withstanding tensile forces.[4,5] Peptidoglycan is comprised of multiple linear polysaccharide strands with alternating β-1,4 linked N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) residues cross-linked by peptide chains of 3-5 amino acids. Secondary modifications to the PG such as deacetylation and variation of the amino acid combination of the peptide or changes in the position of the interpeptide bridge are also possible.[6]

Gram-positive bacteria have carbohydrate based secondary cell wall polysaccharides (SCWP) bound to either the cytoplasmic membrane or the PG.[7] The SCWP of Gram-positive bacteria can be divided into three groups based on their structural characteristics. (i) Teichoic acids are short copolymers of glycerol phosphate or ribitol phosphate and carbohydrates linked *via* phosphodiester bonds. Teichoic acids that are anchored to the lipid membrane with a lipid anchor are referred to as lipoteichoic acids (LTAs) and teichoic acids that are covalently bound to peptidoglycan are classified as wall teichoic acids (WTA).[8-11] (ii) Teichuronic acids comprise the second group and consist of uronic acid-containing heteropolysaccharides.[12,13] (iii) The non-classical group includes neutral and acidic polysaccharides which do not fit the criteria of the other groups.[7,14,15] The SCWP of *B anthracis* falls into the non-classical group since it is covalently attached to the PG through an acid labile phosphate group at the reducing end of the polysaccharide.[16]

Like species from nearly every branch of Bacteria and Archaea, the cell envelope is coated with a monomolecular Surface layer (S-Layer). The S-layer represents an important interface between these cells and their environment.[17-19] S-layers are composed of proteins or glycoproteins that self-assemble into uniform, paracrystalline lattices of square, hexagonal or oblique appearance on the cell surface. Despite the apparently conserved function of providing a 2D array around the cell surface, genetic and functional studies show that there is a wide diversity in both the sequences and the roles of S‑layer proteins.[30] They have been shown to function as protective coats, as molecular sieves, or as molecule and ion traps.[20] They also have been shown to play a role in cell adhesion and surface recognition.[21,22] Based on their involvement in bacteria-host interactions, S-layers have been suggested to play roles in virulence.[18,23-25]

The carbohydrate-containing components typically found on the cell walls of gram-positive bacteria include an extensive peptidoglycan layer, teichoic acids, lipoteichoic acids, capsular polysaccharides, and a paracrystalline S-Layer made up of S-layer proteins that are usually glycosylated.[26] The *B. anthracis* cell wall differs in several key aspects from this generalized description. First, the *B. anthracis* cell is surrounded by a poly-γ-D-glutamate capsule instead of a polysaccharide capsule.[27] Second, the cell wall lacks wall teichoic acid[3], and finally, their S-layer proteins are not glycosylated.[28,29]

BACILLUS ANTHRACIS AND HUMAN HEALTH

The *Bacillus cereus* group of bacteria is a group of phylogenetically closely related species. The group is comprised of seven bacterial species including *B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, and *B. cytotoxicus*.[31,32] The members of this group are Gram-positive, rod-shaped, aerobic or facultative anaerobic bacteria with the ability to form endospores. Bacilli can be observed as single cells or long chains of individual cells with individual cells ranging in size from 1 µm to 10 µm.[33] The *B. cereus* group is comprised of mostly non-pathogenic bacteria, but does have several important pathogenic members.[34] Two bacilli are considered medically

significant namely *Bacillus anthracis,*[35] which causes anthrax in humans and other mammals[36], and *B. cereus*, which can cause food borne illnesses.[37,38] A third species, *B. thuringiensis*, is an insect pathogen commonly used as a biological form of pest control.[39,40]

Anthrax Infection: Anthrax infection is initiated by the introduction of *B. anthracis* spores into the host primarily through minor skin or gastrointestinal tract abrasions. Alternatively, infection may occur *via* inhalation of spores.[35,36,41] Macrophages in the host phagocytize the spores and bring them to nearby lymph nodes where they germinate into vegetative cells. In this environment, the cells can multiply and subsequently move into the bloodstream where they disseminate to distant organs such as the spleen or lungs. If left untreated, the three clinical forms of anthrax; cutaneous, gastrointestinal and pulmonary anthrax are potentially fatal. The cutaneous form accounts for approximately 95% of the incidents and is characterized by a raised bump that develops into a black necrotic ulcer. Without treatment the mortality rate for the cutaneous form is about 20%. Gastrointestinal anthrax produces a general set of symptoms that include nausea, loss of appetite, and fever followed by abdominal cramps, vomiting of blood and severe diarrhea which is fatal in 25-75% of cases. Pulmonary anthrax is the most severe form with initial symptoms similar to a common cold that rapidly progress and can lead to death within a matter of days. Without antibiotics, the mortality rate is around 80%. Delayed or incorrect diagnosis of the disease is a major factor in the mortality rate.[42,43]

The virulence of *B. anthracis* infection is mediated by the production of anthrax toxins. The anthrax toxins are comprised of three distinct proteins: the protective antigen, the lethal factor, and the edema factor. The genes encoding these proteins are located on the pXO1 plasmid, and their transcription is regulated by another pXO1 gene known as the anthrax toxin activator. A second plasmid pXO2, is required for capsule formation.[44-46] The plasmids are not essential for growth, and a number of *B. anthracis* derivative strains do not carry both virulence plasmids. Besides *B. anthracis* Ames strains that contain both pXO1 and pXO2 plasmids, *B. anthracis* Sterne strains contain only pXO1, while *B.*

anthracis Pasteur strains contain only plasmid pXO2. With the exception of avirulent CDC 684, strains with both plasmids are highly pathogenic, although strains with only one plasmid can retain some of their pathogenicity in certain host organisms.[47] *B. anthracis* is endemic in parts of Africa, Asia and South America but can be found in most parts of the world. The spores are highly robust and have been shown to be resistant to chemical agents, heat, ultraviolet and ionizing radiations, and pressure giving them the ability to survive in the environment for decades. *B. anthracis* can be weaponized with relative ease and recognition of inhalation mediated infection can prove difficult due to the non-specific nature of symptoms. This was demonstrated by the death of four people who inhaled spores from contaminated mail.[48-50] Due to the potential risk to public health, there is considerable interest in the development of early disease diagnostics and anthrax vaccines.[51]

CELL WALL ARCHITECTURE OF BACILLUS ANTHRACIS AND RELATED SPECIES

The bacterial cell wall is essential for survival and plays key roles in host-guest interactions. The vegetative cell wall of *B. anthracis* is composed of a thick peptidoglycan layer that attaches a secondary cell wall polysaccharide through an anomeric phosphodiester linkage.[52] *B. anthracis* also elaborates a polyglutamic acid capsule that endows vegetative cells with an ability to escape phagocytic clearance.[27]

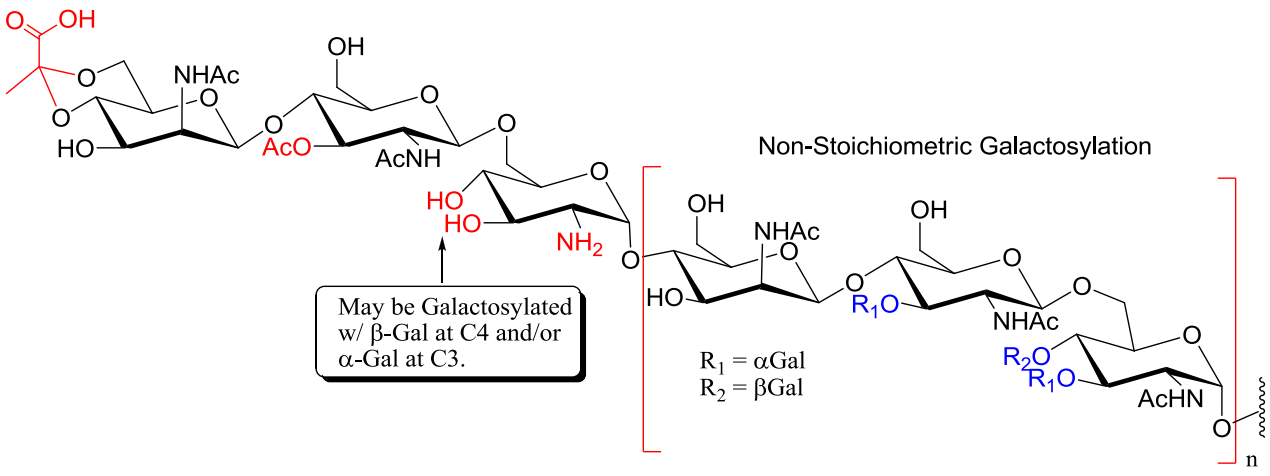


Figure 1.2: *B. anthracis* Secondary Cell Wall Polysaccharide

The secondary cell wall polysaccharide of *B. anthracis* has a [→4)-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→6)-α-D-GlcNAc-(1→)]$_n$, repeating backbone that can be branched at C-3 and C-4 of the α-D-GlcNAc moiety with α-D-Gal and β-D-Gal respectively and at C-3 of the β-GlcNAc residue with a α-Gal (Figure 1.2).[53] These positions are only partially substituted resulting in considerable micro-heterogeneity. Furthermore, it has been found that a single repeating unit located at the distal (non-reducing) end of the polysaccharide is further modified with a 4,6-*O*-pyruvyl ketal on the penultimate β-D-ManNAc moiety. In addition, the β-D-GlcNAc residue is modified by a 3-*O*-acetyl ester and the α-D-GlcNAc moiety is *N*-deacylated to afford a free amine.[54] It is possible that the α-GlcNH$_2$ moiety is further modified by a α-Gal at C-3 and β-Gal at C-4. However, NMR studies have not been able to confirm this modification. CDC 684 is a non-galactosylated and avirulent variant of *B anthracis* that contains both virulence plasmids (pXO1 and pXO2) and a polymerized SWCP backbone identical to that of galactosylated forms; It was found that CDC 684 retained the 4,6-*O*-pyruvyl ketal, O-acetate and amino group modifications found on SCWPs of galactosylated *B. anthracis* strains as well as an intact S-layer.[54] Previous genetic studies have indicated that pyruvylation of *B. anthracis* SCWP is essential for S-Layer protein binding[55], and thus it is fitting that important binding ligands for these proteins would be uniquely located at the non-reducing end of the polysaccharide.

Cell Wall architecture of related species: The structures of secondary cell wall polysaccharides from *B. anthracis Ames*, *Sterne* and *Pasteur* and several phylogenetically related *B. cereus* strains have been elucidated and it was found they have unique and shared structural features (Figure 1.3).[54-58] *B. cereus* G9241 and 03BB87 (human pathogens), *B. cereus* Cameroon and Ivory Coast (great ape pathogens) and *B. cereus* ATCC 10987 (non-pathogenic) have the same backbone as *B. anthracis*, but differ in the pattern of side chain carbohydrates. For example, the β-ManNAc moiety of the internal repeating units of *B. cereus* G9241 and 03BB87 can be modified by an α-Gal moiety, which is not present in *B. anthracis*

species. On the other hand, *B. cereus* Cameroon and Ivory Coast have a unique α-Gal(1→3)α-Gal disaccharide at this position. The non-pathogenic *B. cereus* ATCC 10987 produce a polysaccharide that has a modified backbone repeating unit consisting of a →6)-α-GalNAc-(1→4)-β-ManNAc-(1→4)-β-GlcNAc-(1→). It is substituted with β-Gal at C-3 position of α-GalNAc and non-stoichiometrically 3-*O*-acetylated on β-ManNAc.

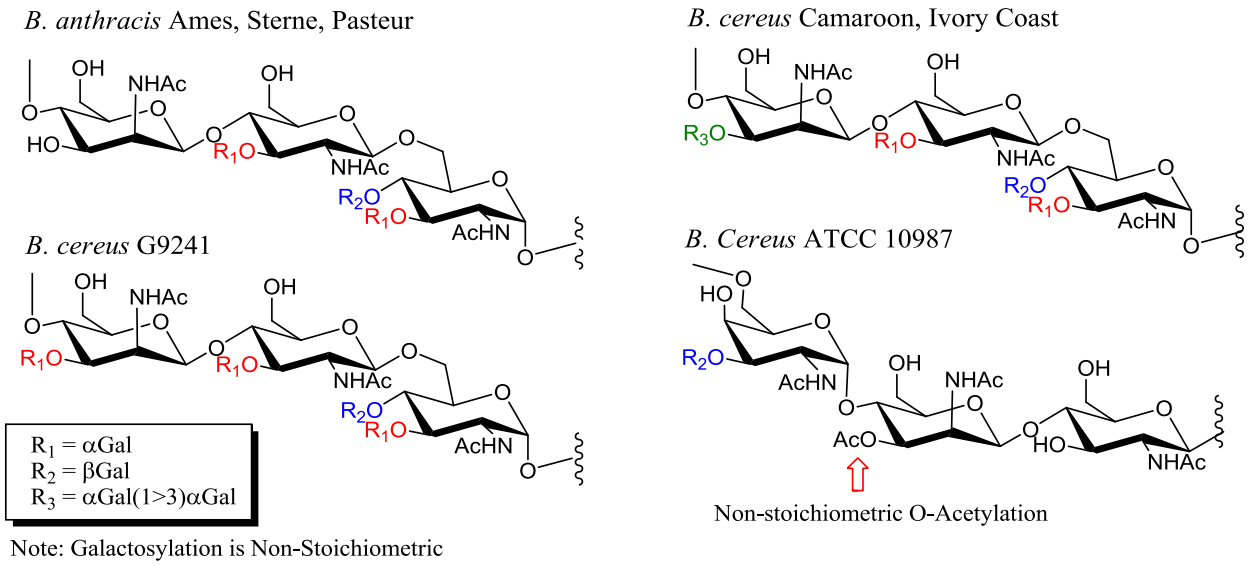


Figure 1.3: SCWP Repeating Units from Various Bacilli.

***B. anthracis* S-Layer:** The surface layer is a monomolecular layer of the bacterial cell envelope composed of identical proteins or glycoproteins that spontaneously self-assemble into a bi-dimensional crystalline array that encases the entire cell surface (Figure 1.1). S-layers have been identified in nearly every taxonomic group of bacteria and represent an almost universal feature of Achaea.[59-61] The S-layer of *B. anthracis* is comprised of two S-Layer proteins (SLPs) namely, Sap and EA1.[62,63] These proteins are anchored to the cell surface through non-covalent interactions with the secondary cell wall polysaccharides (SCWP).[64-67] The S-layer also harbors twenty-two *B. anthracis* S-layer-associated proteins (BSLs) that serve many functions including the uptake of nutrients (BslK), adhesion to host

tissues (BslA), and the separation of cells within chains of vegetative bacilli (BslO).[68-70] Genetic studies have also shown that *B. anthracis* requires S-layer proteins for the pathogenesis of infection.[71]

S-layer proteins (EA1 and Sap) are chimeric proteins possessing a self-assembly module (crystallization domain) that confers paracrystalline properties and a cell wall targeting module that anchors the proteins to the cell wall. The cell wall targeting module is usually made up of three so called S-layer homology (SLH) domains each having approximately 55 amino acids.[62,72,73] In addition to S-layer proteins, SLH domains are found in many other bacterial cell surface proteins including the S-layer-associated proteins. Although it was initially proposed that SLH domains bind peptidoglycan, it is now evident that the SCWP serves as the anchoring structure for these proteins.[65] Furthermore, it has been shown through genetic experimentation that pyruvylation of *B. anthracis* polysaccharide is essential for the attachment of the S-layer proteins EA1 and Sap to the bacterial cell wall.[64-66] Pyruvate alone is not the ligand of SLH domain bearing proteins, and thus it appears that this functional group needs to be presented as part of a specific carbohydrate to mediate binding. The molecular basis of SLH specificity for pyruvylated polysaccharides is not yet known. This knowledge is of great importance because the binding of SLH domains to pyruvylated secondary cell wall carbohydrates is thought to be an ancestral mechanism for the anchoring of S-layer proteins to the envelope of bacteria.[65]

In *Bacillus anthracis*, the secretion of EA1 and Sap is mediated by the accessory Sec secretion system. (Figure 1.4) The proteins contain an amino-terminal signal peptide (white box) that directs the nascent polypeptide to the secretion apparatus and is cleaved upon membrane translocation (at location of black arrow). Translocation requires the accessory ATPase, SecA2. Following recognition by SecA2, the nascent polypeptide is translocated across the membrane through a pore that consists of SecY2, SecE and SecG. After translocation to the cell surface, the proteins self-assemble and anchor to SCWP in order to form a functional S-layer.[30,67,74]

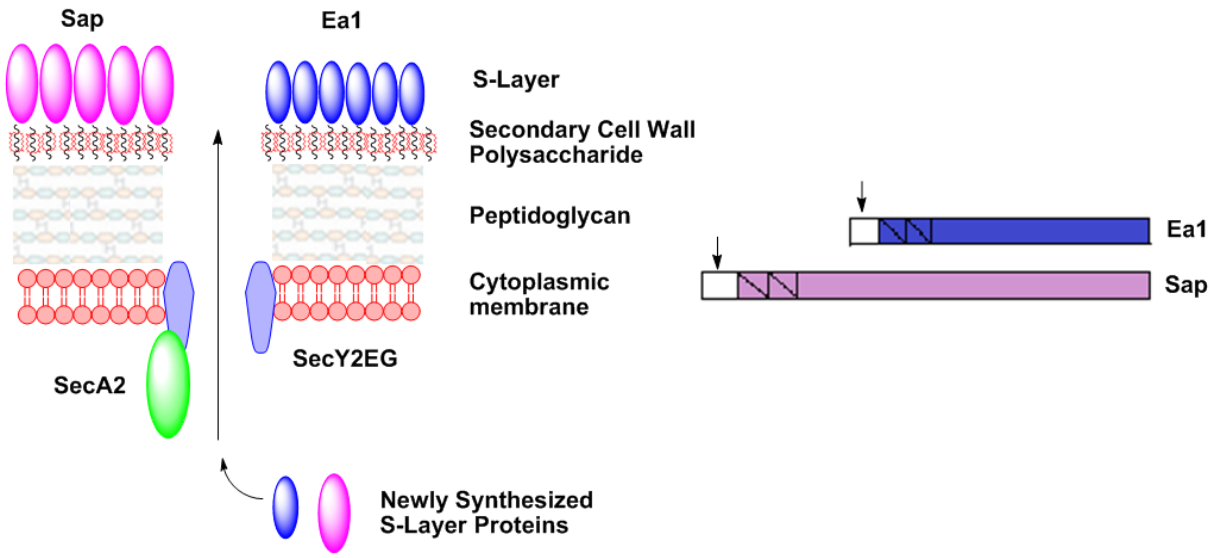


Figure 1.4: *B. anthracis* S-Layer Protein Secretion

MEDICAL SIGNIFICANCE OF CELL-WALL POLYSACCHARIDES IN MEDICINE

The cell walls of many bacterial genera are well established as diagnostic targets,[75-78] carbohydrate-based vaccine antigens,[78,79] and virulence factors.[80] The *B. anthracis* SCWP is known to play a role in the assembly of the S-Layer which has been implicated as a virulence factor.[64,66,67] In addition, *B. anthracis* SCWP was found to provide specific binding sites for the cell wall binding domains of bacteriophage endolysins.[81] Endolysins are hydrolytic enzymes produced during the late stages of bacteriophage infection that act to lyse the bacterial cell and release the viral progeny. Endolysins have the potential to be developed into diagnostic or therapeutic agents. The polysaccharides themselves are also antigenic providing opportunities for the development of vaccines, diagnostics, and therapeutics. Despite the importance of SCWP in physiology and pathology, it has been difficult to identify and characterize important structural features due to the heterogeneity and structural complexity of isolated secondary cell wall polysaccharides. In order to effectively study the functions of these cell wall carbohydrates and their associated proteins, collections of structurally well-defined oligosaccharides are required.

CARBOHYDRATE SYNTHESIS

Carbohydrates are the most complex and diverse class of biopolymers commonly found in nature.[82] Nucleic acids and proteins are linear assemblies connected by a specific bond type. Proteins are assembled from a combination of 20 basic building blocks and connected to one another by amide linkages. Nucleic acids are assembled from a combination of four basic building blocks and are linked *via* [3'-5']-phosphodiester bonds. In contrast, carbohydrates can be linear or highly branched and are assembled from a large number of different monosaccharide building blocks that vary in ring size (5-membered furanose, 6-membered pyranose), configuration (glucose, mannose, etc.), and anomeric configuration. In addition, the building blocks can be further modified (e.g., acylation, sulfation, phosphorylation, etc.) adding more complexity and structural diversity. Chemical synthesis of targeted complex oligosaccharides, though challenging, presents several advantages including well-defined structure, larger quantities of final product, and flexible target modifications.[83]

The majority of oligosaccharide synthetic procedures are comprised of three distinct stages. The first stage involves the protection of free hydroxyls and amines with protecting groups. Depending on the complexity of the desired product, the protecting groups can be orthogonal in nature to allow for selective deprotection and modification of those positions. The second step involves stereoselective glycosylation reactions between a glycosyl donor and acceptor. The process of deprotecting specific hydroxyls and subsequent glycosylations can be repeated in an iterative fashion until the desired structure is obtained. The third step involves removal of the remaining protecting groups (deprotection) to afford the final product. There are a number of synthetic steps, purifications and characterizations involved in a typical oligosaccharide synthesis. For this reason, it is important to design a synthetic strategy that minimizes the number of steps.[84,85] One way to achieve this is through the use of common intermediates. This can be achieved in modular synthesis where a small number of building block intermediates can be joined together in various ways in order to yield a large library of synthetic

targets.[86-88] In addition, one-pot multistep glycosylations have been developed in order to reduce the number of glycosylation and purification steps.[89-94] One-pot reactions have also been developed that allow for the introduction of multiple protecting groups onto monosaccharide building blocks in a single step.[95-98]

Stereoselective synthesis of the glycosidic bond: The glycosylation reaction involves the coupling of a glycosyl donor and acceptor and is one of the key steps in oligosaccharide synthesis (Figure 1.5). The glycosyl donors are equipped with a leaving group at the anomeric center and typically have protecting groups installed in order to prevent side reactions with exposed hydroxyls.[99,100] The glycosyl acceptor is usually protected with the exception of a single free hydroxyl group that can be utilized to form the glycosidic linkage. During a typical glycosylation reaction, an activator/promoter, usually electrophilic in nature, is utilized to activate the donor. After activation, the donor becomes a good leaving group and departs leaving a positive charge at the anomeric center.

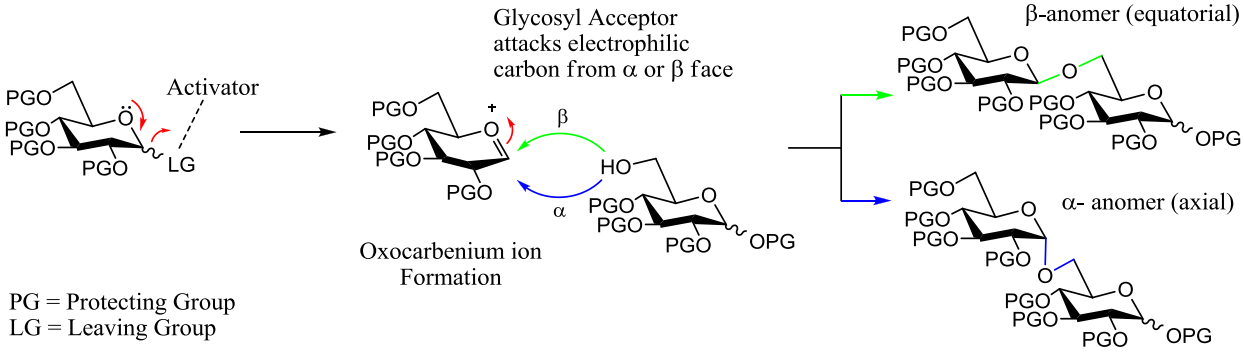


Figure 1.5. Glycosyl Donor Activation and Reaction with Acceptor

Depending on the molecularity and the type of activation, the anomeric carbocation can be stabilized by the lone pair electrons on the endocyclic oxygen, resulting in the formation of an oxocarbenium ion as a reactive species. Absent any intramolecular participation, the planar oxocarbenium ion can be readily

attacked by a nucleophilic acceptor in an S_N1 type manner from either the top (β) or bottom face (α) resulting in the formation of two possible diastereomers (anomers).

Anomeric Effect: The anomeric effect can significantly influence the resulting stereoselectivity at the anomeric center during glycosylation reactions.[101] The effect can be seen when comparing cyclohexanes to oxanes (Figure 1.6). A cyclohexane with an electronegative substituent prefers equatorial configuration due to unfavorable 1,3-diaxial interactions that would result from the axial configuration. Substitution of a heteroatom into the ring adjacent to the endocyclic oxygen shifts the thermodynamic preference to axial configuration.

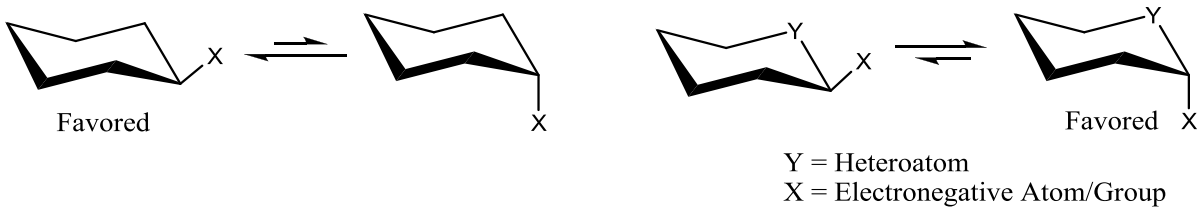


Figure 1.6. Demonstration of the Anomeric Effect

In carbohydrates the estimated magnitude of the anomeric effect is ~1.5kcal/mol.[102] In the axial configuration, the endocyclic oxygen lone pair is oriented antiperiplanar to the anti-bonding C-X σ* orbial and is able to donate electron density lowering the overall energy of the system (Fig 1.7).

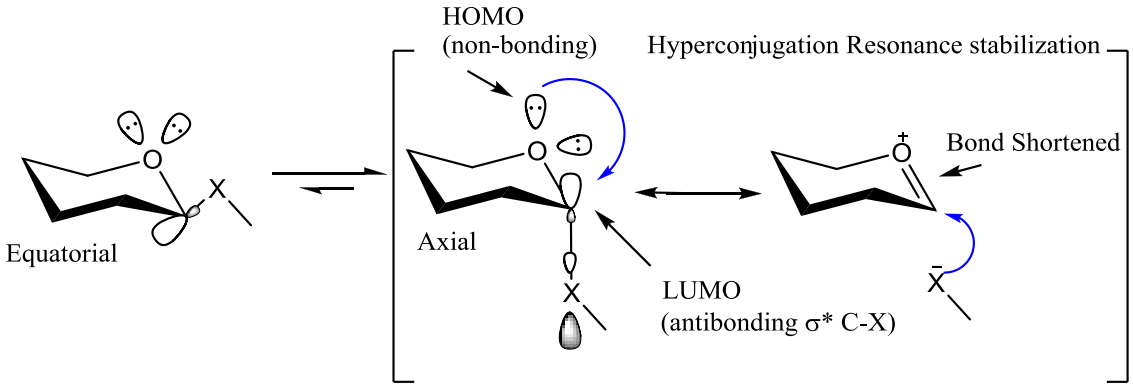


Figure 1.7: Stabilization due to electron donation and hyperconjugation resonance stabilization

This results in the shortening of C-O bond and lengthening of the C-X bond. The hyperconjugation resonance form can further stabilize the axial configuration.[103] Other possible contributors to the anomeric effect include minimization of unfavorable dipole interaction as well as minimization of electrostatic repulsion (Figure 1.8). Opposing dipoles are energetically more favorable than aligned dipoles. The beta anomer will produce two similarly oriented dipoles resulting in a less stabilized configuration than the alpha form. It has also been proposed that electrostatic repulsion found in the β-anomer is destabilizing, making alpha the preferred form.[103]

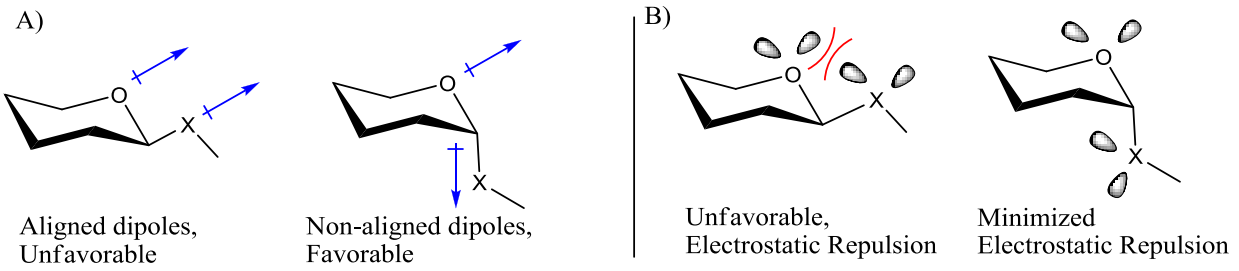


Figure 1.8: Anomeric Effect A) Dipole stabilization B) Stabilization *via* decreased electrostatic repulsion.

Neighboring group participation: During the synthesis of glycans, the sterochemical outcome of glycosylation must be controlled in order to efficiently produce the desired product. Neighboring group participation is one of the most common methods for influencing the stereochemistry at the anomeic center during glycosylation reactions (Figure 1.9).

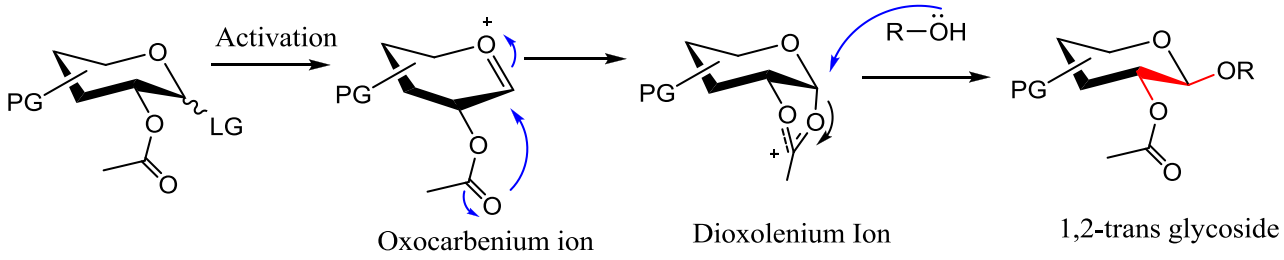


Figure 1.9: Neighboring group participation for the selection of 1,2-trans glycosides.

Neighboring group participation involves the use of specific functional groups at the C-2 position of the donor that allow for the stabilization of the oxocarbenium ion intermediate. The most common utilization of this method involves the use of esters at the C-2 position of the donor as a means to form 1,2-trans-glycosides. If an amine is present at the C-2 position, cyclic imides (*e.g.* phthalimides), carbamates, and protected amides substituted with deactivating electron withdrawing groups can also be utilized for 1,2-trans-selectivity. Following activation of the donor, the anomeric leaving group departs resulting in the formation of an oxocarbenium ion which is quickly trapped by the 2-*O*-acetyl ester forming a more stable five-membered intermediate. The resulting dioxolenium ion intermediate acts to block the bottom face of the ring forcing the acceptor to attack from the top face resulting in the formation of a 1,2 trans linkage.[104,105] In addition to C-2 neighboring groups, remote protecting groups at positions C-3, C-4 and/or C-6 may affect stereoselectivity in certain cases. It is believed that this phenomena is mediated by varying means including participation, H-bond mediated aglycone delivery, steric hindrance and/or electron withdrawal.[106-115]

Neighboring group participation using chiral auxiliaries to obtain 1,2-cis glycosides: The formation of 1,2-cis glycosides or C-2 deoxy glycosides usually requires the absence of a C-2 participating functionality. In these cases, the resulting stereochemistry is a summation of many factors, including the spatial orientation of donors and acceptors, protecting groups, promoters, and solvents.[116] As an alternative, chiral auxillaries can be installed at the C-2 position of the glycosyl donor to gain stereoselectivity in these reactions (Figure 1.10).[117-120]

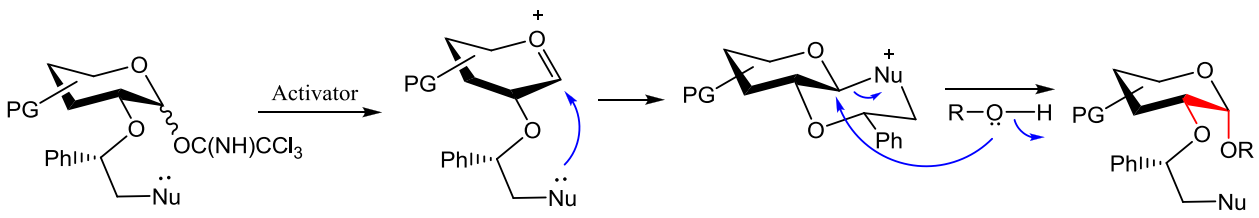


Figure 1.10: C-2 Chiral auxillary mediated installation of 1,2-cis glycosides.

Following activation, the nucleophilic group on the chiral auxillary attacks the oxocarbenium ion intermediate and forms a 6-membered cyclic intermediate. The resulting trans-decalin intermediate is favored over the cis-decalin intermediate due to unfavorable 1,4-diaxial steric clashing that occurs with the latter. The trans-decalin structure acts to block the top face of the ring forcing acceptor attack from the bottom face resulting in 1,2-cis glycosidic bond formation.

Solvent Effects on Glycosylation: The stereoselectivity of glycosylation reactions can be influenced by the use of solvents (Figure 1.11). Diethyl ether is known to assist in the formation of α-glycosides *via* formation of an equatorial diethyl oxonium ion intermediate that blocks the top face thereby forcing the acceptor to attack from the bottom face. In contrast, acetonitrile is used to assist in the formation of β-glycosides. It is suspected that acetonitrile forms an α-linked nitrilium ion at the anomeric center that serves to block the bottom face forcing the acceptor to attack from the top face.

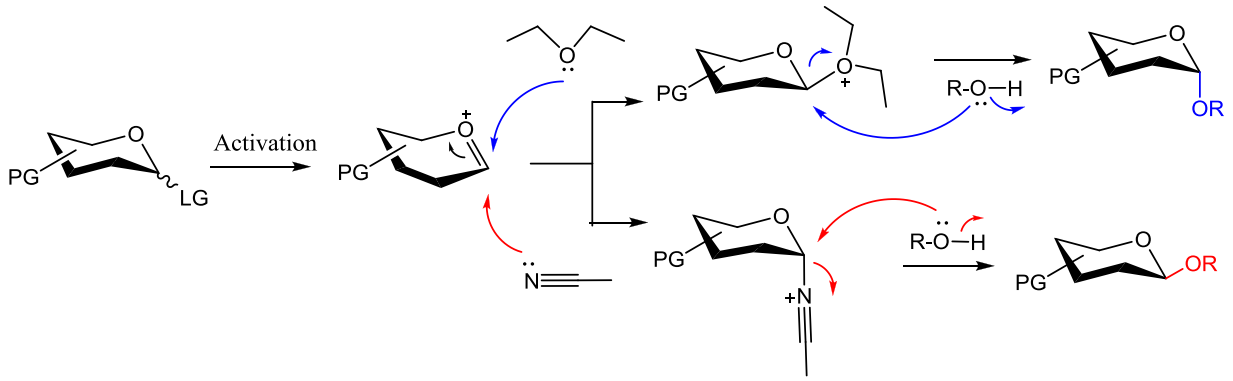


Figure 1.11: Solvent effect on the stereochemical outcome of glycosylation reactions.

Glycosyl Donors: The choice of leaving group can affect reactivity and stereoselective outcome of a glycosylation reaction (Figure 1.12). Although a large number of donor types exist, the most utilized donor groups include halides, imidates, thioglycosides, and sulfoxide. The activation conditions for each donor are different. The Koenigs-Knorr method employs glycosyl bromides and chlorides, as donors for

glycosylation reactions (Figure 1.12-A). Mercury and silver salts are typically used as promoters in these reactions.[121] Anomeric fluorides have also been used along with a $SnCl_2$-$AgClO_4$ promotor system.[122]

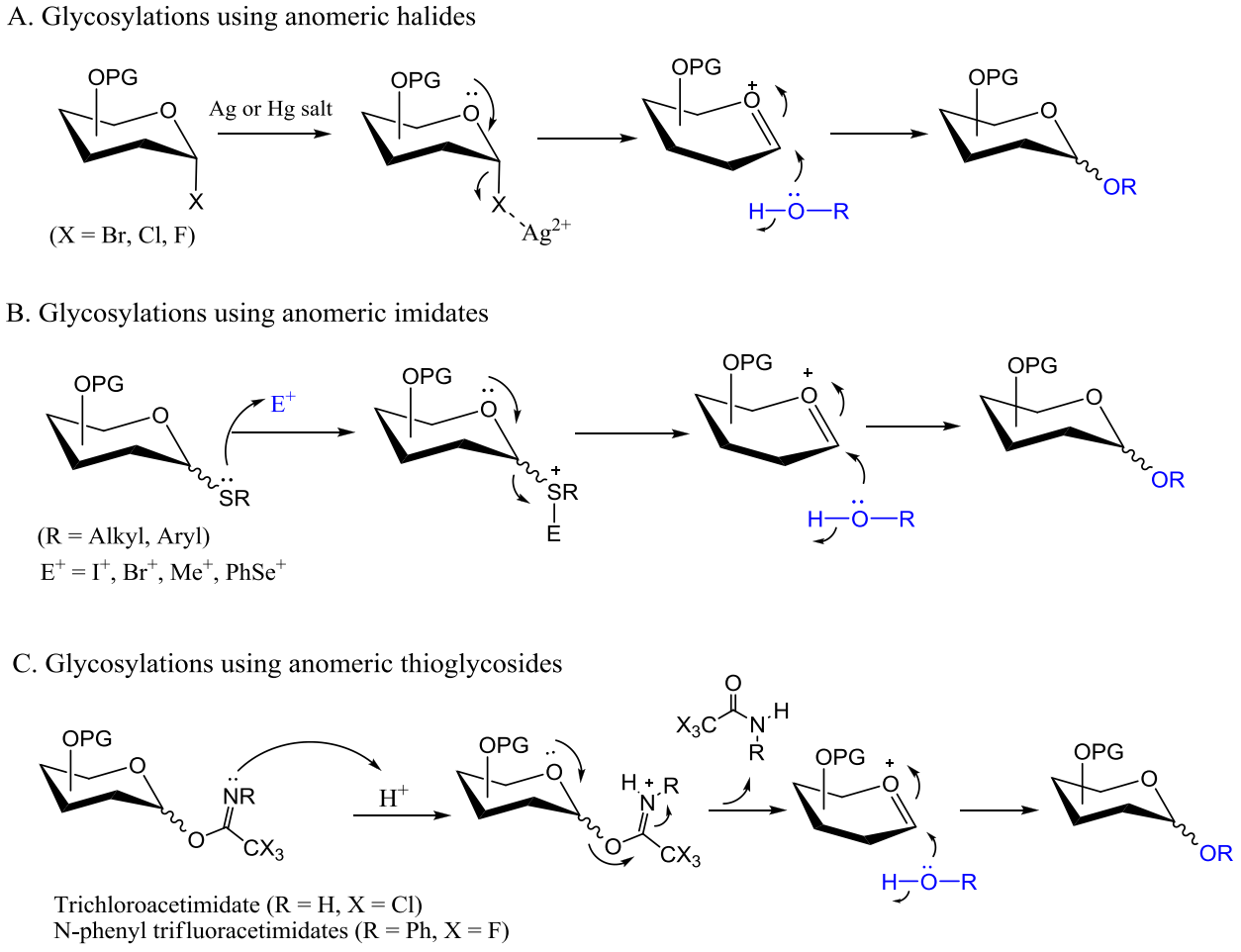


Figure 1.12: Glycosylations using various glycosyl donors.

Alkyl or aryl thioglycosides are another commonly used donor type (1.12-B). Soft electrophiles such as iodonium or bromonium are used to activate the thioglycoside converting it into a labile sulfonium intermediate that subsequently departs leading to the formation of the oxocarbenium ion intermediate. The acceptor can then attack the electrophilic intermediate at the anomeric center forming the glycosidic bond. Several common promotor systems for thioglycosides include i) N-iodosuccinimide and triflic acid, ii) N-iodosuccinimide and Trimethylsilyl trifluoromethanesulfonate

(TMSOTf), and iii) methyl triflate (MeOTf). In addition, thioglycosides can be pre-activated by first oxidizing to sulfoxide and then activating at low temperature with triflic anhydride.[123]

Another commonly utilized donor group is the imidate (Figure 1.12-C). Two types of imidates are commonly employed; they are the trichloroacetimidate and the N-phenyl-trifluoroacetimidate donors. The imidates are readily activated in the presence of catalytic amounts of Brønsted acid (*e.g*. triflic acid) or Lewis acid (*e.g.* TMSOTf). The glycosidic bond is formd by way of the oxocarbenium intermediate.

Anomeric control by electronic and steric effects: It was discovered by Crich and co-workers that stereoselective formation of β-mannosides could be achieved by pre-activation of 4,6-*O*-benzylidene protected mannosyl sulfoxide donors at low temperature followed by addition of a glycosyl acceptor (Figure 1.13).[124] It was found that locking the pyranose ring with 4,6-*O*-benzylidene acetal was important for high β-selectivity.[125] It was proposed that under pre-activation conditions, the oxacarbenium ion is trapped by a triflate anion which leads to the formation of the more stable α-triflate. This α-triflate intermediate can then be displaced in S_N2 fashion after addition of the acceptor to afford the β-mannoside product. It was hypothesized that the α-triflate can form a contact ion pair (CIP) where the triflate anion remains on the bottom face acting to block attack from that direction.

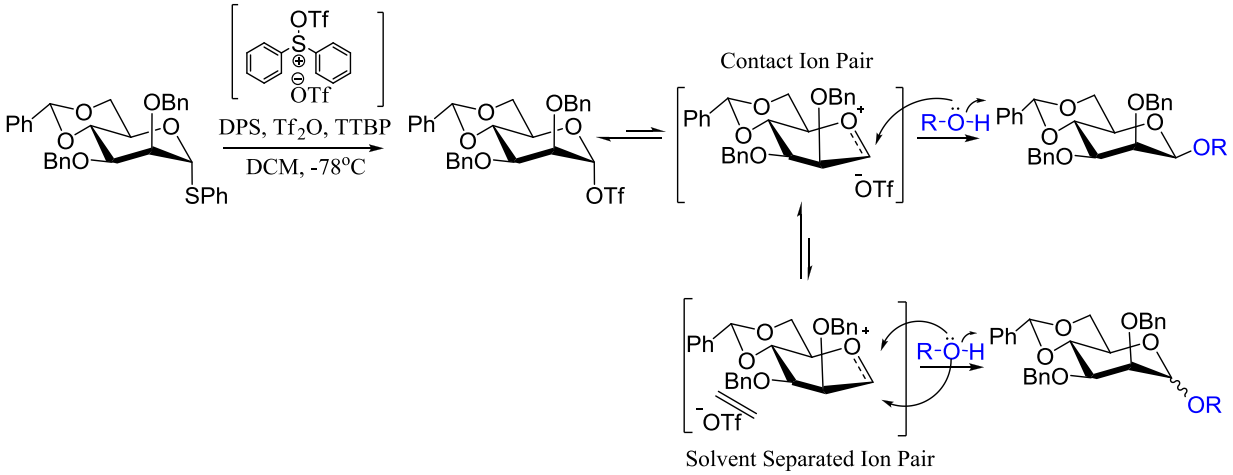


Figure 1.13: Mechanistic Detail of the Crich Beta Mannosylation Reaction

It has also been proposed that the nulceophile can form a loose association with the oxacarbenium ion as the α-triflate departs. The formation of α-product is thought to occur due to the formation of a solvent separated ion pair (SSIP) where the counter-ion is solvated resulting in a loss of facial selectivity. The 4,6-*O*-benzylidene acetal is thought to lock the pyranose ring in a stable chair conformation.[125] This is unfavorable for the formation of the intermediate oxacarbenium ion and subsequent S_N1 glycosylation since it requires rehybridization and flattening of the ring. The combination of the torsional disarming effect afforded by the 4,6-*O*-benzylidene acetal combined with the strong endo-anomeric effect help to produce the α-triflate as the favored intermediate.

Additonal factors that can affect the efficiency and stereoselectivity of glycosylation reactions include competing side reactions such as hydrolysis of the donor, orthoester formation, migration of functional groups, etc.[126] In addition, the temperature, concentration, type of acceptor, type of promotor, and amount of promotor can have significant effect on glycosylation reactions.

Amine Protecting Groups in Carbohydrate Chemistry: An amino sugar is a sugar molecule in which a hydroxyl group has been replaced with an amine group. There are a wide variety of amino sugars found in nature with N-Acetyl-D-glucosamine being one of the most abundant (Figure 1.14).

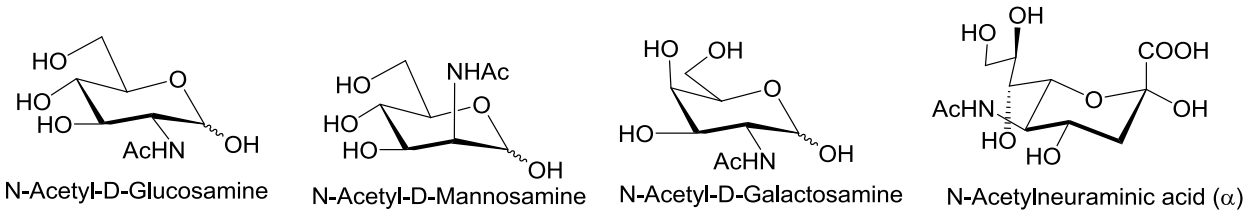


Figure 1.14: Examples of Common Amino Sugars

Each amino sugar can adopt different forms adding to their complexity. For instance, N-Acetyl-D-Glucosamine can be linked *via* an alpha or beta linkage. In addition, the sugar can be N-deacetylated to yield N-Acetyl-D-Glucosamine. The trisaccharide backbone of *B.Cereus* group SCWP is composed of

amino sugars and a thorough understanding of amine protection is necessary in order to design a successful synthetic strategy for SCWP derived compounds. The structural complexity of naturally occurring oligosaccharides makes the choice of protecting group an important part of the process. These groups must be orthogonal to other protecting groups and stable under the various reactions conditions utilized in the synthesis. In addition, a function providing neighboring group participation should be used in certain instances and avoided in others. Unfortunately, there is no ideal protecting group and choices must be made on a case by case basis. Monosaccharides bearing a 2-acetamido are abundant building blocks in naturally occurring oligosaccharides, but their direct incorporation by chemical glycosylation is typically low yielding. This is mainly due to formation of the stable 1,2-oxazoline intermediate (Figure 1.15).[127,128] Although, there are some chemical glycosylation methods that utilize oxazoline in reactions, these reactions tend to work only in special cases.[129,130] In addition, N-acetyl deprotection requires harsh basic conditions for removal and is not a practical choice for protection if a free amine is desired.

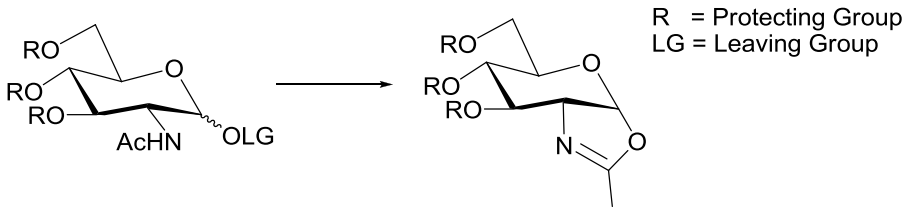


Figure 1.15: Oxazoline Formation

Fortunately, oxazoline formation can be bypassed by utilizing amino protecting groups in place of NH-Acetyl during glycosylation reactions (Figure 1.16). The replacement of the NH-Acetyl group with strongly electron-withdrawing groups has been shown to prevent the formation of oxazoline intermediates which can adversely affect glycoside bond formation.[131-139] Amine protecting groups commonly used in carbohydrate chemistry include 2,2,2-trichloroethoxycarbonyl (Troc), trichloroacetyl (TCA), phthalimide (Phth), azide (N_3), and carboxybenzyl (Cbz / Z). Less common amino protecting

groups in carbohydrate synthesis include 9-Fluorenylmethyl carbamate (Fmoc), trifluoroacetyl (TFA), and t-Butoxycarbonyl (BOC).

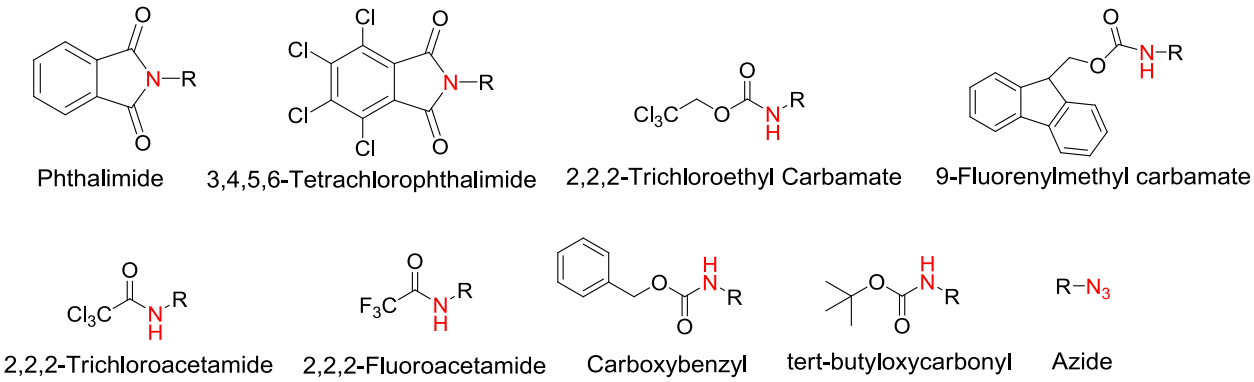


Figure 1.16: Common Amine Protecting Groups:

The phthalimide protecting group (Phth) is used for the bisprotection of primary amines (Figure 1.17). It is often utilized at the C-2 position and allows for the assembly of 1,2-transglycosidic linkages. Phthalimide is typically installed using phthalic anhydride and K_2CO_3 and be removed using a variety of conditions including treatment with hydrazine, sodium borohydride, butylamine, hydroxylamine, and ethylenediamine. The phthalimide group is stable to a wide range of conditions including acidic (HBr, HOAc), oxidative (H_2O_2, O_3, Jones oxidation), and mild transesterification conditions used to remove O-acetyl. It is typically not affected by hydrogenation, but it was reportedly converted to a lactam by hydrogenation in acetic acid over Pd/C.[140-146] The main drawback associated with the phthalimide group is its potential for ring opening in the presence of nucleophilic reagents or in basic conditions. In addition, it was shown to be susceptible to reduction in the presence of standard benzyl installation conditions with NaH in DMF.[140] Many conditions in the building block synthesis are basic and this presents a problem especially because the amine protecting group is usually installed at an early stage of the synthesis and does not get removed until after the glycosylation reactions are completed.

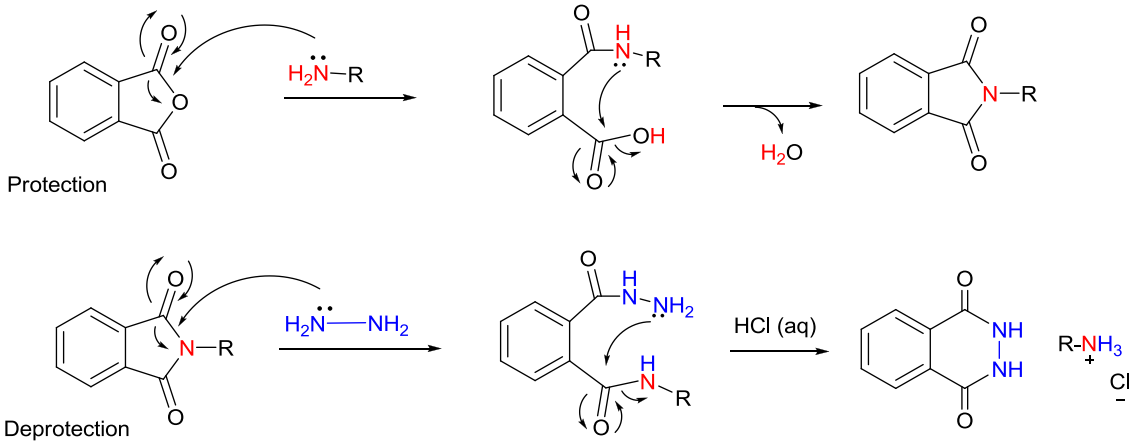


Figure 1.17: Installation and Deprotection of the Phthalamide Protecting Group.

Furthermore, the phthalamide group has been previously shown in literature to be difficult to remove and oftentimes requires harsh conditions.[147] A more base labile alternative is the tetrachlorophthaloyl (TCP). The TCP group can be installed using Tetrachlorophthaloyl anhydride and removed with ethylenediamine and DMF (1:200) at 40 °C. TCP is stable to piperidine and can be used with Fmoc.[148,149] In addition O-acetates can be removed in its presence with $Mg(OMe)_2$ in methanol.[150] Like the Phth group, TCP is not stable to standard benzyl installation conditions using NaH and BnBr in DMF.[146]

The trichloroethoxycarbonyl (Troc) group is also commonly utilized as a C-2 amine protecting group in oligosaccharide syntheses (Figure 1.18). Its use has been shown to provide 1,2-trans selectivity and high yields in both thioglycoside and trichloroacetimidate based glycosylations.[151-153]

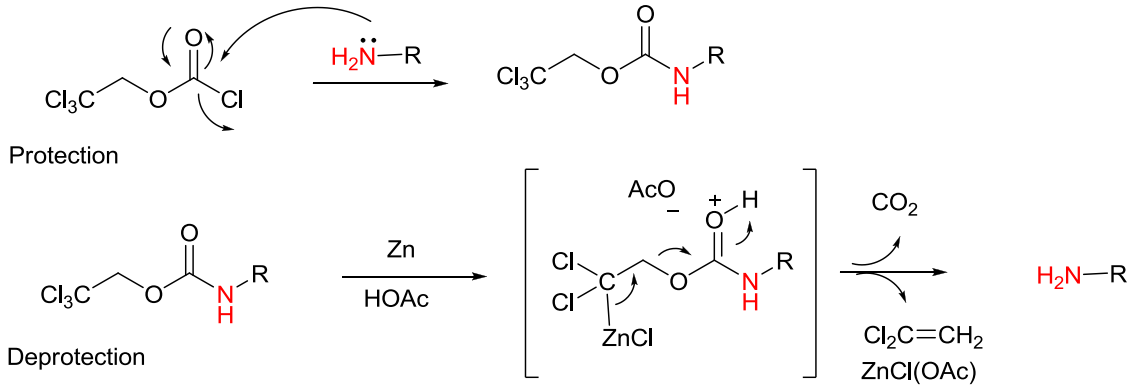


Figure 1.18: Installation and Deprotection of 2,2,2-Trichloroethyl Carbamate Protecting Group.

The Troc group can be readily installed using 2,2,2-Trichloroethyl chloroformate (Troc-Cl) in aqueous solution with potassium carbonate. Troc is typically cleaved *via* Grob fragmentation with Zn dust in the presence of acetic acid, resulting in elimination of 1,1 dichloroethylene and decarboxylation.[154] Deprotection can be performed selectively in the presence of Cbz, Boc, Fmoc, and Alloc groups.[155,156] However, It is not compatible for use with catalytic hydrogenolysis conditions.

The utility of the trichloroacetyl (TCA) group as an amino protecting group has been previously demonstrated along with its ability to produce a high degree of 1,2-trans stereoselectivity.[133] TCA is easily installed using trichloroacetyl chloride (TCA-Cl) or trichloroacetic anhydride (Figure 1.19). The options for deprotection and differentiation make the TCA group one or the more versatile amine protecting groups. It can either be cleaved under basic conditions with NaOH to afford a free amine or reduced to acetamide under neutral conditions using hydrogenolysis or reductive dehalogenation[157,158] In addition, it can be converted to a carbamate by first treating with Na_2CO_3 in DMF to form an isocyanate and then reacting with a suitable alcohol.[159]

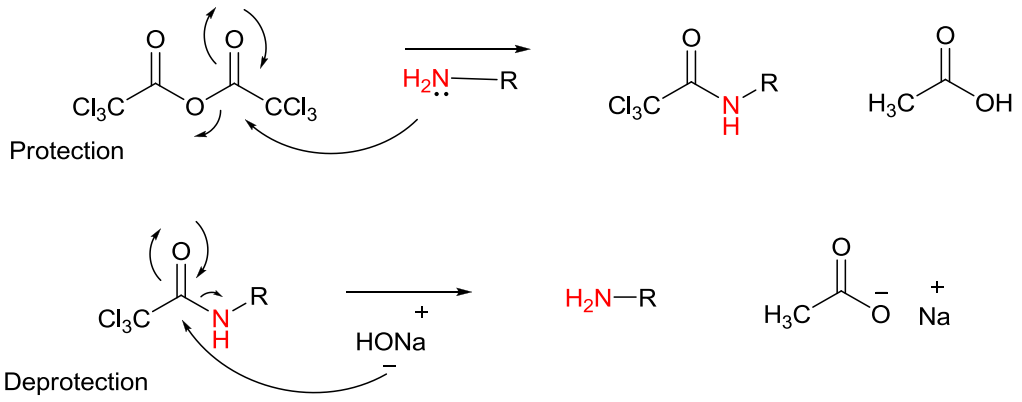


Figure 1.19: Installation and Deprotection of Trichloroacetyl Protecting Group.

Trifluoroacetyl (NH-TFA) is a more base-labile alternative to TCA.[160] The NH-TFA group is more commonly utilized in peptide chemistry due to its resistance to strong acids like trifluoroacetic acid

making it attractive since it can survive standard BOC deprotection conditions. However, the trifluoroacetyl protecting group does find occasion use in carbohydrate synthesis. NH-TFA is typically installed using trifluoroacetic anhydride (Tf_2O) and can be removed by treatment with base (0.2 N NaOH in 10 min), aqueous piperidine,[161-164] or sodium borhydride.[165] The base sensitivity of the group does preclude its use over a large number of synthetic steps.

The 9-fluorenylmethoxycarbonyl (Fmoc) goup is another amine protecting group that has found widespread use in peptide and carbohydrate chemistry. The group is easily installed in high yield using Fmoc-Cl, Et_3N, and DCM. It can be easily removed by way of β-elimination using mildy basic conditions (Figure 1.20). The fluorene group has a labile proton at its tertiary center with a pKa of 23. Loss of the proton is facilitated by the formation of dibenzocyclopentadienide anion which is aromatic in nature. The slower step involves elimination and subsequent formation of dibenzofulvene and a carbamate that readily loses CO_2 to afford the free amine. The dibenzofulvene is reactive and can readily attach to available nucleophiles making it prone to form side products.[154]

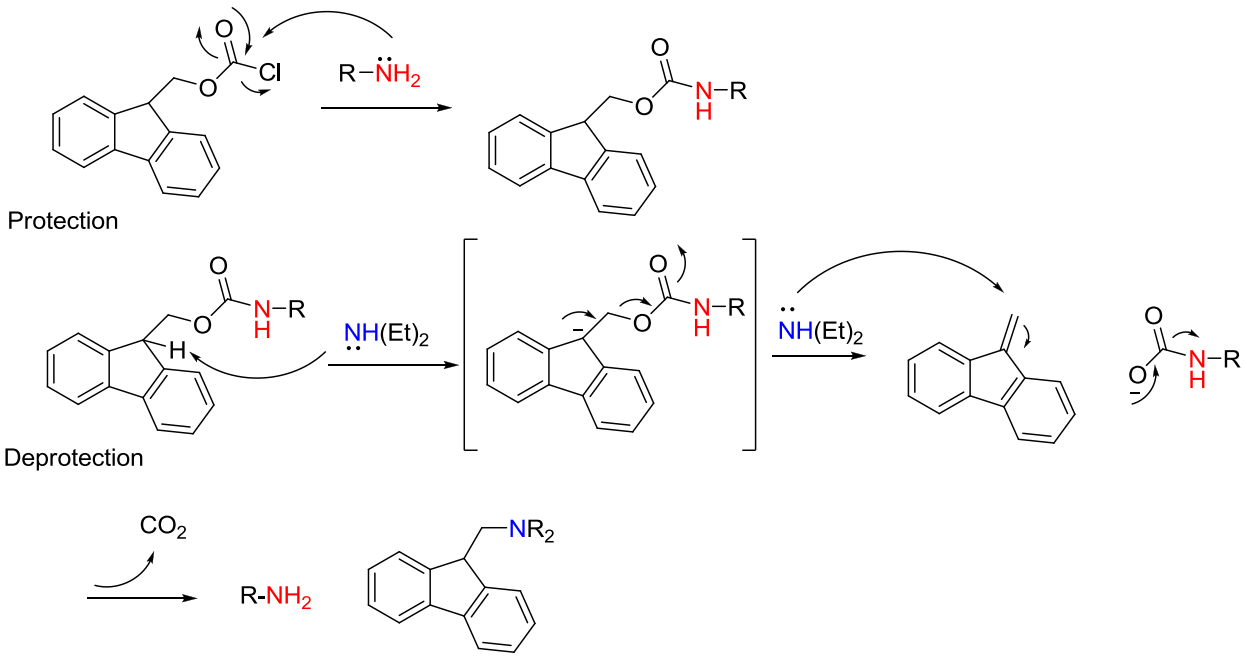


Figure 1.20: Installation and Deprotection of 9-Fluorenylmethyl carbamate Protecting Group.

Secondary amines are typically used in the reaction since they are better at capturing the dibenzofulvene generated during deprotection. Typical bases used for removal include morphiline, piperidine, and piperazine in organic solvent. Fmoc is stable to acids, however, it is not completely stable to the catalytic hydrogenation conditions typically used to remove benzyl ethers especially when Pd/C or PtO_2 are used as catalysts. Fmoc can sometimes survive treatment with Pd/BaSO4.[166-171]

The azide function can be considered a masked amino group and is commonly used to protect the amine in carbohydrate synthesis. While there are a number of known 1,2-trans-stereodirecting amine protecting groups, few non-participating protecting groups are available. For this reason, the azide group has almost exclusively been used for the installation of 1,2-cis linkages.[172-175] A number of conditions and methods can be used to install the azide group (Figure 1.21). Diazotransfer reactions using either triflyl azide or imidazole-1-sulfonyl azide in the presence of copper(II)sulfate are commonly used to install the azide.[176-179, 183] The azide can also be introduced *via* nucleophilic displacement of a halide or other leaving group.[153] Azides are relatively robust and can be reduced under a wide variety of conditions making them a common sight in many synthetic methods.

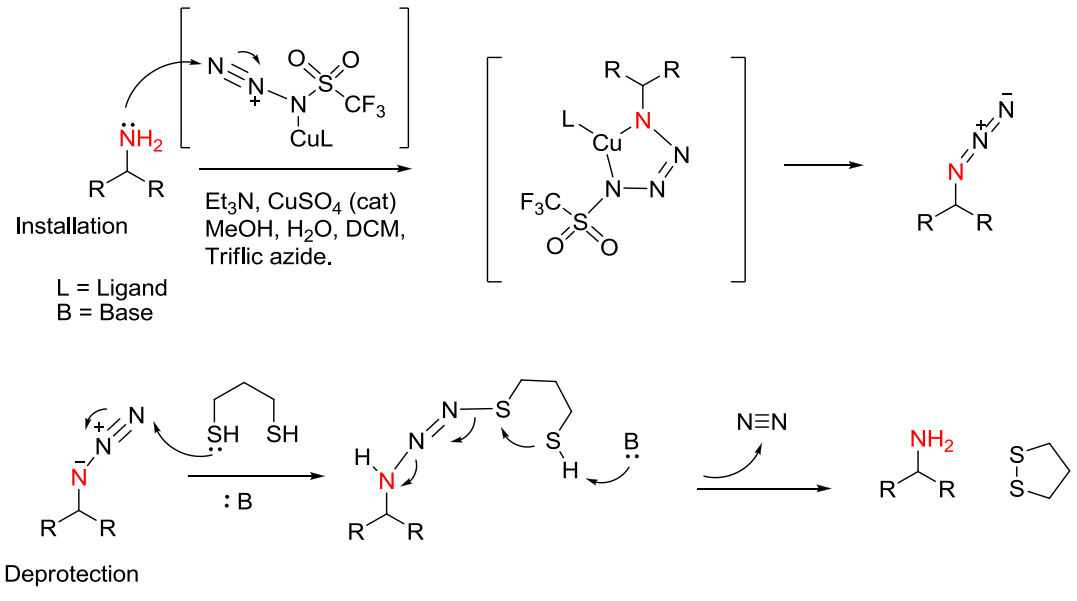


Figure 1.21: Installation and Reduction of the Azide Functional Group.

They can be easily reduced using Pd/C hydrogenation conditions. In circumstances where C=C bonds or benzyl groups are present in the same molecule, hydrogen sulfide in pyridine allows for the chemoselective reduction of azide in their presence. Azide reduction can also be achieved using Staudinger conditions, Zinc in acetic acid, Tin(II)Chloride, propanedithiol in Et_3N, and AIBN/tributyltin hydride radical reduction conditions. This is not an exhaustive list of reduction conditions as many additional methods are also available.[133,177a,b,180]

Carboxybenzyl (Cbz, Z) is a carbamate originally used as an amine protecting group in peptide synthesis, but has since found use in carbohydrate synthesis.[153] It is typically not used to protect C-2 amines due to its tendency for benzyl migration and oxazolidinone formation. Cbz can be installed with benzyl chloroformate and sodium carbonate in aqueous solution and can be removed with catalytic hydrogenation in the presence of Pd/C or by treatment with HBr (Figure 1.22).[181,182] Deprotection proceeds *via* formation of a terminal carbamic acid that is readily decarboxylated to afford the free amine.[154] It is a robust and resistant to a wide variety of conditions and is commonly utilized as a permanent protecting group.

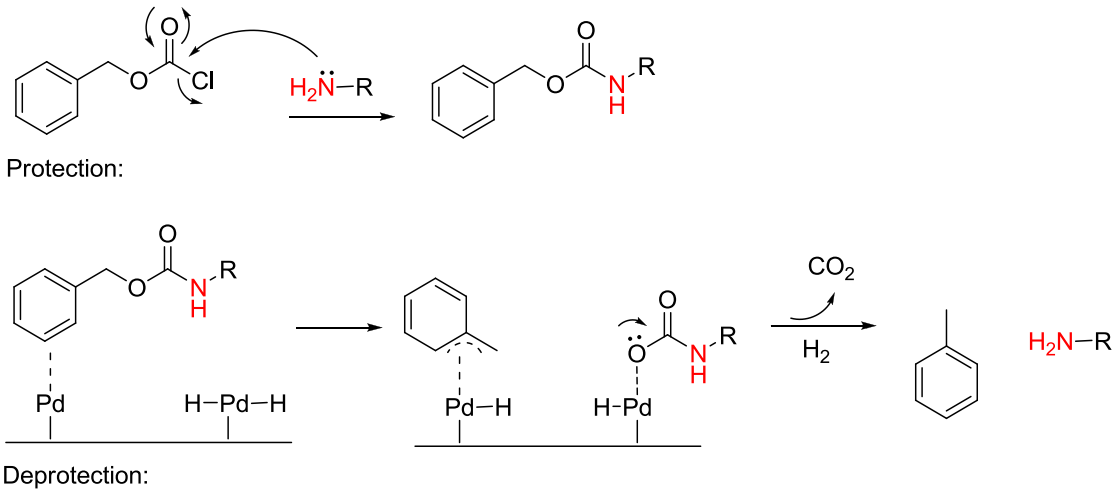


Figure 1.22: Installation and Palladium Catalyzed Hydrogenation of the Carboxybenzyl Protecting Group.

The *tert*-butyloxycarbonyl (Boc) protecting group is an amine protecting group primarily used in peptide synthesis, but has found some occasional use in carbohydrate chemistry.[183] The Boc group can be added to the amine under aqueous conditions using di-tert-butyl dicarbonate (Boc_2O) in the presence of a base such as sodium bicarbonate. Another common installation condition is Boc_2O in THF. (Figure 1.23). The Boc group is resistant to catalytic hydrogenation and stable towards most nucleophiles and bases. Therefore, an orthogonal protection strategy using a base-labile protection group such as Fmoc is possible. *Tert*-butyl carbamates are cleaved under acidic conditions (*e.g*. 25-50% TFA/DCM) with the production of tert-butyl cations. Scavengers such as thiophenol can prevent nucleophilic substrates from being alkylated.

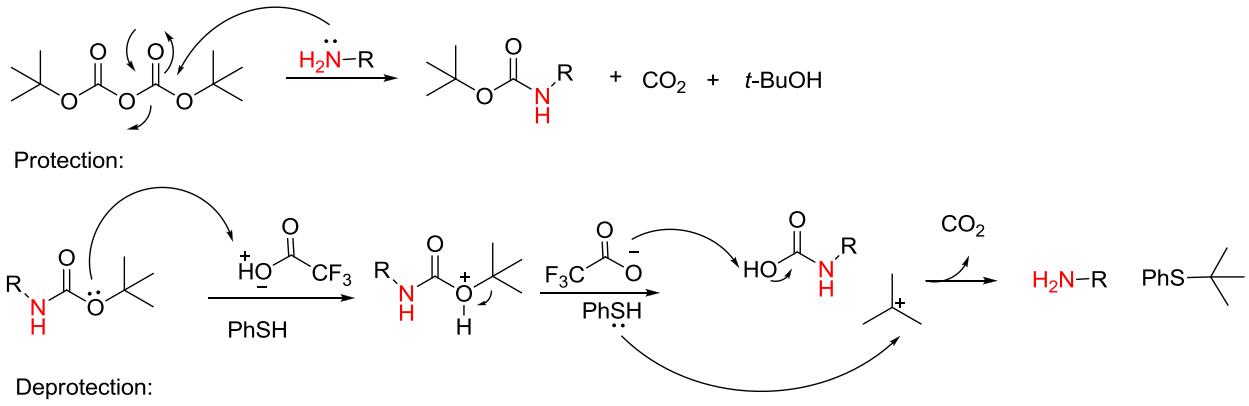


Figure 1.23: Installation and Deprotection of the tert-Butoxycarbonyl Protecting Group

CONCLUSIONS

The secondary cell wall polysaccharide of *B. anthracis* plays a key role in the organization of the envelope of vegetative cells and is intimately involved in host-guest interactions. The characterization of SCWP from *B. anthracis* and other strains from the *B. cereus* group is important and could be used for the identification of potential vaccine antigens, the generation of antibody based diagnostics, and for the study of various cell wall binding proteins. Research in this area has proven difficult due to the lack of well defined SCWP derived oligosaccharides. Naturally sourced cell wall polysaccharides occur in

complex mixtures making it difficult to determine structural features that are important for carbohydrate protein interactions. The aims of the work described in this dissertation are to optimize synthetic strategies for accessing complex oligosaccharides derived from the SCWP of *B. anthracis*. The library of compounds will be utilized to probe the molecular mechanisms of binding between SCWP structural features and various cell wall binding proteins. Genetic evidence suggests that S-layer and BSL proteins are essential for pathogenesis, cell maintenance, and nutrient uptake. A better understanding of SLH binding interactions with SCWP will contribute vital knowledge towards the design of therapeutic compounds acting to disrupt such interactions. In addition, the compounds will be used to probe the binding and activity of proteins that are believed to play a role in SCWP assembly. Certain structural modifications, such as O-acetylation, are thought to play a role in the site specific deposition of particular S-Layer and BSL proteins. Better understanding of these carbohydrate protein interactions will provide insight on how the cell modulates protein binding on the outer envelope and could lead to new ideas about how we may regulate these processes by external means.

REFERENCES

1) Keppie, J.; Smith, H.; Harris-Smith, W.: *The chemical basis of the virulence of Bacillus anthracis. II. Some biological properties of bacterial products*, Vol. 34, (1953).
2) Makino, S.; Uchido, I.; Terakado, N.; Sasakawa, C.; and M. Yoshikawa. Molecular characterization and protein analysis of the cap region, which is essential for encapsulation in *Bacillus anthracis. J. Bacteriol*. **1989**, *171*, 722-730.
3) Sutcliffe, I. C.; Russell, R., R. Lipoproteins of Gram positive bacteria. *J. Bacteriol*. **1995**, *117*, 1123-1128.
4) Koch, A. L. The surface stress theory of microbial morphogenesis. *Adv. Microb Physiol*. **1983**, *24*, 301-366.
5) Shockman, G. D.; Barrett, J. F. Structure, function, and assembly of the cell walls of Gram positive bacteria. *Annu. Rev. Microbiol*. **1983**, *37***,** 501-527.
6) Vollmer, W. Structural variation in the glycan strands of bacterial peptidoglycan. *FEMS Microbiology Reviews*. **2008**, *32*(2), 287-306.
7) Schäffer, C.; Messner, P. The structure of secondary cell wall polymers: how Gram-positive bacteria stick their cell walls together. *Microbiology*. **2005**, *151***,** 643-651.
8) Swoboda J.G.; Campbell, J.; Meredith T.C.; Walker, S. Wall Teichoic Acid Function, Biosynthesis, and Inhibition. *Chembiochem : a European journal of chemical biology*. **2010**, *11*(1): 35-45.
9) Archibald, A. R.; Baddiley, J.; Blumson, N.L. The Teichoic acids. *Adv. Enzymol*. **1968**, *30***,** 223-253.
10) Archibald, A. R.; Hancock, I.C.; Harwood, C.R. Cell Wall Structure, Synthesis, and Turnover. In Sonenshein A, Hoch J, Losick R (ed), Bacillus subtilis and Other Gram-Positive Bacteria. ASM Press, Washington, DC, 381-410 (1993).

11) Fischer, W. Lipoteichoic acid and lipids in the membrane of *Staphylococcus aureus*. *Med Microbiol Immunol*. **1994**, *183,* 61-76.

12) Hancock, I. C.; Baddiley, J**.** Biosynthesis of the bacterial envelope polymers teichoic acid and teichuronic acid, 2nd ed, vol. 2. Plenum, New York (1985).

13) Ward, J. B. Teichoic and teichuronic acids: Biosynthesis, assembly, and location. *Microbiol. Rev*. **1981**, *45*, 211-243.

14) Araki, Y.; Ito., E. Linkage units in cell walls of Gram-positive bacteria. *CRC Crit. Rev. Microbiol*. **1989**, *17***,** 121-135.

15) Naumova, I. B.; Shashkov, A.S. Anionic polymers in cell walls of Gram positive bacteria. *Biochemistry*. **1997**, *62,* 809-840.

16) Messner, P.; Schäffer, C.; Kosma, P. Bacterial Cell-Envelope Glycoconjugates. *Advances in carbohydrate chemistry and biochemistry*. **2013**, *69*, 209–272.

17) Beveridge, T. J., Graham, L.L. Surface layers of bacteria. *Microbiol. Mol. Biol. Rev*. **1991**, *55***,** 684-705.

18) Messner, P.; Sleytr, U.B. Crystalline bacterial cell surface layers. *Adv. Microb Physiol*. **1992**, *33***,** 212-275.

19) Sleytr, U. B.; Messner, P.; Pum, D.; Sara, M. Crystalline Bacterial Cell Surface Layer Proteins. R.G. Landes / Academic Press, Austin, TX (1996).

20) Ahn, J. S., Chandramohan, I.; Liou, L.E.; Bayles, K.W. Characterization of CidR-mediated regulation in *Bacillus anthracis* reveals a previously undetected role of S-layer proteins as murein hydrolases. *Mol. Microbiol*. **2006**, *62*, 1158-1169.

21) Kern, J. W.; Schneewind, O. BslA, a pXO1 encoded adhesin of *Bacillus anthracis*. *Mol. Microbiol*. **2008**, *68*, 504-515.

22) Sleytr, U. B**.** Basic and applied S-layer research: an overview. *FEMS Microbiol. Rev*. **1997** *20*, 5-12.

23) Blaser, M. J.; Pei, Z. Pathogenesis of Campylobacter fetus infections: critical role of high-molecular weight S-layer proteins in virulence. *J. Infect. Dis*. **1993**, *167*, 372-377.

24) Kay, W. W., Trust, T.J. Form and functions of the regular surface array (S-layer) of *Aeromonas salmonicida*. *Experientia*. **1991**, *47*, 412-414.

25) Kotiranta, A. H. M; Kari, K; Kerosuo, O; Olsen, I; Sorsa, T; Meurman, JH; Lounatmaa, K. Surface structure, hydrophobicity, phagocytosis and adherence to matrix proteins of *B. cereus* cells with and without the crystalline surface protein layer. *Infect Immun*. **1998**, *66*, 4895-4902.

26) Agata, N.; Mori, M.; Ohta, M.; Suwan, S**.** A novel dodecadepsipeptide, cereulide, isolated from *Bacillus cereus* causes vauole formation in HEp-2 cells. *FEMS Microbiol Lett*. **1994**, *121*, 31-34.

27) Uchida, I. M. S; Sasakawa, C; Yoshikawa, M; Sugimoto, C; Terakado, N. Identification of a novel gene dep, associated with depolimerization of the capsular polymer in *Bacillus anthracis*. *J Gen Microbiol*. **1993**, *131*, 363-367.

28) Ada, G., Isaacs, D. Carbohydrate-protein conjugate vaccines. *Clin Microbiol. Infect*. **2003**, *9*, 79-85.

29) Ahn, J. S.; Chandramohan, I; Liou, L.E.; Bayles, K.W. Characterization of CidR-mediated regulation in *Bacillus anthracis* reveals a previously undetected role of S-layer proteins as murein hydrolases. *Mol. Microbiol*. **2006**, *62*, 1158-1169.

30) Fairweather, N. F., Fagan, R. P. Biogenesis and functions of bacterial S‐layers. *Nature Reviews. Microbiology*. **2014**, *12*(3): 211-22.

31) Fekete, T.; Bacillus species and related genera other than Bacillus anthracis. In: Principles and Practice of Infectious Diseases, Mandell GL, Bennett JE, Dolin RM (Eds), Churchill Livingston, New York City, 2727 (2010).

32) Drobniewski F.A. *Bacillus cereus* and related species. *Clin Microbiol Rev*. **1993**, *6*, 324-638.

33) Baillie, L.; Read T.D.; *Bacillus anthracis*: a bug with attitude. *Curr Opin Microbiol.* **2001**, *4*, 78–81.

34) Griffiths, M. Understanding Pathogen Behaviour: Virulence, Stress Response and Resistance. CRC Press (2005).

35) Hanna P.C.; Ireland J.A. Understanding *Bacillus anthracis* pathogenesis. *Trends. Microbiol*. **1999**, *7*(5), 180-182.

36) Mock M.; Fouet A. Anthrax. *Annu. Rev*. *Microbiol*. **2001**, *55*, 647-671.

37) Bottone E.J. *Bacillus cereus*, a volatile human pathogen. *Clin. Microbiol. Rev.* **2010**, *23*(2), 382-398.

38) Pexara A.; Govaris A. *Bacillus cereus*: an important foodborne pathogen. *J. Hellenic Vet. Med. Soc.* **2010**, *61*(2), 127-133.

39) Romeis J.; Meissle M.; Bigler F.; Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol*. **2006**, *24*(1), 63-71.

40) Nielsen-LeRoux C.; Gaudriault S.; Ramarao N.; Lereclus D.; Givaudan A.; How the insect pathogen bacteria Bacillus thuringiensis and Xenorhabdus/Photorhabdus occupy their hosts. *Curr. Opin. Microbiol*. **2012**, *15*(3), 220-231.

41) Weiner Z.P.; Glomski I.J.; Updating perspectives on the initiation of *Bacillus anthracis* growth and dissemination through its host. *Infect. Immun.* **2012**, *80*(5), 1626-1633.

42) Brachmann, P. Inhalation anthrax. *Ann. NY Acad*. *Sci*. **1980**, 353: 83-93.

43) Dixon, T.; Meselson, M.; Guillemin, J.; Hanna, P. Anthrax. *N. Engl. J. Med*. **1999**, *341*, 815-826.

44) Mock, M.; Fouet, A. Anthrax. *Annual Review of Microbiology.* **2001**, *55*, 647-671.

45) Okinaka, R. T.; Cloud, K.; Hampton, O.; Hoffmaster, A.R.; Hill, K.K.; Keim, P.; Koehler, T.M.; Lamke, G.; Kumano, S.; Mahillon, J.; Manter, D.; Martinez, Y.; Ricke, D.; Svensson, R.; Jackson, P.J. Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. *J Bacteriol.* **1999**, *181***,** 6509-6515.

46) Smith, H., Williams, A.E.; Pearce, J.H.; Keppie, J.; Harris-Smith, P.W.; Fitsgeorge, R.B.; Witt, K. The chemical basis of the virulence of *Bacillus anthracis*. VII. Two components of the anthrax toxin: their relationship to known immunizing aggressins. *Br J Exp Pathol.* **1956**, *37*, 263-271.

47) Welkos, S. L. Plasmid-associated virulence factors of non-toxigenic (pX01-) *Bacillus anthracis*, *Microbial Pathogenesis*. **1991**, *10*, 183-198.

48) Jernigan J.A., Stephens D.S., Ashford D.A., Omenaca C., Topiel M.S., Galbraith M., Tapper M., Fisk T.L., Zaki S., Popovic T., Meyer R.F., Quinn C.P., Harper S.A., Fridkin S.K., Sejvar J.J., Shepard C.W., McConnell M., Guarner J., Shieh W.J., Malecki J.M., Gerberding J.L., Hughes J.M. & Perkins B.A. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg. Infect. Dis.* **2001**, *7*(6), 933-944.

49) Jernigan D.B., Raghunathan P.L., Bell B.P., Brechner R., Bresnitz E.A., Butler J.C., Cetron M., Cohen M., Doyle T., Fischer M., Greene C., Griffith K.S., Guarner J., Hadler J.L., Hayslett J.A., Meyer R., Petersen L.R., Phillips M., Pinner R., Popovic T., Quinn C.P., Reefhuis J., Reissman D., Rosenstein N., Schuchat A., Shieh W.J., Siegal L., Swerdlow D.L., Tenover F.C., Traeger M., Ward J.W., Weisfuse I., Wiersma S., Yeskey K., Zaki S., Ashford D.A., Perkins B.A., Ostroff S., Hughes J., Fleming D., Koplan J.P. & Gerberding J.L. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. *Emerg. Infect. Dis.* **2002**, *8*(10): 1019-1028.

50) Webb G.F. A silent bomb: the risk of anthrax as a weapon of mass destruction. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*(8), 4355-4356.

51) Irenge L.M., Gala J.L. Rapid detection methods for *Bacillus anthracis* in environmental samples: a review. *Appl. Microbiol. Biotechnol.* **2012**, *93*(4), 1411-1422.

52) Fouet A. The surface of *Bacillus anthracis*. *Mol. Aspects. Med.* **2009**, *30*(6), 374-385.

53) Choudhury B., Leoff C., Saile E., Wilkins P., Quinn C.P., Kannenberg E.L., Carlson R.W. The structure of the major cell wall polysaccharide of *Bacillus anthracis* is species-specific. *J. Biol. Chem.* **2006**, *281*(38), 27932-27941.

54) Forsberg L.S., Abshire T.G., Friedlander A., Quinn C.P., Kannenberg E.L. & Carlson R.W. Localization and structural analysis of a conserved pyruvylated epitope in *Bacillus anthracis* secondary cell wall polysaccharides and characterization of the galactose-deficient wall polysaccharide from avirulent *B. anthracis* CDC 684. *Glycobiology.* **2012**, *22*(8), 1103-1117.

55) Mesnage S, Fontaine T, Mignot T, Delepierre M, Mock M, Fouet A. Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *The EMBO Journal*. **2000**, *19*(17), 4473-4484.

56) Leoff C., Choudhury B., Saile E., Quinn C.P., Carlson R.W., Kannenberg E.L. Structural elucidation of the nonclassical secondary cell wall polysaccharide from *Bacillus cereus* ATCC 10987. Comparison with the polysaccharides from *Bacillus anthracis* and *B. cereus* type strain ATCC 14579 reveals both unique and common structural features. *J. Biol. Chem.* **2008**, *283*(44), 29812-29821.

57) Leoff C., Saile E., Rauvolfova J., Quinn C.P., Hoffmaster A.R., Zhong W., Mehta A.S., Boons G.J., Carlson R.W. & Kannenberg E.L. Secondary cell wall polysaccharides of *Bacillus anthracis* are antigens that contain specific epitopes which cross-react with three pathogenic *Bacillus cereus* strains that caused severe disease, and other epitopes common to all the *Bacillus cereus* strains tested. *Glycobiology.* **2009**, *19*(6), 665-673.

58) Forsberg L.S., Choudhury B., Leoff C., Marston C.K., Hoffmaster A.R., Saile E., Quinn C.P., Kannenberg E.L., Carlson R.W. Secondary cell wall polysaccharides from *Bacillus cereus* strains G9241, 03BB87 and 03BB102 causing fatal pneumonia share similar glycosyl structures with the polysaccharides from *Bacillus anthracis*. *Glycobiology.* **2011**, *21*(7), 934-948.

59) Sara M., Sleytr U.B. S-Layer proteins. *J. Bacteriol.* **2000**, *182*(4), 859-868.

60) Albers S.V., Meyer B.H. The archaeal cell envelope. *Nat. Rev. Microbiol.* **2011**, *9*(6), 414-426.

61) Ilk N., Egelseer E.M., Sleytr U.B. S-layer fusion proteins--construction principles and applications. *Curr. Opin. Biotechnol.* **2011**, *22*(6), 824-831.

62) Etienne-Toumelin I., Sirard J.C., Duflot E., Mock M. & Fouet A. Characterization of the *Bacillus anthracis* S-layer: cloning and sequencing of the structural gene. *J. Bacteriol.* **1995**, *177*(3), 614-620.

63) Mesnage S., Tosi-Couture E., Mock M., Gounon P., Fouet A. Molecular characterization of the *Bacillus anthracis* main S-layer component: evidence that it is the major cell-associated antigen. *Mol. Microbiol.* **1997**, *23*(6), 1147-1155.

64) Mesnage S., Fontaine T., Mignot T., Delepierre M., Mock M. & Fouet A. Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *EMBO J.* **2000**, *19*(17), 4473-4484.

65) Schaffer C., Messner P. The structure of secondary cell wall polymers: how Gram-positive bacteria stick their cell walls together. *Microbiology.* **2005**, *151*(Pt 3), 643-651.

66) Kern J., Ryan C., Faull K., Schneewind O. *Bacillus anthracis* surface-layer proteins assemble by binding to the secondary cell wall polysaccharide in a manner that requires csaB and tagO. *J. Mol. Biol.* **2010**, *401*(5), 757-775.

67) Kern V.J., Kern J.W., Theriot J.A., Schneewind O., Missiakas D. Surface-layer (S-layer) proteins Sap and EA1 govern the binding of the S-layer-associated protein BslO at the cell septa of *Bacillus anthracis*. *J. Bacteriol.* **2012**, *194*(15), 3833-3840.

68) Kern J.W. & Schneewind O. BslA, a pXO1-encoded adhesin of *Bacillus anthracis*. *Mol. Microbiol.* **2008**, *68*(2), 504-515.

69) Tarlovsky Y., Fabian M., Solomaha E., Honsa E., Olson J.S., Maresso A.W. A *Bacillus anthracis* S-layer homology protein that binds heme and mediates heme delivery to IsdC. *J. Bacteriol.* **2010**, *192*(13), 3503-3511.

70) Anderson V.J., Kern J.W., McCool J.W., Schneewind O., Missiakas D. The SLH-domain protein BslO is a determinant of *Bacillus anthracis* chain length. *Mol. Microbiol.* **2011**, *81*(1), 192-205.

71) Wang Y.T., Oh S.Y., Hendrickx A.P., Lunderberg J.M., Schneewind O. *Bacillus cereus* G9241 S-layer assembly contributes to the pathogenesis of anthrax-like disease in mice. *J. Bacteriol.* **2013**, *195*(3), 596-605.

72) Fujino T., Beguin P., Aubert J.P. Organization of a *Clostridium thermocellum* gene cluster encoding the cellulosomal scaffolding protein CipA and a protein possibly involved in attachment of the cellulosome to the cell surface. *J. Bacteriol.* **1993**, *175*(7), 1891-1899.

73) Lupas A., Engelhardt H., Peters J., Santarius U., Volker S. & Baumeister W. Domain structure of the *Acetogenium kivui* surface layer revealed by electron crystallography and sequence analysis. *J. Bacteriol.* **1994**, *176*(5), 1224-1233.

74) Nguyen-Mau, S. M., Oh, S. Y., Kern, V. J., Missiakas, D. M. & Schneewind, O. Secretion genes as determinants of *Bacillus anthracis* chain length. *J. Bacteriol*. **2012**, *194*, 3841–3850.

75) Allison, G. E., and N. K. Verma. Serotype-converting bacteriophages and O-antigen modification in Shigella flexneri. *Trends Microbiol*. **2000**, *8*, 17-23.

76) Mignot, T., B. Denis, E. Couture-Tosi, A.-B. Kolsto, M. Mock, and A. Fouet. Distribution of S-layers on the surface of *Bacillus cereus* strains: phylogenetic origin and ecological pressure. *Environ. Microbiol*. **2001**, *3*, 493-501.

77) Venezia, R. A., S. Ryan, S. Alward, and W. A. Koston. Evaluation of a rapid method for the detection of streptococcal group A antigen directly from throat swabs. *J. Clin. Microbiol*. **1985**, *21*, 395-398.

78) Weintraub, A. Immunology of bacterial polysaccharide antigens. *Carbohydr. Res*. **2003**, *338*, 2539-2547.

79) Makino, S., I. Uchido, N. Terakado, C. Sasakawa, and M. Yoshikawa. Molecular characterization and protein analysis of the cap region, which is essential for encapsulation in *Bacillus anthracis*. *J. Bacteriol*. **1989**, *171*, 722-730.

80) Novak, R. T., Hoffmaster, A.R., and Wilkins, P.P.. 2005. Abstr. 105th Gen. Meet. Am. Soc. Microbiol., abstr. C277. American Society for Microbiology, Washington, DC. (2005)

81) Clark J.R. & March J.B. Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *Trends Biotechnol*. **2006**, *24*(5), 212-218.

82) Haase, C.; Seitz, O. Glycopeptides and Glycoproteins: Synthesis, Structure, and Application, 267: (2007).

83) Muthana, S.; Cao, H.; Chen, X. Recent progress in chemical and chemoenzymatic synthesis of carbohydrates. Invited review. *Curr. Opin. Chem. Biol*. **2009**, *13*, 573-581.

84) Zhu, X. M.; Schmidt, R. R. New principles for glycoside-bond formation. *Angew. Chem., Int. Ed.* **2009**, *48*, 1900.

85) Boons, G. J. Strategies in oligosaccharide synthesis. *Tetrahedron.* **1996**, *52*, 1095-1121.

86) Arungundram, S.; Al-Mafraji, K.; Asong, J.; Leach, F. E., 3rd; Amster, I. J.; Venot, A.; Turnbull, J. E.; Boons, G. J. Modular synthesis of heparan sulfate oligosaccharides for structure-activity relationship studies. *J. Am. Chem. Soc.* **2009**, *131*(47), 17394.

87) Zhang, Y.; Gaekwad, J.; Wolfert, M. A.; Boons, G. J. Modulation of innate immune responses with synthetic lipid A derivatives. *J. Am. Chem. Soc.* **2007**, *129*, 5200.

88) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R.; Palmacci, E. R.; Seeberger, P.H. Modular Synthesis of Heparin Oligosaccharides. *Chem. Eur. J.* **2003**, *9*, 140.

89) Tanaka, H.; Yamada, H.; Takahashi, T. *Trends in Glycoscience and Glycotechnology.* **2007**, *19*,183.

90) Zhu, X. M.; Schmidt, R. R. Enzymes in the synthesis of glycoconjugates. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900.

91) Codee, J. D. C., van den Bos, L. J.; Litjens, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. Sequential one-pot glycosylations using 1-hydroxyl and 1-thiodonors. *Org. Lett.* **2003**, *5*, 1947.

92) Wasonga, G.; Zeng, Y. L.; Huang, X. F. *Sci. Chi. Chem.* **2011**, *54*, 66.

93) Wang, Z.; Xu, Y. M.; Yang, B.; Tiruchinapally, G.; Sun, B.; Liu, R. P.; Dulaney, S.; Liu, J. A.; Huang, X. F. Preactivation-based, one-pot combinatorial synthesis of heparin-like hexasaccharides for the analysis of heparin-protein interactions. *Chem. Eur. J.* **2010**, *16*, 8365.

94) Sun B, Srinivasan B, Huang X. Pre-activation-based one-pot synthesis of an alpha-(2,3)-sialylated core-fucosylated complex type bi-antennary N-glycan dodecasaccharide. *Chemistry Eur J*. **2008**, *14*(23), 7072–7081.

95) Wang, C. C.; Lee, J. C.; Luo, S. Y.; Kulkarni, S. S.; Huang, Y. W.; Lee, C. C.; Chang, K. L.; Hung, S. C. Regioselective one-pot protection of carbohydrates. *Nature*. **2007**, *446*, 896.

96) Francais, A.; Urban, D.; Beau, J. M. Tandem catalysis for a one-pot regioselective protection of carbohydrates: the example of glucose. *Angew. Chem. Int. Ed.* **2007**, *46*, 8662.

97) Chang K.-L., Zulueta M. M. L., Lu X.-A., Zhong Y.-Q. & Hung S.-C. Regioselective one-pot protection of D-glucosamine. *J. Org. Chem*. **2010**, *75*, 7424–7427.

98) Jones, R. A.; Davidson, R.; Tran, A. T.; Smith, N.; Galan, M. C. *Carb. Res.* **2010**, *345*, 1842.

99) Ernst, B.; Hart, G. W.; Sinay, P. In Carbohydrate in Chemistry and Biology; Ernst, B.; Hart, G. W.; Sina, P., Eds.; Wiley-VCH Verlag GmbH: Weinheim, Germany, (2000).

100) Ranade, S. C.; Demchenko, A. V. J. Carbohydr. Chem. **2013**, *32*, 1–43.

101) Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C. *Proc. Natl. Acad. Sci*. **2004**, *101*: 17033.

102) Chi-Huey Wong, Carbohydrate-based Drug Discovery. John Wiley & Sons, p 306 (2006)

103) Cuevas, E. Juaristi, G.. The anomeric effect. Boca Raton: CRC Press. (1995)

104) Crich, D., Dai, Z., Gastaldi, S. On the Role of Neighboring Group Participation and Ortho Esters in β-Xylosylation: ^{13}C NMR Observation of a Bridging 2-Phenyl-1,3-dioxalenium Ion *J. Org. Chem.* **1999**, *64*(14), 5224-5229.

105) Paulsen, H.; Herold, C. P. *Chem. Ber. Recl*. **1970**, *103*, 2450.

106) Demchenko, A. V. ; Rousson, E. ; Boons, G. J. Stereoselective 1,2-cis-galactosylation assisted by remote neighboring group participation and solvent effects. *Tetrahedron Lett*. **1999**, *40*: 6523-6526.

107) Mukaiyama, T.; Suenaga, M.; Chiba, H.; Jona, H. *Chem. Lett*. **2002**, 56-57.

108) Cheng, Y. P.; Chen, H. T.; Lin, C. C. *Tetrahedron Lett*. **2002**, *43*, 7721.

109) De Meo, C.; Kamat, M. N.; Demchenko, A. V. Remote Participation-Assisted Synthesis of β-Mannosides. *Eur. J. Org. Chem.* **2005**, *4*, 706-711.

110) Vanboeckel, C. A. A.; Beetz, T.; Vanaelst, S. F. Substituent effects on carbohydrate coupling reactions promoted by insoluble silver salts. *Tetrahedron.* **1984**, *40*, 4097.

111) Smid, P.; Deruiter, G. A.; Vandermarel, G. A.; Rombouts, F. M.; Vanboom, J. H. Iodorium-ion assisted stereospecific glycosylation synthesis of oligosaccharides containing α(1→4)-linked l-glycopyranosyl units. *J. Carb. Chem*. **1991**, *10*, 833.

112) Ustyuzhanina, N.; Komarova, B.; Zlotina, N.; Krylov, V.; Gerbst, A.; Tsvetkov, Y.; Nifantiev, N. *Synlett*. **2006**, 921-923.

113) Crich, D.; Hu, T. S.; Cai, F. Does Neighboring Group Participation by Non-Vicinal Esters Play a Role in Glycosylation Reactions? Effective Probes for the Detection of Bridging Intermediates. *J. Org. Chem*. **2008**, *73*(22), 8942.

114) Baek, J. Y.; Lee, B. Y.; Jo, M. G.; Kim, K. S. Beta-directing effect of electron-withdrawing groups at O-3, O-4, and O-6 positions and alpha-directing effect by remote participation of 3-O-acyl and 6-O-acetyl groups of donors in mannopyranosylations. *J. Am. Chem. Soc*. **2009**, *131*(48), 17705.

115) Baek, J. Y.; Lee, B. Y.; Jo, M. G.; Kim, K. S. β-Directing Effect of Electron-Withdrawing Groups at O-3, O-4, and O-6 Positions and α-Directing Effect by Remote Participation of 3-O-Acyl and 6-O-Acetyl Groups of Donors in Mannopyranosylations. *J. Am. Chem. Soc*. **2010**, *132*(20), 7229.

116) Demchenko, A. 1,2-cis O-Glycosylation: Methods, Strategies, Principles. *Curr. Org. Chem*. 2003, *7*(1), 35–79.

117) Kim, J. H. ; Yang, H. ; Boons, G. J. Stereoselective glycosylation reactions with chiral auxiliaries. *Angew Chem Int Ed Engl*. **2005**, *44*, 947-949.

118) Kim, J. H. ; Yang, H. ; Khot, V. ; Whitfield, D. ; Boons, G. J. Stereoselective glycosylations using (R)- or (S)-(ethoxycarbonyl)benzyl chiral auxiliaries at C-2 of glycopyranosyl donors. *Eur. J. Org. Chem*. **2006**, *22,* 5007-5028.

119) Kim, J. H.; Yang, H.; Park, J.; Boons, G. J. A general strategy for stereoselective glycosylations. *J. Am. Chem. Soc.* **2005**, *127*(34), 12090.

120) Kim, J. H. ; Yang, H. ; Khot, V. ; Whitfield, D. ; Boons, G. J. Stereoselective glycosylations using (R)- or (S)-(ethoxycarbonyl)benzyl chiral auxiliaries at C-2 of glycopyranosyl donors. *Eur. J. Org. Chem*. **2006**, *22*, 5007-5028.

121) Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges*, **1901**, *34*, 957.

122) Mukaiyama, T.; Murai, Y.; Shoda, S. An Efficient Method For Glucosylation Of Hydroxy Compounds Using Glucopyranosyl Fluoride. *Chem. Lett*. **1981**, *10*(3), 431.

123) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. Glycosylation of unreactive substrates. *J. Am. Chem. Soc*. **1989**, *111*(17), 6881.

124) Crich, D.; Sun, S. Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates. *Tetrahedron*. **1998**, *54*(29): 8321–8348.

125) Fraserreid, B.; Wu, Z. F.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. Torsional effects in glycoside reactivity: saccharide couplings mediated by acetal protecting groups. *J. Am. Chem. Soc*. **1991**, *113*, 1434.

126) Bochkov A.F., Zaikov G.E. Chemistry of the O-glycosidic bond: formation and cleavage. Pergamon Press; Oxford - New York - Toronto - Sydney - Paris - Frankfurt: (1979).

127) Banoub, J.; Boullanger, P.; Lafont, D. Synthesis of oligosaccharides of 2-amino-2-deoxy sugars. *Chem. Rev*. **1992**, *92*, 1167-1195.

128) Lafont, D.; Boullanger, P. J. Syntheses of N-Acyl and N-Alkoxycarbonyl Derivatives of 2-Alkoxycarbonylamino-2-deoxy-D-glucose. *Carbohydr. Chem*. **1992**, *11*: 567-586.

129) Xolin, A., Stévenin, A., Pucheault, M., Norsikian, S., Boyer F.D., Beau, J.M. Glycosylation with N-acetyl glycosamine donors using catalytic iron(III) triflate: from microwave batch chemistry to a scalable continuous-flow process. *Org. Chem. Front*. **2014**, *1*, 992-1000.

130) Christensen, H., Christiansen, M.S., Petersena, J., Jensen H.H., Direct formation of β-glycosides of N-acetyl glycosamines mediated by rare earth metal triflates. *Org. Biomol. Chem*. **2008**, *6*, 3276-3283.

131) Wolfrom, M. L.; Conigliaro, P. Trifluoroacetyl as an N-Protective Group in the Synthesis of Purine Nucleosides of 2-Amino-2-Deoxy-Saccharides. *J. Carbohydr. Res*. 1969, *11*, 63-76.

132) Israel, M.; Murray, R. J. Adriamycin analogs. 1. Preparation and antitumor evaluation of 7-O-(.beta.-D-glucosaminyl)daunomycinone and 7-O-(.beta.-D-glucosaminyl)adriamycinone and their N-trifluoroacetyl derivatives *J. Med. Chem*. **1982**, *25*, 24-28.

133) Blatter, G.; Beau, J. M.; Jacquinet, J. C. The use of 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives in syntheses of oligosaccharides. *Carbohydr. Res*. **1994**, *260*, 189-202.

134) Donohoe, T. J.; Logan, J. G.; Laffan, D. D. P. Trichloro-oxazolines as Activated Donors for Aminosugar Coupling. *Org. Lett.* **2003**, *5*, 4995-4998.

135) Imoto M., Yoshimura H., Shimamoto T., Sakaguchi N., Kusumoto S., Shiba T. Total synthesis of Escherichia coli lipid A, the endotoxically active principle of cell-surface lipopolysaccharide. *Bull Chem Soc Jpn*. **1987**, *60*, 2205-2214.

136) Paulsen, H.; Krogmann, C. *Liebigs Ann. Chem*. **1989**, 1203-1213.

137) Qiu, D.; Koganty, R. R. *Tetrahedron Lett.* **1997**, *38*, 45-48.

138) Nilsson, K. G. I. Glycosidase-Catalysed Synthesis of Di- and Trisaccharide Derivatives Related to Antigens Involved in the Hyperacute Rejection of Xenotransplants. *Tetrahedron Lett*. **1997**, *38*, 133-136.

139) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. N-trichloroethoxycarbonyl-glucosamine derivatives as glycosyl donors. *Carbohydr. Res*. **1996**, *296*, 135-147.

140) Meng, X.-B.. Unexpected reductions of glycosyl spacer-armed phthalimides. *Carbohydrate Res*. **2004**, *339*, 1497-1501.

141) Bundle D.R., and Josephson, S., Artificial carbohydrate antigens: synthesis of rhamnose disaccharides common to ShigellaflexneriO-antigen determinants. *Can. J. Chem*. **1979**, *57*(6), 662.

142) Durette P.L., Meitzner E.P., and Shen, T.Y. Bacterial cell wall constituents. II.1 synthesis of O-(N-acetyl-β-muramyl-L-alanyl-D-isoglutamine)-(1→4)-N-acetyl-D-glucosamine *Tetrahedron Lett*. **1979**, *42*, 4013.

143) Madsen, R., Udodong, U.E., Roberts, C., Mootoo, D.R., Konradsson, P., and Fraser-Reid, B., *J. Am. Chem. Soc*. **1995**, *117*, 1554.

144) Osby J.O., Martin M.G., and Ganem, B., An exceptionally mild deprotection of phthalimides. *Tetrahedron Lett*. **1984**, *25*, 2093-2096.

145) Stangier P., and Hindsgaul O., Solid-Phase Transimidation for the Removal of N-Phthalimido- and N-Tetrachlorophthalimido Protecting Groups on Carbohydrates. *Synlett,* **1996**, *2*, 179-181.

146) Geert-Jan Boons, Karl J. Hale, Organic Synthesis with Carbohydrates, John Wiley & Sons, p 52 (2008).

147) Huang, L.; Lu, X.; Huang, X.: Chemical syntheses of hyaluronic acid oligosaccharides. *ACS Symp. Ser.* **2008**, *990*, 29-53.

148) Cros E., Solid-phase synthesis of C-terminal peptide amides from N-tetrachlorophthaloyl protected amino acids. *Tetrahedron Lett*. **2001**, *42*, 610-6107.

149) Monroc S., Feliu L., Serra J., Planas M., Bardají E. Synthesis and Solid-Phase Applications of N-Tetrachlorophthaloyl (TCP) Side-Chain-Protected Amino Acids. *Synlett,* **2006**, *17,* 2743.

150) Qin Z.H., Meng H.L., Zhong C.S., Li J. Selective De-O-acetylation in TCP-containing Carbohydrate Structures with Magnesium Methoxide. *Chin. Chem Lett*. **2000**, *11*, 941.

151) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. N-trichloroethoxycarbonyl-glucosamine derivatives as glycosyl donors. *Carbohydr. Res*. **1996**, *296***,** 135-147.

152) Bryan K. S. Yeung , Daniel C. Hill , Maria Janicka , and Peter A. Petillo, Synthesis of Two Hyaluronan Trisaccharides, *Org. Lett*. **2000**, *2*(9): 1279–1282.

153) Mo, Kai-For, Li X., Li H., Low L.Y., Quinn C.P., Boons G.J. Endolysins of Bacillus Anthracis Bacteriophages Recognize Unique Carbohydrate Epitopes of Vegetative Cell Wall Polysaccharides with High Affinity and Selectivity. *J. Am. Chem. Soc*. **2012**, *134*(37): 15556–15562.

154) Kocienski P.J. Protecting Groups, 3rd Edition (2005)

155) Woodward, R.B.; Heusler, K.; Gosteli, J.; Naegeli, P.; Oppolzer, W.; Ramage, R.; Ranganathan, S.; Vorbrüggen, H. The Total Synthesis of Cephalosporin C1. *J. Am Chem. Soc*. **1966**, *88*, 852-853.

156) Carson, J. F. *Synthesis* **1981**, 268-270.

157) Donohoe, T. J.; Logan, J. G.; Laffan, D. D. P. Trichloro-oxazolines as Activated Donors for Aminosugar Coupling. *Org. Lett.* **2003**, *5*, 4995-4998.

158) Seeberger, K. R. Love. Solution syntheses of protected type II Lewis blood group oligosaccharides: study for automated synthesis. *J org. chem*. **2005**, *70*(8): 3168.

159) Nishikawa T., Urabe D., Tomita M., Tsujimoto T., Iwabuchi T., Isobe M. One-Pot Transformation of Trichloroacetamide into Readily Deprotectable Carbamates. *Org. Lett*. **2006**, *8*(15): 3263-3265.

160) Schallenberg, E. E.; Calvin, M. Ethyl Thioltrifluoroacetate as an Acetylating Agent with Particular Reference to Peptide Synthesis. *J. Am. Chem. Soc*. **1955**, *77*(10), 2779-2783.

161) Weygand, F.; Csendes, E. N-Trifluoracetyl-aminosäuren. *Angew. Chem*. **1952**, *64*, 136.

162) Goldberger, R. F.; Anfinsen, C. B. The Reversible Masking of Amino Groups in Ribonuclease and Its Possible Usefulness in the Synthesis of the Protein. *Biochemistry*. **1962**, *1*, 401.

163) Ohno, M.; Eastlake, A.; Ontjes, D.; Anfinsen, C. B. Synthesis of the fully protected, carboxyl-terminal tetradecapeptide sequence of staphylococcal nuclease. *J. Am. Chem.Soc*. **1969**, *91*, 6842-6847.

164) Moroder, L.; Filippi, B.; Borin, G.; Marchiori, F. Studies on cytochrome c. X. Synthesis of Nα-benzyloxycarbonyl-[Thr^{107}]-dotetracontapeptide (sequence 67–108) of baker's yeast iso-1-cytochrome c. *Biopolymers.* **1975**, *14*, 2061-2074.

165) Weygand, F.; Frauendorfer, E. [Reductive elimination of the N-trifluoroacetyl and N-trichloroacetyl group by sodium boron hydride and applications in peptide chemistry]. *Chem. Ber*. **1970**, *103*, 2437.

166) Carpino, L. A.; Han, G. Y. 9-Fluorenylmethoxycarbonyl function, a new base-sensitive amino-protecting group. *J. Am. Chem. Soc*. **1970**, *92*, 5748.

167) Carpino, L. A.; Han, G. Y. 9-Fluorenylmethoxycarbonyl amino-protecting group. *J. Org. Chem*. **1972**, *37*, 3404.

168) Rabanal, F.; Haro, I.; Reig, F.; Garcı´a-Anto´n, J.M. *An. Quim*. **1990**, *86*, 84.

169) Butwell, F. G. W.; Haws, E. J.; Epton, R. Surface Structures of Polymers for Biomedical Applications. *Makromol. Chem*., *Macromol. Symp.* **1988**, *19*, 69.

170) Carpino, L. A.; Mansour, E. M. E.; Cheng, C. H.; Williams, J. R.;MacDonald, R.; Knapczyk, J.; Carman, M.; Lopusinski, A. Polystyrene-based deblocking-scavenging agents for the 9-fluorenylmethyloxycarbonyl amino-protecting group. *J. Org.Chem*. **1983**, *48*(5), 661-665.

171) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. Piperazino-functionalized silica gel as a deblocking-scavenging agent for the 9-fluorenylmethyloxycarbonyl amino-protecting group. *J. Org. Chem*. **1983**, *48*, 666.

172) Paulsen, H. Advances in Selective Chemical Syntheses of Complex Oligosaccharides. *Angew. Chem. Int. Ed. Engl*. **1982**, *21*, 155-173.

173) Schmidt, R. R.; Kinzy, W. Anomeric-oxygen activation for glycoside synthesis: the trichloroacetimidate method. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123.

174) Garg, H. G.; Von Dem Bruch, K.; Kunz, H. *Adv. Carbohydr. Chem. Biochem*. **1994**, *50*, 227-310.

175) Mayer, T. G.; Kratzer, B.; Schmidt, R. R. Synthesis of a GPI Anchor of Yeast (Saccharomyces cerevisiae) *Angew. Chem. Int. Ed. Engl*. **1994**, *34*, 2177-2181.

176) Seeberger, P.H., Baumann M., Zhang G., Kanemitsu, T., *et al*. Synthesis of Neomycin Analogs to Investigate Aminoglycoside-RNA Interactions. *Synlett*. **2003**, *9***,** 1323.

177) a) Luo S.-Y., Thopate S.R., *et al*., *Tetrahedron Lett*., **43**: 4889 (2002). b) J. Liu, M. M. D. Numa, H. Liu,et al, J. *Org. Chem*., **69**: 6273 (2004). c) B. Yu, J. Yang, et al., *Org. Lett*., **4**: 3455 (2002). d) J.T. Lundquist and J.C. Pelletier, *Org. Lett*. **2002**, *4*, 3219.

178) Nyffeler P.T., Liang C.-H., The Chemistry of Amine−Azide Interconversion: Catalytic Diazotransfer and Regioselective Azide Reduction. *J. Am. Chem. Soc*. **2002**, *124*, 10773.

179) Lartia R., Murat P., Dumy P., Defrancq P. *et al*., Versatile Introduction of Azido Moiety into Oligonucleotides through Diazo Transfer Reaction *Org. Lett.* **2011**, *13*(20): 5672.

180) Alper P.B., Hendrix M., *et al*. Probing the Specificity of Aminoglycoside−Ribosomal RNA Interactions with Designed Synthetic Analogs. *J. Am. Chem. Soc*. **1998**, *120*, 1965.

181) Bergmann M., and Zervas L., *Ber*. **1932**, *65*, 1192.

182) Atwell G.J., and Denny A. Monoprotection of α,ω-Alkanediamines with the N-Benzyloxycarbonyl Group. *Synthesis*, **1984**, *12*, 1032-1033.

183) Bayley H., Standring D.N., Knowles J.R. Propane-1,3-Dithiol - Selective Reagent for Efficient Reduction of Alkyl and Aryl Azides to Amines. Tetrahedron Lett. **1978**, *39*, 3633-3634.

184) Galonić, D.P., and Gin D.Y.. Chemical Glycosylation in the Synthesis of Glycoconjugate Antitumour Vaccines. *Nature.* **2007**, *446*(7139), 1000–1007.

185) Cai, C., Dickinson, D. M., Li, L., Masuko, S., Suflita, M., Schultz, V., Linhardt, R. J. Fluorous-Assisted Chemoenzymatic Synthesis of Heparan Sulfate Oligosaccharides. *Organic Letters*. **2014**, *16*(8), 2240–2243.

186) Adachi M., Tanaka H., Takahashi T. An effective sialylation method using N-Troc- and N-Fmoc-protected β-thiophenyl sialosides and application to the one-pot two step synthesis of 2,6-sialyl-T Antigen. *Synlett.* **2004**, *35*(4), 609-614.

187) Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Jacquinet, J.-C.; Sinay, P.; Torri, G. Synthesis of heparin fragments: a methyl alpha-pentaoside with high affinity for antithrombin III. *Carbohydr. Res*. **1987**, *167*, 67-75.

188) Heyns, K.; Harrison, R.; Paulsen, H. Synthese eines 1→3-verknüpften Disaccharids aus zwei D-Glucosamin-Einheiten. *Chem. Ber*. **1967**, *100*(1): 271-279.

189) Kulkarni, S. R.; Zimmermann, H. K. Derivate des D-Glucosamins, VII. Darstellung und Eigenschaften von 3.4.6-Tri-O-benzoyl-D-glucopyrano-[1.2:4'.5']-oxazolidon-(2') *Liebigs Ann. Chem*. **1965**, *684*, 223-226.

CHAPTER 2

PROBING BINDING INTERACTIONS BETWEEN S-LAYER PROTEINS AND SYNTHETIC OLIGOSACCHARIDES DERIVED FROM BACILLUS ANTHRACIS SECONDARY CELL WALL POLYSACCHARIDE

Chapman R.N., Liu L., Lunderberg J., Schneewind O., and Boons, G.J.
To be submitted to Chem. Sci.

ABSTRACT

The outer surface of *Bacillus anthracis* is coated with a proteinaceous two-dimensional surface layer (S-layer), formed by the self-assembly of monomeric S-layer proteins (SLPs) and a smaller number of S-layer associated proteins (BSLs). S-Layer and BSL proteins both bind secondary cell wall polysaccharide (SCWP), but their deposition on the cell surface is not random. Phenotypic evidence from *B. anthracis* genetic mutants suggests that specific functionalities on the terminal non-reducing SCWP unit including a 4,6-*O*-linked ketal pyruvate, a free amine , and O-acetylation play a role in SLP and BSL protein binding and could allow for selective binding of proteins that act to modulate downstream cellular processes. In the present study, a library of compounds based on the terminal unit of *B. anthracis* SCWP was synthesized in order to probe the relationship between the aforementioned functional groups and a series of mCherry fusion proteins constructed from the SLH domains of S-Layer and BSL proteins. A number of synthetic challenges had to be addressed, including the development of synthetic procedures for the unique pyruvylated monosaccharides, the selection of appropriate orthogonal protecting groups, the choice of stereoselective and robust activating conditions for the poorly reactive pyruvylated donor, and the selection of appropriate conditions for the reduction of trichloroacetyl and azide functions. Microarray and ELISA based binding experiments were developed to examine the binding interactions between the synthetic oligosaccharides and the mCherry fusion proteins. It was found that the 3-*O*-acetyl function on the β-GlcNAc and distal ManNAc did not significantly affect binding affinity relative to non-O-acetylated compounds. The presence of a free amine on α-GlcNH_2 produced a small increase in binding affinity for particular proteins. The 4,6-*O*-pyruvyl ketal was found to be essential for SLP and BSL binding in all cases. Unexpectedly, the C-2 acetamido function of the non-reducing ManNAc was found to be esential for the binding of SLPs and several BSL proteins. Interestingly, some BSL proteins could bind in high affinity in the absence of the C-2 acetamido indicating differences in binding requirements

between SLH domains and showing that in principle, modifications to the C-2 position could be used as a means to modulate protein binding.

INTRODUCTION

Bacillus anthracis is a Gram positive, spore forming bacterium known to cause anthrax in humans and animals.[1] Like species from nearly every branch of Bacteria and Archaea, the cell envelope is coated with a monomolecular Surface layer comprised of S-Layer proteins (SLPs) and a smaller number of S-layer associated proteins (BSLs).[2a-e] The SLPs are synthesized in the cytoplasm and translocated across the cell membrane where they bind secondary cell wall polysaccharide (SCWP) through non-covalent interactions and self-assemble into a two-dimensional para-crystalline lattice across the cell surface.[3-5] (Figure 2.1) The surface layer serves a number of important functions ranging from mediating the infection of host cells to nutrient uptake.[6-9]

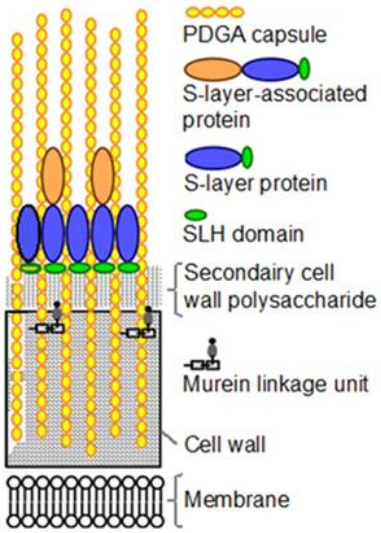


Figure 2.1 The Cell Wall of *B. anthracis*

Despite the involvement of the S-layer in many important biological events, very little is known about the molecular mechanisms that govern these processes. The structural heterogeneity of *B. anthracis*

SCWP has made it difficult to perform detailed binding studies and identify structural features important for S-layer protein and S-layer associated protein binding interactions.[10] Until recently, the structural features of *B. anthracis* SCWP were not well characterized leaving genetic experimentation and the use of heterogeneous cell wall extracts as the only way to probe carbohydrate protein interactions.[11-13] NMR studies on extracted *B. anthracis* SCWP have since provided valuable structural information about the non-reducing trisaccharide unit that has been utilized to synthesize a library of well-defined polysaccharides to study features important for SLP and BSL carbohydrate interactions.[14]

The S-Layer of *B. anthracis* is made up of surface array protein (Sap) and extractable antigen 1 (EA1).[15,16] Sap predominates during the early and logarithmic growth phase and gives way to EA1 as the cell enters the stationary phase. Sap and EA1 S-layers do not overlap and form distinct patches on the surface.[17,18] The S-Layer can be further modified with up to 22 additional S-layer associated proteins as minor constituents that may impart additional functions to the cell.[19] For example, BslK has been shown to scavenge heme in order to utilize its iron; [20] BslA mediates the adherence of vegetative forms to host cells;[21] BslO has peptidoglycan hydrolase activity and may play a role in the separation of bacilli from elongating chains;[22] and BSLs R, S, T, and U are predicted murein hydrolases.[23,24] The function and regulation of a number of BSLs has yet to be elucidated. Both SLPs and BSL proteins are tethered to the cell envelope *via* non-covalent binding interactions between their S-Layer homology (SLH) domain and the secondary cell wall polysaccharide. The SLH domain is made up of three consecutive ~55 amino acid SLH motifs that fold into a pseudo-trimer which functions to bind SCWP. Although divergent in primary sequence, it has been proposed through computational modeling that all SLH domains within the *B. anthracis* genome likely adopt a similar fold.[25-27] Unlike BSL proteins, SLPs have an additional domain at the C-terminal known as a crystallization domain that allows the proteins to bind one another and form a contiguous layer across the cell surface. [28,29,2a]

The *B. anthracis* SCWP is made up of a trisaccharide repeat [→4)-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→6)-α-D-GlcNAc-(1→)]$_n$, where α-GlcNAc is substituted with α-Gal and β-Gal at the C-3 and C-4 positions respectively, and β-GlcNAc is substituted with α-Gal at the C-3 position (Figure 2.2).[30] The SCWP is bound to peptidoglycan *via* phosphodiester linkage to the C-6 hydroxy of MurNAc.[26,31] The *csaB* gene product was found to be necessary for the addition of pyruvyl onto the *B. anthracis* SCWP. Genetic studies involving the knockout of *csaB* resulted in a phenotype lacking the S-layer.[25] In addition, a study involving the knockout of *csaB* in a closely related *B. cereus* G9241 mutant produced a phenotype that failed to retain an S-layer and showed a concomitant decrease in virulence thereby illustrating the importance of proper S-layer assembly for pathogenesis.[32]

NMR studies on isolated SCWP from *B. anthracis* and several phylogenetically related strains were used to identify several unique sturtural features on the non-reducing trisaccharide unit including a 4,6-*O*-pyruvyl ketal on β-ManNAc, a 3-*O*-acetyl on β-GlcNAc, and a C-2 free amino function on the α-GlcNH$_2$ (Figure 2.2). It is possible that the GlcNH$_2$ is further modified by an α-Gal at C-3 and β-Gal at C-4 positions. However, NMR studies have not been able to confirm this modification.[14]

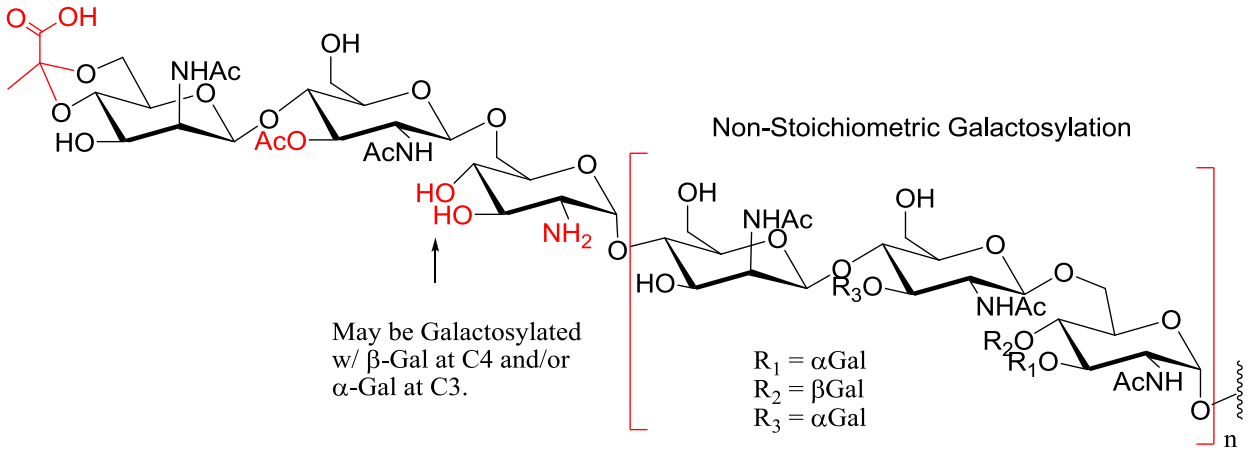


Figure 2.2: The *Bacillus anthracis* Secondary Cell Wall Polysaccharide.

Despite this ambiguity, evidence indicates that galactose modifications do not play a significant role in SLP binding. CDC-684 is a non-galactosylated avirulent variant of *B anthracis* with an identical SWCP

backbone. The CDC-684 variant retained the pyruvate acetal, O-acetate, and amino group modifications found on SCWPs from galactosylated B*. anthracis* strains as well as an intact S-layer. It has been surmised that the modifications present on non-reducing SCWP unit could function as directive elements for glycan-protein interactions.[14]

Although S-layer and BSL proteins both bind SCWP, their deposition on the cell surface is not random. O-acetylation of SCWP has been proposed as a means to enable the deposition of certain SLP and BSL proteins at specific sites on the cell envelope.[13,34] Two putative acetyltransferase systems identified in *B. anthracis* include the PatA1/PatB1 and PatA2/PatB2 systems. PatA1 and PatA2 are membrane bound *O*-acetyltransferases that are suspected to play roles in modifying SCWP and possibly peptidoglycan.[13,35] The *patA1*, *patA2*, and *patA1patA2* deletion mutants produced elongated chains of vegetative cells with SCWP quantities comparable to those of WT cells, but with fewer *O*-acetyl modifications. It was demonstrated that the *patA2* mutant assembled significantly less EA1 into the envelope relative to the wild type strain while the *patA1patA2* double mutant showed a complete loss of EA1 from the S-layer. Interestingly, it was found that the ability to form a Sap based S-Layer was retained in mutant strains. The findings indicated that *O*-acetylation of SCWP was necessary for EA1 binding, but not for Sap binding.

It is known that *B. anthracis* vegetative cells have the ability to form elongated chains within host organisms to exceed the size of macrophages or granulocytes to avoid being engulfed. It was suggested that O-acetylation of SCWP may be a way for the cell to modulate this process.[13,34] In wild type cells, BslO murein hydrolase has been shown to localize around the septal regions of the cell envelope in order to facilitate the separation of daughter cells.[22] The *patA1patA2* deletion mutants failed to incorporate BslO or BslA into the S-layer. It was suggested that the absence of BslO at the cell septa was the cause of the elongated phenotype seen in the *patA1/patA2* mutants. A model was proposed suggesting that *patA1* and *patA2* mediated *O*-acetylation of SCWP is a prerequisite for EA1,

BslO, and BslA binding and can be utilized by the cell to promote their deposition to localized regions of the cell envelope.[13]

Despite the genetic evidence in support of SCWP functional groups playing a role in SLP and BSL binding and their potential for regulating downstream cellular processes, no experiments have been performed using well defined oligosaccharides. Herein, we report the synthesis of a library of novel oligosaccharides based on the terminal trisaccharide unit of *B. anthracis* SCWP (Figure 2.3). The library contains six trisaccharide members (**1**-**6**) with modifications including a 4,6-*O*-pyruvyl ketal as well as varying patterns of O-acetylation and N-deacetylation.

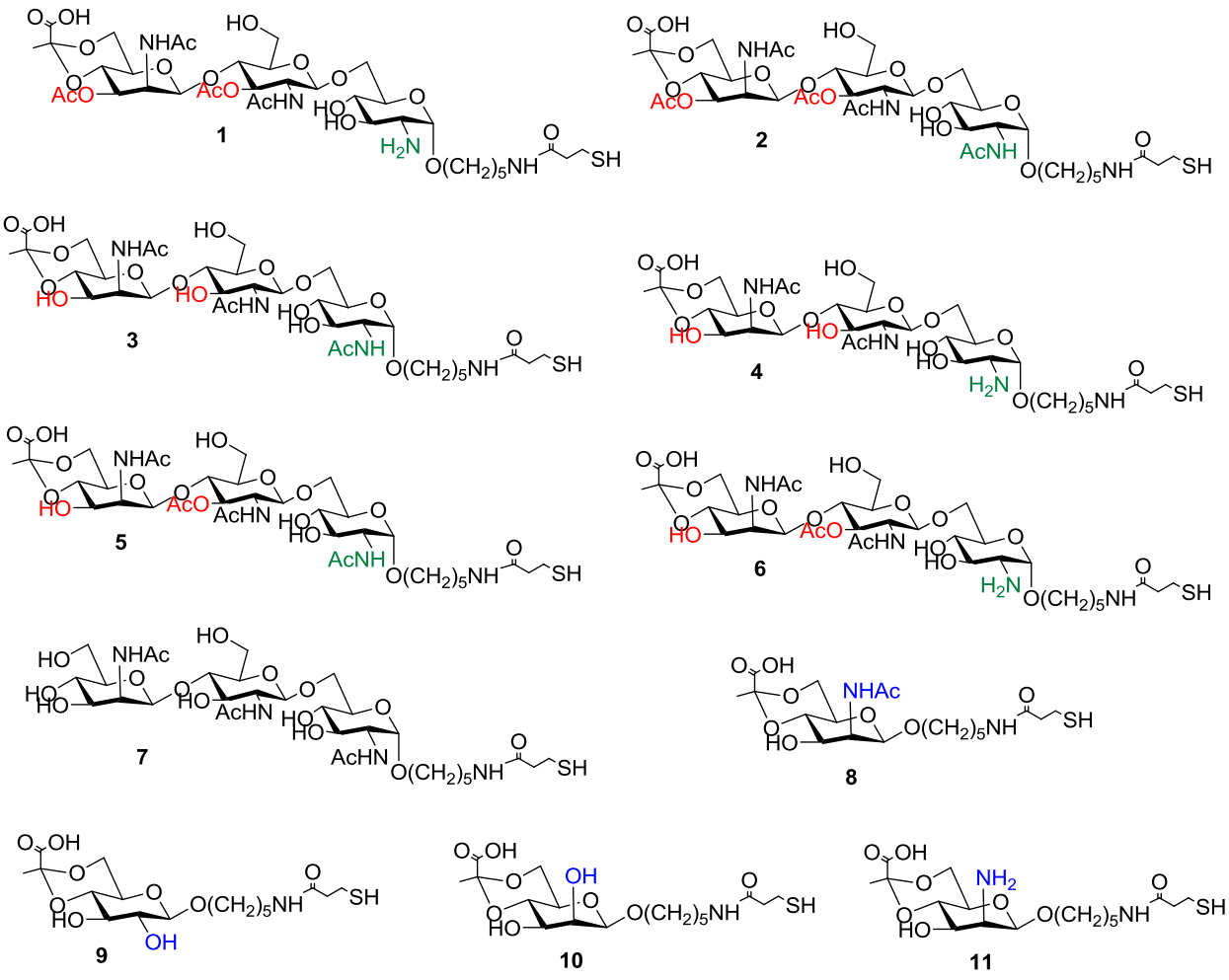


Figure 2.3: Synthetic Target Compounds

In addition, a trisaccharide lacking the 4,6-O-pyruvyl ketal (**7**) was also included in order to determine the importance of pyruvylation for SLH-binding and provide definitive proof to verify the findings in the *csa* genetic experiments. The role of the 3-*O*-acetyl function on β-GlcNAc was assayed by comparing compounds with and without the O-acetyl modification at this position. The idea was entertained that additional acetyl modifications may occur at positions other than the internal β-GlcNAc on a scale too small to be detected by NMR. Furthermore, the closely related *B. Cereus* ATCC 10987 has a one residue substitution on its SCWP polymer repeat, but is known to acetylate the O-3 position of ManNAc.[36] For these reasons, compounds **1** and **2** were made bearing a 3-*O*-acetyl modification on the non-reducing α-ManNAc to test the importance of this position for SLH mediated binding. The role of the free amine was probed by replacing the α-$GlcNH_2$ with α-GlcNAc in select target compounds (**2, 3**, **5**). A truncated structure consisting of a 4,6 pyruvlated ManNAc was also synthesized (**8**) in order to determine the minimum epitope for binding. In addition, a glucoside (**9**), mannoside (**10**), and mannosamine (**11**) target compound bearing the 4,6-*O*-pyruvyl ketal were synthesized in order to assess the importance of the C-2 acetamido function on ManNAc for binding. In the event that **8** can bind an SLH protein, but neither **9** nor **10** can bind, the importance of the acetamido function can be established. If targets **8** and **10** can bind the protein, but **9** cannot bind, the importance of stereochemistry at the position will be apparent. Analysis of the *B. anthracis* genome indicates the existence of 10 N-deacetylases. Some are known to act on peptidoglycan, but the substrates of others are still unknown.[92] If additional N-deacetylation exists on small quantities of SCWP or occurs during a particular part of the growth cycle, it may prove difficult to detect. For these reasons, target **11** was synthesized to see if N-deacetylation at the C-2 position of ManNAc has the potential to play a role in modulating the binding of SLP and BSL proteins.

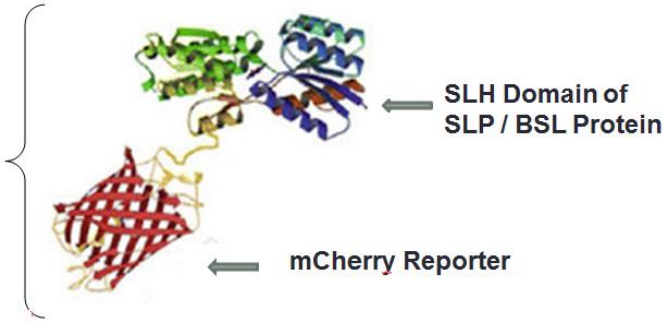
Following the challenging synthesis of target compounds **1**-**11**, carbohydrate microarray and ELISA based experiments were designed and utilized to probe binding interactions between the synthetic targets and a panel of mCherry fusion proteins constructed from the SLH binding domains of

various SLP and BSL proteins (Table 2.1). The overarching questions that were addressed utilizing the oligosaccharide library include: 1) What functionalities or combinations of functionalities are important for SLP/BSL binding? 2) Do all S-layer and S-layer associated proteins have the same ligand requirements for biding? 3) Can O-acetylation be used as a means to modulate the binding of EA1, BslA, and BslO as demonstrated in the genetic studies?

Table 2.1: mCherry Fusion Proteins

Protein	***Description***	***Reference***
Sap	SLH Domain+mCherry	**12**
EA1	SLH Domain+mCherry	**12**
BslA	SLH Domain+mCherry	
BslI	SLH Domain+mCherry	
BslK	SLH Domain+mCherry	
BslM	SLH Domain+mCherry	
BslO	SLH Domain+mCherry	**9**
BslP	SLH Domain+mCherry	
BslR	SLH Domain+mCherry	
BslS	SLH Domain+mCherry	
BslT	SLH Domain+mCherry	
BslU	SLH Domain+mCherry	
PlyG	CBD Domain+mCherry	**12**

mCherry fluorophore + SLH/CBD domain



RESULTS AND DISCUSSION

Chemical Synthesis: A library of oligosaccharides derived from the non-reducing terminal unit of *B. anthracis* SCWP was synthesized (Figure 2.3). The target compounds contain an orthogonal five carbon linker that allows for conjugation to any desired carrier protein or surface. The chemical synthesis of the library was challenging due to the presence of a 1,2-*cis*-glycosidic linkage with β-configuration, acetyl ester(s), free amine(s), and a carboxylic acid. (Figure 2.4) In this respect, the base labile acetyl ester demands that base cleavable protecting groups are avoided. In addition, the carboxylic acid and free

amine complicates protein conjugation chemistry. Usually, this step involves a late stage modification of an amino or carboxylic acid containing anomeric spacer with a thiol moiety for reaction with maleimide or bromoacyl modified carrier proteins. Early stage installation of a thiol containing spacer would prove problematic due to sulfur's ability to poison the Pd-catalyst required for the removal of the commonly utilized benzyl ethers. In addition, installation of the 4,6-*O*-pyruvyl ketal must also be done in a manner that selectively yields the (S)-configuration. Further complicating the synthesis is the poor reactivity of pyruvylated donors.[37]

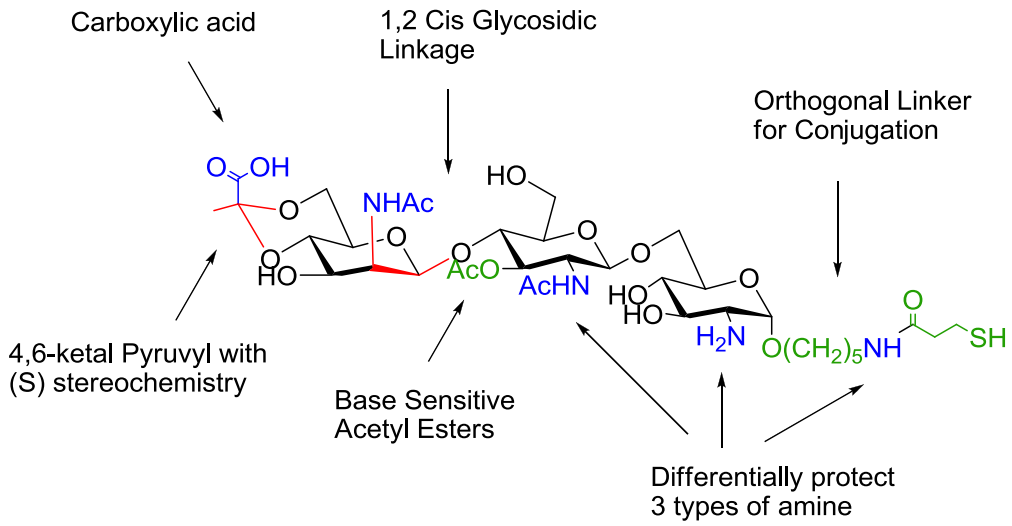


Figure 2.4: Synthetic challenges for *B. anthracis* SCWP derived compounds

The chemical synthesis was demanding and required many changes to the protecting groups and the synthetic strategy. It was envisioned that target compounds **1**-**6** would be synthesized from two common trisaccharide intermediates (Figure 2.5). One of the intermediates has a permanent protecting group (*e.g*. Benzyl) at the O-3 position of ManNAc in order to provide access to compounds **5** and **6**. The second intermediate was acetylated at the O-3 position of ManNAc to afford compounds **1** and **2**. Completion of the disaccharide intermediate was considered a key step in the synthesis since the 4,6-*O*-pyruvyl ketal with (S) stereochemistry was already installed as well as the 1,2-*cis*-glycosidic linkage.

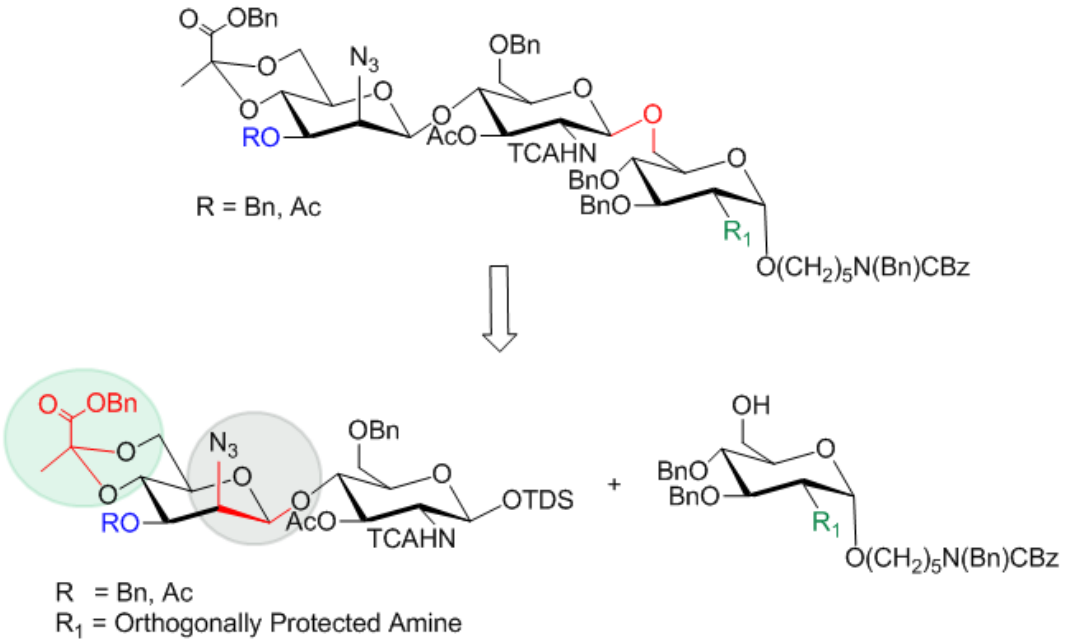


Figure 2.5: General Synthetic Strategy for Assembly of Common Trisaccharides for Targets **1**-**6**

The 1,2-*cis*-glycosidic bond with beta configuration is one of the most difficult linkages to install because its formation is thermodynamically unfavorable due to an absence of stabilization from the anomeric effect and kinetically unfavorable due to steric clashing from the C-2 axial substituent. By contrast, 1,2-*trans*-linkages can be easily formed using neighboring group participation (Figure 2.6). Two overarching synthetic strategies were designed that utilized differing methods to introduce the challenging 1,2-*cis*-glycosidic linkage with beta configuration. The first strategy (**Method 1**) involves utilization of an O-2 participating group (alloxycarbonyl, levulinate, etc.) on the donor to first install the beta linked glucoside. Following disaccharide formation, deprotection of the O-2 protecting group on the non-reducing glucoside will take place, followed by installation of a leaving group, and subsequent S_N2 inversion of the chiral center to yield a β-linked mannoside. The second strategy (**Method 2**) involves a direct introduction of the 1,2-*cis*-linkage using Crich β-Mannosylation conditions.[38]

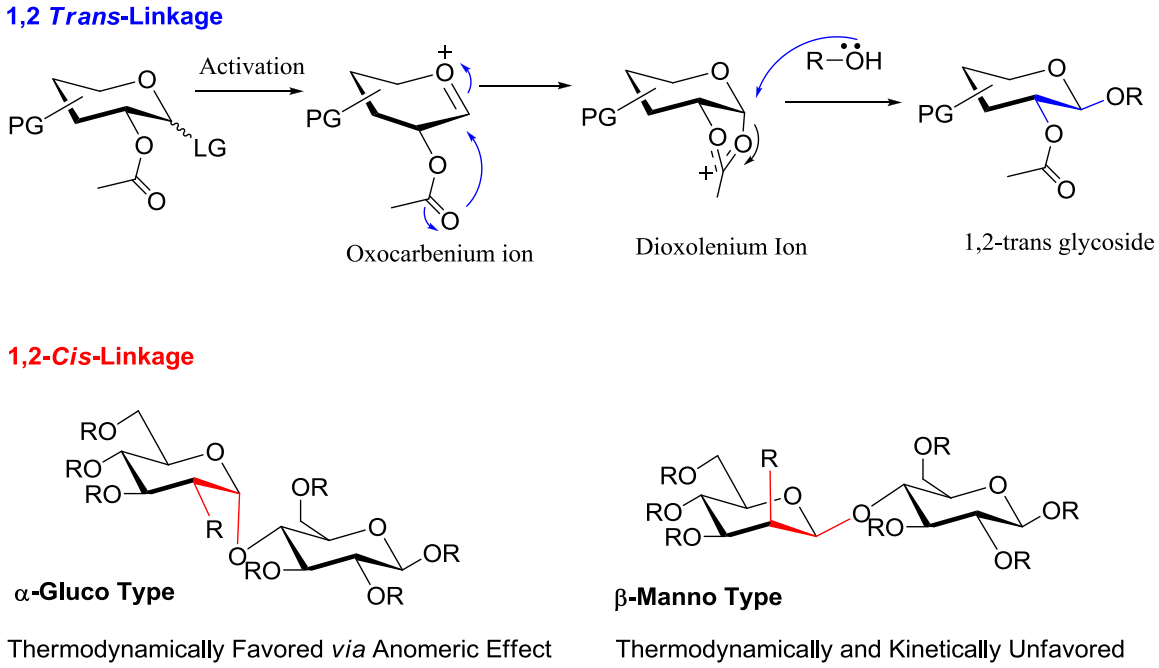
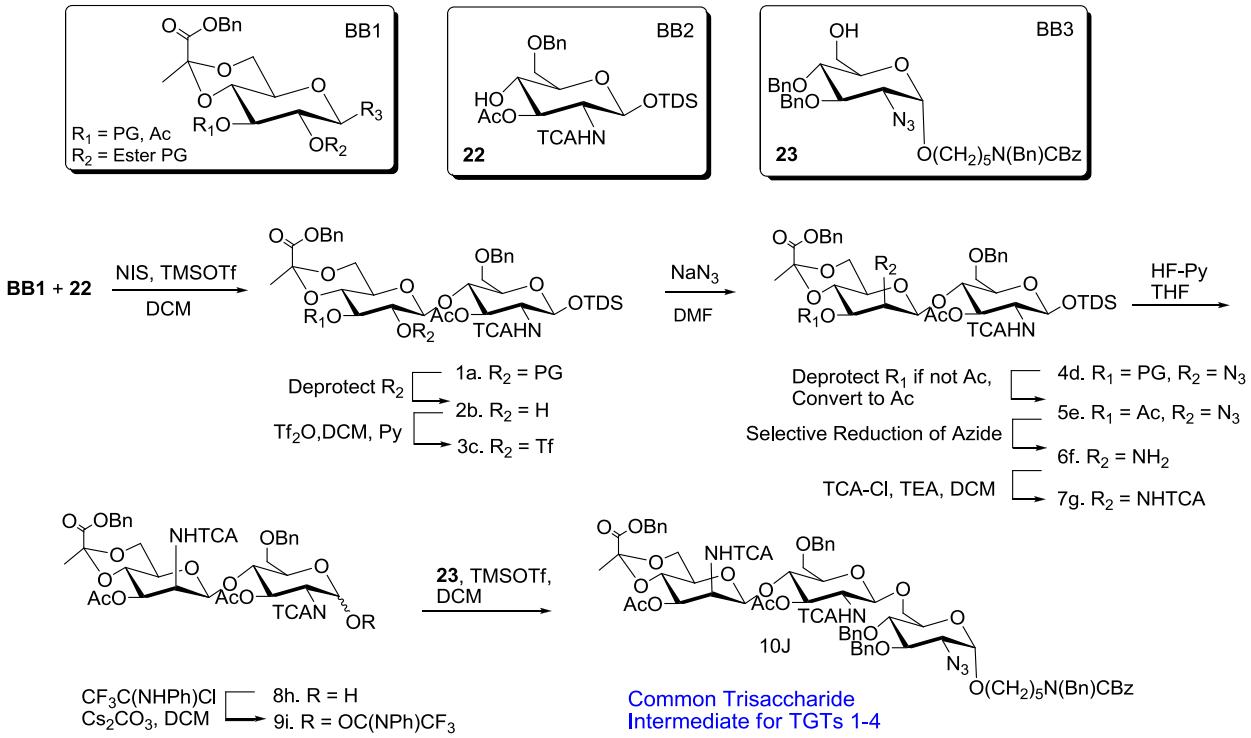


Figure 2.6: Synthetic Challenges: Preparation of 1,2-*cis*-Beta-mannoside

Method 1: C-2 Inversion Strategy

It was envisaged that the target compounds would be assembled using four monosaccharide building blocks (BBs). A key feature of this approach is the use of trichloroacetyl (TCA), trifluoroacetyl (TFA), and the benzyloxycarbonyl (Cbz)/Benzyl (Bn) pair as amino protecting groups.[39] The differential reactivities of these protecting groups make it possible to selectively modify the various amino groups of the target compounds. In particular, the TCA at C-2 of a 2-amino glycosyl donor can perform neighboring group participation during glycosylations to give selectively a 1,2-*trans*-glycoside. The TCA can be subsequently reduced to an acetamido function by treatment with Bu_3SnH and AIBN.[40] The purpose of the TFA protecting group is to enable the preparation of a compound bearing a single free amine on the linker. The Bn/Cbz protecting groups can be removed using catalytic hydrogenation in the presence of TFA allowing for selective installation of a thiol spacer for protein conjugation. Afterwards, cleavage of the *N*-TFA group under very mild basic conditions with triethylamine and methanol reveals the free amine on

the final target without affecting the base labile acetyl ester.[41] A general description of the synthetic strategy for the preparation of target compounds **1**-**4** is described below (Scheme 2.1) and serves to demonstrate how the various chemical reactivities can be exploited.

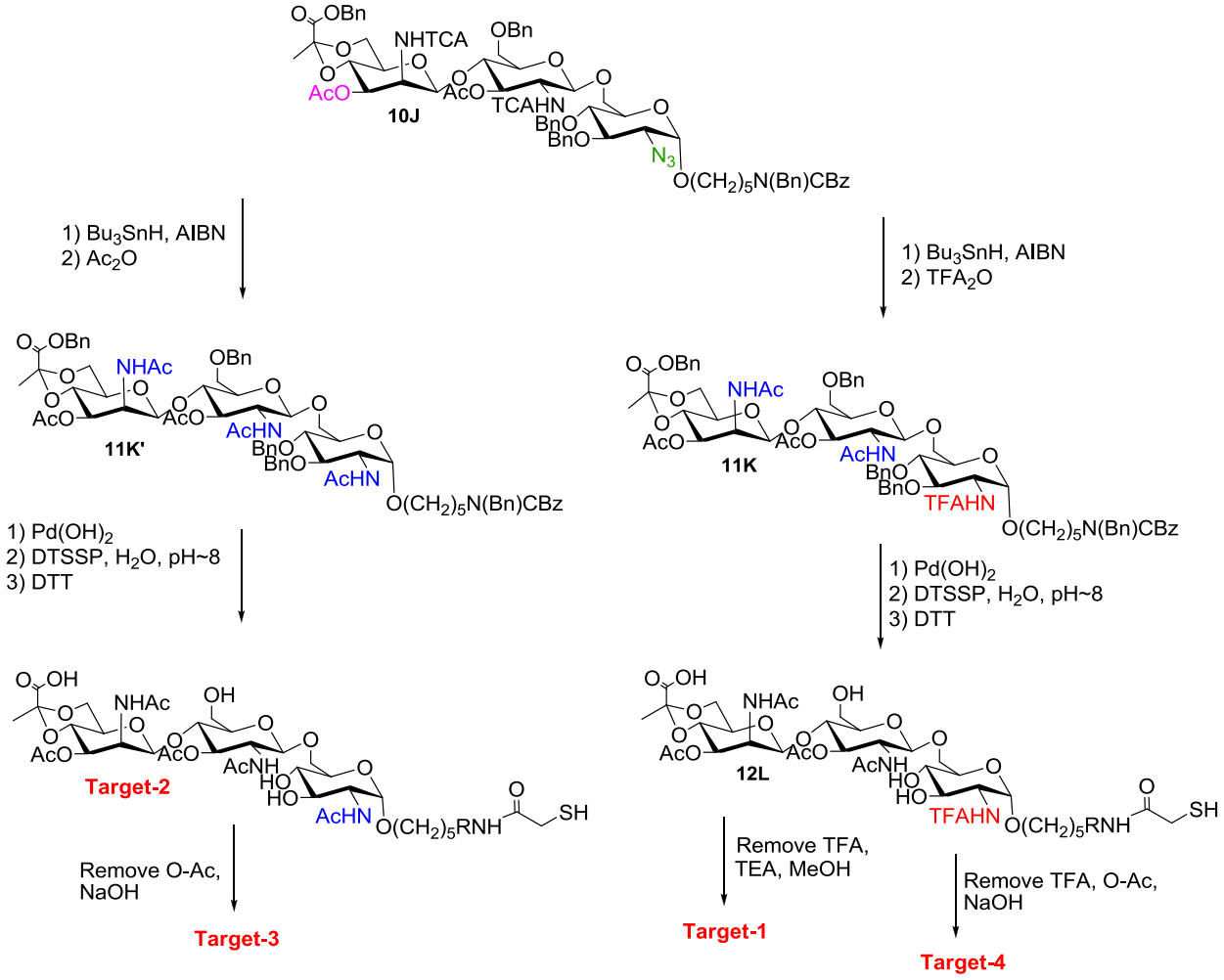


Scheme 2.1: Method 1: General Overview of Synthetic Strategy.

Method 1: Planned Synthetic Procedure: The first step involves a TMSOTf catalyzed glycosylation between glucosyl donor (**BB1**) and acceptor **22**. **BB1** will have a participating protecting group (e.g. Alloc) at C-2 to give selectively the 1,2-*trans*-glycoside.[42] Next, the C-2 protecting group of **1a** will be removed and a triflate will be installed on the resulting C-2 hydroxy of compound **2b** to provide intermediate **3c**. Next, the triflate will be displaced by sodium azide in S_N2 fashion to yield the 2-azido-β-D-mannoside (**4d/5e**).[43] If the C-3-position is not yet O-acetylated (**4d**), the temporary protecting group will be removed and the resulting 3-hydroxy will be acetylated. Next, the azido function of **5e** will be selectively reduced to amine **6f** (*e.g.* propanedithiol, TEA) and protected with TCA using standard

conditions to provide compound **7g**. The TDS can then be removed using HF-pyridine complex yielding **8h.**[44] The lactol will be converted into glycosyl donor **9i** using with *N*-phenyltrifluoroacetimidoyl chloride and Cs_2CO_3.[45] A TMSOTf catalyzed glycosylation of imidate **9i** with glycosyl acceptor **56** will provide the common trisaccharide intermediate **10J** as only the 1,2-*trans*-glycoside by way of neighboring group participation of the TCA function.

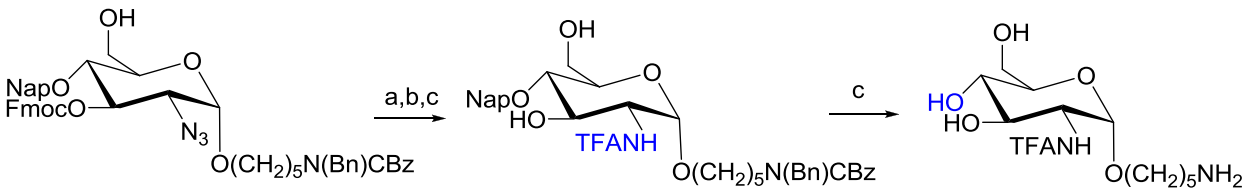
The common trisaccharide can be further differentiated to afford target compounds **1**-**4** (Scheme 2.2). Tributyltin hydride and a catalytic amount of AIBN can be used to reduce the TCA and azide functions of **10J** to the corresponding acetamido and amine functions.[40]



Scheme 2.2: General Synthetic Strategy for Compounds **1**-**4**.

The resulting amine can be protected with a TFA function to afford compound **11K** as a precursor for Targets **1** and **4**. Alternatively, the amine can be acetylated using Ac_2O to provide **11K'** as a precursor for Target compounds **2** and **3**. The reduction of azide and TCA can also be accomplished Zn-Cu couple in acetic acid.[46] Afterwards, catalytic hydrogenation of **11K/11K'** can be utilized for the removal of the benzyl ester, ethers, and Cbz groups. The thiol spacer can then be installed onto the linker amine using DTSSP (3,3'-dithiobis(sulfosuccinimidyl propionate)) to afford compounds **12L** and **2**. Treatment of **12L** with triethylamine in methanol will remove the TFA[41] and afford target compound **1**. Treatment of **12L** with a stronger base will remove both the TFA and O-acetyl groups affording target compound **4**. To obtain target **3**, compound **2** can be deacetylated with NaOH. Access to the additional target compounds (**5** and **6**) can be provided *via* modification of the O-3 position on the Mannoside donor (BB1) with a permanent protecting group such as benzyl.

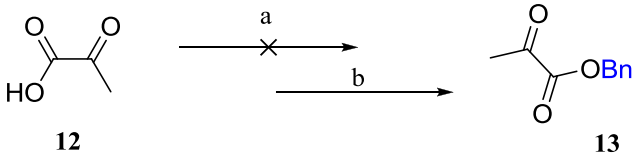
A model study was conducted to determine if the *N*-TFA function would be stable under the catalytic hydrogenation conditions used to remove benzyl and Cbz protecting groups (Scheme 2.3).



Scheme 2.3: TFA Stability study using benzyl deprotection conditions. (a) i) Zn-Cu Couple, HOAc; (b) TEA, DCM; (c) Pyridine, Trifluoroacetic anhydride, 0°C; (d) $Pd(OH)_2$, H_2, t-BuOH, H_2O, HOAc.

In the model study, an N-TFA bearing monosaccharide was made from a readily available building block. The TFA bearing compound was reacted under catalytic hydrogenation conditions with $Pd(OH)_2$. After stirring for 18 hrs, MALDI-TOF MS detected several peaks representing partially deprotected intermediates and residual starting material. The reaction mixture was filtered and concentrated. Fresh

solvent and catalyst was added and the reaction was allowed to stir for an additional 6hrs. At this point, the major peak was that of the product. No changes in the TFA protecting group could be detected.



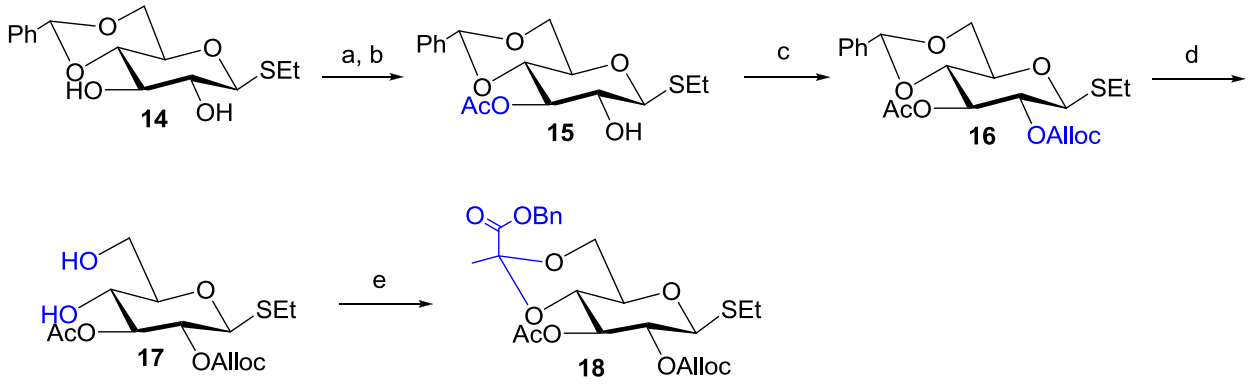
12 **13**

Scheme 2.4: Synthesis of Benzyl Pyruvate.(a) Benzyl Bromide, Cs_2CO_3, DMF; (b) Benzyl Alcohol, Pyridine, THF, Mesyl chloride, 0^{o}C (46%).

Synthesis of Benzyl Pyruvate: The first attempt to make benzyl pyruvate (**13**) involved dissolving pyruvic acid (**12**) in DMF along with benzyl bromide and cesium carbonate (Scheme 2.4). Unfortunately a complex mixture was obtained containing decarboxylation side products and other impurities. In order to prevent side reactions, an alternate method was attempted in which a mesylate was generated *in situ* in the presence of benzyl alcohol at 0^{o}C.[47] The alcohol serves as a readily available nuclephile that can displace the mesylate before decarboxylation can occur. Utilizing this method, benzyl pyruvate could be obtained in 46% yield in high purity. The product showed no degradation by NMR after storage at -20^{o}C for 8 weeks.

Synthesis of Building Block 1 Version 1: Starting with intermediate **14**, organotin-mediated regioselective protection was utilized to selectively install acetyl at the C-3 position. The dibutyltin oxide reacts with the diol to give the corresponding stannylene acetal. A complex interplay of structural and electronic factors helps to increase the nucleophilicity of one of the oxygen atoms in the stannylene ring thereby enhancing O-2 selectivity.[48] Compound **15** was obtained in 69% yield along with a small quantity of inseparable di-acetylated impurity (Scheme 2.5). Afterwards, Alloc was selectively installed at the C-2 position to provide compound **16**. Next, compound **16** was heated in an 80% aqueous solution of acetic

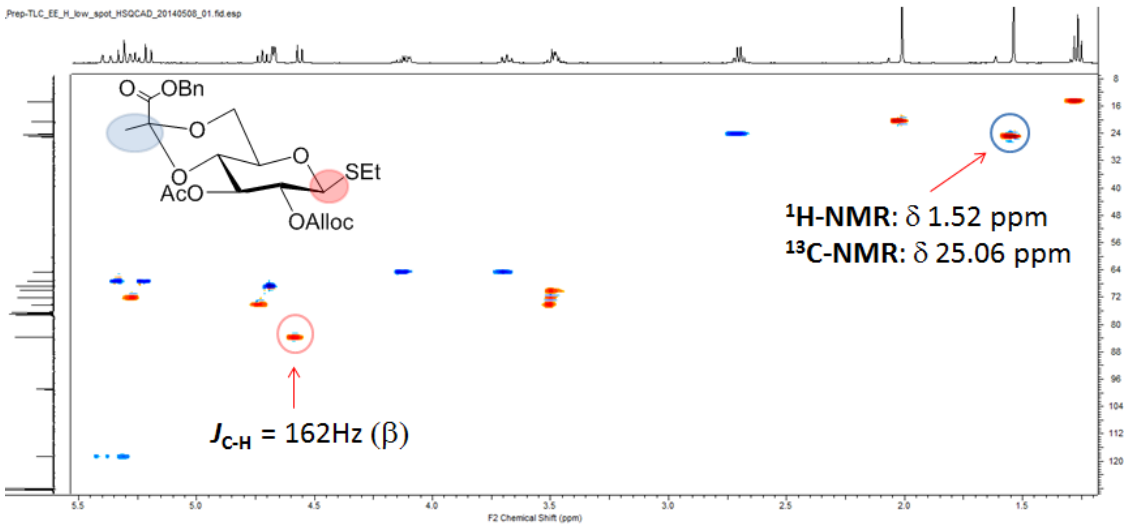
acid to afford diol **17** in 83% yield over two steps. At this point, the diacetylated impurity could be separated out *via* silica gel chromatography (40% Acetone/tolene).



Scheme 2.5: Synthesis of Building Block 1 Version 1. (a) i) Dibutyltin oxide, MeOH, 65°C; (b) AcCl, DCM, 0°C, 69%; (c) Allyl chloroformate, DMAP, DCM; (d) 80% HOAc (aq), 50°C, 83%, 2-steps; (e) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 66%.

In order to find appropriate conditions for the installation of pyruvyl, a test reaction was set up. The solvents typically used in reported literature are dichloromethane and acetonitrile. The reactions were found to proceed faster in acetonitrile than in DCM. In addition, reactions in acetonitrile produced only the thermodynamic product. However, the thermodynamically less stable isomers in DCM reactions would eventually rearrange to the thermodynamically favored forms under acidic conditions. For 4,6-*O*-acetals the thermodynamic products were the isomers having an axial methoxycarbonyl group (i.e. (S)-isomers for D-Glucopyranosyl and D-Mannopyranosyl forms and (R)-isomers for D-Galactopyranosyl derivatives.)[49] In this case, the S-stereoisomer is the desired thermodynamic product; therefore conditions using a longer reaction time in acetonitrile at room temperature were attempted. Diol **17** was dissolved in acetonitrile along with benzyl pyruvate.[50,51] $BF_3(OEt)_2$ was added *via* dropwise addition and the reaction was allowed to proceed for 18hrs. Following column chromatography, compound **18** was successfully obtained in 66% yield as a 1:4 (α:β) mixture. The anomerization of pyruvylated thioglycosides has been documented in literature where a pyruvylated 1-thiophenyl-galactoside, usually

anomerically stable under acidic condition, was prone to anomerization and gave an anomeric mixture of the corresponding (S)-pyruvylated D-galactosides.[52] NMR was used to confirm that the product was indeed a mixture of anomers as opposed to a mixture of (R) and (S) stereoisomers. The anomers were separated using preparatory TLC by triply eluting with 30% Et_2O/Hexanes and S-stereochemistry was confirmed by NMR (Figure 2.7).

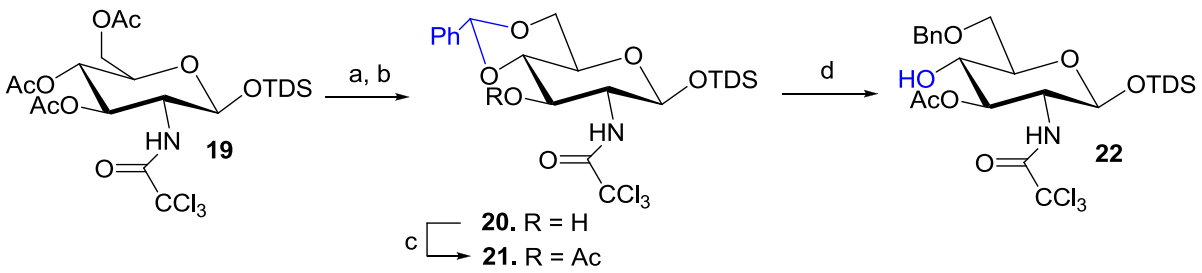


Expected:

	^{1}H-NMR:	^{13}C-NMR:
Equatorial methyl (S):	1.5 ppm	25 ppm
Axial methyl (R):	1.7 ppm	17 ppm

Figure 2.7: Characterization of Pyruvylated Building Block (β-anomer): HSQC NMR Spectra

An equatorially oriented methyl group (i.e. (S)-configuration of acetal carbon in D-glucose and D-mannose) shows an up-field shift in ^{1}H-NMR spectrum compared to an axially oriented methyl (1.5 ppm vs. 1.7 ppm). Also, the equatorial methyl group shows a down-field shift in ^{13}C-NMR compared to an axial methyl (25 ppm vs. 17 ppm).[53,54] In addition, the coupling constants for the anomeric protons were J_{H-H} = 4.8 Hz and J_{C-H} = 171 Hz for alpha anomer and J_{H-H} = 9.8 Hz and J_{C-H} = 162 Hz for the beta anomer.

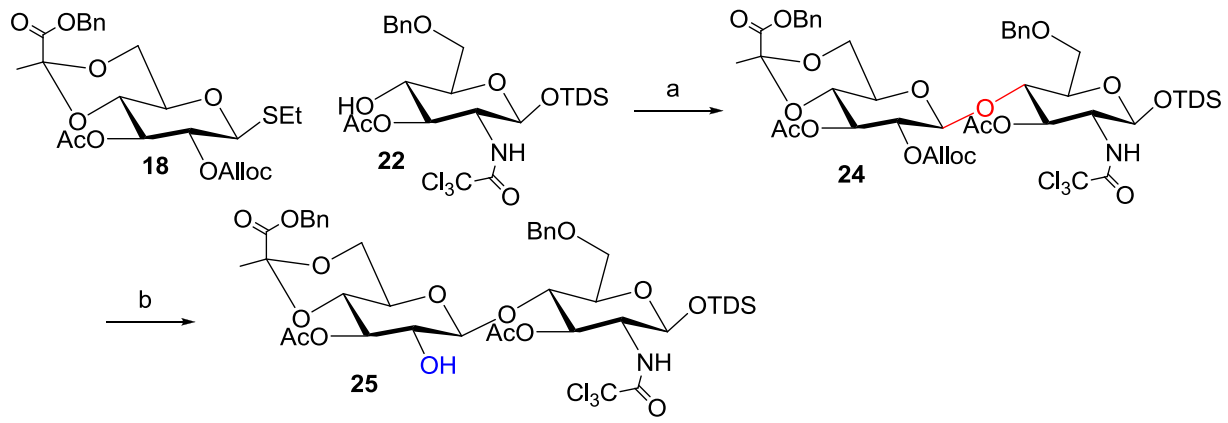


Scheme 2.6: Synthesis of Building Block 2 Version 1. (a) NaOMe, MeOH; (b) $PhCH(OMe)_2$, CH_3CN, CSA, 66% 2-steps; (c) Ac_2O, Pyridine, 95%; (d) Triethylsilane, TfOH, DCM, -78°C, 92%.

Synthesis of Building Block 2 Version 1: The next step of the synthesis involved the preparation of the second building block (Scheme 2.6). Compound **19** was synthesized using a previously described method.[55] Intermediate **19** was deacetylated under Zemplen conditions to yield a 3,4,6 triol. Without further purification, the benzylidene was installed using benzaldehyde dimethyl acetal and CSA to afford compound **20** in 66% yield over 2 steps. The C-3 hydroxyl was then acetylated with Ac_2O to provide **21** in 95% yield. Afterwards, the benzylidene was selectively opened at the O-4 position using triethylsilane, TfOH conditions to give **22** in 92% yield.

Building Block 3 Version 1: Compound (**23**) was synthesized using a previously published procedure.[56]

Disaccharide Synthesis: For the synthesis of disaccharide **24**, neighboring group participation from the 2-*O*-Alloc was utilized to selectively form the beta product (Scheme 2.7).[42] Several conditions were attempted (Table 2.2). The first attempt employed NIS (1.5eq) and TMSOTf (0.26eq) activation conditions at -20°C. After stirring for 1hr, the reaction was warmed to 0°C. A small amount of product was seen by TLC, but the major components of the reaction mixture were hydrolyzed donor and unreacted starting material. The yield of **24** was low at 13%. It has been mentioned in literature that pyruvylated donors are deactivated and can be difficult to activate during glycosylation reactions.[37]



Scheme 2.7: Disaccharide Synthesis with Building Block 1 Version 1. (a) ***Method 1***: NIS, TMSOTf, DCM, -20 to 0°C; ***Method 2***: i. DPS, TTBP, Tf_2O, DCM, -78°C to -55°C ii. Add Acceptor dropwise, Warm to RT. ***Method 3***: NIS, TfOF, DCM, 0°C, (b) $(PPh_3)_4Pd$, Morpholine, THF, H2O.

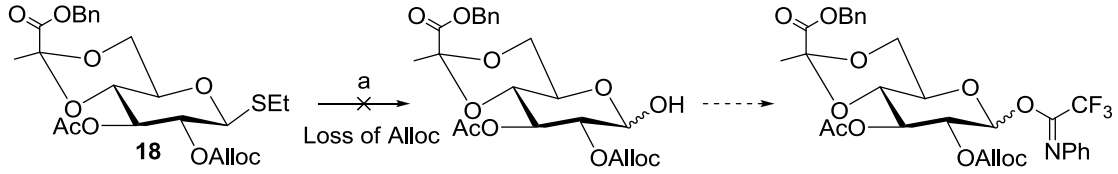
Table 2.2: Glycosylation Conditions for Donor **18** and Acceptor **22**.

	Conditions	**Yield**
Method 1:	NIS (1.5 eq), TMSOTf (0.26 eq), DCM, -20°C to 0°C	13% Mostly SM and hydrolyzed donor
Method 2:	DPS, TTBP, Cyclooctene, Tf_2O, DCM, -60°C to 0°C	21% Mostly SM and hydrolyzed donor
Method 3:	NIS (1.5 eq), TfOH (0.26-1.0 eq), DCM, -20°C to 0°C	16% Loss of Alloc from donor

The next attempt at disaccharide formation involved the use of preactivation conditions.[57] Donor **18** was combined with diphenylsulfoxide (DPS), 2,4,6-tri-t-butylpyrimidine (TTBP), and cyclooctene scavenger to protect the Alloc double bond. The mixture was cooled to -60°C. Tf_2O was added *via* dropwise addition and the mixture was allowed to warm to -50°C. Acceptor **22** was added by dropwise addition and the mixture was allowed to warm to 0°C. The reaction yield of **24** had improved, but the 21% yield was not optimal. The next glycosylation procedure attempted utilized NIS and TfOH activation conditions. It was apparent that donor **18** was not very reactive so an attempt was made to push the reaction to

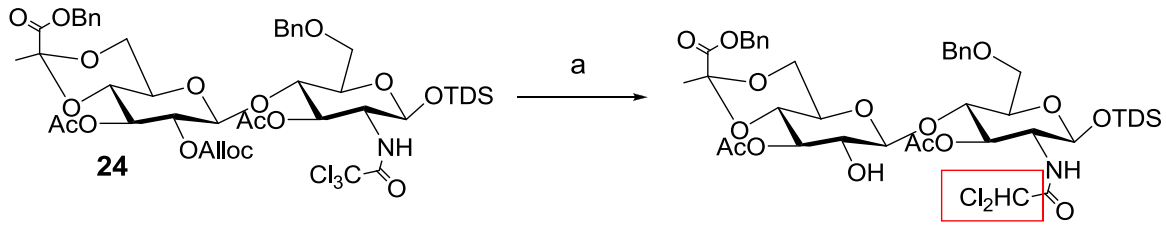
completion by adding additional triflic acid until the starting material was completely consumed. Donor **18** and acceptor **22** were dissolved in DCM and cooled to -20°C. Next, NIS (1.5eq) was added followed by TfOH (0.26eq). The reaction was warmed to 0°C. Triflic acid was added in 0.2 eq increments. Following each addition of triflic acid, TLC was taken in order to monitor the progress of the reaction. Once a total of 1eq of triflic acid had been added, the reaction was stirred for an additional 30 minutes and quenched. Even at this time, residual donor remained as well as donor without Alloc.

Due to the low reactivity of donor **18**, a decision was made to convert the thioglycoside to imidate to see if reactivity could be improved. An attempt was made to hydrolyze the thioglycoside directly to lactol using NIS, TFA, H_2O, and acetone[58] (Scheme 2.8). However, this method resulted in the loss of Alloc.



Scheme 2.8: Hydrolysis of Thioglycoside **18** and Conversion to Imidate. (a) NIS, Trifluoroacetic acid, Acetone, water; (b) 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride, K_2CO_3, DCM.

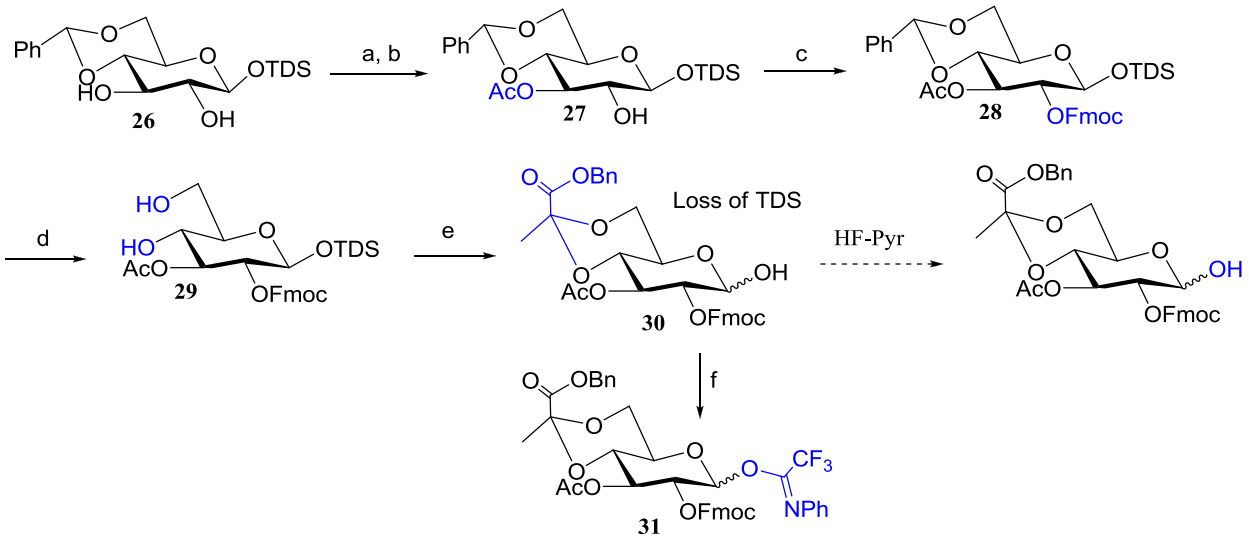
Before proceeding with the design and synthesis of an alternative building block, the disaccharide (**24**) from the separate reactions above was combined and used to test out the proceeding reactions involving the removal of Alloc, installation of triflate, and S_N2 inversion to the 2-Azido Mannopyranoside (Scheme 2.9).



Scheme 2.9: Removal of Alloc from disaccharide **22**. (a) $(PPh_3)_4Pd$, Morpholine, THF, H_2O.

To remove Alloc, disaccharide **24** was reacted with $(PPh_3)_4Pd$ in THF and water with a morpholine scavenger to prevent transallylation.[59] Unfortunately, only trace amounts of **25** were detected. The majority of **24** had been converted to a side-product with partially reduced TCA as verified by MALDI-TOF MS and NMR. It was apparent that the deprotection conditions for Alloc would not be compatible with the TCA protecting group.

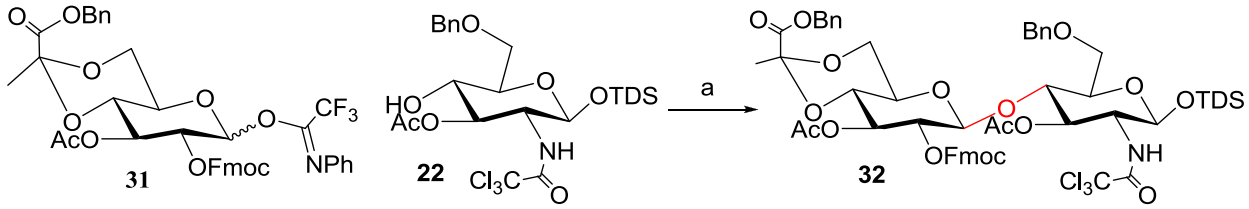
Due to the combination of poor reactivity of the thioglycoside donor (**18**) and the incompatibility of $(PPh_3)_4Pd$ with TCA, a decision was made to try an imidate based glycosylation strategy using an alternative protecting group in place of Alloc. The Seeberger group had reportedly used a benzylated galactoside with Fmoc protection at the O-2 postion and an anomeric imidate.[60] The Fmoc would allow for easy deprotection under mild basic conditions. It has also been shown that an imidate could be formed in the presence of Fmoc.[61]



Scheme 2.10: Synthesis of Building Block 1 Version 2. (a) i) Dibutyltin oxide, MeOH, 65°C; (b) AcCl, DCM, 0°C, 70%; (c) Fmoc-Cl, Pyridine, DCM, 100%; (d) 80% HOAc (aq), 50°C, 89% (e) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 15%; (f) 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride, K_2CO_3, DCM, 78%.

Building Block 1 Version 2: Compound **26** was synthesized using a previously published procedure.[62] Dibutyltin oxide conditions were employed to first form the stannylene acetal with **26** (Scheme 2.10).

This was followed by selective installation of acetyl at the O-3 position using acetyl chloride in DCM to afford compound **27** in 70% yield. Next, Fmoc was installed at the O-2 position using Fmoc-Cl to provide compound **28** in quantitative yield. Removal of the benzylidene acetal was accomplished by heating in 80% Acetic acid (aq) to give **29** in 89% yield. Installation of the 4,6-*O*-pyruvyl ketal was attempted under $BF_3(OEt)_2$ conditions.[50,51] Unexpectedly, a pyruvylated product with cleaved TDS (**30**) was obtained in 15% yield. Although this synthetic scheme appears to be a dead end, the product obtained was converted to an imidate (**31**) in 78% yield for use in a test reaction to determine if imidate based glycosylation can produce better yields than the thioglycoside based method.



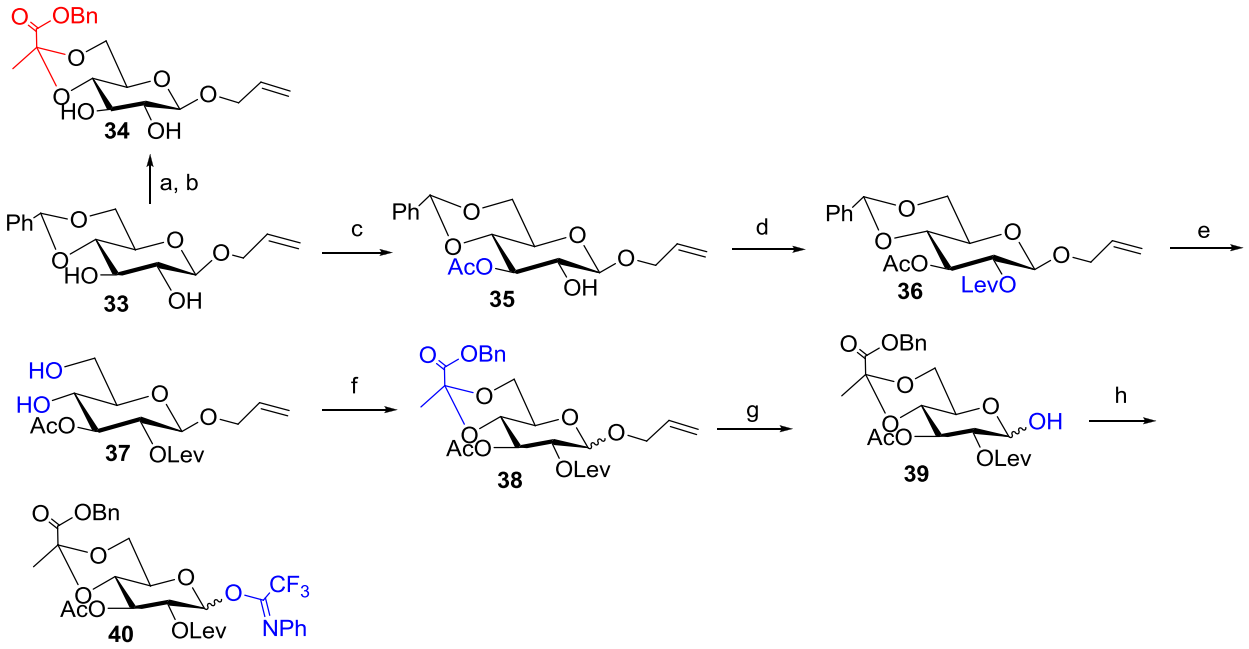
Scheme 2.11: Synthesis of Disacharide **32**. (a) TMSOTf, DCM, -30°C, 20%.

During the TMSOTf catalyzed glycosylation of donor **31** with acceptor **22**, it was noticed that **31** was completely consumed within 20 minutes indicating a more reactive donor in comparison to the thioglycoside (Scheme 2.11). There was, however, a significant quantity of hydrolyzed donor and the yield of **32** was only 20%. In an effort to replace the labile TDS protecting group of **29** with a more robust alternative, compound **28** was treated with HF-Pyridine. Unexpectedly, the reaction proceeded very slowly. After stirring for 2 days, a mixture of starting material and product along with side products lacking benzylidene were detected in the mixture. A decision was made to further modify the donor by replacing the anomeric TDS with an Allyl ether and by using a levulinoyl ester in place of the 2-*O*-Fmoc.

Building Block 1 Version 3: Compound **33** was synthesized according to a previously published procedure[63] A short cut in the synthesis was attempted *via* early installation of the 4,6-*O*-pyruvyl ketal

on the non-protected glucoside (Scheme 2.12). Unfortunately, the yield of **34** was too low (~12%) to proceed in this direction.

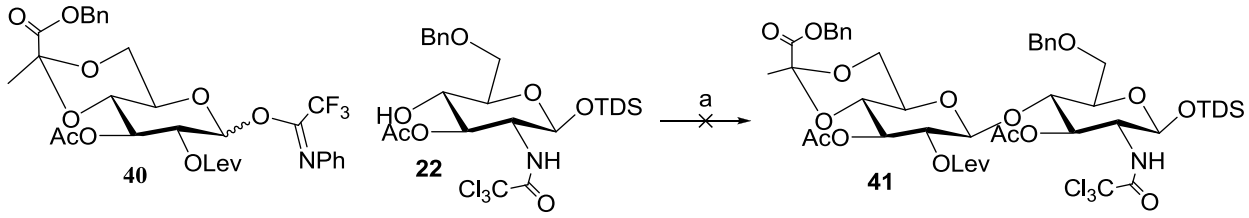
Dibutyltin oxide conditions were employed to selectively install the 3-*O*-acetyl providing compound **35** in 27% yield along with a separable 2-*O*-acetyl impurity in 24% yield. Next, the levulinoyl ester was installed at the O-2 position to afford **36** in quantitative yield.



Scheme 2.12: Synthesis of Building Block 1 Version 3. (a) 80% HOAc (aq); (b) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 12%; (c) i) Dibutyltin oxide, MeOH, 65°C; ii) AcCl, DCM, 0°C, 27%; (d) Levulinic acid, DCC, DMAP, DCM, 100%; (e) 80% HOAc (aq), 50°C, 91%; (f) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 33%; (g) i) $C_{34}H_{38}F_6IrP_3$, THF ii) $HgO,HgCl_2$ Acetone, H_2O, 93%; (h) 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride, K_2CO_3, DCM, 99%.

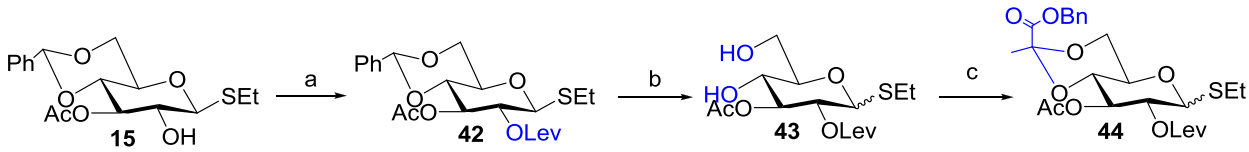
The benzylidene acetal was removed using 80% HOAc (aq) to give **37** in 91% yield. Next, the pyruvyl ketal was installed using $BF_3(OEt)_2$ conditions to provide compound **38** in 33% yield.[50,51] The yield for pyruvylation was significantly lower for this building block than for the previous thioglycosides. This could be due to the presence of Lev at O-2 in place of the previous Alloc or the anomeric O-Allyl in place of the thioglycoside. Afterwards, 1,5 Cyclooctadienebis-(methyldiphenylphosphine)iridium(I)-

hexafluorophosphate catalyst was used to isomerize the allyl. This was followed by cleavage of the resulting enol-ether under HgO/$HgCl_2$ conditions to afford **39** in 93%.[64] Lactol **39** was then converted to imidate donor **40** in 99% yield.



Scheme 2.13: Glycosylation reaction with imidate **40**. (a) TfOH, DCM, -20 to 0°C.

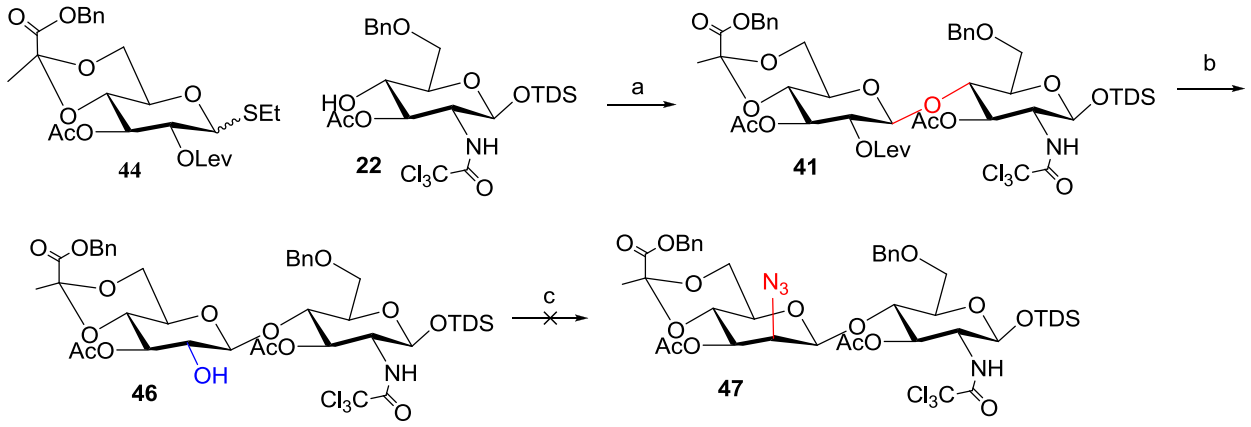
Donor **40** and acceptor **22** were used in a triflic acid catalyzed glycosylation in an attempt to make disaccharide **41** (Scheme 2.13). The reaction was initiated at -20°C and allowed to come to room temperature over 1hr. No product was detected in the reaction mixture by TLC or MALDI-TOF MS analysis of the reaction mixture. Only hydrolyzed donor and residual acceptor were present. Due to a lack of disaccharide formation using donor **40** and the low yield with imidate donor **31** in the previous reaction, a decision was made to retain the C-2 Lev, but revert back to the thioglycoside based donor.



Scheme 2.14: Synthesis of Building Block 1 Version 4. (a) Levulinic acid, DCC, DMAP, DCM, 97%; (b) 80% HOAc (aq), 50°C, 82%; (c) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 38%.

Building Block 1 Version 4: Starting from intermediate **15**, Lev was installed at the O-2 position to give compound **42** in 97% yield (Scheme 2.14). Afterwards, benzylidene was removed using 80% HOAc (aq) to provide diol **43** in 82% yield. The 4,6-*O*-pyruvyl ketal was installed using $BF_3(OEt)_2$ conditions to afford an α:β mixture of compound **44** in 38%.[50,51] The yield for pyruvylation was low in comparison to the reaction involving thioglycoside **18** (~68%). It was initially thought that having an anomeric O-allyl in

place of the thioglycoside in BB1-V3 may be the cause of the low yields for pyruvylation, however the results seen here seem to indicate that replacing the 2-*O*-Alloc with 2-*O*-lev may contribute to the lower yield as this is the only diference between building blocks **18** and **44**. A triflic acid catalyzed glycosylation of donor **44** with acceptor **22** yielded disaccharide **41** in 37% yield (Scheme 2.15). This was the highest yield obtained thus far for disaccharide formation. The disaccharide product was moved forward in order to test out the proceeding reactions. The 2-*O*-Lev group on **41** was removed using hydrazine acetate to afford compound **46** in 82% yield.[65] The next step involved inversion of the C-2 position by first installing a leaving group and then displacing the leaving group in S_N2 fashion with an azide nucleophile to produce the 2-Azido-β-Mannoside (**47**).[43] Unfortunately, triflate proved difficult to install.

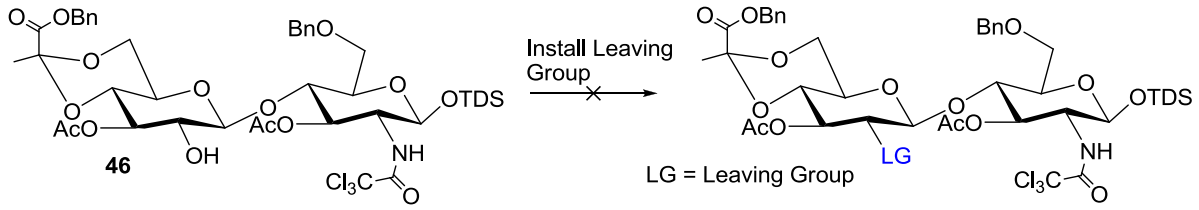


Scheme 2.15: Synthesis of Disaccharide **41** with thioglycoside donor **44**. (a) NIS, TfOH, DCM, -20 to 0°C, 37%; (b) Hydrazine Acetate, MeOH, DCM, 82%; (c) i) Tf_2O, DCM, 0°C, ii) NaN_3, DMF.

The triflate intermediate typically produces a significant shift on TLC to a higher *Rf* value in comparison to the starting material. Initially, it was thought that the unusual nature of the pyruvylated donor may have reduced the *Rf* shift that was normally observed and that the starting material and product were overlapping. The reaction was pushed forward to the S_N2 inversion step with sodium azide, but no product was detected. At this point, a failed installation of triflate was suspected. The reaction was repeated with fresh reagents, but still no product was obtained. Installation of alternative leaving groups

was attempted under a variety of conditions (Table 2.3). An effort was made to install Mesylate and Tosylate using an excess of MsCl or TsCl at room temperature. After stirring overnight, additional equivalents of MsCl or TsCl were added and the reactions were heated. Unfortunately, installation of the leaving groups failed in all cases.

Table 2.3: O-2 Leaving Group Installation Conditions Attempted on Disaccharide **46**.

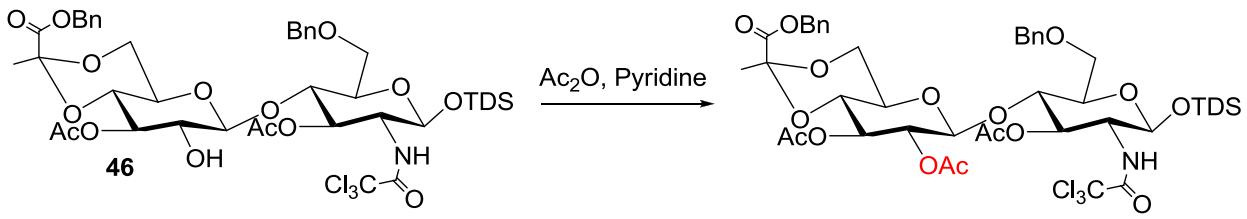


Equivalents Tf_2O:	Base:	Conditions:	Yield:
7 eq.	Pyridine (42 eq.)	Stir 1hr at 0°C and 1 hr at Room Temperature	No Rxn
7 eq.	Pyridine (42 eq.)	Stir 1hr at 0°C and stir at Room Temperature (~16 hrs)	No Rxn
Additional 7 eq.	Pyridine (42 eq.)	Heat at reflux-Monitor reaction over 16 hr	No Rxn

Equivalents MsCl:	Base:	Conditions:	Yield:
2.5 eq.	Et_3N (2.5 eq.)	Stir at Room Temperature (~16 hr)	No Rxn
Additional 2.5 eq.	Et_3N (Aditional 2.5 eq.)	Heat at reflux-Monitor reaction over 16 hr	No Rxn

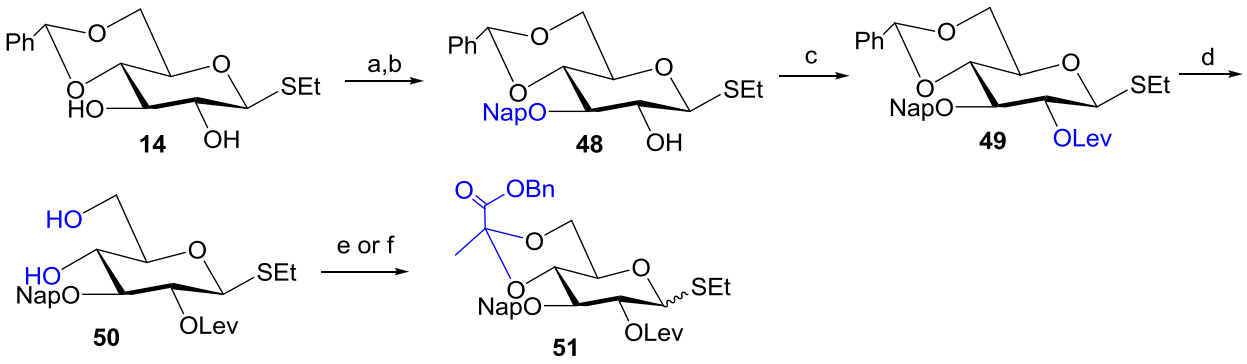
Equivalents TsCl:	Base:	Conditions:	Yield:
3 eq.	Pyridine (solvent)	Stir at Room Temperature (~16 hr)	No Rxn
Additional 3 eq.	Pyridine (solvent)	Heat at reflux-Monitor reaction over 16 hr	No Rxn

In order to verify that the O-2 position was indeed available for installation of a leaving group, a small quantity of disaccharide was reacted with Ac_2O and Pyridine (Scheme 2.16). Within 15 minutes, acetylated product formation could be seen by MALDI-TOF MS indicating that the 2-*O*-hydroxy was indeed available.



Scheme 2.16: Actylation of the O2 Position on Disaccharide **46**.

It was thought that the strong electron withdrawing nature of the leaving groups may be contributing to unfavorable dipole interactions and subsequent failure of the reaction. It was also suspected that the 3-*O*-acetyl may be problematic so a decision was made to protect the O-3 position with a non-electron withdrawing functionality to eliminate these possibilities. On the modified building block the 2-*O*-Lev was retained and the 3-*O*-acetyl was converted to a 3-*O*-(2-Naphthylmethyl) ether (Nap).

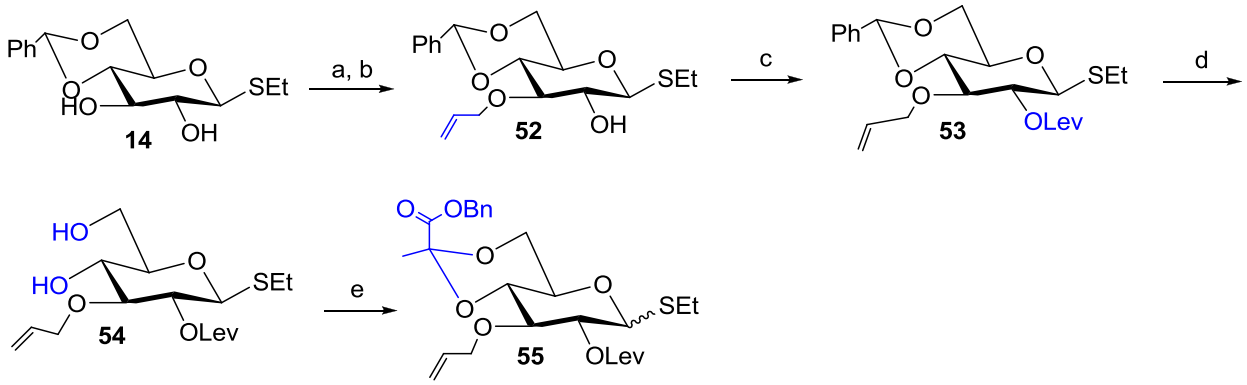


Scheme 2.17: Synthesis of Building Block 1 Version 5. (a) i) Dibutyltin oxide, MeOH, 65°C; (b) Nap-Br, CsF, DMF, 43%; (c) Levulinic Acid, DCC, DMAP, DCM, 98%; (d) 80% HOAc (aq), 50°C, 82% (e) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 8%; (f) i) TMS-Cl, pyridine ii) Benzyl Pyruvate TMSOTf, DCM -20°C to RT.

Building Block 1 Version 5: Compound **14** was reacted with dibutyltin oxide to form the stannylene acetal followed by reaction with CsF and NapBr to selectively install the 3-*O*-(2-methylnaphthyl) and afford compound **48** in 43% yield (Scheme 2.17). Next, the levulinoyl ester was installed at the O-2 position to provide **49** in 98% yield. The benzylidene acetal was removed by heating in 80% HOAc (aq) to give **50** in 82% yield. During the installation of pyruvyl using $BF_3(OEt)_2$ conditions,[50,51] the Nap protecting

group was unexpectedly lost contributing to the low 8% yield of **51**. An alternative method was attempted for installation of the 4,6-*O*-pyruvyl ketal.[66] In this method, the free hydroxyl positions were first silylated with TMS-Cl in pyridine. Afterwards, TMSOTf catalyzed formation of the 4,6-*O*-pyruvyl ketal was attempted in the presence of benzyl pyruvate. Unfortunately, this method yielded only 4% product. At this point, a decision was made to retain the C-2 Lev but replace the labile 3-*O*-Nap group with a more robust allyl ether.

Building Block 1 Version 6: Compound **14** was reacted under dibutyltin oxide conditions to install the stannylene acetal. The tin intermediate then was stirred with with CsF and allyl bromide to selectively install the 3-*O*-Allyl and afford compound **52** in 77% yield (Scheme 2.18). Next, the levulinoyl ester was installed at the O-2 position providing compound **53** in 96% yield. Afterwards, the benzylidene acetal was removed by heating **53** in 80% HOAc (aq) to give **54** in 94% yield.

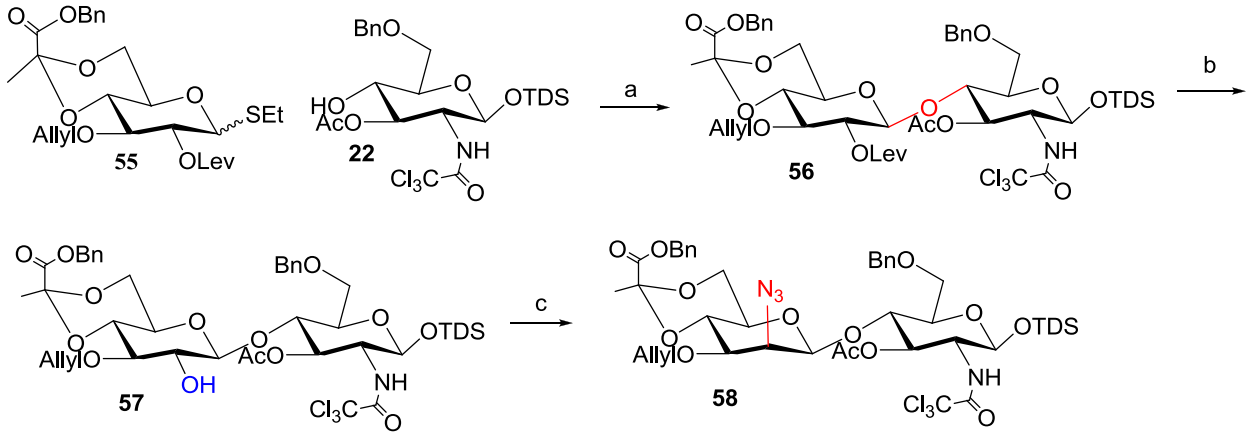


Scheme 2.18: Synthesis of Building Block 1 Version 6. (a) i) Dibutyltin oxide, MeOH, 65°C; (b) Allyl-Br, CsF, DMF, 77%; (c) Levulinic Acid, DCC, DMAP, DCM, 96%; (d) 80% HOAc (aq), 50°C, 94% (e) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 20%.

The pyruvyl ketal was subsequently installed using $BF_3(OEt)_2$ conditions affording compound **55** in 20% yield. Previous reports have shown that installation of pyruvyl is typically higher yielding when an electron withdrawing substituent is present at the O-3 position.[50,51] Ideally, it would be better to first install pyruvyl with an electron withdrawing substituent at O-3, and afterwards, replace that function

with the desired protecting group. As indicated in previous reactions, the 2-*O*-Lev may also be playing a role in the poor pyruvylation yield as well.

During the first attempt at disaccharide formation with donor **55** and acceptor **22**, an orthoester side product formed as the major product (Scheme 2.19). The reaction was repeated and an additional 0.1eq of TfOH was added after 15 mins in order to prevent orthoester formation. The yield of **56** was still relatively low at 24% and a trehalose side product was also present. Next, the disaccharide was stirred with hydrazine acetate to remove the Lev affording **57** in quantitative yield.



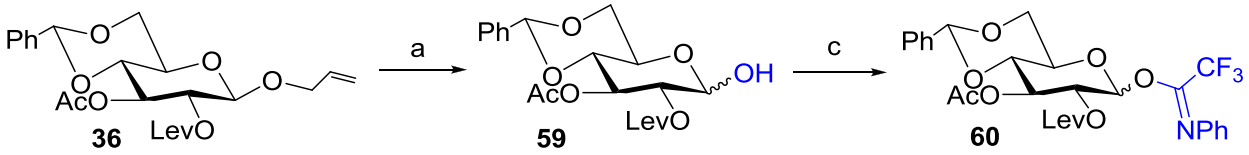
Scheme 2.19: Synthesis of Disaccharide **58** and conversion to 1,2-cis glycoside **58** *via* C-2 inversion. (a) NIS, TfOH, DCM, -20 to 0°C, 24%; (b) Hydrazine Acetate, MeOH, DCM, 100%; (c) i)Tf_2O, DCM, Pyr, 0°C, ii) NaN_3, DMF, 55°C, 68%.

The installation of triflate on **57** was successfully performed using Tf_2O. Next, inversion of the C-2 position was performed by heating with sodium azide in DMF to give **58** in 68% yield.[43] The results demonstrate that by replacing the 3-*O*-acetyl ester on the pyruvylated glucoside with a 3-*O*-allyl ether, the triflate can be successfully installed at the O-2 position for subsequent S_N2 inversion. The success of this strategy allowed for the key disaccharide intermediate in the synthesis to be obtained. However, this synthetic route was not without drawbacks necessitating the exploration of alternative strategies. In addition to the low yield of the glycosylation reaction, installation of pyruvyl onto the donor was also

low yielding (20%). In any future strategy, an ester substituent would need to be in place at the O-3 position of the glucoside prior to pyruvlation to increase the efficiency of the acetalization reaction. Afterwards, this function would need to be selectively removed in the presence of other base labile functions such as the benzyl and levulinoyl esters. Next, an ether based substituent would need to be installed at the O-3 position using mild conditions. In addition, a change of protecting group at O-2 may also be necessary due to the low pyruvylation yields experienced with 2-*O*-Lev donors. Due to the many variables and potential problems, alternative strategies were explored.

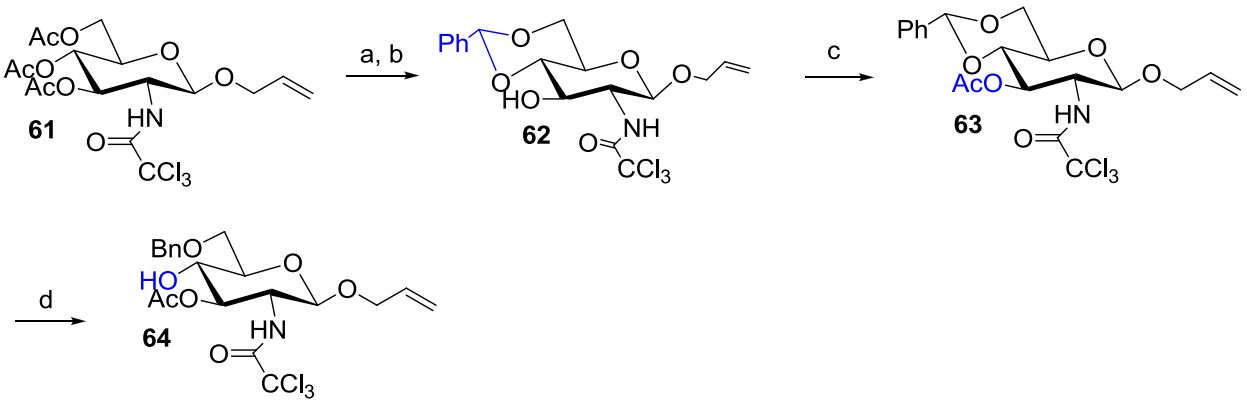
Pyruvyl Installation on a Disaccharide: A synthetic procedure involving the pyruvylation of a disaccharide was previously reported.[67] Installation of the 4,6-*O*-pyruvyl ketal at the disaccharide stage would allow for use of more reactive non-pyruvylated donors during glycosylations. The strategy involves first synthesizing a benzylidene protected disaccharide and then removing the benzylidene to make available the O-4 and O-6 hydroxys for installation of the pyruvyl.

Building Block 1 Version 7: The anomeric Allyl was removed from intermediate compound **36** using 1,5 Cyclooctadienebis-(methyldiphenylphosphine)iridium(I)hexafluorophosphate catalyst to first isomerize the allyl. The resulting enol-ether was cleaved under $HgO/HgCl_2$ conditions to provide **59** in 93% yield[64] (Scheme 2.20). Next, the lactol was converted to an N-phenyl trifluoracetimidate donor (**60**) in 93% yield.



Scheme 2.20: Synthesis of Building Block 1 Version 7. (a) i) $C_{34}H_{38}F_6IrP_3$, THF ii) HgO, $HgCl_2$, H_2O, Acetone, 93%; (b) 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride, K_2CO_3, DCM, 93%.

Due to the fact that installation of the pyruvyl occurs after disaccharide formation, the anomeric TDS on the acceptor had to be replaced with a more robust allyl. It was previously shown in the synthesis of Building Block 1 Version 2 that $BF_3(OEt)_2$ conditions can cleave the anomeric TDS.

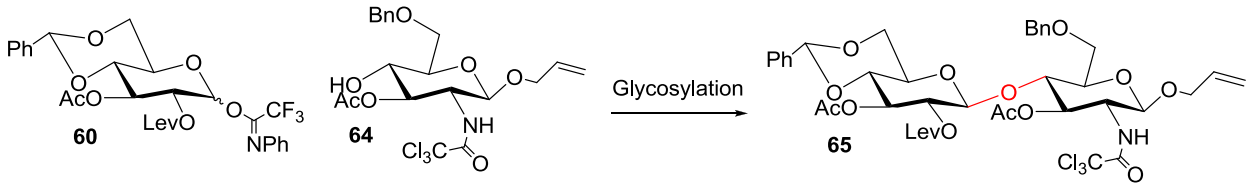


Scheme 2.21: Synthesis of Building Block 2 Version 2. (a) i) NaOMe, MeOH ii) Dowex Acidic resin; (b) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile; (c) Ac_2O,Pyridine, 78% 3-steps; (d) Triethylsilane, Triflic acid, DCM, 63%.

Building Block 2 Version 2: Compound **61** was synthesized according to a previously published procedure.[68] Intermediate **61** was treated with methanolic sodium methoxide to remove the acetyl protecting groups (Scheme 2.21). Next, benzylidene was installed using benzaldehyde dimethyl acetal and CSA to afford **62**. The 3-*O*-hydroxy was acetylated using Ac_2O and pyridine to provide compound **63** in 78% yield over three steps. Afterwards, reductive ring opening of benzylidene at the O-4 position was performed with triflic acid and triethylsilane to give compound **64** in 63% yield.[69] With donor **60** and acceptor **64** in hand, disaccharide formation was attempted. The glycosylation was attempted using two different conditions (Table 2.4). The lower temperature condition (-50°C to -35°C) gave a better yield, but the product was a mixture of alpha and beta anomers. The higher temperature reaction (-40°C to -10°C) provided better Beta selectivity, but at a lower yield. It was expected that the replacement of the deactivating 4,6-*O*-pyruvyl ketal with 4,6-*O*-benzylidene would provide a more reactive donor and a significant improvement in yield. However, only a modest improvement in yield was seen. The

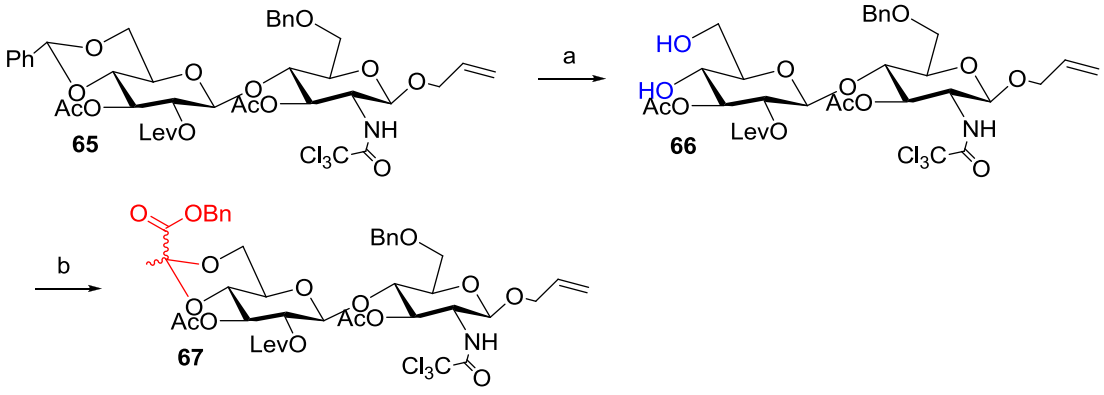
combination of acetyl and levulinate ester may have a deactivating effect on the donor. No further optimization of the reaction was performed as this was proof of principle reaction for the purpose of seeing if the pyruvyl could be installed in good yield with (S) stereochemistry at the disaccharide stage.

Table 2.4: Glycosylation Conditions for the Syntheis of Disaccharide **65**.



Conditions:	Results:
TMSOTf (0.23eq), DCM, -40°C to -10°C	25% Yield (Beta Only)
TMSOTf (0.23eq), DCM, -50°C to -35°C	43% (α:β, 2:3)

The benzylidene on disaccharide **65** was removed using 80% acetic acid to give diol **66** in 78% yield (Scheme 2.22). Afterwards, 4,6-*O*-pyruvyl ketal was installed using $BF_3(OEt)_2$ conditions[50,51] to afford **67** as a mixture of (R) and (S) stereoisomers in 28% yield. NMR was used to verify that the impurity was indeed the undesired (R) stereoisomer located at the chiral carbon of pyruvyl ketal (Figure 2.8).[53]



Scheme 2.22: Installation of 4,6-*O*-Pyruvyl Ketal on Disaccharide. (a) 80% HOAc (aq), 78%; (b) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 28% (R/S mixture).

It could be seen that the product had two β-linkages *via* C-H coupling constants as well as a mixture of the axial and equatorial pyruvyl metyl. Due to the low yield and purity resulting from pyruvyl installation at the disaccharide stage, the β-Mannosylation based strategy (Method 2) was attempted.

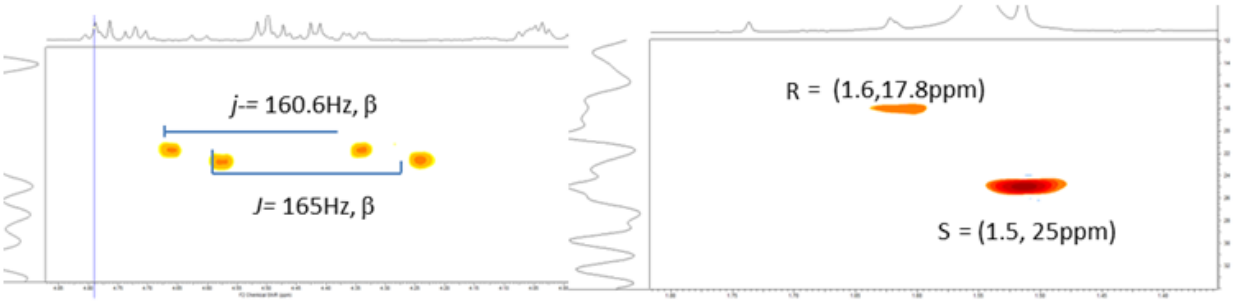
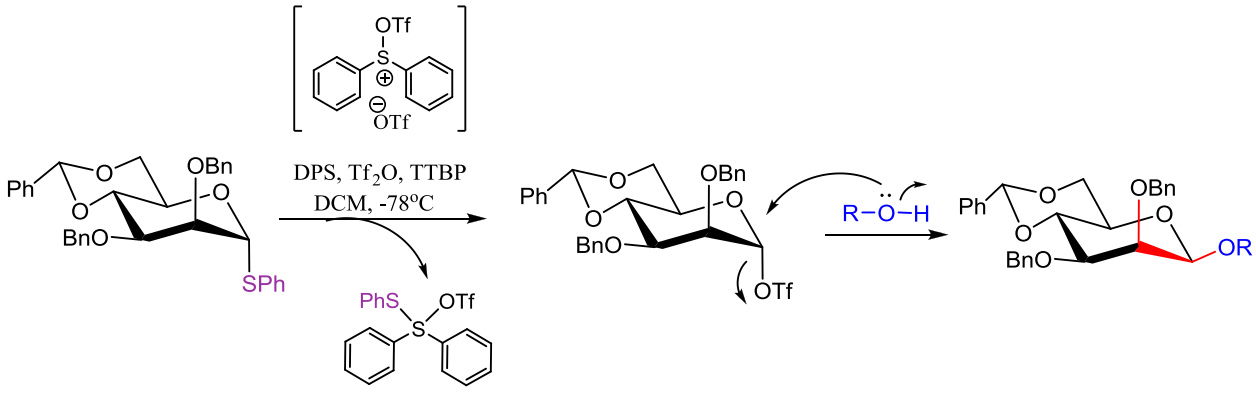


Figure 2.8: HSQC Spectra Showing Beta Product with R and S mixture at Pyruvyl stereocenter.

Method 2: Beta Mannosylation Strategy

The Method 2 strategy utilizes Crich β-mannosylation to directly install the 1,2-*cis*-glycosidic linkage with beta configuration (Scheme 2.23). Crich's method involves preactivation of mannosyl sulfoxides or thioglycosides with triflic anhydride or benzenesulfenyl triflate, respectively, which results in the formation of an α-triflate. Afterwards, an acceptor can be added to displace the α-triflate in S_N2 fashion to afford the desired β-mannoside.[70]



Scheme 2.23: Preparation of 1,2-cis-Beta-mannosides using Crich β-Mannosylation:

The α-triflate intermediate has been observed in glycosylations employing the mannuronic acid donor.[71] The adoption of a high-energy conformation for the α-triflate with mannuronic donors may contribute to the increased reactivity and beta slectivity observed for this type donor compared to other uronic acid donors.[72] Mannuaronic acid has some similarities in structure to the 4,6-*O*-pyruvylated donors with the exception of the locked ring conformation (Figure 2.9). It was hoped that the successful activation and subsequent formation of beta product seen in mannuronic acid would translate to some degree to the pyruvylated donors.

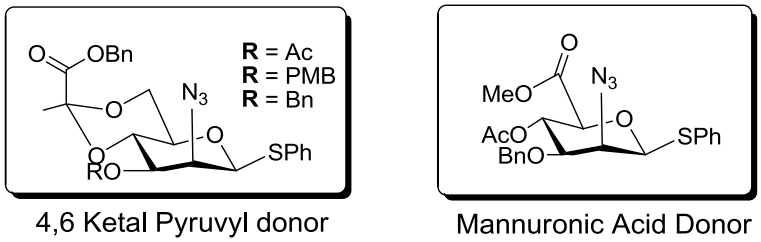
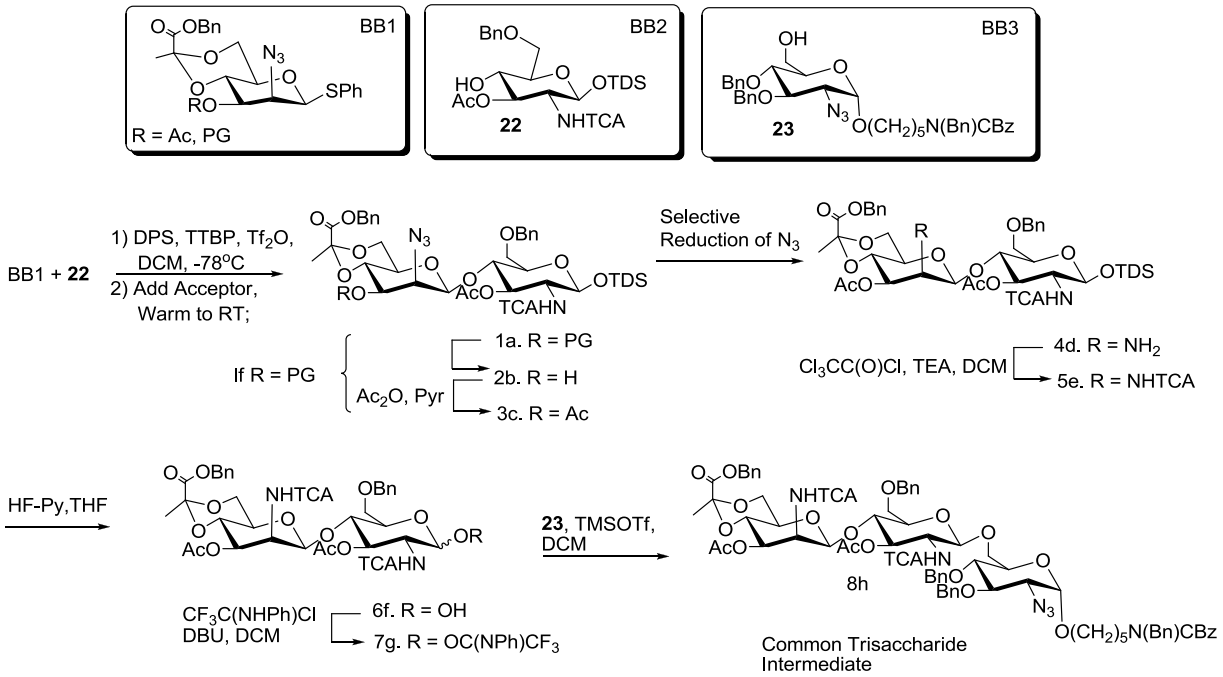


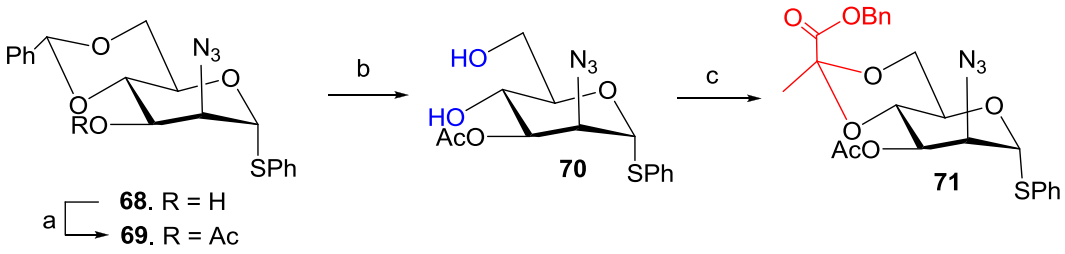
Figure 2.9: Comparison between Manuronic Acid and 4,6-*O*-Pyruvylated donors.

Method-2: Planned Synthetic Procedure: The use of TCA, TFA, and Cbz/Bn amino protecting groups was retained from the previous strategy.[39] The formation of disaccharide **1a** or **3c** will be accomplished using Crich beta mannosylation conditions (Scheme 2.24).[38,70] If a protecting group is used at the O-3 position of the Mannoside donor (BB1), it will be selectively removed and the resulting hydroxy will be acetylated. Next, the azide will be selectively reduced in the presence of TCA (*e.g*. 1,3-Propanedithiol, TEA) to yield **4d**. The resulting free amine will be protected with TCA to yield **5e**. Afterwards, the TDS can be removed with HF-Pyridine complex[44] to afford **6f**. The resulting lactol can then be converted to N-phenyl trifluoracetimidate donor (**7g**). A TMSOTf catalyzed glycosylation of donor **7g** with glycosyl acceptor **23** should yield the common trisaccharide intermediate **8h** as only the β-glucoside due to neighboring group participation of the TCA function.



Scheme 2.24: Method 2: General Synthestic Strategy For Common Trisaccharide Intermediate (1 of 2).

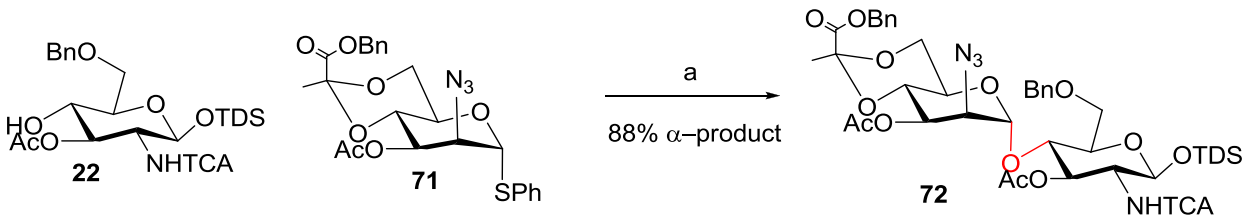
At this point the synthesis converges with synthetic route laid out in Method 1. The deprotection strategy in Scheme 2.2 (Pg. 60) can be followed for access to target compounds **1**-**4**. Intstallation of a benzyl at the O-3 position of the Mannoside donor (BB1) will provide access to targets **5** and **6**.



Scheme 2.25: Synthesis of β-Mannosyl Donor 1 Version 1 (a) Ac_2O, Pyridine; (b) 80% HOAc (aq), 70°C, 91% 2-steps; (c) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 74%.

β-Mannosyl Donor 1 Version 1: Compound **68** was synthesized according to a previously published procedure.[73] The C-3 hydroxy on compound **68** was acetylated using Ac_2O and pyridine to give compound **69** (Scheme 2.25). Next, the benzylidene acetal was hydrolyzed by heating in 80% HOAc(aq)

to provide compound **70** in 91% yield over two steps. Finally, the 4,6-*O*-pyruvyl ketal was installed using $BF_3(OEt)_2$ conditions[50,51] to afford compound **71** in good yield at 74%.

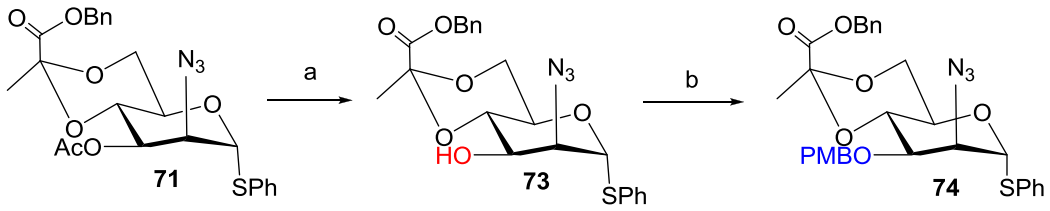


Scheme 2.26: Glycosylation with Donor **71** using β-Mannosylation Conditions. (a) i) DPS, TTBP, Tf_2O, DCM (-78°C to -55°C); ii) Cool to -78°C, Add **22**, warm to RT, 88% (α).

Donor **71** was dissolved in DCM along with diphenyl sulfoxide (DPS) and 2,4,6-Tri-tert-butylpyrimidine (TTBP) and cooled to -78°C (Scheme 2.26). Triflic anhydride (Tf_2O) was added and the reaction was allowed to warm to -55°C to allow for anomeric triflate formation. The reaction mixture was re-cooled to -78°C and acceptor **22** in DCM was added *via* dropwise addition, Afterwards, the reaction mixture was allowed to warm to 0°C. The reaction produced the highest yield of any reaction attempted thus far at 88%. Unfortunately, the product was exclusively in alpha configuration. The conditions proved effective for activating the donor, but the streoselectivity needed to be improved. It has been reported in literature that an electron drawing substituent at the O-3 position of a benzylidene bearing thioglycoside also yielded an alpha product under β-Mannosylation conditions.[74] However, the unique nature of the donor made the attempt worthwhile.

β-Mannosyl Donor 1 Version 2: In an attempt to gain Beta selectivity, a modified building block was made with a benzyl type protecting group in place of the 3-*O*-acetyl. The donor was modified by first removing the 3-*O*-acetyl using Guanidine buffered sodim methoxide to provide compound **73** in 88% yield (Scheme 2.27).[75] Next, a para-methoxybenzyl ether was installed to provide compound **74**. The presence of the base sensitive benzyl ester complicated the installation of PMB. Several different conditions were attempted (Table 2.5). Initially, freshly made Ag_2O was used along with PMB-Cl in

DCM[76]. Product formation was observed but a large amount of starting material remained. Additional equivalents of Ag_2O and PMB-Cl were added but the reaction did not proceed much further.

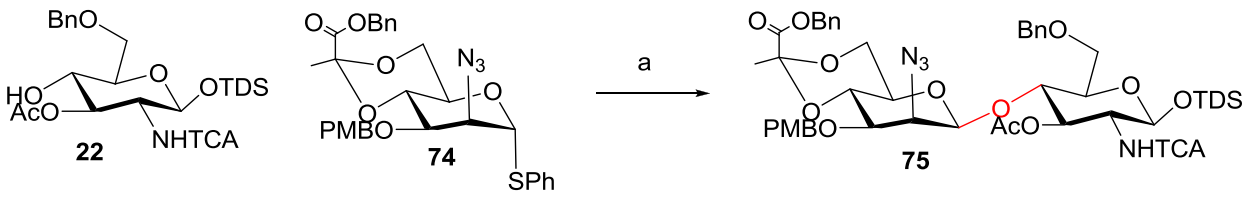


Scheme 2.27: Synthesis of β-Mannosyl Donor 1 Version 2. (a) Guanidine-HCl, NaOMe, DCM, MeOH, 88%; (b) PMB Installation.

Table 2.5: PMB Ether Installation Conditions

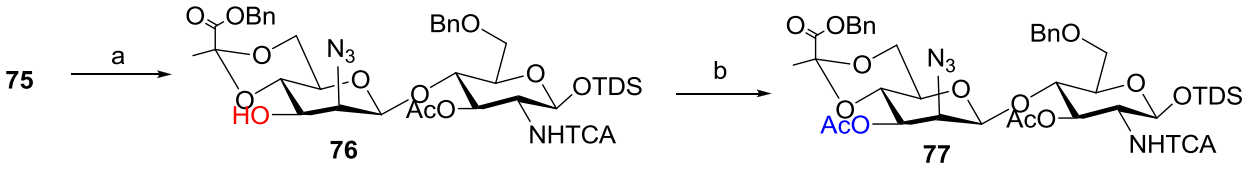
Conditions	Results
Ag_2O, PMB-Cl, DCM, 16hrs	33%, large excess PMB-Cl and Ag_2O, difficult purification
PMB-Imidate (4eq), TfOH (0.2eq), DCM, -20-0°C, 1.0hr	60-70%, on 100mg scale. Yield drops significantly on larger scale (1g) to 30-40%
NaH, PMB-Cl, DMF, 0°C, 3hr	78%,, Best overall, worked on gram scale

The purification was difficult due to excess PMB-Cl and the yield was only 33%. Next, a para-methoxy-benzyltrichloroacetimidate was made from p-methoxybenzyl alcohol, trichloroacetonitrile, and DBU.[77] The imidate was added to a solution of **73** in DCM and cooled to -20°C. Triflic acid was added and the mixture was allowed to warm to 0°C. On a 100mg scale, the reaction yield was relatively high at ~70%. However, in larger reactions approaching 1g, the yield dropped to 30-40%. Afterwards, standard benzyl installation conditions were attempted. The starting material was dissolved in dry DMF and cooled to 0°C. Sodium hydride was added portion-wise to avoid significant exotherm and the mixture was stirred for 5 mins. Next, PMB-Cl was added to the mixture *via* dropwise addition. A yield of 78% was obtained and the yield remained relatively high on gram scale.



Scheme 2.28: Glycosylation with Donor **74** using β-Mannosylation Conditions. (a) i) DPS, TTBP, Tf_2O, DCM (-78°C - 55°C); ii) Cool -78°C, **12**; iii) Warm to RT, 71% 1.2:1 (β:α).

Crich Beta mannosylation conditions were attempted using the new PMB modified donor **74** and acceptor **22** (Scheme 2.28). Fortunately, the modified donor afforded some β-selectivity and yielded a 1.2:1 (β:α) mixture of disaccharide **75** in 71% yield. The yield of subsequent reactions reached as high as 85%, however the α:β ratio remained the same. The β-Mannosylation strategy (Method 2) proved to be the best overall method despite the loss of some material as alpha product. Redeeming aspects of this strategy include the high yielding synthesis of the Mannoside donor and the reduced number of steps afforded by pre-installation of the C2-azide. With some disaccharide product in hand, the material was pushed forward in order to conduct frontier reactions on the proceeding steps.

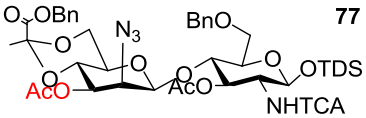
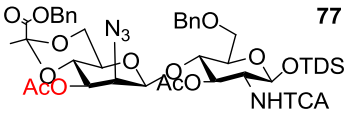
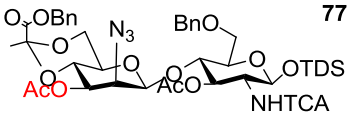
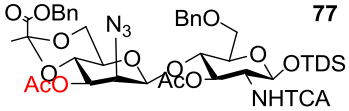
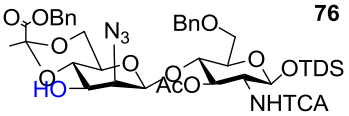
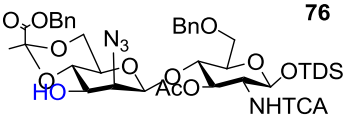
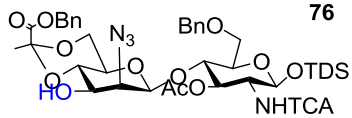
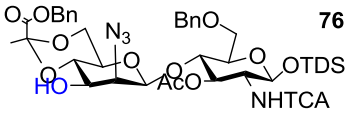
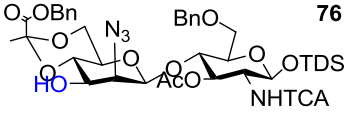
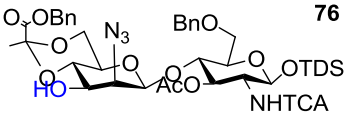


Scheme 2.29: Removal of PMB from disaccharide **76** and Acetylation of free hydroxy. (a) DDQ, H_2O, DCM, 84%; (b) Ac_2O, Pyridine, 100%.

The PMB of **75** was removed using DDQ[78] to afford **76** in 84% yield (Scheme 2.29). A portion of compound **76** was set aside in order to test out various azide reducing conditions (Table 2.6). A second portion of **76** was acetylated to provide **77** in quanitative yield. Early on in the synthesis, it was found that the 3-*O*-acetyl on ManNAc was more base labile than the 3-*O* acetyl on GlcNAc. Unfortunately, the

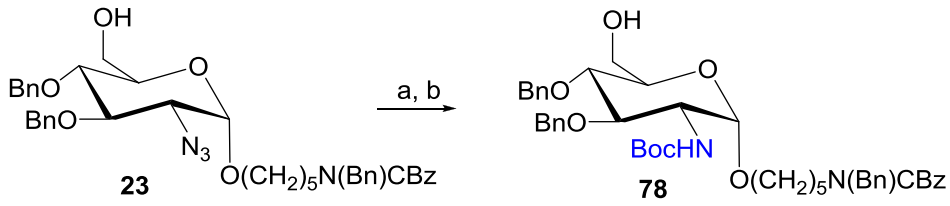
selectivity of deprotection was not good enough to utilize in a synthetic strategy that would have provided easy access to compounds **5** and **6**.

Table 2.6: Azide Reduction Conditions Attempted on Disaccharides **76** and **77**.

Starting Material	Reaction Conditions:	Result:
 **77**	1) PPh_3 (1.5eq), THF 50°C 2) H_2O 50°C *Ref: 79*	a) PPh_3, iminophosphorane b) Loss of TCA
 **77**	1) PMe_3 (1.5eq), THF 50°C 2) a) H_2O 50°C b) 0.1M NaOH (2eq) *Ref: 80*	a) PMe_3, iminophosphorane b) Loss of TCA
 **77**	Propanedithiol (6eq), TEA (6eq), MeOH a) RT 60hrs b) 50°C 24hrs *Ref: 81*	a) Loss of Ac from SM b) Loss of Ac from SM and some partial reduction of TCA
 **77**	$SnCl_2$ (1.5eq), PhSH (4.5eq) TEA (5eq), ACN 0°C *Ref: 82*	Within 30mins, azide reduced but TCA reduced to dichloro form. Confirmed with NMR study of BB2 Acceptor
 **76**	1) PMe_3 (1.5eq), THF 50°C 2) 0.1M NaOH (2eq) *Ref: 80*	Appears to have reduced TCA, also unknown major peak at 917(m/z)
 **76**	Propanedithiol (6eq), TEA (6eq), MeOH 50°C 12hrs *Ref: 81*	Mixture of SM and Product with partially reduced TCA
 **76**	$NiCl_2$-$(H_2O)_6$ (5eq), $NaBH_4$ (8eq), MeOH 0°C *Ref: 83*	a) Azide Reducedin 20mins, TCA in various staged of reduction. b) After 16hrs, added more $NiCl_2$, $NaBH_4$ to push for full reduction of TCA. TCA slow to reduce.
 **76**	BH_3-THF (4eq), THF, 50°C *Ref: 84*	No Reaction (36hrs)
 **76**	Thioacetic Acid (2.5eq), 2,6 Lutidine (2.6eq), Chloroform 50°C *Ref: 85*	No reaction after 24hrs. Additional 6eq of Thioacetic acid, Lutidine added and still no reaction after additional 16hrs
 **76**	Lindlar's Catalyst, H_2, MeOH *Ref: 86*	No Reaction after 16hrs. Additonal Catalyst added and began to see partial reduction of TCA and unknown peak at 909 (m/z)

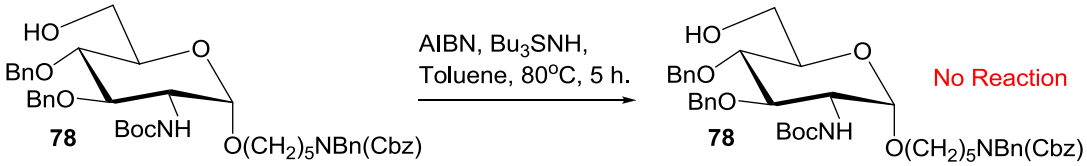
Some of the reducing conditions are basic in nature so reactions were attempted on both **75** and **76** to determine if azide reduction should be attempted before or after the installation of 3-*O*-Acetyl on ManNAc. A number of reduction methods were attempted, but it proved difficult to selectively reduce the azide in the presence of TCA (Table 2.6). The use of Staudinger conditions resulted in the loss of TCA and produced an iminophosphorane intermediate that proved difficult to hydrolyze. Reduction with propanedithiol and triethylamine was attempted, but the conditions resulted in the loss of acetyl and partial reduction of TCA. Thioacetic acid and BH_3-THF reduction conditions were also attempted, but no reaction took place with either condition. In addition, $SnCl_2$, $NiCl_2$, and Lindlar catalyst based methods resulted in partial reduction of TCA. At this point, a modified β-Mannosylation based strategy was designed.

Due to the iniability to selectively reduce the azide in the presence of TCA, it was thought that pre-reduction of the azide on compound **23** prior to glycosylation and protection of the resulting amine with an orthogonal protecting group would provide a viable alternative to the current strategy.



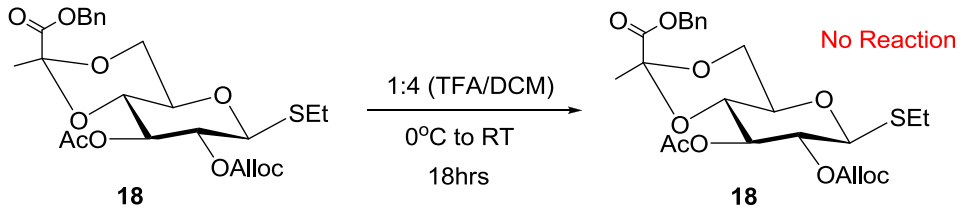
Scheme 2.30: Synthesis of Building Block 3 Version 2. a) Zn-Cu couple, HOAc; (b) Boc_2O, THF, 72% 2-steps.

Building Block 3 Version 2: Compound **23** was first treated with Zinc-Copper couple to reduce the azide and then treated with Boc_2O to afford compound **78** in 72% yield (Scheme 2.30). Before proceeding with the new strategy, it was necessary to verify that the Boc group would be stable under AIBN and Bu_3SnH reduction conditions.[40] Compound **78** was subjected to the radical reduction conditions for 5hrs after which no loss of Boc could be detected (Scheme 2.31).



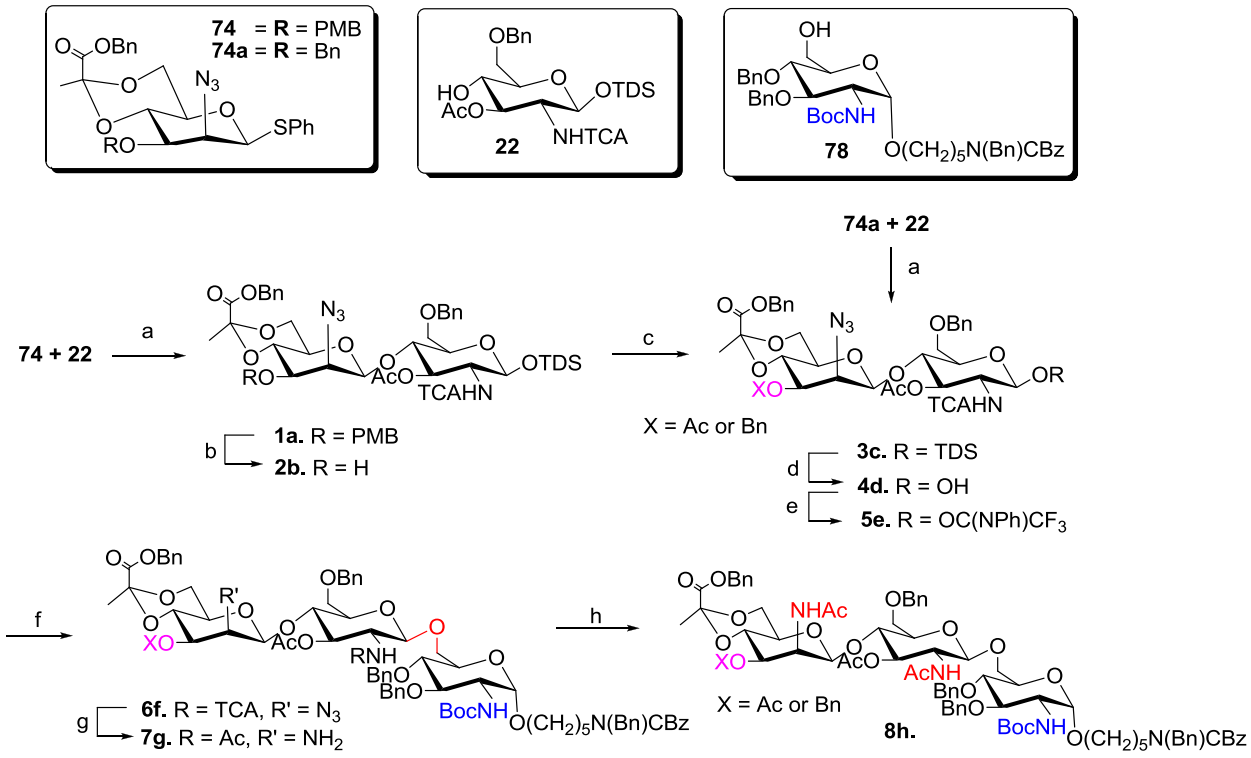
Scheme 2.31. Stability of Boc under TCA and Azide Reducing Conditions.

To test the stability of 4,6-*O*-pyruvyl ketal under the Boc deprotection conditions used in the new strategy, a thioglycoside building block (**18**) was subjected to the TFA deprotection conditions (Scheme 2.32). The reaction was allowed to proceed for 1.5 h at 0°C after which no loss of pyruvyl was detected by MALDI MS. The mixture was allowed to warm to room temperature and stir for an additional 18 hr but still no loss of pyruvyl was detected.



Scheme 2.32: Stability of 4,6-*O*-Pyruvyl Ketal in the presence of TFA.

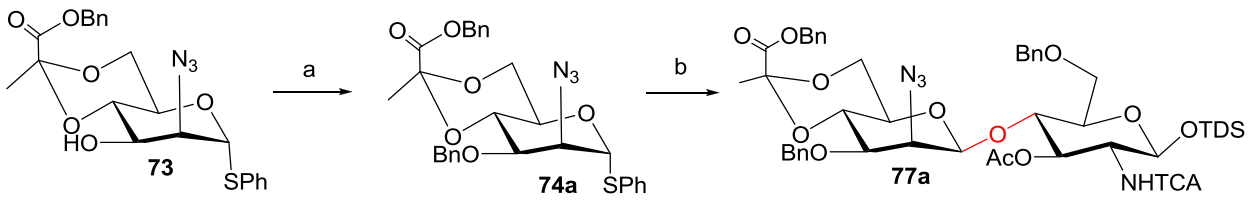
Method-2 *Revised*: **Planned Synthetic Procedure**: In the revised strategy, the target compounds were assembled using monosaccharide building blocks **74**, **74a**, **22** and **78** (Scheme 2.33). As in the previous strategy, the β-mannosamine moiety was introduced using Crich β-mannosylation conditions.[38,70] Two versions of the thioglycoside donor were synthesized. One version has a 3-*O*-PMB ether (**74**) that allows for selective deprotection and acetylation of the O-3 hydroxyl for access to target compounds **1**-**2**. The other version (**74a**) has a 3-*O*-benzyl ether that can be carried through the synthesis and deprotected at a late stage to afford a free hydroxy for target compounds **5** and **6**. As previously mentioned, the azide function of building block **23** from the previous strategy was pre-reduced to amine and Boc protected at the building block stage to provide compound **78**.



Scheme 2.33: General Synthetic Strategy: Method 2; Revised. (a) i. DPS, TTBP, Tf_2O, DCM, -78°C to -55°C; ii. Add Acceptor dropwise, Warm to RT; (b) DDQ, DCM, PBS, pH 7.4; (c) Ac_2O, Pyridine; (d) HFPy, THF; (e) $CF_3C(NHPh)Cl$, Cs_2CO_3, DCM; (f) **78**, TMSOTf, DCM, -20 to 0°C; (g) AIBN, Bu_3SnH, Toluene; (h) Ac_2O/Pyr.

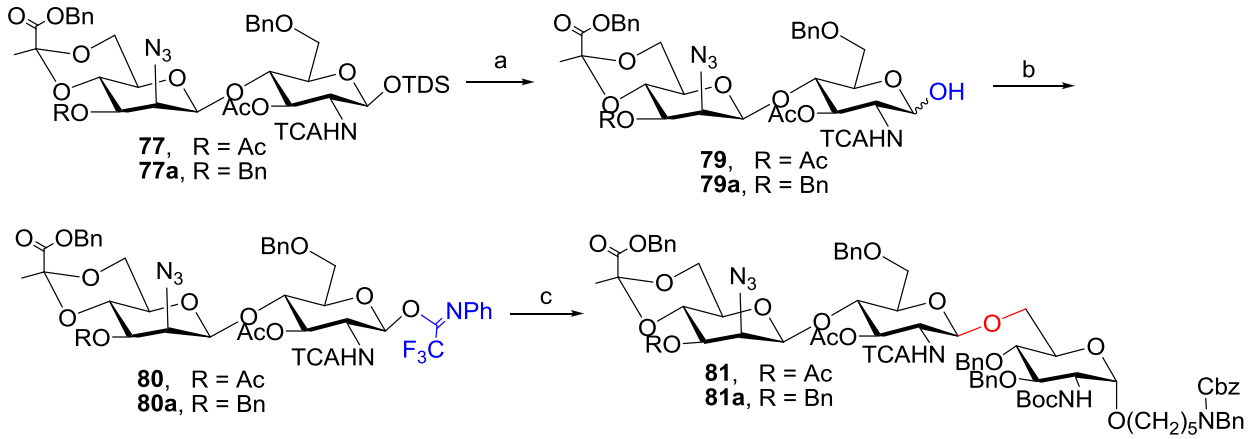
The use of TCA and Cbz/Bn as amino protecting groups was retained from the previous strategy. The Boc group is reportedly stable to catalytic hydrogenation allowing for selective removal of Benzyl/Cbz and modification of the linker amine. Afterwards, Boc can be removed to provide access to the free amine on select target compounds. Deprotection can be performed rapidly using 1:4 TFA/DCM at 0°C without affecting the acetyl esters.

β-Mannosyl Donor 1 Version 3: A 2-azido mannoside donor with a 3-*O*-benzyl (**74a**) was synthesized in order to provide a precursor to target compounds **5** and **6** (Scheme 2.34). Compound **73** was reacted with NaH and BnBr in DMF at 0°C to provide **74a** in 84% yield. Donor **74a** and acceptor **22** were then reacted under Crich β-Mannosylation conditions to afford a 1.1:1 (β:α) mixture of **77a** in 74% yield.



Scheme 2.34. Glycosylation with Donor **74a** using β-Mannosylation Conditions. (a) NaH, BnBr, DMF, 0°C, 3hr, 84%; (b) i) DPS, TTBP, Tf_2O, DCM (-78°C - 55°C); ii) Cool -78°C, **22**; iii) Warm to RT, 74% 1.1:1 (β:α).

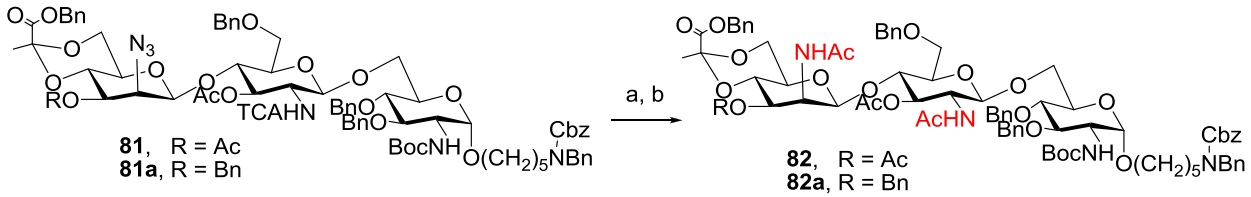
The next hurdle in the synthesis was the formation of trisaccharides **81** and **81a** (Scheme 2.35). The TDS was cleaved from **77**(**a**) with HF-Pyr complex[44] to afford lactol **79** in 90% yield and **79a** in 88% yield. Next, the lactol was converted to N-phenyl trifluoracetimidate donor **80** in 78% yield and **80a** in 82% yield.



Scheme 2.35: Synthesis of Trisaccharide **81**/**81a**. (a) HF, Pyridine, THF, **79**/**79a** 90%/88%; (b) Cs_2CO_3, Trifluoro-N-phenyl imidoyl Chloride, DCM, **80**/**80a** 78%/82%; (c) **78**, TMSOTf, DCM, -20 to 0°C, **81**/**81a** 67%/56%.

The imidate donor **80**(**a**) and acceptor **78** were utilized in a TMSOTf catalyzed glycosylation reaction to give trisaccharide **81** in 67% yield and **81a** in 56% yield. Fortunately, the Boc group remained intact despite the acidic glycosylation conditions. This was likely enabled by the low temperature of the reaction mixture and the catalytic amount of acid used in the glycosylation. The next major hurdle was the reduction of TCA to acetyl and azide to amine in the presence of the Boc function (Scheme 2.36).

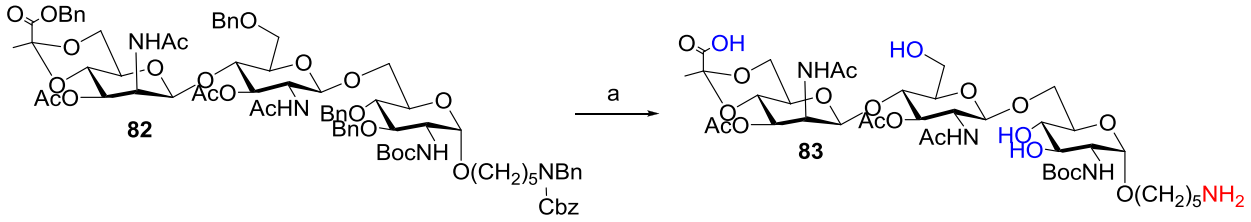
The radical reduction of **81(a)** using AIBN and Bu_3SnH and subsequent acetylation with Ac_2O and pyridine proceeded in good yield affording compound **82** in 96% over two steps and **82a** in 75% yield over two steps. After the two common trisaccharide intermediates (**82**, **82a**) for targets **1**-**6** were successfully obtained, they were further diversified into the final target compounds.



Scheme 2.36: TCA and Azide Reduction on Trisaccharide **81**/**81a**. (a) AIBN, Bu_3SnH, Toluene, 80°C; (b) Ac_2O, Pyridine, **82**/**82a** 96%/75%.

Synthesis and Deprotection of Target Compounds 1-6

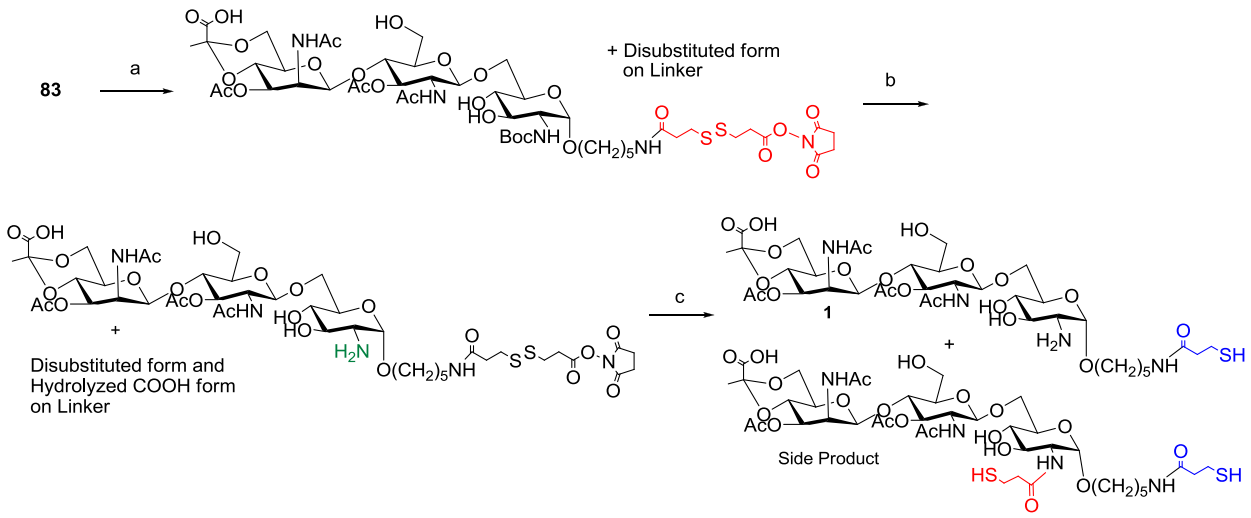
Target Compound 1: For target compound **1**, the next step involved removal of benzyl/Cbz groups using catalytic hydrogenation (Scheme 2.37). Again, it was imperative that the Boc remain intact under these conditions in order for this synthetic route to remain viable.



Scheme 2.37: Synthesis of Target Compound **1**. (a) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 67%.

Aromatic deprotection of **82** was accomplished using $Pd(OH)_2$ under hydrogen to obtain **83** in 67% yield**.** The use of acetic acid in the solvent mixture was avoided during hydrogenation due to the presence of the acid sensitive Boc. It was found that using a larger 1:1 ratio of $Pd(OH)_2$ to starting material ensured the reaction would be complete after stirring for ~16 hrs. In one instance, the reaction was only partially

complete, so the mixture was filtered and fresh solvent and $Pd(OH)_2$ was added. After ~14hrs, it was found that loss of Boc began to occur. For this reason, it is best to use an excess of catalyst in order to prevent this complication. The next step involved the installation of the disulfide linker (Scheme 2.38).



Scheme 2.38: Compound **1**: Addition of Thiol spacer and deprotection. (a) DSP, DIPEA, DMF; (b) 30% TFA/DCM, Triethylsilane, 0°C; (c) DTT, PBS buffer (pH 7.4).

The protected thiol linker was successfully installed onto the free amine of **83** using (dithiobis(succinimidyl propionate) (DSP; Figure 2.10). The product was obtained as a mixture with either one or two compounds substituted per linker. Afterwards, the Boc was cleaved revealing the free amine. Unexpectedly, some succinimidyl ester was seen in MALDI. It was expected that the remaining succinimidyl ester would have been hydrolyzed. During the reduction of disulfide with DTT in phosphate buffered saline (PBS; pH 7.4), LC/MS indicated the presence of product with two bound linkers. It turns out that the residual activated ester was reacting with the free secondary amine in the basic PBS buffer. For this reason, a decision was made to switch to 3,3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP). DTSSP is water soluble and the reaction can be performed in aqueous solution which should lead to hydrolysis of all of the residual activated ester during the course of the reaction. Following addition of

the linker, the product was treated with DTT and purified by C18 silica gel chromatography to remove any unreacted linker and prevent future side reactions.

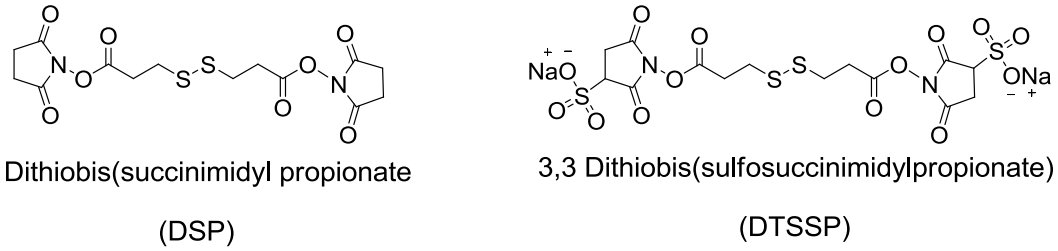
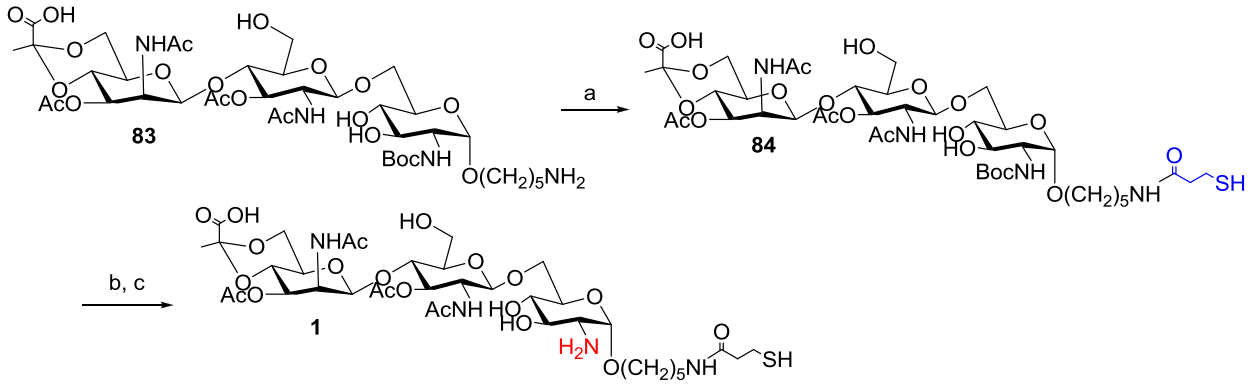


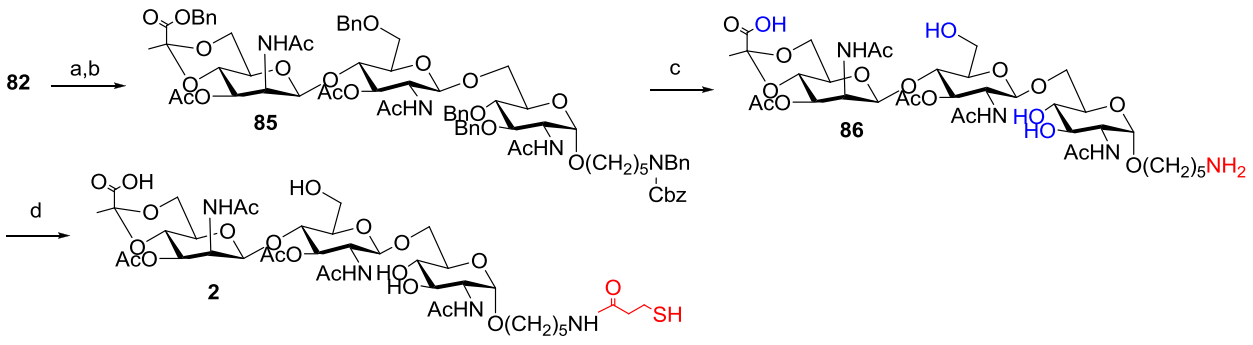
Figure 2.10: Comparison of DSP and DTSSP Linkers

The protected thiol linker was first installed onto compound **83** (Scheme 2.39) using DTSSP in PBS buffer (pH 7.4). Next, DTT was added to the reaction mixture in order to remove residual activated ester and reduce the disulfide bridge linking disubstituted product so purification could be simplified.



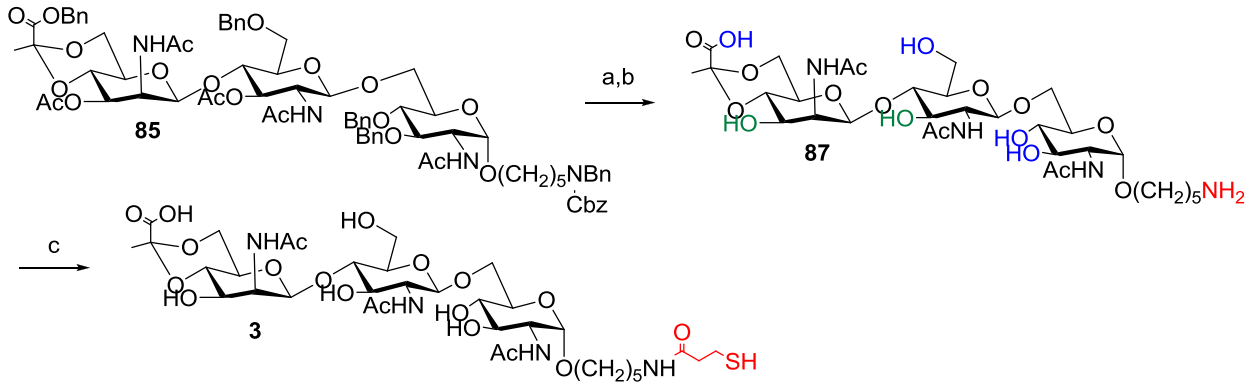
Scheme 2.39: Compound **1**: Revised Method; Addition of Thiol spacer and deprotection. (a) i) DTSSP, H_2O, pH ~8; ii) DTT, PBS (pH 7.4), 73%; (b) 1:3 TFA/DCM, Triethylsilane, 0°C; (c) DTT, PBS buffer (pH 7.4), 97% 2-steps.

The crude was purified by C18 silica gel column to give **84** in 73% yield. Afterwards, the Boc was cleaved with 1:3 TFA/DCM revealing the free amine. The crude was stirred in PBS with DTT and then purified using a P2 size exclusion column to afford target **1** in 97% yield.



Scheme 2.40: Synthesis of Target Compound **2**. (a) 1:3 TFA/DCM, 0°C; (b) Ac_2O, Pyridine, 91% 2-steps; (c) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 62%; (d) DTSSP, PBS (pH 7.4); (e) DTT, PBS, 40°C, 91%.

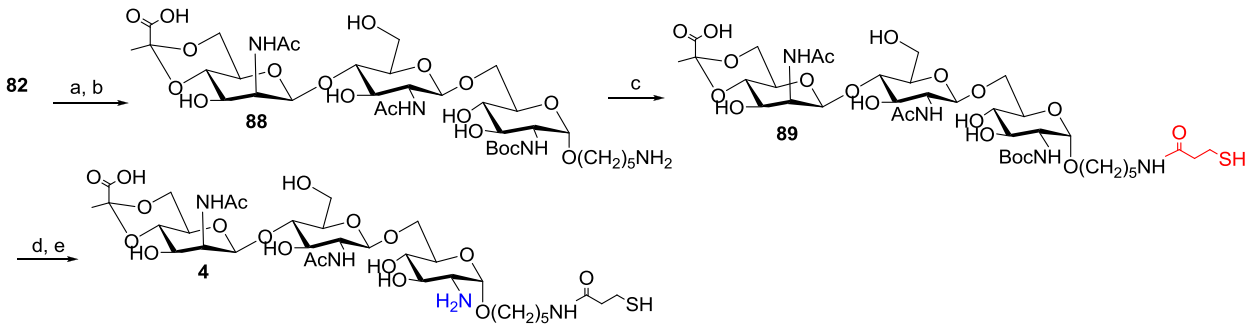
Synthesis of Target Compound 2: The Boc protecting group on compound **82** was removed by treatment with a 1:3 TFA/DCM solution at 0°C. The resulting amine was acetylated with Ac_2O to provide **85** in 91% yield (Scheme 2.40). Next the aromatic protecting groups were removed with $Pd(OH)_2$ under H_2 to afford **86** in 62% yield. The protected thiol linker was then installed using DTSSP in PBS (pH 7.4). Following treatement with DTT, target **2** was obtained in 91% yield.



Scheme 2.41: Synthesis of Target Compound **3**. (a) LiOH, THF, H_2O; (b) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 53% 2-steps; (c) i) DTSSP, H_2O, pH ~8; ii) DTT, PBS (pH 7.4), quant.

Synthesis of Target Compound 3: A key step in the deprotection of target compounds **3** and **4** involves removal of the O-acetyl functions on the trisaccharide. Initially, Zemplen deacetylation and Guanidine buffered sodium methoxide conditions were attempted; however, extensive transesterification of the

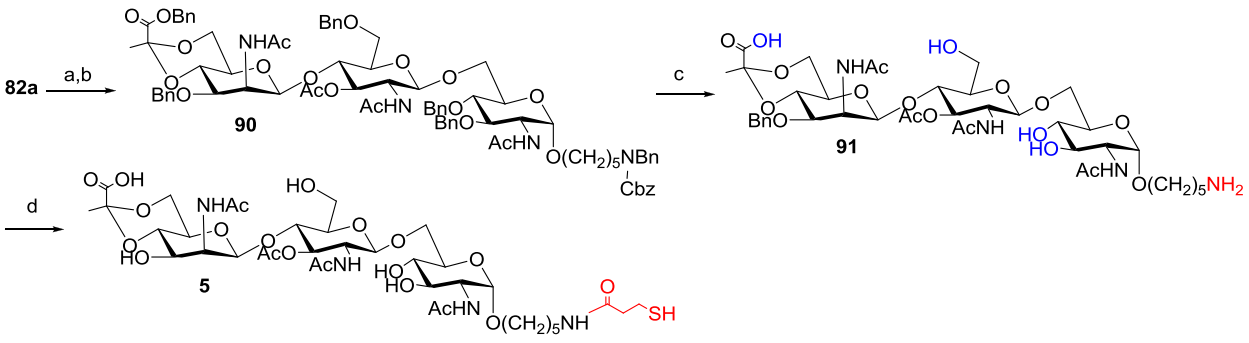
pyruvyl ester was taking place. A decision was made to switch to LiOH(aq) conditions in order to hydrolyze the benzyl and acetyl esters simultaneously.[87] Compound **85** was deacetylated using LiOH(aq) in THF at 35°C (Scheme 2.41). Next, the intermediate was reacted with $Pd(OH)_2$ under H_2 to obtain **87** in 53% yield over two steps. Afterwards, the protected thiol spacer was installed using DTSSP in PBS buffer (pH 7.4). Following treatment with DTT, target compound **3** was obtained in quantitative yield.



Scheme 2.42: Synthesis of Target Compound **4**: (a) LiOH, THF, H_2O; (b) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 61% 2-steps; (c) i) DTSSP, H_2O, pH~8; ii) DTT, PBS buffer (pH 7.4), 99%; (d) 1:3 TFA/DCM, Triethylsilane, 0°C; (e) DTT, PBS buffer (pH 7.4) (84%).

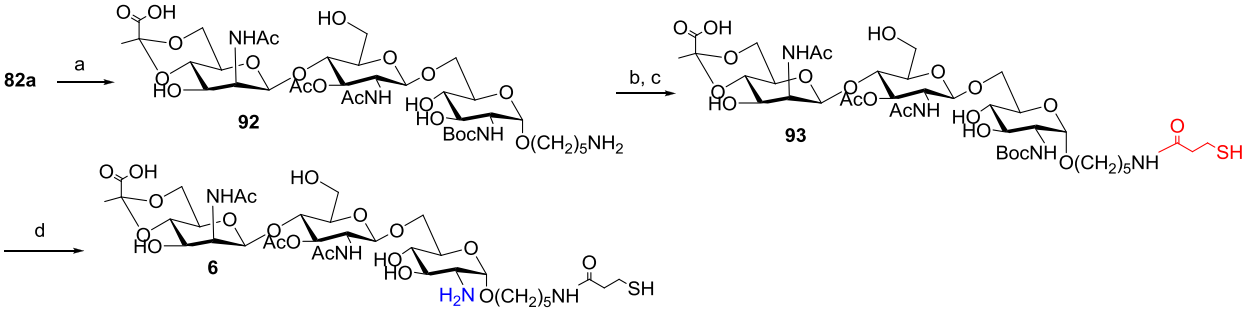
Synthesis of Target Compound 4: Compound **82** was deacetylated using LiOH in THF at 35°C. The benzyl and Cbz functions were removed from the intermediate using catalytic hydrogenation to afford **88** in 61% yield over two steps (Scheme 2.42). Next, the protected thiol linker was installed onto **88** using DTSSP in PBS (pH 7.4). Afterwards, the disulfide was converted to free thiol with DTT to provide **89** in 99% yield. The Boc protecting group was then removed with 1:3 TFA/DCM to give target **4** in 84% yield.

Synthesis of Target Compound 5: The Boc protecting group was removed from compound **82a** with 1:4 TFA/DCM solution at 0°C and the resulting amine was acetylated with Ac_2O to provide **90** in 97% yield (Scheme 2.43). Next the benzyl and Cbz protecting groups were removed with $Pd(OH)_2$ under H_2 to afford **91** in 74% yield. The protected thiol linker was then installed onto **91** using DTSSP in PBS (pH 7.4). Afterwards, the disulfide was converted to free thiol with DTT to give target **5** in 82% yield.



Scheme 2.43: Synthesis of Target Compound **5**. (a) 1:3 TFA/DCM; (b) Ac_2O, Pyridine, 97%; (c) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 74%; (d) DTSSP, PBS (pH 7.4); (e) DTT, PBS, 40°C, 82%.\

Synthesis of Target Compound 6: Compound **82a** was reacted with $Pd(OH)_2$ under H_2 to remove benzyl and Cbz functions and give **92** in 57% yield (Scheme 2.44). The protected thiol linker was then installed onto **92** using DTSSP in PBS (pH 7.4). Afterwards, the disulfide was converted to free thiol with DTT to provide **93** in 91% yield. Next, the Boc protecting group was removed with 1:3 TFA/DCM. The resulting intermediate was stirred with DTT in PBS (pH 7.4) and purified to afford target **6** in 91% yield.



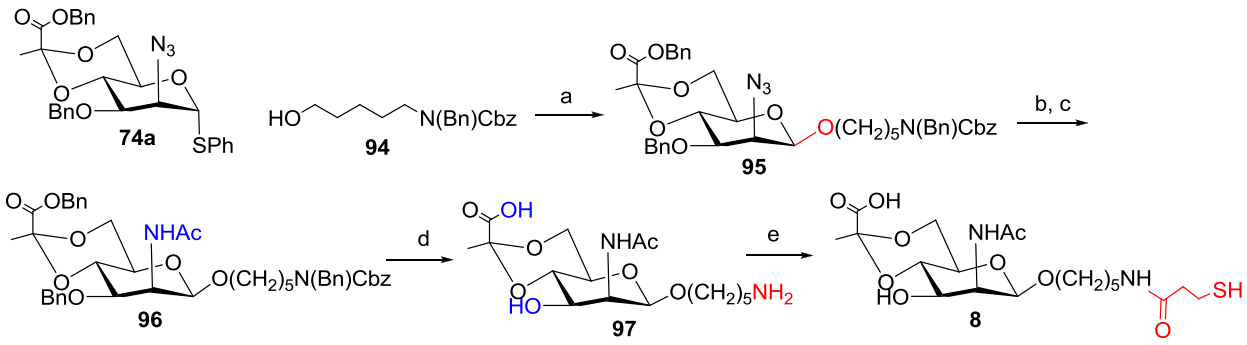
Scheme 2.44: Synthesis of Target Compound **6**: (a) $Pd(OH)_2$, tBuOH, H_2O, H_2 (1 atm), 57%; (b) i) DTSSP, H_2O, pH ~8; ii) DTT, PBS (pH 7.4), 91&; (c) 33% TFA/DCM, Triethylsilane, 0°C; (d) DTT, PBS buffer (pH 7.4), 91%.

Synthesis of Target Compound 7: The non-pyruvylated trisaccharide (**7**) was synthesized as previously described.[56] The free amine was conjugated to the DTSSP linker and converted to free thiol by reduction with DTT.

Synthesis and Deprotection of Target Compounds 8-11

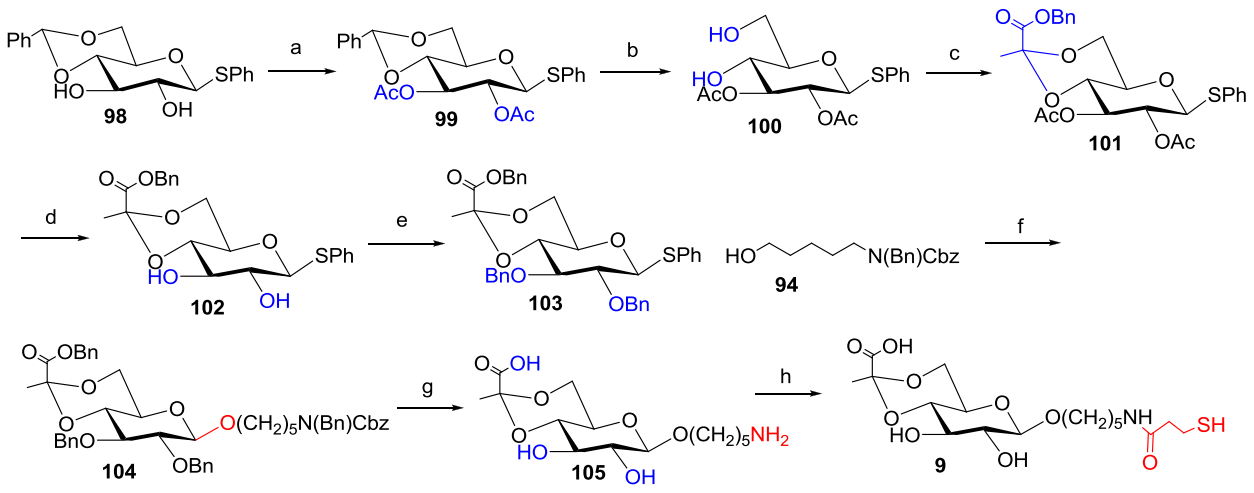
Following the completion of target compounds **1**-**6**, an additional series of targets were synthesized (**8**-**11**). The first compound synthesized was target compound **8** and it represents a truncated version of the trisaccharide consisting of only the pyruvylated ManNAc. Targets **9-11** are derivatives of compound **8** with modifications at the C-2 position. These compounds were used to determine the minimum epitope for binding and the importance of the C-2 acetamido for SLH-carbohydrate binding interactions.

Synthesis of Target Compound 8: Crich β-Mannosylation conditions were used for glycosylation of donor **74a** and the linker acceptor (**94**). Compound **95** was formed in 87% as a (1.1:1, β:α) mixture (Scheme 2.45). After separation of the beta product, the azide was reduced using Zn-Cu couple in acetic acid and acetylated with Ac_2O to give **96** in 76% yield. The benzyl protecting groups were removed using $Pd(OH)_2/H_2$ reduction conditions to afford **97** in 77% yield. The DTSSP linker was installed onto the resulting free amine and the thiol was liberated by reduction with DTT to give target compound **8** in 81% yield.



Scheme 2.45: Synthesis of Target Compound **8**. (a) i. DPS, TTBP, Tf_2O, DCM, -78°C to -55°C ii. Add Acceptor **94** dropwise, Warm to RT, 87% 1.1:1 (β:α); (b) Zn-Cu Couple, HOAc (c) Ac_2O, Pyridine, 76% 2-steps; (d) $Pd(OH)_2$, tBuOH,H_2O, H_2 (1 atm), 77%; (e) i), DTSSP, H_2O, pH~8; ii) DTT, PBS (pH 7.4), 81%.

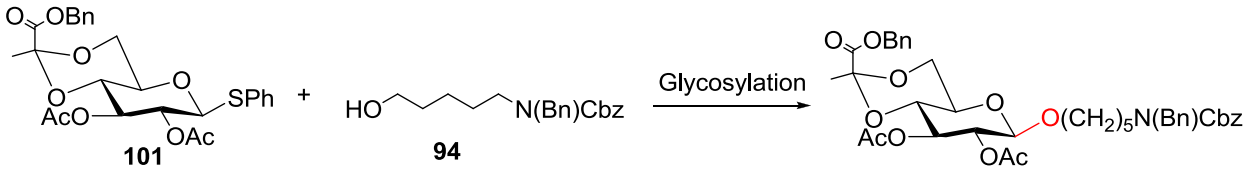
Synthesis of Target Compound 9: Compound **98** was synthesized according to a previously published procedure.[88] Thioglycoside **98** was treated with Ac_2O to give compound **99** in 99% yield. (Scheme 2.46).



Scheme 2.46: Synthesis of Target Compound **9**. (a) Ac_2O, Pyridine, 99%; (b) 80% HOAc (aq), THF, 55°C, 86%; (c) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 69%; (d) Guanidinim Chloride, NaOMe, MeOH, DCM, 85%; (e) NaH, DMF, 0°C, 60%; (f) i. DPS, TTBP, Tf_2O, DCM, -78°C to -55°C ii. Add Acceptor **94** dropwise, Warm to RT, 88% 1:1 (β:α); (g) $Pd(OH)_2$, tBuOH,H_2O, H_2 (1 atm), 92%; (h) i), DTSSP, H_2O, pH~8; ii) DTT, PBS (pH 7.4). 95%.

Next, the benzylidene was removed by heating **99** in 80% HOAc (aq) to afford diol **100** in 86% yield. The 4,6-*O*-pyruvyl ketal was installed using benzyl pyruvate and $BF_3(OEt)_2$ providing compound **101** in 69% yield. A shortcut in the synthetic method was attempted that entailed early glycosylation of **101** with linker **94** (Table 2.7). It was hoped that the pyruvylated glucoside donor would provide better reactivity and beta selectivity than the O-3 acetylated Mannoside donor. Several reaction conditions were attempted including the preactivation conditions used for the successful activation of the 3-*O*-acetylated mannoside donor. Unfortunately, only trace product was obtained due to the low reactivity of the donor. A decision was made to replace the O-acetyl functions with benzyl ethers to produce a more reactive donor. The O-2 and O-3 acetyl functions were removed with guanidinium and sodium methoxide conditions to afford compound **102** in 85% yield. Next, The O-2 and O-3 positions were benzylated using NaH and benzyl bromide at 0°C to provide compound **103** in 60% yield.

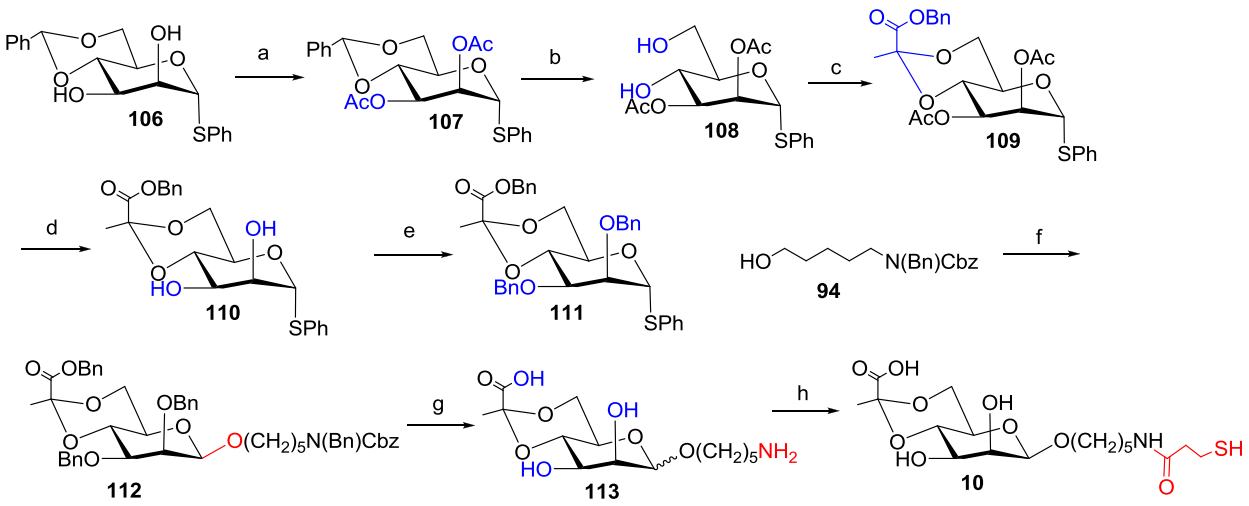
Table 2.7: Glycosylation Conditions for Linker Installation.



Conditions:	Result:
NIS (1.5 eq), TMSOTf (0.26 eq), -20°C to 0°C	Trace Pr
NIS (1.5 eq), TfOH (0.2-1 eq), -20°C to RT	Trace Pr
i) DPS, TTBP, Tf_2O, DCM, -78°C to -55°C ii) Add acceptor **94** dropwise, Warm to RT	Trace Pr

Donor **103** and acceptor **94** were used in a Crich β-Mannsoylation reaction to afford a 1:1 (β:α) mixture of **104** in 88% yield. The benzyl protecting groups were removed using $Pd(OH)_2$ conditions to give **105** in 92% yield. The resulting free amine on the linker was then modified using DTSSP followed by disulfide reduction with DTT to provide target **9** in 95% yield.

Synthesis of Target Compound 10*:* Compound **106** was synthesized according to a previously published procedure.[89] Thioglycoside **106** was acetylated with Ac_2O in Pyridine to give **107** in quantitative yield (Scheme 2.47). Next, the benzylidene was removed by heating in 80% HOAc (aq) to afford diol **108** in 66% yield over two steps. The 4,6-*O*-pyruvyl ketal was installed using benzyl pyruvate and $BF_3(OEt)_2$ to provide compound **109** in 65% yield. The O-2 and O-3 acetyl functions were removed with guanidinium buffered sodium methoxide to produce **110** in 70% yield. Next, The C-2 and C-3 hydroxy positions were benzylated using NaH and benzyl bromide at 0°C to provide compound **111** in 65% yield. Donor **111** and acceptor **94** were glycosylated under Crich β-Mannsoylation conditions to give a 2:1 (β:α) mixture of **112** in 66% yield.

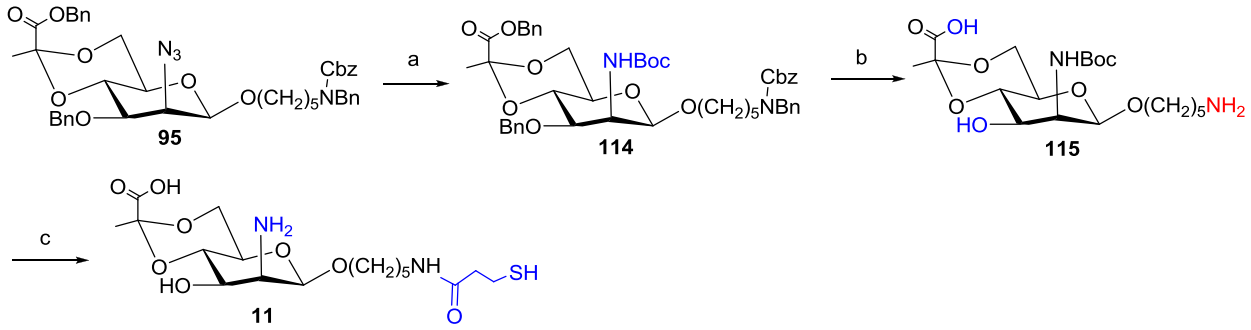


Scheme 2.47: Synthesis of Target Compound **10**. (a) Ac_2O, Pyridine, quant; (b) 80% HOAc (aq), THF, 55°C, 66%; (c) Benzyl Pyruvate, $BF_3(OEt)_2$, Acetonitrile, 65%; (d) Guanidinim Chloride, NaOMe, MeOH, DCM, 70%; (e) NaH, BnBr DMF, 0°C, 65%; (f) i. DPS, TTBP, Tf_2O, DCM, -78°C to -55°C ii. Add Acceptor **94** dropwise, Warm to RT, 66% 2:1 (β:α).; (g) $Pd(OH)_2$, tBuOH,H_2O, H_2 (1 atm), 77% 2.3:1 (β:α); (h) i), DTSSP, H_2O, pH~8; ii) DTT, PBS (pH 7.4), 95%.

The benzyl protecting groups from compound **112** were removed using catalytic hydrogenation to afford **113** in 77% yield. Unexpectedly, the product formed as a 2.3:1 (β:α) mixture. The reaction was repeated but the results remained the same. Separation of the mixture proved difficult at this late stage. Fortunately, a method was devised using a ZIC-HILIC semi-prep column in line with an LCMS. The product was eluted with ammonium bicarbonate and acetonitrile solvents. The first peak to elute was determined to the the alpha product as determined by NMR. The free amine on the linker of the β anomer was modified using DTSSP and treated with with DTT to provide target **10** in 95% yield.

Synthesis of Target Compound 11*:* The azide on compound **95** was reduced to amine using Zn-Cu couple in acetic acid (Scheme 2.48). The amine was then Boc protected with Boc anhydride to provide compound **114** in 81% yield over 2 steps. Next, the aromatic protecting groups were removed using catalytic hydrogenation to give compound **115** in 74% yield. The resulting free amine on the linker was

then modified using DTSSP and treated with DTT. Afterwards, the Boc was removed using 1:3 TFA/DCM to afford compound **11** in 67% yield.



Scheme 2.48: Synthesis of Target Compound **11**: (a) i) Zn-Cu Couple, HOAc; ii) Boc_2O, Et_3N, THF, 81%; (b) $Pd(OH)_2$, tBuOH,H_2O, H_2 (1 atm), 74%; (c) i), DTSSP, H_2O, pH~8; ii) 33% TFA/DCM, 0°C; iii) DTT, PBS (pH 7.4) 67%.

Binding Studies with S-Layer and S-Layer Associated Proteins

S-Layer Proteins: The mCherry fusion proteins were provided by the lab of Dr. Olaf Schneewind. The proteins were fusions between the mCherry Fluorescent reporter and the SLH domains of various SLP and BSL proteins. The concentration of each mCherry protein in mg/mL was determined using a standard Bicinchoninic acid protein assay (BCA)[90] and a fluorescence based assay (Appendix). In the fluorescence based assay, a known concentration of mCherry protein was serially diluted and the fluorescence intensity was determined at each concentration. A standard curve was made and a linear regression equation was obtained. Next, the mcherry proteins were diluted with known volumes of diluent and the fluorescence of each sample was measured and analyzed using the linear regression equation of the standard. It was found that the protein analysis using the BCA method was generally higher. This was likely due to non-mCherry protein contaminants that made it through the purification process. Since the mCherry fluorescence based method only accounted for mCherry fusion proteins, it was deemed more accurate and these values were used for the experiments.

The working concentration range for the mCherry proteins was determined using an ELISA based method. For these experiments, several target compounds were conjugated to BSA using thiol-maleimide click conditions (Figure 2.11). The loading efficiency of each BSA-conjugate was determined by MALDI-TOF MS.[93]

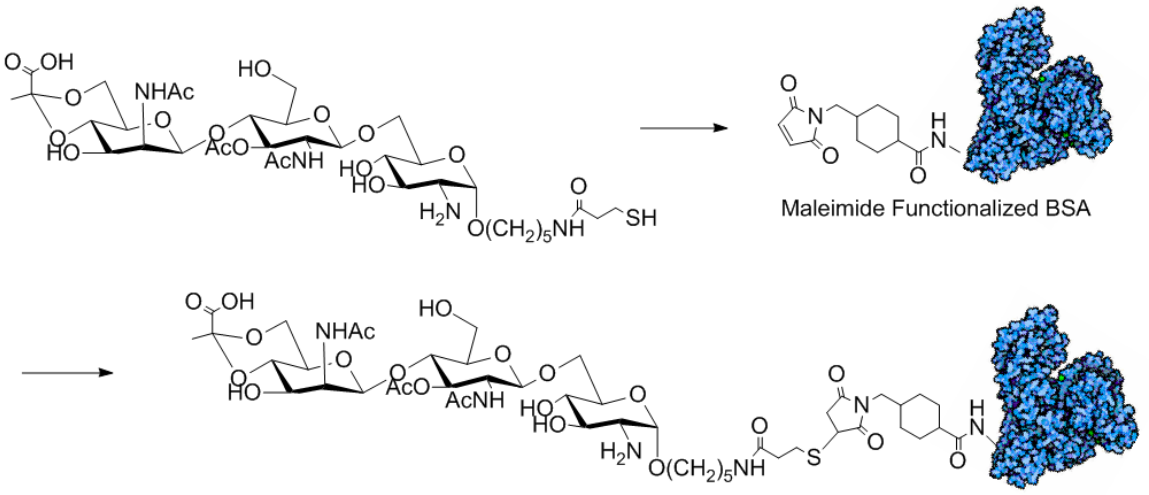


Figure 2.11: Conjugation of Synthetic Oligosaccharides to BSA using Thiol-Maleimide Coupling.

Several concentrations of the BSA conjugates were adsorbed onto 96-well ELISA plates and screened against serial dilutions of Sap mCherry fusion protein. It was hoped that the fluorescence generated by the mCherry moiety would be sufficient for the experiments (Figure 2.12). Although mCherry fluorescence could be detected, the working concentration of protein was very high and the fluorescence intensity was not much higher than the background as indicated with data from the BSA negative control. In an attempt to boost the fluorescence signal, an anti-mCherry Rabbit polyclonal antibody was obtained for the purpose of binding the mCherry portion of the fusion protein. A Goat anti-Rabbit Alexa Fluor 488 secondary antibody was then used to bind the anti-mCherry antibody and visualize binding. Using the same concentrations, the AlexaFluor saturated the detector (Figure 2.12).

	Sap		mCherry Fluorescence			Gain 5% A-12	
	ug/mL	20	10	5	2.5	1.25	Blank
TGT-1 (2ug/mL)		8704	8548	8426	6068	4538	3332
TGT-2 (2ug/mL)		9589	9919	9276	6094	4778	3394
TGT-7 (2ug/mL)		3632	3247	3838	3859	3472	3425
BSA (2ug/mL)		3298	3678	3654	3343	3703	3216
	Sap		Alexa488 Fluorecsence			Gain 5% A-12	
	ug/mL	20	10	5	2.5	1.25	Blank
TGT-1 (2ug/mL)		56157	61141	63705	64764	65000	2871
TGT-2 (2ug/mL)		61957	62744	62767	63128	62968	3191
TGT-7 (2ug/mL)		25081	15540	11272	8514	6407	3857
BSA (2ug/mL)		8428	6685	5372	4654	4359	3628

Figure 2.12: mCherry Fluorescence versus Alexa Fluor 488 Fluorescence

An updated experiment was conducted to find the optimal working concentration of the mCherry fusion proteins using the Alexa Fluor 488 antibody (Figure 2.13). With the modified experiment, the maximum end of the working concentration range was ultimately lowered from 20μg/mL to 0.5μg/mL and serial dilutions were made from that stock. This allowed for more efficient use of the valuable mCherry fusion proteins. In the previous mCherry fluorescence based experiments, the signal was only ~2.5 to 3 times higher than the background at the highest concentration of protein. With the AlexaFluor based experiments, the signal was boosted to 25 to 30 times above background at peak concentrations.

	Alexa488 Gain 50% A6									
	sap									
	ug/mL	0.625	0.3125	0.156	0.078	0.0391	0.0195	0.00977	0.00488	0.00244	Blank
TGT-2 (2ug/mL)		45901	46258	42543	32762	21662	12962	8114	5884	3756	1290
TGT-2 (1ug/mL)		45145	46505	43499	34079	23054	14093	9330	6161	4266	1226
TGT-2 (0.5ug/mL)		42863	45365	43527	34328	23879	15717	10283	6651	4701	1222
Neg (2ug/mL)		2274	1917	1823	1777	1412	1512	1493	1420	1305	1326
Neg (1ug/mL)		2295	1961	1775	1723	1647	1367	1518	1514	1454	1320
Neg (0.5ug/mL)		2235	1757	1630	1578	1457	1464	1331	1376	1409	1223
BSA (2ug/mL)		1747	1682	1605	1224	1611	1423	1304	1226	1306	9700
BSA (0.5ug/mL)		1950	1642	1467	1576	1523	1466	1628	1574	1417	1287

Figure 2.13: Determination of the Working Concentration for mCherry proteins with Alexa Fluor 488.

Microarray: A microarray screening was employed as a quick and efficient method to identify any binding interactions between the proteins and target compounds. Aminosilane glass slides were

functionalized with maleimide using a previously published method.[91] The synthetic compounds were converted to free thiol form using immobilized TCEP prior to printing (Figure 2.14).

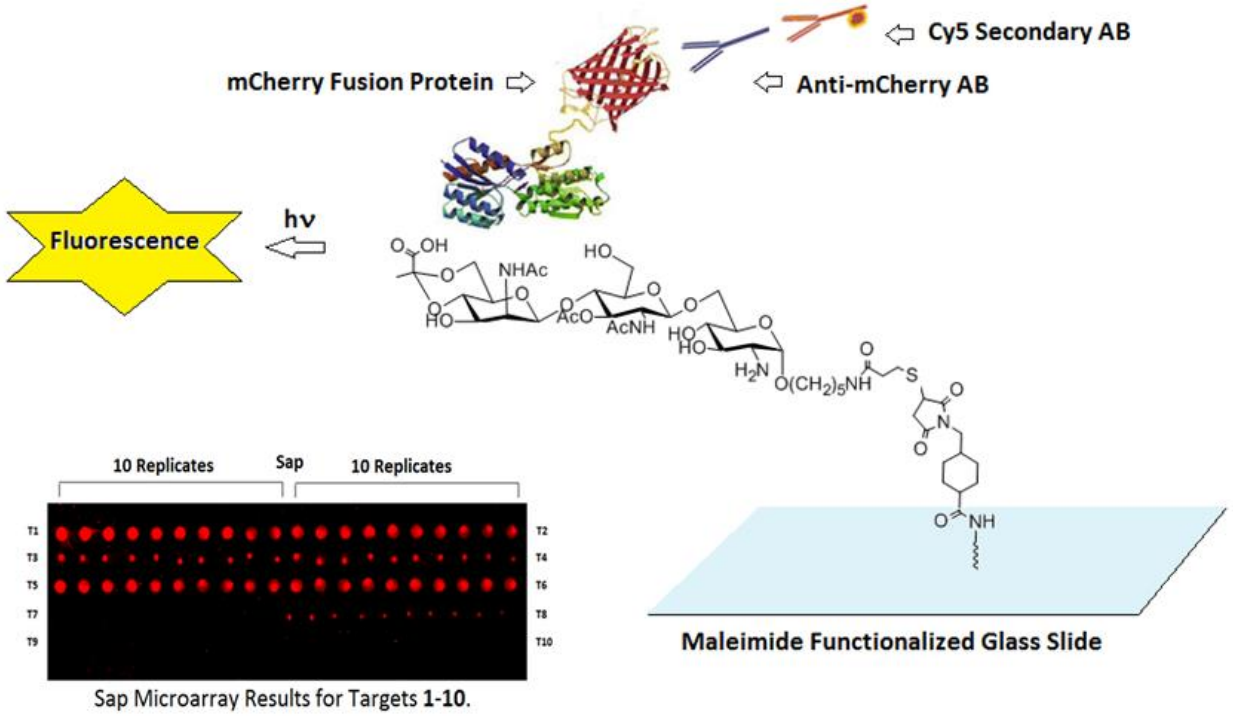


Figure 2.14: Microarray Experimental Procedure.

The compounds were printed at 2 concentrations (500μM, 100μM) and screened against two concentrations of each mCherry fusion protein (0.5μg/mL and 2μg/mL). The 100μM target concentration paired with the 0.5μg/mL protein concentration provided the best results. Due to the large number of proteins, only the microarray data related to EA1, Sap, BslA, and BslO will be displayed. The complete data is accessable in the Appendix. The microarray data is to be used as a qualitative measure of binding. Surprisingly, trisaccharide targets **1**-**6** were found to bind all SLP and BSL proteins in the panel. (Figures 2.15, Appendix) The data indicates that neither free amine presentation nor O-acetylation is requisite for binding SLH bearing proteins. As expected, no binding occurred between the PlyG endolysin and any of the synthetic oligosaccharides due to a lack of galactosylation on the target

compounds. Also, the mCherry protein control did not show any binding (Appendix). It was found that the lack of 4,6-*O*-pyruvyl ketal on the distal ManNAc (**7**) completely abolished binding thus providing direct evidence for previous *csa* genetic experiments and demonstrating it's vital role in S-Layer assembly. Truncated target **8** showed some degree of positive binding for all proteins suggesting that the terminal unit alone was capable of producing binding interactions with SLH bearing proteins. The 4,6-*O*-pyruvylated glucoside (**9**) and Mannoside (**10**) were synthesized for the purpose of probing the importance of the C-2 acetamido and C-2 stereochemistry for carbohydrate-protein binding interactions.

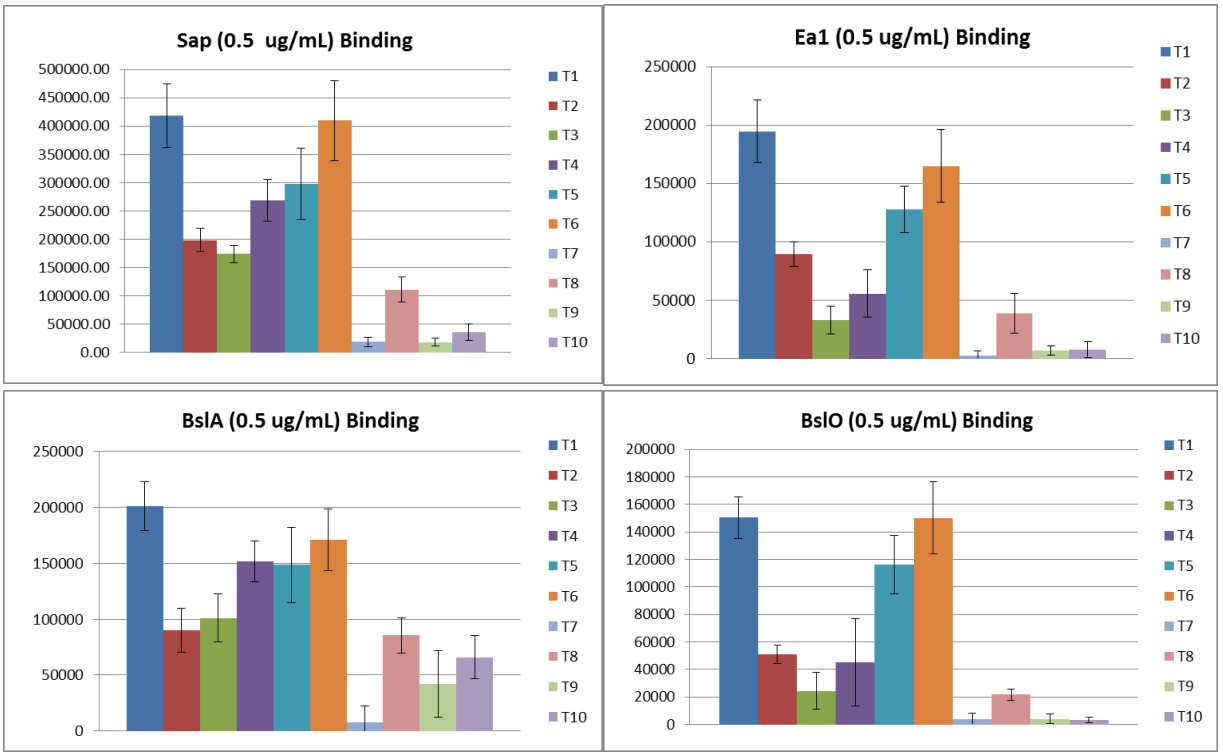


Figure 2.15: Microarray Screening Results for Sap, EA1, BslA, BslO. (Error bar: mean s.d., n=10)

Binding with **9** was completely abolished in both SLPs and in several BSL proteins. The pyruvylated Mannoside (**10**) produced similar results indicating that loss of binding is not due to steric clashes in the binding pocket resulting from epimerization at the C-2 position. The results demonstrate that in addition

to the 4,6-O-pyruvyl ketal, the C-2 acetamido function on the distal ManNAc may also play an important role in the binding of SLP and BSL proteins. Interestingly, some BSL proteins (*e.g*. BslA, BslM) were able to bind targets **9** and **10** indicating that there may be differences in binding requirements between proteins.

ELISA Binding Experiments: For the purpose of determining differences in binding affinity between each particular protein and target, an ELISA based dose-response experiment was designed (Figure 2.16). The EC_{50} values for each target-protein combination were obtained and used to quantify differences in binding affinity. For these experiments, the target compounds were conjugated to BSA using thiol-maleimide conjugation (Figure 2.11). A BSA conjugate made with the non-binding target **7** was utilized as a negative control. The loading efficiency of each BSA conjugate was determined by MALDI-TOF-MS.[93]

A series of Micro-titer plate wells were coated with the BSA-target conjugates. As previously mentioned, the inherent fluorescence of the mCherry reporter was to be used in order to determine binding. Due to weak signal, a rabbit derived anti-mCherry antibody was used followed by a goat anti-rabbit Alexafluor488 antibody to visualize binding. Surprisingly, it was found that the trisaccharides bearing the 4,6-*O*-pyruvyl ketal (Targets **1**-**6**) were able to bind the entire panel of S-Layer and BSL proteins in high affinity with EC_{50} values in the ng/mL range (Figure 2.17, Appendix).

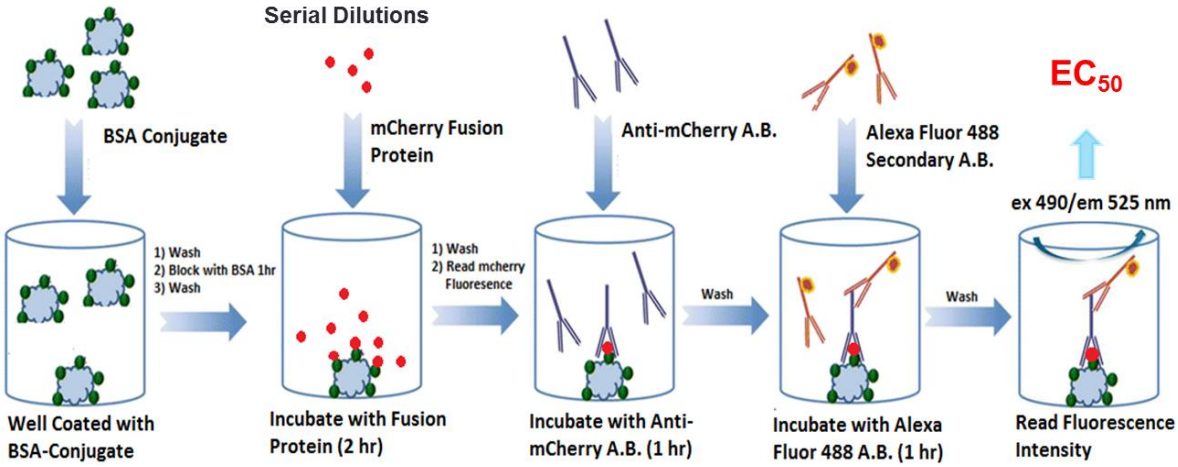


Figure 2.16: ELISA Experiemental Procedure

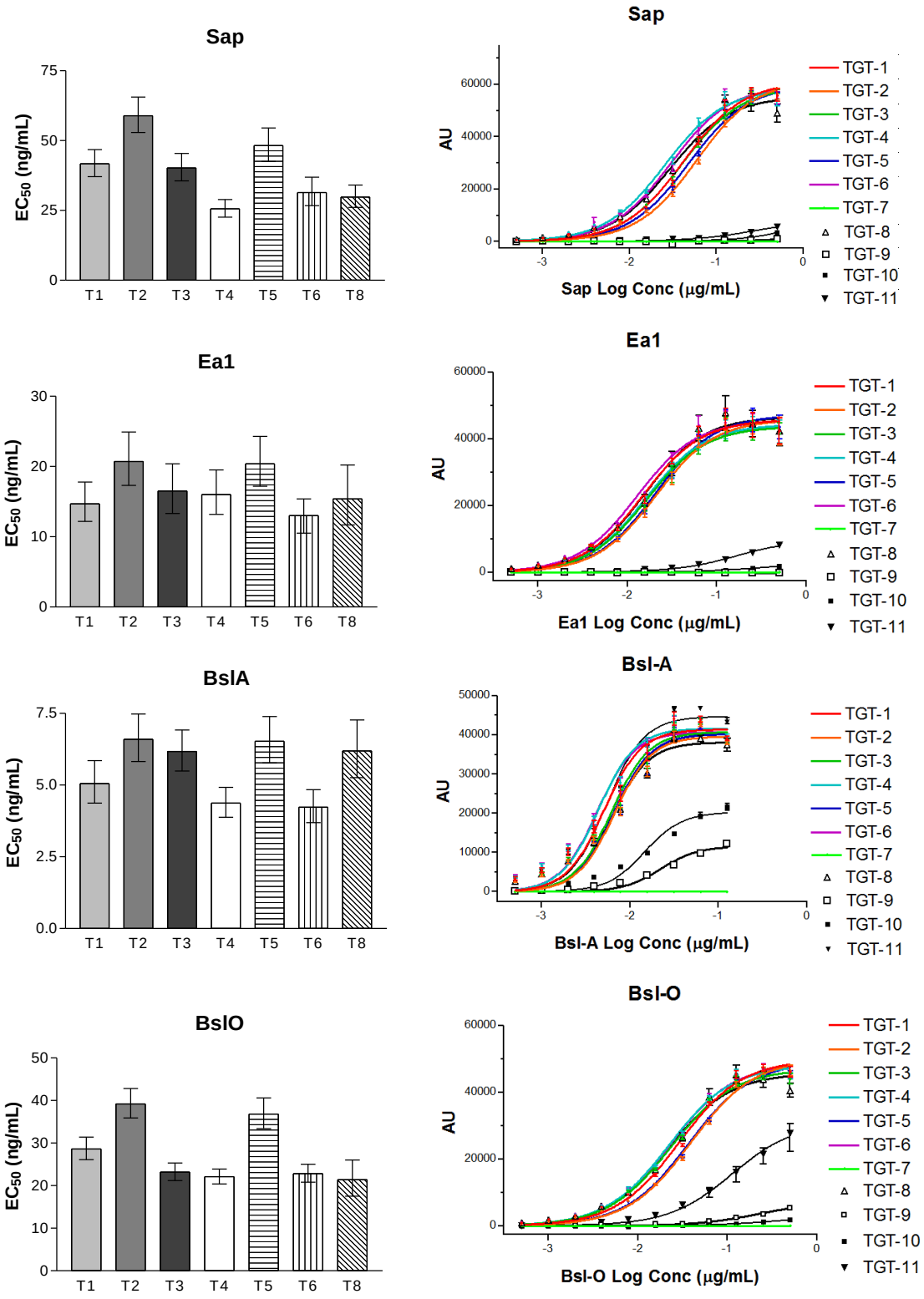


Figure 2.17: Dose Response Curves and EC_{50} values for Sap, EA1, BslO, and BslA. Error displayed as 95% Confidence Intervals, n=6 replicates for TGTs 1-6, n=3 replicates for TGT-8.

The presence of the free amine on the α-GlcNH$_2$ produced a small increase in affinity between SCWP and the SLH proteins (Figure 2.17, Tables 2.8, Appendix). On average, the free amine bearing compounds **1**, **4**, and **6** showed a minor improvement in binding relative to their acetamido bearing counterparts **2**, **3**, and **5** respectively. However, the magnitude of this effect is small and is likely not significant enough to have a major impact on S-Layer assembly.

Table 2.8: Effects of Structural Features On Binding Affinity for Targets **1**-**6**. Based on ELISA EC$_{50}$ values.

Functional Group Of Interest:	Constant:	Variable:	General Effect:
C3-OAc ManNAc:	C2-NH2 (α-GlcNAc)	+OAc vs -OAc T1 T6	Little/No Difference in SLP/Bsl Binding
C3-OAc ManNAc:	NHAc (α-GlcNAc)	+OAc vs -OAc T2 T5	Little/No Difference in SLP/Bsl Binding
C3-OAc β-GlcNAc:	NH2 (GlcNAc)	+OAc vs -OAc T4 T6	Little/No Difference in SLP/Bsl Binding
C3-OAc β-GlcNAc:	NHAc (GlcNAc)	+OAc vs -OAc T3 T5	Little/No Difference in SLP/Bsl Binding
C2-NH2 α-GlcNAc:	C3-OAc (GlcNAc/ManNAc)	+NH2 vs NHAc T1 T2	Marginal Increase in binding with NH$_2$ 0.4 fold Avg increase
C2-NH2 α-GlcNAc:	No-OAc	+NH2 vs NHAc T4 T3	Marginal Increase in binding with NH$_2$ 0.45 fold Avg increase
C2-NH2 α-GlcNAc:	C3-OAc (GlcNAc)	+NH2 vs NHAc T6 T5	Marginal Increase in binding with NH$_2$ 0.6 fold Avg increase

The O-acetylated target compounds did not show any appreciable changes in binding relative to their non O-acetylated counterparts. In contrast to previous genetic studies, EA1, BslO, and BslA were able to bind to target compounds lacking the 3-*O*-acetyl modification on the β-GlcNAc in high affinity.

The findings indicate that O-acetylation of SCWP is not a direct means for modulating protein binding and an alternative mechanism could be responsible for the effects seen in the *patA1/patA2* knockout studies. Perhaps, O-acetylation acts in an indirect manner or in combination with other proteins. Alternative models have been put forth to explain site specific S-layer deposition. It has been shown that EA1 and Sap form non-overlapping patches on the cell surface and that EA1 is located predominately in the septal region. It was proposed that the dynamic Sap/EA1 S-layer coverage of the cell surface restricts the deposition of BslO to the SCWP at septal rings.[95] In a related study, three genes were identified that were found to play a part in controlling the chain-length of vegetative cells. The genes included an accessory secretion ATPase (*secA2*) and two S-layer assembly genes (*slaP*, *slaQ*). The three genes reside within the S-layer gene cluster that includes genes for the installation of 4,6-*O*-pyruvyl ketal onto the SCWP (*csa*) and the genes encoding EA1 and Sap S-layer proteins, (*eag* and *sap*). Both *secA2* and *slaP* are required for the efficient secretion of Sap and EA1 S-layer proteins, but not for the secretion of BSLs or other secreted products. It is known that S-layer assembly *via* secA2 and slaP was shown to contribute to the proper positioning of BslO murein hydrolase, which cleaves septal peptidoglycan in order to separate chains of bacilli. Additional studies will have to be performed in order to determine if this same mechanism of site specific protein deposition is applicable to other BSL proteins.[94]

Compounds bearing the 3-*O*-acetyl on the terminal ManNAc did not show any marked differences in binding relative to their non 3-*O*-acetylated counterparts suggesting that the position is likely not important for SLH-carbohydrate binding interactions (Figure 2.17, Tables, 2.8, Appendix). In a broader context, it does not appear that O-acetylation serves as a direct means to modulate the binding of the SLH domain bearing proteins used in this study.

The truncated target **8** consists of only the 4,6-*O*-pyruvylated ManNAc and was found to bind the entire panel of SLP and BSL proteins in high affinity indicating that this terminal residue plays the most significant role in binding interactions between SCWP and the SLH binding domain (Table 2.8,

Figure 2.17, Appendix). The strong binding of this terminal unit suggests that any structural functions used for modulation would most-likely reside on this portion of the SCWP. As seen in the microarray, the 4,6-*O*-pyruvylated glucoside (**9**) mannoside (**10**), and mannosamine (**11**) had a complete loss of binding to both EA1 and sap S-layer proteins and binding was either partially or completely abolished in BSL proteins. However, target **11** was found to have better binding relative to both **9** and **10.** Interestingly, the importance of the C-2 acetamido on the ManNAc varied between different BSLs. For instance, BslP, BslA, BslI, and BslM retained some significant capacity to bind target compounds **9** -**11** despite the absence of the acetamido function. The results suggest that the SLH domains of various proteins may utilize different structural features for binding and show that structural modifications at the C-2 position could in principle be used to modulate SLP and BSL binding (Figure 2.18).

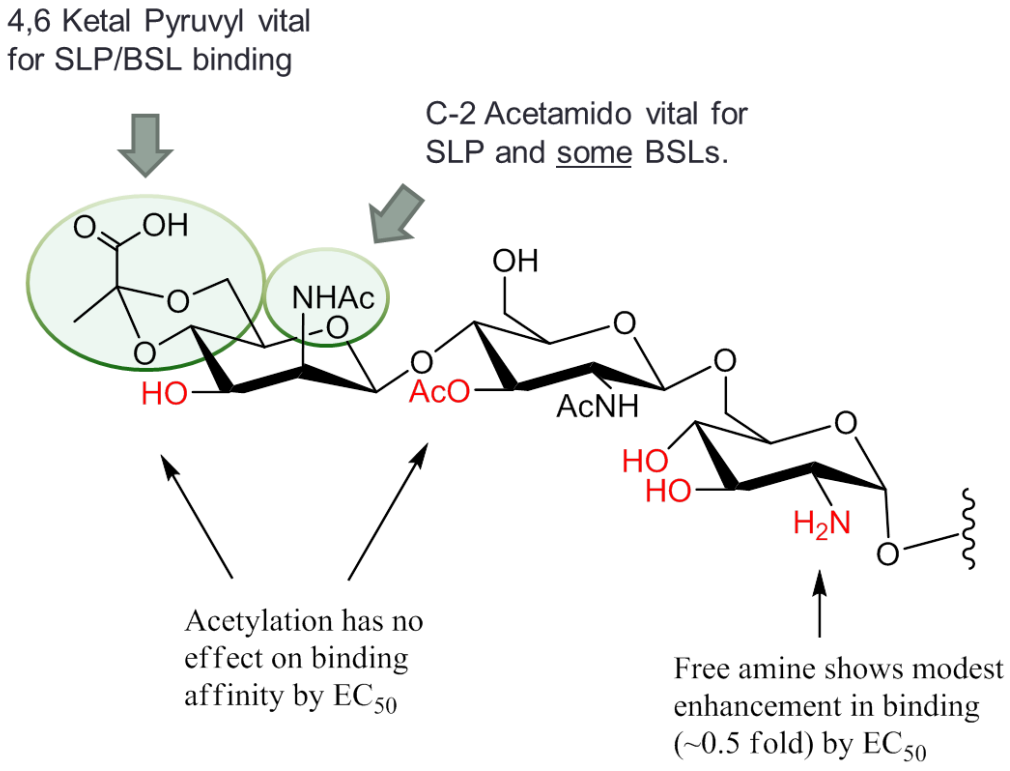


Figure 2.18: Summary of the Effects of Various SCWP Structural Features on SLP/BSL Binding Affinity.

CONCLUSIONS

In summary, an efficient synthetic strategy was developed for the purpose of producing a library of complex oligosaccharides derived from the terminal unit of *B. anthracis* SCWP. A number of synthetic challenges were addressed, including the development of synthetic procedures for unique pyruvylated building blocks, the choice of appropriate orthogonal protecting groups, the selection of robust activating conditions for the poorly reactive pyruvylated donor, the choice of glycosylation method for the installation of the 1,2-*cis*-glycosidic linkage with Beta configuration, and the selection of appropriate conditions for the reduction of trichloroacetyl and azide functions. The use of a mannosyl thioglycoside donor in combination with Crich β-Mannosylation conditions was found to be essential for effective activation of the donor and good glycosylation yields. It was found necessary to use an ether based protecting group at the O-3 position of the donor in order to obtain beta selectivity. This proved challenging since ideal installation conditions for the 4,6-*O*-pyruvyl ketal with benzyl pyruvate required an acetyl ester at the O-3 position. Fortunately, a condition was found using guanidine buffered sodium methoxide that allowed for selective removal of the 3-*O*-acetyl function in the presence of the pyruvyl benzyl ester without transesterification side products. Furthermore, pre-installation of the 4,6-*O*-pyruvyl ketal at the building block stage was found to be crucial for obtaining optimal yields of the acetal and absolute (S)-stereochemistry. The choice of amino protecting groups was also a key part of the strategy. A non-participation function had to be used on the mannoside donor at the C-2 position in order to prevent formation of the α-product so the azide was chosen. The TCA function was used at the C-2 amine position of the second building block in order to promote β-selectivity during glycosylation *via* neighboring group participation. The third building block was Boc protected at the C-2 amine position and bisprotected at the aglycon amine with Benzyl and Cbz. Following completion of the glycosylation reactions, the azide and TCA functions were reduced and converted to acetamido functions. The acid labile nature of Boc necessitated that Zn-Cu couple in acetic acid was avoided so AIBN and Bu_3SnH

radical conditions were used. Following the reduction, the remaining Boc and Cbz/Benzyl amine protecting groups could be manipulated independently of one another. For instance, the aromatic protecting groups could be removed using catalytic hydrogenation conditions without affecting Boc or acetyl esters. This allowed for the selective installation of the thiol spacer. In addition, the Boc could be removed using acidic conditions without affecting the aromatic protecting groups or the acetyl esters. Also, both Boc and the aromatic protecting groups are resistant to the basic LiOH conditions used to remove the benzyl and acetyl esters.

Microarray and ELISA based binding assays were designed that utilized the synthetic target compounds to dissect the highly complex carbohydrate binding interactions between SCWP and S-layer proteins. The overarching questions that were answered include: **1) What functionalities or combinations of functionalities are important for SLP/BSL binding?** The 4,6-*O*-pyruvyl ketal was found to be essential for the binding of all SLP and BSL proteins thereby providing definitive evidence in support of previous *csaB* genetic experiments. Unexpctedly, the C-2 acetamido on ManNAc was found to be an important feature for SLH binding interactions and its substitution with hydroxy or amine resulted in a complete loss of binding to SLPs and a partial or complete loss of binding to most BSLs. Interestingly, the presentation of the free amine on the α-GlcNH$_2$ led to a small increase in binding affinity across the set of proteins. However, the magnitude of the effect was small and not likely enough to affect S-Layer formation or BSL deposition in any appreciable way. **2) Do all S-layer and S-layer associated proteins have the same ligand requirements for biding?** It was found that several BSL proteins could bind relatively well in the absence of the C-2 acetamido on ManNAc demonstrating that SLH binding requirements can vary and showing that in principle, modifications to the C-2 position could be used as a means to modulate protein binding. **3) Can O-acetylation be used as a means to modulate the binding of EA1, BslA, and BslO as demonstrated in the genetic studies?** It was found that the presence of a 3-*O*-acetyl function on the β-GlcNAc of the trisacchsaride compounds did not directly

modulate the binding of EA1, BslO, or BslA proteins as previously suggested in the *patA1*/*patA2* genetic studies. In addition, compounds with 3-*O*- acetylation on the terminal ManNAc did not show any appreciable differences in binding relative to their non 3-*O*-acetylated counterparts indicating that the position is likely not important for SLH-carbohydrate binding interactions.

EXPERIMENTAL

General Synthetic Methods: Reactions were performed using flame-dried glassware under an atmosphere of argon using anhydrous solvents unless otherwise noted. Ambient temperature in the laboratory was usually 20°C. CH_2Cl_2 and CH_3CN were distilled freshly from CaH_2. Other commercially available reagents were obtained from Aldrich, Fisher or TCI and used as received. Thin layer chromatography (TLC) was performed using aluminium backed Silica Gel 60 TLC plates w/UV254 from Merck KGaA. Visualization of TLC plates was performed by UV, 5% sulfuric acid/ethanol, or cerium molybdate stain. Silica gel flash chromatography was performed using silica gel (60 Å, 40-63 μm) from Silicycle, Canada. NMR spectra were recorded in the NMR facility of Complex Carbohydrate Research Center, UGA, on a Varian Mercury 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C), Varian Inova 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C), Varian Inova 600 with cryoprobe (600 MHz for ^{1}H, 150 MHz for ^{13}C), Varian VNMRS 600 with cryoprobe (600 MHz for ^{1}H, 150 MHz for ^{13}C) or Varian Inova 800 with cryoprobe (800 MHz for ^{1}H, 200 MHz for ^{13}C). Chemical shifts are reported in parts per million (ppm) on the δ scale. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ was referenced the solvent peak at 7.260 ppm (^{1}H) and 77.16 ppm (^{13}C), HOD (4.79). The assignments of ^{1}H NMR peaks were made from 2D ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY spectra. High resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF system (ESI) or an AB SCIEX TOF/TOF™ 5800 System (MALDI) with Ultramark 1621 as the internal standard. Protein mass spectrometry data were obtained using a Bruker Microflex MALDI-TOF spectrometer.

mCherry fusion proteins: The mCherry fusion proteins were obtained from the lab of Olaf Schneewind. The proteins are mCherry translational fusions. The proteins contain a decahistidine tag and the SLH domain of either an S-layer or BSL protein at the N terminus fused to an mCherry fluorophore at the C-terminus. The PlyG fusion protein has an N-terminal carbohydrate binding domain (CBD) fused with an mCherry fluorophore at the C-terminus. The "mCherry" protein was included as a negative control. It consists of only the decahistidine tag used for purification and the mCherry protein. Each mcherry fusion protein was quantified using a standard curve generated by plotting known concentrations of the standard mCherry protein against the fluorescence generated at 584 ex/612 em. This method was preferred over BCA analysis as it did not erroneously incorporate non-mCherry protein impurities into the quantity of mCherry fusion protein.

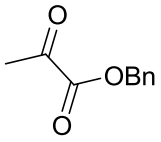
Microarray Study: Materials: Aminosilane glass Slides, Scienion sciFLEXARRAYER Microarray Printer, GenePix 4000B Microarray Scanner, GenePix Pro 7 Software, Anti-mChery (Rabbit) AB (Rockland inc.), Goat anti-Rabbit IgG (H+L) Secondary Antibody, Cy5® conjugate (Sigma). Procedure: Amine functionalized slides were made to display maleimide by incubation for 3hr in a 20mM solution of bifunctional linker in 1:10 (DMF: 50mM $NaHCO_3$(aq), pH 8.5).[91] The Target compounds bearing the thiol linker were dissolved in degassed and deionized water to make a 500μM solution. Immobilized TCEP was added to an Eppindorf tube and the resin was vortexed with PBS buffer (pH 7.4), centrifuged, and removed. An aliquot of the Target compound solution was added to the Epindorff tube and incubated. LC/MS was used in order to confirm that the compound was in free thiol form. If the reaction is proceeding slowly, the reaction mixtures can be gently warmed (~40^{o}C). The sample tubes were centrifuged in order to pull down the immobilized TCEP. An aliquot of each sample was then taken off the top and added to the sample chamber on the plate printer. The plates were printed with 100uM and 500uM concentrations of each target compound. After printing, the slides were allowed to incubate in a humidity chamber for 16hrs. Next, the slides were blocked using a 50 mM aqueous solution of

Mercaptoethanol for 1hr. The slides were thoroughly rinsed and stored at -20^{o}C prior to use. Each subarray was incubated for 2hr two concentrations (0.5 and 2ug/mL) of each SLP or BSL protein in dilution buffer. Afterwards the plates were washed in washing buffer and incubated for 1hr with using a ratio of 1μL (1mg/mL rabbit derived Anti-mCherry antibody) per 2mL of diluent. Afterwards, the plates were washed in washing buffer and incubated for 1hr using a ratio of 1μL (2mg/mL Goat-Anti-Rabbit Cy5 antibody) per 1mL of diluent. After incubation, the plate was washed with washing buffer and analyzed using a fluorescence plate reader (650 ex/ 670 em).

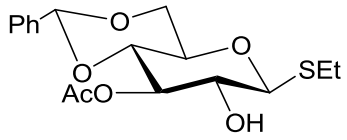
BSA Conjugation: Materials: Thermo Scientific™ Pierce™ Imject™ Maleimide-Activated BSA and Kit - Maleimide Conjugation Kit. Procedure: Before conjugation, the mercaptan was stirred for ~1 h with DTT (20 eq) in a warm water bath (~40 ^{o}C). The mixture was loaded directly onto a P2 size exlusion column for purification. The fractions with product were combined and lyophized. Conjugation was carried out according to the instructions in the BSA-Maleimide kit.

ELISA: Materials: ELISA plates: black 96F Maxisorp Microwell plates (Nunc 437111), Wash buffer: PBS containing 0.1% Tween 20 and 0.5M NaCl; Blocking buffer: PBS containing 1% BSA; Diluent buffer: PBS containing 1% BSA and 0.5% Tween 20; POLARstar Optima plate reader, Anti-mChery (Rabbit) AB (Rockland inc.), Goat Anti-Rabbit IgG (H+L), Alexa Fluor® 488 conjugate AB. Procedure: 1) Coat ELISA plate with the appropriate BSA conjugates (118 nM sugar final) in coating buffer (100 μL per well). Incubate overnight at 4°C. 2) Wash with wash buffer. Wash with plate-washer settings 5.1.1.3. This corresponds to 3 washes with 250 μL and 10 sec soak time. 3) Block with blocking buffer (200 μL/well). Incubate for 1 h at RT. Afterwards, wash with plate-washer settings 5.1.1.3. This corresponds to 3 washes with 250 μL and 10 sec soak time. 3) Prepare serial dilutions in a 96 well plate starting with an initial concentration of 0.5μg/mL. Add 250 μL into the first well of a 96-well assay plate, make two-fold serial dilutions (125 μL + 125 μL) in dilution buffer. Repeat until 11 different concentrations are

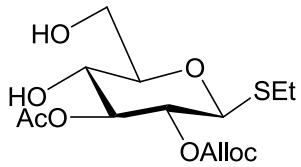
obtained. Transfer sample dilutions from the dilution plate to the coated/blocked plate (100 µL/well). Incubate for 2 h at RT. Afterwards, wash with plate-washer settings 5.1.1.3. This corresponds to 3 washes with 250 µL and 10 sec soak time. 4) Incubate with anti-mCherry (1mg/mL) antibody for 1h. (11uL Antibody stock/22mL Diluent) 100uL/well. Afterwards, wash with plate-washer settings 5.1.1.3. This corresponds to 3 washes with 250 µL and 10 sec soak time. 5) Incubate with Anti-Rabbit Alexafluor488 secondary antibody (2mg/mL) for 1h. (22uL Antibody stock/22uL Diluent) 100uL/well. Afterwards, wash with plate-washer settings 5.1.1.3. This corresponds to 3 washes with 250 µL and 10 sec soak time. 6) Add 50 µL PBS and Read Alexafluor488 fluorescence.



Benzyl Pyruvate (**13**): Pyruvic Acid (**12**, 5 g, 56.8 mmol) was taken up in THF (57 mL), along with benzyl alcohol (11.81 mL, 114 mmol) and pyridine (11.5 mL). The mixture was cooled to 0^{o}C and methanesulfonyl chloride (5.31 mL, 68.1 mmol) was added via dropwise addition. Afterwards, the mixture was allowed to warm to room temperature and stir for an additional 16 hr. The reaction mixture was quenched by the dropwise addition of water and concentrated under vacuum. The crude was diluted with ethyl acetate (30 mL) and washed with water (50 mL, 3x) and brine (30 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (5% EtOAc/Hexanes) to give **13** (4.6 g, 26.0 mmol, 46%) as a colorless oil. R_f = 0.6 (25% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.45-7.31 (m, H, aromatic), 5.28 (s, 2H, CH_2), 2.47 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.72, 160.69, 128.96, 128.85, 128.79, 68.16, 26.90. HR MALDI-TOF MS: m/z: calcd for $C_{10}H_{10}O_3$ $[M+Na]^+$: 201.0522; found: 201.0555.

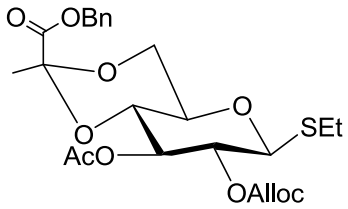


Ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**15**): Compound **14** (4.12 g, 13.19 mmol) was dissolved in methanol (142 mL) along with dibutyltin(IV) oxide (4.10g, 16.49 mmol). The mixture was heated at reflux (65°C) for 16 hr. Afterwards, the reaction mixture was cooled, concentrated, and dried under high vacuum for 4 h. The crude intermediate was taken up in DCM (535 mL) and cooled to 0°C. acetyl chloride (1.17 mL, 16.49 mmol) was added *via* dropwise addition and the mixture was allowed to stir at 0°C for 2 hr. The reaction mixture was transferred to a separatory funnel and washed with 1M KF (aq) (150 mL), water (200 mL), and brine (100 mL). The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (30% EtOAc/Hexanes) to give an inseparable 9:1 mixture of **15** (3.25 g, 9.16 mmol, 69%) and a 2-*O*-acetyl impurity as a colorless syrup. R_f = 0.5 (40% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): (β - Major Product) δ 7.45-7.23 (m, 2H, aromatic), 7.38-7.33 (m, 3H, aromatic), 5.50 (s, 1H, CH), 5.24 (t, 1H, J = 9.27 Hz, H-3), 4.53 (d, 1H, *J* = 9.76 Hz, H-1), 4.36 (dd, 1H, J = 4.88, 10.74 Hz, H-6a), 3.77 (t, 1H, J = 10.25 Hz, H-6e), 3.66 (t, 1H, J = 9.76 Hz, H-4), 3.60-3.54 (m, 2H, H-2, H-5), 2.81-2.73 (m, 2H, CH_2), 2.67 (d, 1H, J = 2.93 Hz, OH), 2.14 (s, 3H, Ac), 1.33 (t, 3H, J = 7.32 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.13, 137.02, 129.26, 128.39, 126.29, 101.66, 87.42, 78.49, 72.31, 70.98, 68.70, 25.15, 21.15, 15.44. HR MALDI-TOF MS: m/z: calcd for $C_{17}H_{22}O_6S$ $[M+Na]^+$: 377.1029; found: 377.1047.

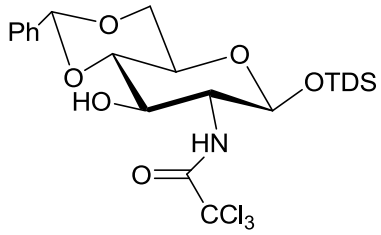


Ethyl 2-*O*-allyloxycarbonyl-3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**17**): Compound **15** (3.25 g, 9.16 mmol) with the 2-*O*-acetyl impurity was dissolved in DCM (53 mL) along with N,N,N',N'-tetramethyletylenediamine (2.06 mL, 13.74 mmol) and dimethylaminopyridine (336 mg, 2.75 mmol).

allyl chloroformate (2.44 mL, 22.90 mmol) was added and the reaction mixture was stirred at room temperature for 6 hr. Afterwards, the reaction mixture was diluted in DCM (300 mL) and washed with $NaHCO_3$ (sat. aq, 100 mL) and brine (100 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (10% EtOAc/hexanes) to give impure **16** (4.01 g, 9.14 mmol, 100%) as an amorphous white solid. R_f = 0.5 (25% EtOAc/hexanes). The residual 10% 2-*O*-acetyl impurity from the preceding reaction was still present and could not be separated. Compound **16** (4.0 g, 9.12 mmol) was taken up in 80% HOAc (aq) (228 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. The residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. At this point it was discovered that the 3-*O*-acetyl product could be separated from the 2-*O*-acetyl impurity. The resulting residue was purified by silica gel column chromatography (40% Acetone/Toluene) to give **17** (2.67 g, 7.61 mmol, 83%) as a colorless oil. R_f = 0.5 (40% Acetone /Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 5.96-5.88 (m,1H, =CH-), 5.36 (dd, 1H, J = 1.46, 17.57 Hz =C*H*H), 5.27 (dd, 1H, J = 1.00, 10.74 Hz, =C*H*H), 5.09 (t, 1H, J = 9.27 Hz, H-3), 4.73 (t, 1H, J = 9.76 Hz, H-2), 4.69-4.62 (m, 2H, CH_2), 4.56 (d, 1H, *J* = 10.25 Hz, H-1), 3.93 (d, 1H, J = 11.22 Hz, H-6a), 3.82 (d, 1H, J = 10.74 Hz, H-6e), 3.72 (t, 1H, J = 9.27 Hz, H-4), 3.47-3.43 (m, 1H, H-5), 3.20 (br.s, 1H, OH), 2.74-2.70 (m, 2H, CH_2), 2.29 (br.s, 1H, OH), 2.10 (s, 3H, CH_3), 1.27 (t, 3H, J = 7.32 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.01, 154.20, 131.35, 129.17, 128.36, 125.43, 119.10, 83.60 (C-1), 79.98 (C-5), 77.28 (C-3), 73.92 (C-2), 69.86 (C-4), 69.07, 62.42 (C-6), 24.77, 21.04, 15.03. HR MALDI-TOF MS: m/z: calcd for $C_{14}H_{22}O_8S$ $[M+Na]^+$: 373.0928; found: 373.0944.

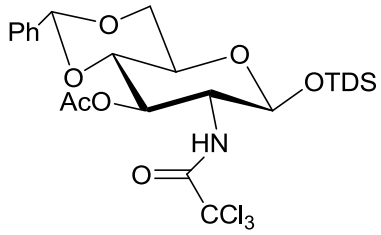


Ethyl 2-*O*-allyloxycarbonyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-β-D-glucopyranoside (**18**): Compound **17** (2.21 g, 6.30 mmol) and Benzyl pyruvate (2.25 g, 12.61 mmol) were dissolved in dry acetonitrile (7.37 mL) under argon atmosphere. $BF_3(OEt)_2$ (1.55 mL, 12.61 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ (sat. aq). The mixture was diluted with DCM (150 mL) and washed with saturated $NaHCO_3$ (75 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/hexanes) to give a 1:4 (α:β) mixture of **18** (2.13 g, 4.17 mmol, 66%) as an amorphous white solid. R_f = 0.7 (35% EtOAc/Hexanes). Separation of the anomers could be performed using preparatory TLC (30% Ether/Hexanes; eluted 3x). H^1NMR (500 MHz, $CDCl_3$): Beta δ 7.39-7.33 (m, 5H, aromatic), 5.95-5.87 (m, 1H, -CH=), 5.37-5.17 (m, 3H, H3, =CH_2), 4.69 (t, 1H, J = 9.76 Hz, H-2), 4.64 (d, 2H, J = 4.88 Hz, CH_2), 4.54 (d, 1H, *J* = 9.76 Hz, H-1), 4.14-4.07 (m, 1H, H-6a), 3.66 (t, 1H, J = 9.76 Hz, H-6e), 3.49-3.42 (m, 2H, H-4, H-5), 2.67 (q, 2H, J = 7.32 Hz, CH_2), 1.98 (s, 3H, Ac), 1.51 (s, 3H, CH_3), 1.24 (t, 3H, J = 7.32 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.10, 169.31, 154.19, 135.20, 131.34, 128.84, 128.79, 125.46, 118.98, 99.35, 84.07 (C-1), 74.64 (C-5), 74.53 (C-2), 72.51 (C-3), 70.32 (C-4), 69.04, 67.70, 64.90 (C-6), 25.22, 24.54, 20.81, 14.94. HR MALDI-TOF MS: m/z: calcd for $C_{24}H_{30}O_{10}S$ $[M+Na]^+$: 533.1452; found: 533.1477.

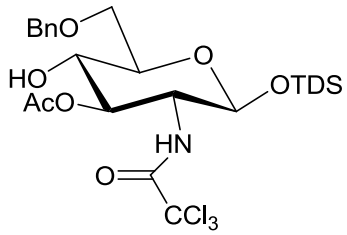


Dimethylthexylsilyl 2-trichloroacetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (20):

Compound **19** (8.1 g, 16.51 mmol) was dissolved in methanol (50 mL) and NaOMe (50 mL, 0.2 M solution in MeOH) was added and the reaction was stirred for 4 h. Afterwards, the reaction was neutralized with Dowex 50 W (H+) acidic resin, filtered, and concentrated under vacuum. The residue was coevaporated with dichloromethane and toluene and dried under high vacuum for 3 h. The residue was dissolved in acetonitrile (35 mL). Benzaldehyde dimethyl acetal (4.12 mL, 27.3 mmol) and CSA (635 mg, 2.73 mmol) were added. After stirring at room temperature for 3 h, EtOAc was added and the mixture was extracted with saturated $NaHCO_3$ solution. The organic phase was dried over MgSO4, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography (5-25% EtOAc/Hexanes) to give **20** (5 g, 9.01 mmol, 66 %) as an amorphous white solid. R_f = 0.3 (40% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.5-7.47 (m, 2H, aromatic), 7.40-7.36 (m, 3H, aromatic), 6.90 (d, 1H, J = 7.03 Hz, NH), 5.55 (s, 1H, CH), 5.12 (d, 1H, J = 7.62 Hz, H-1), 4.35-4.29 (m, 2H, H-3, H-6a), 3.80 (t, 1H, J = 9.96 Hz, H-6e), 3.61-3.44 (m, 3H, H-2, H-5, H-4), 2.86 (d, 1H, J = 2.93 Hz, OH), 1.68-1.56 (m, 1H, CH), 0.88-0.85 (m, 4H, 4xCH_3), 0.17 (s, 3H, CH_3), 0.15 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 162.28, 137.09, 129.49, 128.53, 126.44, 102.11, 95.28 (C-1), 81.81 (C-4), 69.88 (C-3), 68.77 (C-6), 66.39 (C-5), 61.97 (C-2), 34.06, 24.90, 20.25, 20.07, 18.74, 18.65, -1.68, -3.12. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{34}Cl_3NO_6Si$ $[M+Na]^+$: 576.1113; found: 576.1130.

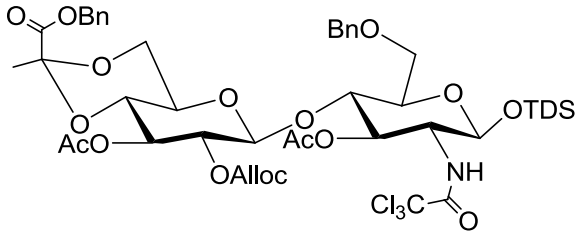


Dimethylthexylsilyl 2-trichloroacetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (21): Compound **20** (5 g, 9.01 mmol) was dissolved in a 1:1 mixture of pyridine and acetic anhydride (50 mL). The reaction was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated under vacuum. The residue was taken up in DCM (100 mL) and transferred to separatory funnel. The organic layer was washed with water (100 mL), dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (35% EtOAc/hexanes) to give **21** (5.1 g, 8.54 mmol, 95%) as an amorphous white solid. R_f = 0.5 (40% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.47-7.44 (m, 2H, aromatic), 7.37-7.35 (m, 3H, aromatic), 7.15 (d, 1H, J = 9.96 Hz, NH), 5.51 (s, 1H, CH), 5.41 (t, 1H, J = 9.96, H-3), 4.77 (d, 1H, J = 7.62 Hz, H-1), 4.13-4.02 (m, 2H, H-2, C-6a), 3.77-3.70 (m, 2H, H-4, H-6e), 3.49-3.40 (m, 1H, H-5), 2.06 (s, 3H, OAc), 1.64-1.55 (m, 1H, C*H*), 0.86-0.81 (m, 4H, 4xCH_3), 0.11 (s, 3H, CH_3), 0.08 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.69, 162.14, 137.17, 129.22, 128.36, 126.23, 101.46, 96.63 (C-1), 92.68, 78.82 (C-4), 71.53 (C-3), 68.61 (C-6), 66.72 (C-5), 58.34 (C-2), 33.98, 24.87, 20.97, 20.13, 19.99, 18.69, 18.63, -1.73, -3.25. HR MALDI-TOF MS: m/z: calcd for $C_{25}H_{36}Cl_3NO_7Si$ $[M+Na]^+$: 618.1219; found: 618.1230.



Dimethylthexylsilyl 2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (22): Compound **21** (0.5 g, 0.838 mmol) was taken up in DCM (11.5 mL) along with activated 4Å

molecular sieves and stirred for 30 minutes under argon. The mixture was cooled to -78^{o}C and triethylsilane (0.268 mL, 1.675 mmol) was added followed by the dropwise addition of triflic acid (0.112 mL, 1.256 mmol). After 15 mins, the reaction mixture was quenched by the addition of 1:1 triethylamine/MeOH (2 mL), filtered, and concentrated under vacuum. The residue was taken up in DCM (50 mL) and washed with water (50 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (30% EtOAc/hexanes) to give **22** (459 mg, 0.766 mmol, 92%) as an amorphous white solid. R_f = 0.35 (35% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.37-7.29 (m, 5H, aromatic), 6.84 (d, 1H, J = 8.80 Hz, NH), 5.14 (t, 1H, J = 9.39, H-3), 4.78 (d, 1H, J = 7.63 Hz, H-1), 4.58 (q, 2H, J = 8.22, 12.33 Hz, CH_2), 3.96-3.91 (m, 1H, H-2), 3.83-3.79 (m, H-4, H-6a), 3.75-3.73 (m, 1H, H-6e), 3.59-3.56 (m, 1H, H-5), 3.02 (d, 1H, J = 2.93 Hz, O*H*), 2.09 (s, 3H, OAc), 1.62-1.58 (m, 1H, C*H*), 0.85-0.82 (m, 4H, 4xCH_3), 0.16 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 172.03, 161.95, 137.58, 128.69, 128.12, 127.83, 96.11 (C-1), 92.64, 74.70 (C-3), 74.03, 73.94 (C-5), 71.17 (C-4), 70.79 (C-6), 57.70 (C-2), 34.01, 24.89, 21.02, 20.13, 20.00, 18.68, 18.64, -1.69, -3.21. HRMS(ESI): m/z: calcd for $C_{25}H_{38}Cl_3NO_7Si$ $[M+Na]^+$: 620.1375; found: 620.1391.

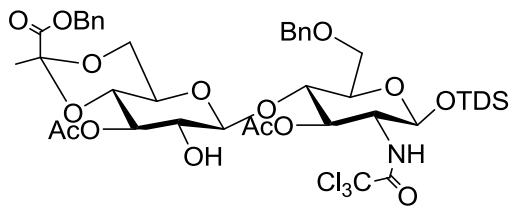


Thexyldimethylsilyl 2-*O*-allyloxycarbonyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**24**): ***Method 1:*** Glucosyl donor **18** (159 mg, 0.311 mmol) and glucosyl acceptor **22** (242 mg, 0.404 mmol) were taken up in toluene (5 mL) and concentrated under vacuum (3x). The mixture was taken up in DCM (4 mL) along with activated 4Å molecular sieves (400 mg) and stirred under argon for 30 min.

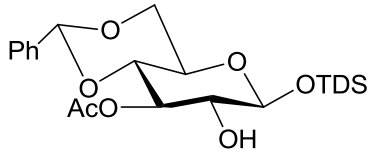
The mixture was cooled to -20°C and N-iodosuccinimide (108 mg, 0.482 mmol) was added followed by dropwise addition of TMSOTf (15 uL, 0.081 mmol). After 1 h, a sizable quantity of donor remained so additional NIS (54 mg, 0.241 mmol) and TMSOTf (7.5 uL, 0.04 mmol) were added and the reaction mixture was warmed to 0°C. After 1 h, much of the donor still remained so the reaction was quenched with pyridine (0.1 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (20 mL) and washed with NaS_2O_3 (sat, aq, 15 mL) and water (15 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (20% EtOAc/hexanes) to give **24** (42 mg, 0.04 mmol, 13%) as an amorphous white solid. R_f = 0.35 (25% EtOAc/hexanes). Unreacted donor and acceptor were recovered.

Method 2: Glucosyl donor **18** (60 mg, 0.118 mmol) and glucosyl acceptor **22** (91 mg, 0.153 mmol) were taken up in toluene (5 mL) and concentrated under vacuum (3x). The mixture was taken up in DCM (2.7 mL) along with activated 4Å molecular sieves (150 mg) and stirred under argon for 30 min. The mixture was cooled to 0°C and N-iodosuccinimide (41 mg, 0.182 mmol) was added followed by dropwise addition of triflic acid (2.1 uL, 0.024 mmol). After 1 h, a sizable quantity of donor remained so additional NIS (20 mg, 0.09 mmol) and TfOH (1 μL, 0.012 mmol) were added. After stirring for 20 mins, the reaction had not proceeded much so additional TfOH (1 μL, 0.012 mmol) was added. After 1 h, much of the donor still remained so the reaction was quenched with pyridine (0.1 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (20 mL) and washed with NaS_2O_3 (sat, aq, 15 mL) and water (15 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (20% EtOAc/hexanes) to give **24** (19.4 mg, 0.019 mmol, 16%) as an amorphous white solid. R_f = 0.35 (25% EtOAc/hexanes). In addition, donor without Alloc was detected as well as disaccharide resulting from the reaction of donor **73** with the donor missing 2-*O*-Alloc.

Method 3: Donor **24** (80 mg, 0.157 mmol), 2,4,6 tri-tert-butylpyrimidine (97 mg, 0.392 mmol), and diphenylsulfoxide (38 mg, 0.188 mmol) were dissolved in toluene and concentrated under vacuum (3x). The dried mixture was taken up in DCM (4.8 mL), cyclooctene (271 mL, 1.567 mmol) and activated 4Å molecular sieves (250 mg) and stirred under argon atmosphere at room temperature for 30 mins. The mixture was cooled to -78^{o}C and trifluoromethanesulfonic anhydride (29 μL, 0.172 mmol) was added via dropwise addition. The mixture was allowed to warm to -50^{o}C in order to activate the donor. The mixture was cooled back to -78^{o}C and compound **18** (141 mg, 0.235 mmol) was dissolved in a minimum amount of DCM and added by dropwise addition. The mixture was allowed to warm to 0^{o}C and stir for an additional 2 h before being quenched with TEA, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/Hexanes) to give **22** (35 mg, 0.033 mmol, 21%) as an amorphous white solid. R_f = 0.35 (25% EtOAc/hexanes). Unreacted donor and acceptor were recovered. H^{1}NMR (600 *MHz*, $CDCl_3$): Beta δ 7.40-7.31 (m, 10H, aromatic), 6.58 (d, 1H, J = 9.76 Hz, NH), 5.92-5.84 (m, 1H, -CH=), 5.34-5.19 (m, 4H, CH_2, =C*H*$_2$), 5.11-5.05 (m, 2H, H-2, H-3^{I}), 4.70 (d, 1H, J = 7.81 Hz, H-1^{I}), 4.66-4.49 (m, 6H, H-1, H-3, 2xCH_2), 4.03 (dd, 1H, J = 5.37, 10.74 Hz, H-6a), 3.95-3.88 (m, 2H, H-2^{I}, H-4^{I}), 3.78 (dd, 1H, J = 3.42, 11.22, H-6a^{I}), 3.64 (d, 1H, J = 10.74 Hz, H-6e^{I}), 3.55 (t, 1H, J = 10.25 Hz, H-6e), 3.40-3.34 (m, 2H, H-4, H-5^{I}), 3.21-3.16 (m, 1H, H-5), 1.94 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.63-1.57 (m, 1H, CH), 1.51 (s, 3H, CH_3), 0.85-0.81 (m, 12H, 4xCH_3), 0.17 (s, 3H, CH_3), 0.11 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.94, 170.15, 169.19, 161.89, 154.05, 135.16, 131.35, 128.81, 128.64, 128.39, 127.97, 127.92, 119.16, 101.01 (C-1), 99.27, 95.99 (C-1^{I}), 92.62, 76.26 (C-3), 76.18 (C-4^{I}), 74.92 (C-5^{I}), 74.46 (C-4), 73.48, 72.25 (C-3^{I}), 71.70 (C-2), 68.87, 67.67 (C-6^{I}), 67.59, 65.74 (C-5), 64.89 (C-6), 57.79 (C-2^{I}), 34.03, 25.21, 24.89, 20.98, 20.75, 20.14, 20.02, 18.68, 18.63, -1.77, -3.18. HR MALDI-TOF MS: m/z: calcd for $C_{47}H_{62}Cl_3NO_{17}Si$ $[M+Na]^+$: 1068.2745; found: 1068.2760.

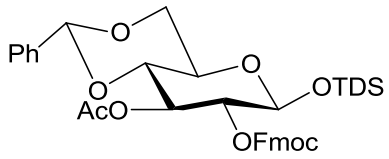


Thexyldimethylsilyl 3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**25**): Compound **24** (89 mg, 0.085 mmol) was dissolved in a mixture of THF (1.67 mL), water (0.167 mL), and morpholine (0.074 mL, 0.850 mmol) along with tetrakis(triphenylphosphine)palladium(0) (49 mg, 0.042 mmol) and stirred under argon for 2 h. The reaction mixture was filtered through a short pad of silica gel and concentrated under vacuum. The residue was purified by silica gel chromatography (15% EtOAc/Hexanes). The major product detected consisted of the product with partially reduced tricloroacetyl functionality (19 mg, 23% $CHCl_2$) as confirmed by MALDI and NMR. It was apparent that alloc deprotection conditions were not compatible with TCA. No trace of the desired product was detected in the crude or purified fractions.



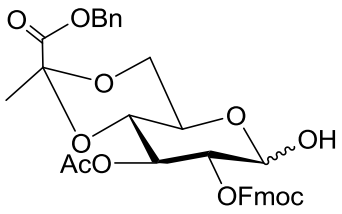
Thexyldimethylsilyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-Glucopyranoside (**27**): Compound **26** (400 mg, 0.974 mmol) was dissolved in methanol (10.5 mL) along with dibutyltin(IV) oxide (303 mg, 1.218 mmol). The mixture was heated at reflux (65^{o}C) for 16 hr. Afterwards, the reaction mixture was cooled, concentrated, and dried under high vacuum for 4 h. The crude intermediate was taken up in DCM (40 mL) and cooled to 0^{o}C. Acetyl chloride (0.087 mL, 1.218 mmol) was added *via* dropwise addition and the mixture was allowed to stir at 0^{o}C for 2 hr. The reaction mixture was transferred to a separatory funnel and washed with 1M KF (aq) (20 mL), water (40 mL), and brine (20 mL). The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by

silica gel column chromatography (20% EtOAc/Hexanes) to give **27** (307 mg, 0.679 mmol, 70%) as a white solid. R_f = 0.85 (50% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.53-7.50 (m, 2H, aromatic), 7.41-7.32 (m, 3H, aromatic), 5.53 (s, 1H, CH), 5.37 (d, 1H, J = 3.52 Hz, H-1), 4.72 (dd, 1H, J = 3.52, 9.96 Hz, H-2), 4.25-4.07 (m, 2H, H-3, H-6a), 3.98-3.90 (m, 1H, H-5), 3.71 (t, 1H, J = 9.96 Hz, H-6e), 3.52 (t, 1H, J= 9.96 Hz, H-4), 2.73 (br.s, 1H, OH), 2.10 (s, 3H, Ac), 1.70-1.61 (m, 1H, CH), 0.93-0.88 (m, 12H, 4xCH_3), 0.15 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.73, 137.11, 129.28, 128.38, 126.33, 101.90, 91.40 (C-1), 81.54 (C-4), 74.87 (C-2), 68.93 (C-6), 68.58 (C-3), 62.37 (C-5), 34.17, 24.99, 20.97, 20.16, 20.10, 18.68, 18.62, -2.31, -3.70. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{36}O_7Si$ $[M+Na]^+$: 475.2123; found: 475.2139.



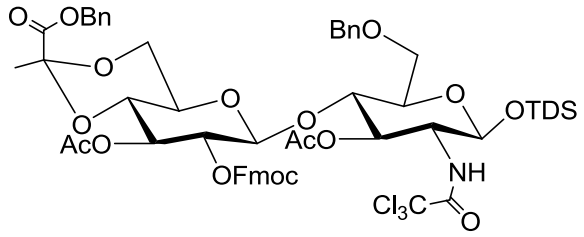
Thexyldimethylsilyl 2-*O*-Fluorenylmethyloxycarbonyl-3-*O*-acetyl-4,6-*O*-benzylidene-β-D-Glucopyranoside (**28**): To a stirring solution of compound **27** (320 mg, 0.706 mmol) in DCM (6.7 mL) and pyridine (3.3 mL) was slowly added a solution of fluorenylmethyloxycarbonyl chloride (548 mg, 2.118 mmol) in DCM (2.3 mL). The reaction was stirred for 16 h and quenched with methanol (1 mL) and concentrated under vacuum. The crude was taken up in DCM (35 mL) and washed with water (35 mL). The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (10% EtOAc/Hexanes) to give **28** (481 mg, 0.713 mmol, 100%) as a white solid. R_f = 0.75 (25% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.80-7.20 (m, 13H, aromatic), 5.56 (s, 1H, CH), 5.46-5.40 (m, 2H, H-1, H-3), 4.93 (dd, 1H, J = 3.52, 9.38 Hz, H-2), 4.47-4.32 (m, 2H, CH_2), 4.30-4.23 (m, 2H, H-6a, C*H*), 4.10-4.02 (m, 1H, H-5), 3.77 (t, 1H, J = 9.96 Hz, H-6e), 2.02 (s, 3H, Ac), 1.70-1.61 (m, 1H, CH), 0.93-0.90 (m, 12H, 4xCH_3), 0.17 (s, 3H, CH_3),0.14 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$): δ 170.23, 154.50, 143.36, 143.19, 141.22, 136.84,129.00, 128.17, 127.90,

127.84, 127.18, 127.13, 126.11, 125.14, 125.04, 120.01, 101.54, 91.50 (C-1), 79.23 (C-4), 73.49 (C-3), 72.68 (C-2), 70.16, 69.90, 68.83 (C-6), 62.56 (C-5), 46.77, 46.57, 34.08, 24.99, 20.76, 20.09, 20.01, 18.58, 18.52, -2.39, -3.69. HR MALDI-TOF MS: m/z: calcd for $C_{38}H_{46}O_9Si$ $[M+Na]^+$: 697.2803; found: 697.2819.



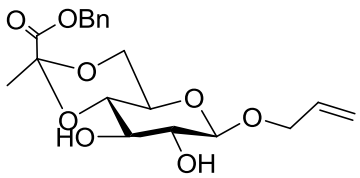
2-*O*-Fluorenylmethyloxycarbonyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranose (**30**): Compound **28** (481 mg, 0.713 mmol) was taken up in 80% HOAc(aq) (18 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. The residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (10% Acetone/Toluene) to give **29** (339 mg, 0.578 mmol, 81%) as a clear oil. R_f = 0.15 (10% Acetone /Toluene). Intermediate compound **29** (332 mg, 0.565 mmol) and benzyl pyruvate (201 mg, 1.13 mmol) were dissolved in dry acetonitrile (0.66 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.139 mL, 1.13 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$, filtered, and transferred to a separatory funnel. The mixture was extracted with DCM (30 mL, 2x) and washed with saturated $NaHCO_3$ (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (20% EtOAc/hexanes) to give **30** (53 mg, 0.087 mmol, 15%) as an amorphous white solid. R_f = 0.4 (35% EtOAc/Hexanes). It was hoped that the TDS protecting group would remain intact or that compound **30** would be formed in higher yield allowing for a step to be removed from the original synthetic strategy. H^1NMR (300 MHz,

$CDCl_3$): Mix of anomers, δ 7.81-7.74 (m, aromatic), 7.66-7.57 (m, aromatic), 7.45-7.22 (m, aromatic), 5.48 (d, J = 2.34 Hz), 5.42-5.33 (m), 5.22-5.15 (m), 5.09 (t, J = 9.38 Hz), 4.87-4.81 (m), 4.74 (d, J = 8.21 Hz), 4.62 (d, J = 7.03), 4.53-4.22 (m), 4.16-3.98 (m), 3.93-3.86 (m), 3.74-3.41 (m), 3.14 (br.s), 2.14 (s), 2.06-2.00 (m), 1.53 (s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.16, 170.27, 170.07, 169.34, 169.10, 154.78, 154.75, 154.32, 143.56, 143.47, 143.37, 143.24, 143.20, 142.95,141.50, 141.43, 141.38, 135.28, 135.20, 128.81, 128.75, 128.70, 128.46, 128.20, 128.03, 127.39, 127.33, 125.45, 125.42, 125.27, 125.13, 120.29, 120.18, 102.02, 99.40, 99.22, 91.08, 79.98, 79.21, 78.79, 77.36, 75.36, 75.08, 74.52, 74.06, 73.09, 71.76, 70.70, 70.49, 70.34, 67.67, 67.59, 67.24, 66.25, 65.24, 64.90, 64.20, 62.16, 46.82, 46.79, 46.70, 46.64, 25.25, 25.18, 20.87. HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{32}O_{11}$ $[M+Na]^+$: 627.1837; found: 627.1849.



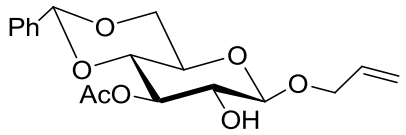
Thexyldimethylsilyl 2-*O*-Fluorenylmethyloxycarbonyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: **(32**): Lactol **30** (0.05 g, 0.082 mmol)was stirred with K_2CO_3 (0.023 g, 0.164 mmol) in DCM along with 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.115 mL, 0.822 mmol). After completion of the reaction, the mixture was filtered and concentrated. The crude was purified by silica gel chromatography (25% EtOAc/ Hexanes + 0.1% pyridine) to afford product **31** in 78% yield (0.05 g, 0.064 mmol). Donor compound **31** (0.05 g, 0.064 mmol) and acceptor **22** (0.050 g, 0.084 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (1.5 mL) along with 4Å molecular sieves (100 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -30^{o}C and TMSOTf (2.7 uL, 0.015 mmol) was added. The

mixture was allowed to warm to -20°C and stir for 1 h. The reaction was quenched with pyridine (100 uL) and diluted in DCM (20 mL). The organic layer was washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% EtOAc/hexanes) to give **32** along with an inseparable impurity (15 mg, 0.013 mmol, 20%) as an amorphous white solid. R_f = 0.4 (25% EtOAc/hexanes). HR MALDI-TOF MS: m/z: calcd for $C_{58}H_{68}Cl_3NO_{17}Si$ $[M+Na]^+$: 1206.3214; found: 1206.3239.

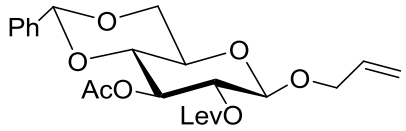


1-*O*-Allyll-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-glucopyranoside: (34): Compound **33** (65 mg, 0.211 mmol) was taken up in 80% HOAc(aq) (7 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. The residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (10% Acetone/Toluene) to give the diol intermediate as an amorphous white solid. R_f = 0.15 (10% MeOH/DCM). Without further purification, the intermediate compound and benzyl pyruvate (74 mg, 0.415 mmol) were dissolved in dry acetonitrile (0.243 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.051 mL, 0.415 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (10 mL) and washed with saturated $NaHCO_3$ (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (45% EtOAc/hexanes) to give **34** along with an inseparable impurity (9.3 mg, 0.024 mmol, 12%) as an amorphous white solid. R_f = 0.2

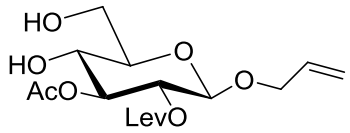
(35% EtOAc/Hexanes). Protection of the C-2 and C-3 alcohol during pyruvylation appears to be essential for good yield. HR MALDI-TOF MS: m/z: calcd for $C_{19}H_{24}O_8$ $[M+Na]^+$: 403.1363; found: 403.1389.



1-*O*-Allyll-3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside: (**35**): Compound **33** (400 mg, 1.297 mmol) was dissolved in methanol (14 mL) along with dibutyltin(IV) oxide (404 mg, 1.622 mmol). The mixture was heated at reflux (65^{o}C) for 16 hr. Afterwards, the reaction mixture was cooled, concentrated, and dried under high vacuum for 4 h. The crude intermediate was taken up in DCM (53 mL) and cooled to 0^{o}C. Acetyl chloride (0.115 mL, 1.622 mmol) was added via dropwise addition and the mixture was allowed to stir at 0^{o}C for 2 hr. The reaction mixture was transferred to a separatory funnel and washed with a solution of 1M KF (aq) (20 mL). The organic layer was washed with water (40 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (25% EtOAc/Hexanes) to give **35** (0.1234 g, 0.352 mmol, 27%) as an amorphous white solid. R_f = 0.4 (30% EtOAc/hexanes). In addition, a 2-*O*-acetyl impurity was isolated (0.1083 g, 0.309 mmol, 24%) as a white solid. R_f = 0.43 (30% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.45-7.43 (m, 2H, aromatic), 7.38-7.35 (m, 3H, aromatic), 5.98-5.90 (m, 1H, =CH-), 5.50 (s, 1H, CH), 5.34 (dd, 2H, J = 1.0, 17.08 Hz, =C*H*H), 5.26-5.21 (m, 2H, H-3, =C*H*H), 4.52 (d, 1H, J = 7.81 Hz), 4.41-4.34 (m, 2H, H-6a, C*H*H), 4.17 (dd, 1H, J = 6.34, 12.69 Hz, C*H*H), 3.80 (t, 1H, J = 10.25 Hz, H-6e), 3.65 (t, 1H, J= 9.76 Hz, H-4), 3.63-3.59 (m, 1H, H-2), 3.54-3.50 (m, 1H, H-5), 2.53 (d, 1H, J = 2.93 Hz, OH), 2.13 (s, 3H, Ac), ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.11, 137.06, 133.48, 129.26, 128.41, 126.30, 118.58, 102.65, 101.69, 78.63, 73.68, 73.57, 70.92, 68.80, 66.72, 21.15. HR MALDI-TOF MS: m/z: calcd for $C_{18}H_{22}O_7$ $[M+Na]^+$: 373.1258; found: 373.1279.

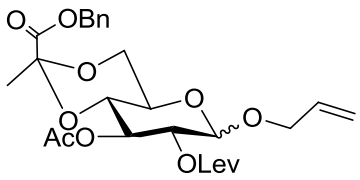


1-*O*-Allyll-2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside: (**36**): To a stirring solution of compound **35** (596 mg, 1.702 mmol) and levulinic acid (1.73 mL, 17.02 mmol) in DCM (43 mL) and was slowly added a solution of DCC (1.76 g, 8.51 mmol) and DMAP (31 mg, 0.255 mmol) in DCM (4.2 mL). The reaction was stirred for 8 h and quenched with methanol (1 mL) and concentrated under vacuum. The crude was taken up in DCM (35 mL) and washed with water (35 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography 2:1 ((30% EtOAc/Hexanes):(DCM)) to give **36** (760 mg, 1.70 mmol, 100%) as a white solid. R_f = 0.4 (35% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.45-7.42 (m, 2H, aromatic), 7.37-7.34 (m, 3H, aromatic), 5.93-5.80 (m, 1H, =CH-), 5.50 (s, 1H, CH), 5.37-5.30 (m, 2H, H-3, =C*H*H), 5.25-5.19 (m, 1H, =C*H*H), 5.07-5.02 (m, 1H, H-2), 4.62 (d, 1H, J = 7.81 Hz), 4.39-4.30 (m, 2H, H-6a, C*H*H), 4.16-4.08 (m, 1H, C*H*H), 3.81 (t, 1H, J = 10.55 Hz, H-6e), 3.70 (t, 1H, J= 9.38 Hz, H-4), 3.56-3.48 (m, 1H, H-5), 2.84-2.49 (m, 4H, 2xCH_2), 2.18 (s, 3H, Ac), 2.09 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.08, 171.51, 170.47, 136.99, 133.29, 129.28, 128.39, 126.30, 117.89, 110.17, 101.67, 100.45, 78.58, 72.46, 71.62, 70.52, 68.74, 66.61, 37.89, 28.03, 20.98. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{28}O_9$ $[M+Na]_+$: 471.1626; found: 471.1639.

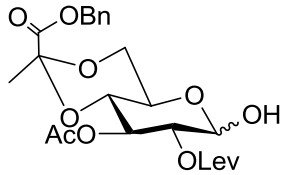


1-*O*-Allyll-2-*O*-levulinyl-3-*O*-acetyl-β-D-glucopyranoside: (**37**): Compound **36** (463 mg, 1.033 mmol) was taken up in 80% HOAc (aq, 26 mL) and heated at 50°C for 16 h. The mixture was concentrated and the residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting crude was purified by silica gel column chromatography (25%

Acetone/Toluene) to give **37** (339 mg, 0.940 mmol, 91%) as a colorless solid. R_f = 0.4 (40% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 5.92-5.79 (m, 1H, =CH), 5.40-5.17 (m, 2H, $=CH_2$), 5.17-4.92 (m, 2H, H-2, H-3), 4.55 (d, 1H, J = 7.62, H-1), 4.34-4.28 (m, 1H, C*H*H), 4.13-4.07 (m, 1H, C*H*H), 3.95-3.59 (m, 3H, H-4, H6ae), 3.45-3.39 (m, 1H, H-5), 2.85-2.44 (m, 6H, 3 x CH_2), 2.17 (s, 3H, CH_3), 2.14 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.26, 171.54, 133.66, 117.71, 99.85, 75.94, 75.74, 71.44, 70.38, 69.91, 62.23, 37.86, 29.88, 28.00, 21.03. HR MALDI-TOF MS: m/z: calcd for $C_{16}H_{24}O_9$ $[M+Na]^+$: 383.1313; found: 383.1333.

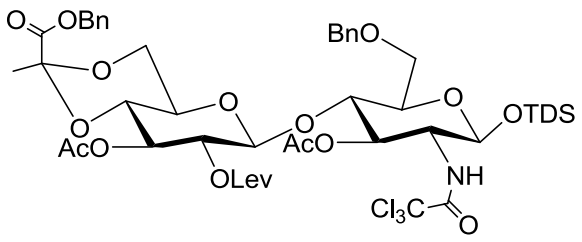


1-*O*-Allyll-2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-glucopyranoside: (**38**): Compound **37** (145 mg, 0.402 mmol) and benzyl pyruvate (143 mg, 0.804 mmol) were dissolved in dry acetonitrile (0.470 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.099 mL, 0.804 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (30% EtOAc/hexanes) to give an inseparable 1:3 (α:β) mixture of **38** (70 mg, 0.134 mmol, 33%) as an amorphous white solid. R_f = 0.4 (35% EtOAc/Hexanes. H^1NMR (500 MHz, $CDCl_3$): alpha/beta; δ 7.37-7.31 (m, 5H, aromatic), 5.89-5.77 (m), 5.47-5.42 (m), 5.30-5.24 (m), 5.21-5.15 (m), 4.98-4.95 (m, H-1α), 4.91 (t, J = 9.76 Hz), 4.78-4.74 (m), 4.53 (d, J = 7.81 Hz, H-1β), 4.26 (dd, J = 4.88, 13.18 Hz), 4.16-4.13 (m), 4.13-4.10 (m), 4.00-3.94 (m), 3.86-3.80 (m), 3.70 (t, J = 10.74 Hz), 3.66-3.60 (m), 3.48 (t, 1H, J= 9.76 Hz), 3.43-3.36 (m), 2.79-2.64 (m), 2.60-2.46 (m), 2.12 (s), 2.10 (s), 2.00 (s), 1.98 (s), 1.50 (s). HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{32}O_{11}$ $[M+Na]^+$: 543.1837; found: 543.1859.



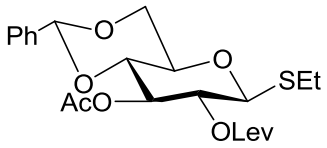
2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-glucopyranoside: (**39**):

Compound **38** (63 mg, 0.120 mmol) and 1,5-Cyclooctadiene-bis[methyldiphenylphosphine]-iridium Hexafluorophosphate (1.02 mg, 1.203 μmol) were dissolved in THF (2.4 mL) to give a pink solution. The solution was placed under H_2 for 5-10 seconds until the mixture turned yellow. The H_2 was flushed out with argon and the reaction was allowed to stir at room temperature until complete as indicated by NMR. The reaction mixture was concentrated and the residue was taken up in 5:1 (Acetone:Water). $HgCl_2$ (20 mg, 0.072 mmol) and HgO (16 mg, 0.072 mmol) were added to the mixture and the reaction was stirred until completion as indicated by TLC. The reaction mixture was filtered through celite and concentrated. The residue was taken up in DCM (15 mL) and washed with sat KI (aq, 15 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to give an anomeric mixture of **39** (0.038g, 0.079 mmol, 65%) as an amorphous white solid. H^1NMR (600 MHz, $CDCl_3$): δ 7.37-7.30 (m, aromatic), 5.46 (t, J = 9.96 Hz), 5.33-5.14 (m), 4.78-4.72 (m), 4.09-3.96 (m), 3.71-3.56 (m), 3.52-3.38 (m), 2.82-2.64 (m), 2.62-2.41 (m), 2.19-2.12 (m), 2.00 (s), 1.98 (s), 1.50 (s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 207.34, 206.67, 172.49, 171.92, 170.55, 170.47, 169.57, 169.31, 135.22, 135.16, 128.76, 128.73, 128.68, 128.38, 128.33, 99.35, 99.32, 96.04, 95.95, 91.09, 90.74, 75.33, 74.69, 74.20, 73.77, 72.14, 71.53, 71.33, 71.07, 68.92, 68.61, 67.65, 67.59, 66.31, 66.22, 65.35, 64.95, 64.55, 62.00, 38.15, 37.92, 37.86, 31.02, 29.83, 29.77, 28.05, 28.00, 25.28, 25.22, 20.87, 20.81. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{28}O_{11}$ $[M+Na]^+$: 503.1524; found: 503.1549.

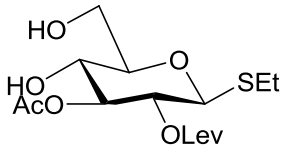


Thexyldimethylsilyl 2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**41): Attempt 1**: To a solution of **39** (38 mg, 0.079 mmol) in DCM (2.3 mL) was added Cs_2CO_3 (52 mg, 0.158 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.111mL, 0.791 mmol). The reaction was stirred at RT until the reaction was complete as verified by TLC. R_f = 0.35 (30% EtOAc/hexanes + 0.1% TEA). Triethylamine (100 μL) was added to the reaction mixture and the mixture was filtered through celite and concentrated. The crude was passed through a short silica gel column (5% to 35% Ea/hex + 0.1% TEA) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride and afford **40** (52 mg, 0.08 mmol, 100%) as an amorphous white solid. Donor compound **40** and acceptor **22** (72 mg, 0.12 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (3.5 mL) along with 4Å molecular sieves (120 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20^{o}C and TfOH (0.5 μL, 5.99 μmol) was added. The mixture was allowed to warm to 0^{o}C and stir for 1 h. The reaction was quenched by the addition of pyridine (100 μL) and diluted in DCM (20 mL). The organic layer was washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. No product could be seen by TLC (40% EtOAc/Hexanes) or detected with MALDI. The donor was consumed within 5 minutes indicating a very reactive donor. The donor and acceptor may have mismatched reactivities.

Attempt 2: Compound **44** (54 mg, 0.103 mmol) and glucosyl acceptor **22** (44 mg, 0.073 mmol) was taken up in toluene (5 mL) and concentrated under vacuum (3x). The mixture was taken up in DCM (3.8 mL) along with activated 4Å molecular sieves (100 mg) and stirred under argon for 30 min. The mixture was cooled to 0^{o}C and N-iodosuccinimide (26 mg, 0.114 mmol) was added followed by dropwise addition of triflic acid (1.3 uL, 0.015 mmol). After stirring for 1 h, the reaction mixture was quenched by the addition of pyridine (0.1 mL), filtered, and concentrated under vacuum. The residue was taken up in DCM (20 mL) and washed with NaS_2O_3 (sat, aq, 15 mL) and water (15 mL). The organic phasre was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (15% Acetone/toluene) to give **45** (29 mg, 0.027 mmol, 37%) as an amorphous white solid. R_f = 0.45 (15% Acetone/Toluene). H^{1}NMR (600 MHz, $CDCl_3$): δ 7.43-7.31 (m, 10H, aromatic), 6.68 (d, 1H, J = 9.38 Hz, NH), 5.27-5.17 (m, 2H, CH_2), 5.08-5.01 (m, 2H, H-3, H-3^{I}), 4.77-4.68 (m, 3H, H-1^{I}, H-2, C*H*H), 4.55-4.47 (m, 2H, H-1, C*H*H), 4.06-4.01 (m, 1H, J = 4.69, 10.55 Hz, C-6a), 3.97-3.88 (m, 2H, H-2^{I}, H-4^{I}), 3.79 (dd, 1H, J = 2.93, 11.72 Hz, H-6^{I}a), 3.67 (d, 1H, J = 11.14 Hz, H-6^{I}e), 3.58-3.47 (m, 2H, H-5^{I}, H-6e), 3.35 (t, 1H, J = 9.96 Hz, H-4), 3.25-3.17 (m, 1H, H-5), 2.72-2.58 (m, 2H, CH_2), 2.39 (t, 2H, J = 6.45 Hz, CH_2), 2.14 (s, 3H, CH_3), 1.96 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.64-1.55 (m, 1H, CH), 1.51 (s, 3H, CH_3), 0.85-0.81 (m, 12H, 4xCH_3), 0.17 (s, 3H, CH_3), 0.11 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 205.91, 171.13, 170.88, 170.52, 169.27, 161.85, 137.99, 135.12, 128.82, 128.75, 128.44, 128.17, 128.04, 100.86 (C-1), 99.29, 96.05 (C-1^{I}), 92.56, 75.44 (C-4^{I}), 74.98 (C-5^{I}), 74.53 (C-4), 73.88, 72.34 (C-2, C-3^{I}), 71.67 (C-3), 67.71 (C-6^{I}), 65.79 (C-5), 64.94 (C-6), 57.81 (C-2^{I}), 37.66, 34.00, 29.88, 27.74, 25.25, 24.87, 20.98, 20.83, 20.13, 19.99, 18.68, 18.63, -1.73, -3.16. HR MALDI-TOF MS: m/z: calcd for $C_{48}H_{64}Cl_3NO_{17}Si$ $[M+Na]_+$: 1082.2901; found: 1082.2928.

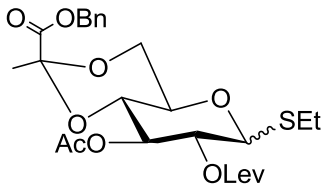


Ethyl 2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**42**): To a stirring solution of compound **15** (3.28 g, 9.24 mmol) and levulinic acid (9.37 mL, 92 mmol) in DCM (231 mL) and was slowly added a solution of DCC (9.53 g, 46.2 mmol) and DMAP (169 mg, 1.386 mmol) in DCM (23 mL). The reaction was stirred for 8 h and quenched with methanol (5 mL) and concentrated under vacuum. The crude was taken up in DCM (35 mL) and washed with water (35 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography 2:1 ((30% EtOAc/Hexanes):(DCM)) to give **42** (4.06 g, 8.97 mmol, 97%) as a white solid. R_f = 0.5 (35% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.44-7.42 (m, 2H, aromatic), 7.37-7.34 (m, 3H, aromatic), 5.50 (s, 1H, CH), 5.36 (t, 1H, J = 9.39 Hz, H-3), 5.06 (t, 1H, J = 9.39 Hz, H-2), 4.58 (d, 1H, J = 9.98 Hz, H-1), 4.37 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.78 (t, 1H, J = 9.98 Hz, H-6e), 3.68 (t, 1H, J = 9.39 Hz, H-4), 3.59-3.55 (m, H-1, H-5), 2.84-2.79 (m, 1H, C*H*H), 2.76-2.66 (m, 3H, C*H*H, CH_2'), 2.64-2.59 (m, 1H, CH*H*''), 2.56-2.51 (m, 1H, C*H*H''), 2.18 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 1.20 (t, 3H, J = 7.63 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.04, 171.55, 170.43, 136.90, 129.27, 128.38, 126.27, 101.60, 84.30, 78.52, 72.55, 70.98, 70.81, 68.63, 37.84, 29.84, 28.08, 24.46, 20.97, 15.01. HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{28}O_8S$ $[M+Na]^+$: 475.1397; found: 475.1489.



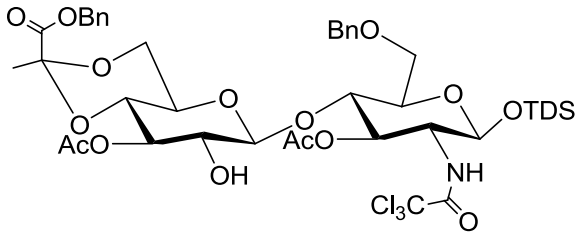
Ethyl 2-*O*-levulinyl-3-*O*-acetyl-1-thio-β-D-glucopyranoside (**43**): Compound **42** (1.45 g, 3.20 mmol) was taken up in 80% HOAc(aq) (80 mL) and heated at 50°C for 16 h. The mixture was concentrated and the residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (25%

Acetone/Toluene) to give **43** (963 mg, 2.64 mmol, 82%) as a colorless solid. R_f = 0.4 (40% Acetone/Toluene). H^1NMR (600 MHz, $CDCl_3$): δ 5.06 (t, 1H, J = 9.39 Hz, H-3), 4.94 (t, 1H, J = 9.39 Hz, H-2), 4.50 (d, 1H, J = 9.98 Hz, H-1), 3.93-3.91 (m, 1H, H-6a), 3.82-3.80 (m, 1H, H-6e), 3.73-3.70 (m, 1H, H-4), 3.45-3.42 (m, 1H, H-5), 3.26 (br.s, 1H, OH), 2.84-2.79 (m, 1H, C*H*H), 2.73-2.63 (m, 3H, C*H*H, CH_2'), 2.62-2.56 (m, 1H, CH*H*''), 2.52-2.47 (m, 1H, C*H*H''), 2.42 (br.s, 1H, OH), 2.16 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 1.25 (t, 3H, J = 7.63 Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.27, 172.04, 171.55, 83.50, 79.93, 70.07, 69.45, 62.33, 37.81, 24.41, 21.04, 15.01. HR MALDI-TOF MS: m/z: calcd for $C_{15}H_{24}O_8S$ $[M+Na]^+$: 387.1084; found: 387.1098.



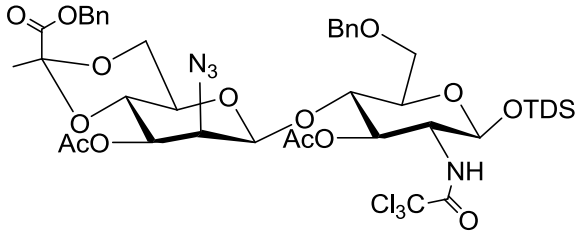
Ethyl 2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α/β-D-glucopyranoside (**44**): Compound **43** (286 mg, 0.785 mmol) and benzyl pyruvate (254 mg, 1.571 mmol) were dissolved in dry acetonitrile (0.918 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.193 mL, 1.571 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (30% EtOAc/hexanes) to give an inseparable 1:2 (α:β) mixture of **44** (155 mg, 0.296 mmol, 38%) as an amorphous white solid. R_f = 0.4 (35% EtOAc/Hexanes). H^1NMR (600 MHz, $CDCl_3$): alpha/beta mix: δ 7.38-7.33 (m, 5H, aromatic), 5.51 (d, 1H, J = 5.87 Hz, H-1α), 5.31-5.27 (m, 1H, C*H*H), 5.21-5.16 (m, 2H, H-3, C*H*H), 4.92-4.89 (m, 1H, H-2), 4.47 (d, 1H, J = 10.56 Hz, H-1), 4.21-4.17 (m, 1H, H-5α), 4.07 (dd, 1H, J = 4.70, 11.74 Hz, H-6a (β), 3.96 (dd, 1H, J = 5.28, 11.15 Hz, H-6a(α)), 3.69-3.64 (m, 1H, H-6e (α.β)), 3.46-3.39 (m, 2H, H-4, H-5), 2.81-2.76 (m, 1H, C*H*H), 2.73-2.47 (m, 5H, C*H*H,

CH_2', CH_2''), 2.16 (s, 3H, CH_3), 2.03 (s, 3H, CH_3 (β)), 1.98 (s, 3H, CH_3 (α)), 1.51 (s, 3H, CH_3 (α)), 1.50 (s, 3H, CH_3 (β)), 1.24-1.20 (m, 3H, CH_3).^{13}C NMR (150 MHz, $CDCl_3$): δ 206.19, 205.96, 171.52, 171.37, 170.53, 170.14, 169.48, 169.28, 135.19, 135.14, 128.78, 128.72, 128.37, 99.36, 99.27, 83.99, 82.67, 75.36, 74.55, 72.39, 71.31, 70.48, 70.41, 69.04, 67.62, 65.05, 64.87, 62.43, 37.80, 37.72, 29.79, 28.08, 28.01, 25.30, 25.19, 24.40, 24.13, 20.85, 14.91, 14.88. HR MALDI-TOF MS: m/z: calcd for $C_{25}H_{32}O_{10}S$ $[M+Na]^+$: 547.1608; found: 547.1618.



Thexyldimethylsilyl 3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**46**): Compound **45** (22 mg, 0.020 mmol) was dissolved in DCM (1.4 mL) and MeOH (0.14 mL) along with hydrazine acetate (3.75 mg, 0.041 mmol). The reaction was allowed to stir at room temperature for 3 h. Completion of the reaction was verified by MALDI since the starting material and product had similar R_f values. The reaction mixture was concentrated under vacuum and the resulting crude was taken up in EtOAc (20 mL) and washed with water (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (35% EtOAc/Hexanes) to give **46** (16 mg, 0.017 mmol, 82%) as a white solid. R_f = 0.5 (35% EtOAc/hexanes). H^{1}NMR (600 MHz, $CDCl_3$): δ 7.79 (d, 1H, J = 9.38 Hz, NH), 7.40-7.26 (m, 10H, aromatic), 5.42 (t, 1H, J = 10.55 Hz, H-3^I), 5.18 (dd, 2H, J = 12.31, 31.65 Hz, CH_2), 5.01 (t, 1H, J = 9.38 Hz, H-3), 4.91 (br.s, 1H, OH), 4.76-4.55 (m, 2H, H-1^I, C*H*H^I), 4.59, 4.55 (d, 1H, J = 13.48 Hz, C*H*H^I), 4.21-4.13 (m, 2H, H-1^I, H-6^{I}a), 4.09-4.00 (m, 2H, H-2^I, H-6a), 3.88 (t, 1H, J = 8.79 Hz, H-4^I), 3.55-3.40 (m, 4H, H-4, H-5^I, H-6e, H-6^{I}e), 3.35-3.21 (m, 2H, H-2, H-5), 1.89 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.66-1.57 (m, 1H, CH), 1.49 (s, 3H, CH_3), 0.88-0.84 (m, 12H, 4xCH_3), 0.20 (s, 3H, CH_3),

0.08 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.92, 170.49, 169.13, 162.26, 136.88, 135.19, 128.78, 128.65, 128.29, 127.83, 105.06 (C-1), 99.15, 95.29 (C-1^I), 92.93, 79.19 (C-5^I), 75.01 (C-4^I), 74.49 (C-4), 73.59 (C-3), 73.15 (C-3^I), 72.52 (C-2), 68.43 (C-6^I), 67.24, 66.14 (C-5), 65.10 (C-6), 56.94 (C-2^I), 34.03, 29.85, 29.41, 25.27, 24.81, 21.07, 20.81, 20.20, 19.97, 18.75, 18.62, -1.61, -3.12. HR MALDI-TOF MS: m/z: calcd for $C_{43}H_{58}Cl_3NO_{15}Si$ $[M+Na]_+$: 984.2534; found: 984.2528.



C-2 Azide installation reactions:

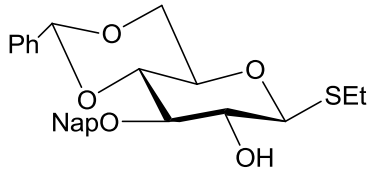
Trial 1: Thexyldimethylsilyl 2-azido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannoopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: **(47**): Compound **46** (4.9 mg, 5.09 μmol) and DMAP (0.075 mg, 0.610 μmol) were dissolved in a mixture of DCM (200 μL) and pyridine (50 μL). The mixture was cooled to 0°C and trifluoromethanesulfonic anhydride (6.02 μL, 0.036 mmol) was added to the stirring mixture *via* dropwise addition. After stirring for 3 h, no product formation could be seen by TLC (35% EtOAc/Hexanes + 1 drop TEA). In the event that the product was of similar R_f to that of the starting material, the reaction was pushed forward to see if any product formation would occur. The reaction mixture was diluted in DCM (20 mL) and transferred to a separatory funnel where it was washed with $NaHCO_3$ (sat, aq, 10mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford a yellow solid. The solid was taken up in DMF (0.1 mL) with Sodium azide (2mg, 0.031 mmol) and heated at 55°C for 18 h. No product formation could be seen by TLC (35% EtOAc/Hexanes + 1 drop TEA) or detected by MALDI.

Trial 2: Compound **46** (2 mg, 2.08 μmol) and DMAP (0.03 mg, 0.249 μmol) were dissolved in a mixture of DCM (200 μL) and pyridine (50 μL). The mixture was cooled to 0°C and trifluoromethanesulfonic anhydride (2.5 μL, 0.015 mmol) was added to the stirring mixture via dropwise addition. After stirring for 1 h 0°C and 16 h at room temperature, no product formation could be seen by TLC (35% EtOAc/Hexanes + 1 drop TEA). The mixture was cooled to 0°C and additional trifluoromethanesulfonic anhydride (2.5 μL, 0.015 mmol) was added via dropwise addition. The reaction was allowed to come to room temperature while stirring but no product formation could be seen. The mixture was then heated (35°C) for 16 h. During the reaction time, no product formation was seen by TLC.

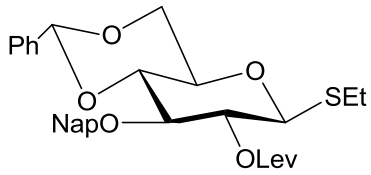
Trial 3: Compound **46** (3.3 mg, 3.43 μmol) was dissolved in a mixture of DCM (300 μL) and cooled to 0°C. methanesulfonyl chloride (0.7 μL, 8.56 μmol) was added to the stirring mixture via dropwise addition followed by triethylamine (1.3 μL, 9.25 μmol). After stirring for 1 h 0°C and 16 h at room temperature, no product formation could be seen by TLC (35% EtOAc/Hexanes + 1 drop TEA). The mixture was cooled to 0°C and additional methanesulfonyl chloride (0.7 μL, 8.56 μmol) and triethylamine (1.3 μL, 9.25 μmol) were added *via* dropwise addition. The reaction was allowed to come to room temperature while stirring but no product formation could be seen. The mixture was then heated (35°C) for 16 h. During the reaction time, no product formation was seen by TLC.

Trial 4: Compound **46** (3.3 mg, 3.43 μmol) and DMAP (0.05 mg, 0.411 μmol) were dissolved in pyridine (300 uL) and cooled to 0°C. p-Toluenesulfonyl chloride (2 mg, 10.28 μmol) was added to the stirring mixture. After stirring for 1 h 0°C and 16 h at room temperature, no product formation could be seen by TLC (35% EtOAc/Hexanes + 1 drop TEA). The mixture was cooled to 0°C and additional p-Toluenesulfonyl chloride (4 mg, 20.5 μmol) were added *via* dropwise addition. The reaction was allowed to come to

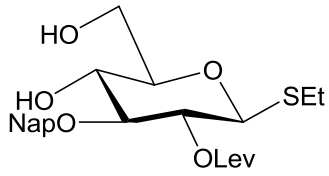
room temperature while stirring but no product formation could be seen. The mixture was then heated (55^{o}C) for 16 h. During the reaction time, no product formation was seen by TLC.



Ethyl 3-*O*-(2-methylnaphthyl)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: (**48**): Compound **14** (796 mg, 2.55 mmol) was dissolved in methanol (27 mL) along with dibutyltin(IV) oxide (793 mg, 3.19 mmol) and heated at reflux (65^{o}C) for 16 hr. Afterwards, the reaction mixture was cooled, concentrated, and dried under high vacuum for 4 h. The crude intermediate was taken up in DMF (15 mL) with cesium fluoride (523 mg, 3.44 mmol) and 2-(Bromomethyl) Naphthalene (704 mg, 3.19 mmol). The mixture was allowed to stir at room temperature for 18 h. The reaction mixture was concentrated under vacuum and the residue was taken up in DCM (100 mL) and transferred to a separatory funnel. The organic phase was washed with 1M KF (aq) (50 mL) and water (200 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (20% EtOAc/Hexanes) to give **48** (491 mg, 1.08 mmol, 43%) as a white solid. R_f = 0.9 (50% EtOAc/Hexanes). H^1NMR (600 MHz, $CDCl_3$): δ 7.86-7.77 (m, 3H, aromatic), 7.77-7.72 (m, 1H, aromatic), 7.54-7.34 (m, 8H, aromatic), 5.60 (s, 1H, CH), 5.13 (d, 1H, J = 11.74 Hz, C*H*H), 5.00 (d, 1H, J = 12.33 Hz, C*H*H), 4.46 (d, 1H, J = 9.39 Hz, H-1), 4.36 (dd, 1H, J = 4.70, 9.98 Hz, H-6a), 3.79 (t, 1H, J = 9.98 Hz, H-6e), 3.77-3.72 (m, 2H, H-3, H-4), 3.63 (t, 1H, J = 8.80 Hz, H-2), 3.52-3.49 (m, 1H, H-5), 2.79-2.72 (m, 2H, CH_2), 2.60 (s, 1H, OH), 1.32 (t, 3H, J = 7.63 Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.34, 135.83, 133.38, 133.15, 129.17, 128.42, 128.36, 128.07, 127.79, 126.97, 126.19, 126.17, 126.11, 126.02, 101.47, 86.76, 81.50, 81.30, 74.80, 73.24, 70.88, 68.77, 24.72, 15.38. HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{28}O_5S$ $[M+Na]^+$: 475.1550; found: 475.1538.

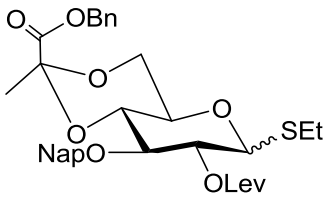


Ethyl 2-*O*-levulinyl-3-*O*-(2 -methylnaphthyl)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: (**49**): To a stirring solution of compound **48** (491 mg, 1.084 mmol) and levulinic acid (1.1 mL, 10.84 mmol) in DCM (27 mL) and was slowly added a solution of DCC (1.12 g, 5.42 mmol) and DMAP (20 mg, 0.163 mmol) in DCM (2.7 mL). The reaction mixture was stirred for 8 h and quenched with methanol (0.5 mL). The mixture diluted with DCM (35 mL) and washed with water (35 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography 2:1 ((20% EtOAc/Hexanes):(DCM)) to give **49** (587 mg, 1.07 mmol, 98%) as an amorphous white solid. R_f = 0.3 (25% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.86-7.75 (m, 4H, aromatic), 7.52-7.38 (m, 8H, aromatic), 5.60 (s, 1H, CH), 5.11-5.00 (m, 2H, H-2, C*H*H), 4.88 (d, 1H, J = 12.31 Hz, C*H*H), 4.45 (d, 1H, J = 9.96 Hz, H-1), 4.37 (dd, 1H, J = 4.69, 10.55 Hz, H-6a), 3.84-3.76 (m, 3H, H-3, H-4, H-6e), 3.53-3.45 (m, 1H, H-5), 2.76-2.63 (m, 4H, 2xCH_2), 2.54-2.49 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.24 (t, 3H, J = 7.62 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.28, 171.57, 137.31, 135.77, 133.35, 133.14, 129.22, 128.46, 128.18, 128.07, 127.81, 126.96, 126.29, 126.20, 126.03, 101.49, 84.35, 81.66, 79.57, 74.50, 71.82, 70.83, 68.75, 37.92, 29.92, 28.14, 24.19, 14.98. HR MALDI-TOF MS: m/z: calcd for $C_{31}H_{34}O_7S$ $[M+Na]^+$: 573.1917; found: 573.1923.



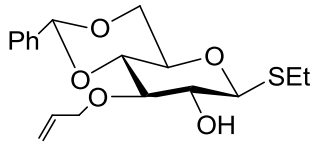
Ethyl 2-*O*-levulinyl-3-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside: (**50**): Compound **49** (587 mg, 1.07 mmol) was taken up in 80% HOAc (aq) (27 mL) and heated at 50°C for 16 h. The mixture was concentrated and the resulting residue was taken up in toluene and concentrated under vacuum (3x) in

order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (25% Acetone/Toluene) to give **50** (405 mg, 0.875 mmol, 82%) as a colorless solid. R_f = 0.2 (15% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 7.85-7.78 (m, 4H, aromatic), 7.49-7.43 (m, 3H, aromatic), 5.05-4.97 (m, 2H, H-2, C*H*H), 4.84 (d, 1H, J = 12.31 Hz, C*H*H), 4.43 (d, 1H, J = 9.96 Hz, H-1), 3.92-3.85 (m, 1H, H-6a), 3.80-3.57 (m, 3H, H-3, H-4, H-6e), 3.41-3.36 (m, 1H, H-5), 2.76-2.61 (m, 4H, 2xCH_2), 2.59-2.51 (m, 2H, CH_2), 2.12 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.24 (t, 3H, J = 7.62 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.40, 171.77, 135.67, 133.38, 133.16, 128.65, 128.10, 127.88, 126.93, 126.43, 126.24, 125.86, 84.00, 83.84, 79.51, 74.96, 72.12, 70.61, 62.75, 37.86, 29.92, 28.20, 24.31, 15.03. HR MALDI-TOF MS: m/z: calcd for $C_{24}H_{30}O_7S$ $[M+Na]^+$: 485.1604; found: 485.1624.



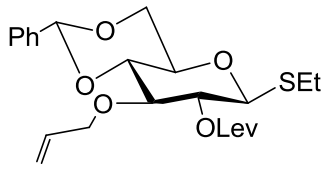
Ethyl 2-*O*-levulinyl-3-*O*-(2-methylnaphthyl)-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α/β-D-glucopyranoside: (51) **Method 1:** Compound **50** (154 mg, 0.333 mmol) and Benzyl pyruvate (119 mg, 0.666 mmol) were dissolved in dry acetonitrile (0.39 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.082 mL, 0.666 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. It was noticed that after addition of $BF_3(OEt)_2$, the reaction mixture was turning a yellow-brown color. The reaction mixture was quenched via dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (30% EtOAc/hexanes) to give an impure mixture of **51** (17.5 mg, 0.028 mmol, 8%) as a pale yellow solid. R_f = 0.65 (35% EtOAc/Hexanes). The major products in the reaction detected using MALDI were missing the 3-*O*-Nap.

Method 2: Compound **50** (46 mg, 0.099 mmol) was dissolved in pyridine (250 μL) and cooled to 0°C. Trimethylsilyl Chloride (0.063 mL, 0.495 mmol) was added to the stirring reaction mixture *via* dropwise addition. The reaction was allowed to warm to room temperature and stir for 16 hrs. The reaction mixture was diluted in DCM (20 mL) and washed with twice with water (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum to give the crude di-silylated intermediate (53.4 mg, 0.088 mmol, 89%) as a white solid. R_f = 0.75 (35% EtOAc/Hexanes). Without further purification, the intermediate and Benzyl pyruvate (31 mg, 0.176 mmol) were dissolved in dry DCM (0.28 mL) under argon atmosphere and cooled to 0°C. TMSOTf (6.36 μL, 0.035 mmol) was added dropwise to the stirring mixture and the reaction was allowed to warm to room temperature and stir at room temperature for 16 h. The reaction mixture was diluted in DCM (20 mL) and washed with twice with water (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (20% EtOAc/Hexanse) to give an impure mixture of **51** (2.1 mg, 3.37 μmol, 4%) as a pale yellow solid. R_f = 0.65 (35% EtOAc/Hexanes). HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{38}O_9S$ $[M+Na]^+$: 645.2129; found: 645.2338.



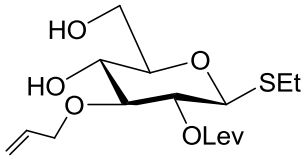
Ethyl 3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: (**52**): Compound **14** (935 mg, 2.99 mmol) was dissolved in methanol (32 mL) along with dibutyltin(IV) oxide (932 mg, 3.74 mmol). The mixture was heated at reflux (65°C) for 16 hr. Afterwards, the reaction mixture was cooled, concentrated, and dried under high vacuum for 4 h. The crude intermediate was taken up in DMF (17 mL) with cesium fluoride (614 mg, 4.04 mmol) and allyl bromide (0.324 mL, 3.74 mmol). The mixture was allowed to stir at room temperature for 18 h. The reaction mixture was concentrated under vacuum and the residue was taken up in DCM (50 mL) and transferred to a separatory funnel. The organic phase was washed with

1M KF (aq) (50 mL) and water (200 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (20% EtOAc/Hexanes) to **52** (813 mg, 2.31 mmol, 77%) as a white solid. R_f = 0.65 (35% EtOAc/Hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.50-7.44 (m, 2H, aromatic), 7.41-7.33 (m, 3H, aromatic), 6.03-5.90 (m, 1H, =CH), 5.56 (s, 1H, CH), 5.31 (d, 1H, J = 17.00 Hz, =C*H*H), 5.20 (d, 1H, J = 9.96 Hz, C*H*H), 4.50-4.42 (m, 2H, H-1, C*H*H), 4.38-4.26 (m, 2H, H-6a, C*H*H), 3.77 (t, 1H, J = 9.96 Hz, H-6e), 3.69-3.45 (m, 4H, H-2, H-3, H-4, H-5), 2.76 (q, 2H, CH_2), 2.59 (1, 1H, OH), 1.33 (t, 3H, J = 7.62 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.35, 134.97, 129.14, 128.39, 126.12, 117.59, 101.40, 86.74, 81.46, 73.82, 73.01, 70.92, 68.78, 24.74, 15.40. HR MALDI-TOF MS: m/z: calcd for $C_{18}H_{24}O_5S$ $[M+Na]^+$: 375.1237; found: 375.1203.

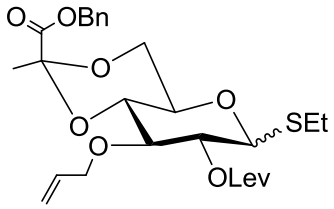


Ethyl 2-*O*-levulinyl-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: (**53**): To a stirring solution of compound **52** (805 mg, 2.28 mmol) and levulinic acid (2.32 mL, 22.83 mmol) in DCM (57 mL) was slowly added a solution of DCC (2.36 g, 11.42 mmol) and DMAP (42 mg, 0.342 mmol) in DCM (5.7 mL). The reaction was stirred for 8 h and quenched with methanol (0.5 mL). The mixture was concentrated under vacuum and the resulting crude was taken up in DCM (75 mL) and washed with water (35 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography 2:1 ((20% EtOAc/Hexanes):(DCM)) to give **53** (984 mg, 2.18 mmol, 96%) as an amorphous white solid. R_f = 0.75 (25% EtOAc/hexanes, eluted 3x). H^1NMR (300 MHz, $CDCl_3$): δ 7.49-7.46 (m, 2H, aromatic), 7.39-7.33 (m, 3H, aromatic), 5.94-5.82 (m, 1H, =CH), 5.55 (s, 1H, CH), 5.27-5.21 (m, 1H, =C*H*H), 5.17-5.13 (m, 1H, C*H*H), 5.04-4.98 (m, 1H, H-2), 4.49 (d, 1H, J = 9.96 Hz, H-1), 4.38-4.31 (m, 2H, H-6a, C*H*H), 4.18-4.12 (m, 1H, C*H*H), 3.77 (t, 1H, J = 9.96 Hz, H-6e), 3.69-3.64 (m, 2H, H-3, H-4), 3.52-3.44 (m, 1H, H-5), 2.87-2.61 (m, 6H, 3 x CH_2), 2.20 (s, 3H, CH_3), 1.25 (t, 3H, J = 7.62 Hz,

CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.31, 171.54, 137.28, 134.91, 129.14, 128.39, 126.10, 117.10, 101.33, 84.30, 81.43, 79.80, 73.63, 71.74, 70.87, 68.72, 38.06, 30.02, 28.17, 24.16, 15.00. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{30}O_7S$ $[M+Na]_+$: 473.1610; found: 473.1587.

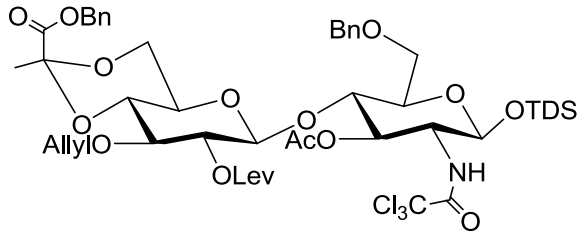


Ethyl 2-*O*-levulinyl-3-*O*-allyl-1-thio-β-D-glucopyranoside: (**54**) Compound **53** (457 mg, 1.014 mmol) was taken up in 80% HOAc(aq) (25 mL) and heated at 50°C for 16 h. The mixture was concentrated and the resulting residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (25% Acetone/Toluene) to give **54** (345 mg, 0.951 mmol, 94%) as an amorphous white solid. R_f = 0.4 (40% Acetone/Toluene). H^{1}NMR (300 MHz, $CDCl_3$): δ 5.94-5.81 (m, 1H, =CH), 5.27-5.22 (m, 1H, =C*H*H), 5.16-5.13 (m, 1H, C*H*H), 4.86 (t, 1H, J = 9.38 Hz, H-2), 4.39 (d, 1H, J = 9.96 Hz, H-1), 4.25-4.14 (m, 2H, CH_2), 3.89-3.84 (m, 1H, C-6a), 3.78-3.72 (m, 1H, H-6e), 3.61 (t, 1H, J = 9.38 Hz, H-3), 3.45-3.32 (m, 2H, H-4, H-5), 2.87-2.50 (m, 6H, 3 x CH_2), 2.16 (s, 3H, CH_3), 1.21 (t, 3H, J = 7.62 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.46, 171.59, 134.83, 117.44, 83.65, 83.49, 79.63, 73.68, 71.96, 70.23, 62.42, 37.89, 29.91, 28.7, 24.16, 14.94. HR MALDI-TOF MS: m/z: calcd for $C_{16}H_{26}O_7S$ $[M+Na]_+$: 385.1291; found: 385.1264.



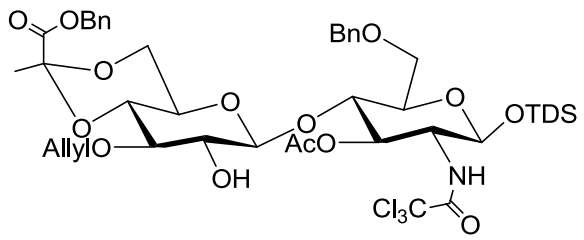
Ethyl 2-*O*-levulinyl-3-*O*-allyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α/β-D-glucopyranoside: (**55**) Compound **54** (144 mg, 0.397 mmol) and benzyl pyruvate (141 mg, 0.794 mmol) were dissolved in

dry acetonitrile (0.464 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.098 mL, 0.794 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (20% EtOAc/hexanes) to give an inseparable 1:1 (α:β) mixture of **55** (42 mg, 0.081 mmol, 20%) as an amorphous white solid. R_f = 0.5 (35% EtOAc/Hexanes). H^1NMR (600 MHz, $CDCl_3$) (α/β mixture) : δ 7.39-7.31 (m, 5H, aromatic), 5.89-5.82 (m, 1H, =CH), 5.49 (d, 1H, J = 5.87 Hz, H-1α), 5.29-5.20 (m), 5.12-5.09 (m), 4.90-4.85 (m), 4.68-4.70 (m), 4.40 (d, 1H, J = 9.98 Hz, H-1β), 4.34-4.31 (m), 4.16-4.10 (m), 4.05 (dd, J = 10.56, 4.70 Hz), 3.94 (dd, J = 5.28, 10.56 Hz), 3.72-3.66 (m), 3.55 (t, J = 9.39 Hz), 3.44-3.39 (m), 3.37-3.33 (m), 2.83-2.72 (m), 2.67-2.59 (m), 2.56-2.45 (m), 2.18 (s), 1.54 (s), 1.53 (s), 1.25-1.19 (m). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.27, 206.20, 171.82, 171.43, 169.59, 169.41, 135.24, 135.17, 135.06, 135.04, 128.77, 128.61, 128.57, 128.41, 128.33, 116.54, 116.51, 116.40, 116.37, 99.15, 99.05, 83.98, 82.86, 82.84, 79.47, 78.19, 75.76, 73.01, 72.56, 72.51, 71.20, 71.17, 70.28, 67.65, 67.62, 67.58, 67.54, 65.19, 64.98, 62.41, 38.06, 37.97, 29.96, 29.95, 28.15, 28.11, 25.47, 25.37, 24.42, 23.84, 14.94, 14.90. HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{34}O_9S$ $[M+Na]^+$: 545.1816; found: 545. 1792.

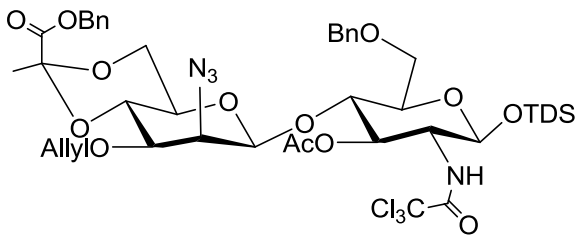


Thexyldimethylsilyl 2-*O*-levulinyl-3-*O*-allyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**56**): Compound **55** (40 mg, 0.077 mmol) and glucosyl acceptor **22** (33 mg, 0.055 mmol) were taken up in toluene (5 mL) and concentrated under vacuum (3x). The mixture was taken up in DCM (2.8 mL) along

with activated 4Å molecular sieves (100 mg) and stirred under argon for 30 min. The mixture was cooled to -20^{o}C and N-iodosuccinimide (19 mg, 0.085 mmol) was added followed by dropwise addition of triflic acid (1.0 μL, 0.011 mmol). Afterwards, the reaction was allowed to warm to 0^{o}C. After stirring for 15 min, donor was still present so additional triflic acid (2.0 μL, 0.022 mmol) was added and the reaction was allowed to stir for an additional 45 mins at 0^{o}C. The reaction mixture was quenched by the addition of pyridine (0.1 mL), filtered, and concentrated. The residue was taken up in DCM (20 mL) and washed with NaS_2O_3 (sat, aq, 15 mL) and water (15 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (10% Acetone/toluene) to give **56** (14 mg, 0.013 mmol, 24%) as an amorphous white solid. R_f = 0.5 (15% Acetone/Toluene). H^1NMR (600 MHz, $CDCl_3$): δ 7.41-7.30 (m, 10H, aromatic), 6.63 (d, 1H, J = 9.38 Hz, NHTCA), 5.86-5.73 (m, 1H, =CH-), 5.25-4.99 (m, 5H, H-3^{I}, 2xCH_2), 4.76-4.67 (m, 3H, H-1^{I}, H-2, C*H*H), 4.53 (d, 1H, J = 11.72 Hz, C*H*H), 4.43 (d, 1H, J = 7.62 Hz, H-1), 4.31-4.25 (m, 1H, C*H*H^{I}), 4.07-3.98 (m, 2H, H-6a, C*H*H), 3.94-3.82 (m, 3H, H-2^{I}, H-4, H-6a^{I}), 3.71 (m, 1H, H-6e^{I}), 3.58 (t, 1H, J = 10.55 Hz, H-6e), 3.52-3.48 (m, 1H, H-5^{I}), 3.42-3.31 (m, 2H, H-3, H-4), 3.19-3.07 (m, 1H, H-5), 2.84-2.41 (m, 4H, 2xCH_2), 1.88 (s, 3H, OAc), 1.62 (s, 1H, CH), 1.53 (s, 3H, CH_3), 0.86-0.82 (m, 12H, 4xCH_3), 0.17 (s, 3H, Si-CH_3), 0.12 (s, 3H, Si-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.21, 171.02, 170.87, 169.40, 161.85, 138.25, 135.14, 135.05, 128.81, 128.71, 128.65, 128.47, 128.06, 128.00, 116.31, 101.14, 99.06, 99.16, 92.59, 78.43, 77.31, 75.37, 75.08, 73.88, 72.78, 72.44, 72.11, 67.91, 67.71, 65.71, 65.09, 57.85, 37.85, 34.01, 27.89, 25.42, 24.90, 20.95, 20.14, 20.00, 18.68, 18.63, -1.70, -3.13. HR MALDI-TOF MS: m/z: calcd for $C_{49}H_{66}Cl_3NO_{16}Si$ $[M+Na]_+$: 1080.3039; found: 1080.2992.

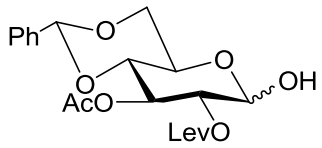


Thexyldimethylsilyl 3-*O*-allyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**57**): Compound **56** (12 mg, 0.011 mmol) was dissolved in DCM (0.78 mL) and MeOH (0.078 mL) along with hydrazine acetate (2 mg, 0.023 mmol). The reaction was allowed to stir at room temperature for 3 h. Completion of the reaction was verified by MALDI since the starting material and product had similar R_f values. The reaction mixture was concentrated under vacuum and the resulting crude was taken up in EtOAc (20 mL) and washed with water (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (35% EtOAc/Hexanes) to give **57** (11 mg, 0.011 mmol, 100%) as a white solid. R_f = 0.25 (25% EtOAc/hexanes). H^1NMR (600 MHz, $CDCl_3$): δ 7.77 (d, 1H, J = 8.80 Hz, NHTCA), 7.39-7.30 (m, 10H, aromatic), 5.83-5.77 (m, 1H, =CH-), 5.42 (t, 1H, J = 9.98 Hz, H-3^I), 5.22-5.14 (m, 3H, CH_2, C*H*H), 5.02 (d, 1H, J = 9.98 Hz, C*H*H), 4.75 (d, 1H, J = 7.63 Hz, H-1^I), 4.72 (s, 2H, CH_2), 4.36-4.33 (m, 1H, C*H*H^I), 4.17-4.12 (m, 3H, H-1, H-6a^I, C*H*H^I), 4.07-4.01 (m, 2H, H-6a, H-2^I), 3.89 (t, 1H, J = 8.80 Hz, H-2), 3.60-3.56 (m, 2H, H-6e, H-6e^I),3.50 (t, 1H, J = 9.98 Hz, H-4^I), 3.38-3.32 (m, 1H, H-3, H-4), 3.26-3.19 (m, 2H, H-5, H-5^I), 1.86 (s, 3H, OAc), 1.59-1.54 (m, 1H, CH), 1.51 (s, 3H, CH_3), 0.84-0.80 (m, 12H, 4xCH_3), 0.17 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.87, 169.38, 162.28, 137.67, 135.45, 135.12, 128.78, 128.58, 128.51, 128.08, 127.96, 127.79, 116.33, 104.81, 98.95, 95.33, 92.92, 80.54, 78.79, 77.62, 75.36, 73.70, 73.57, 73.23, 68.15, 67.26, 65.95, 65.23, 57.04, 53.91, 33.99, 25.44, 24.78, 21.08, 20.16, 19.98, 18.71, 18.61, -1.54, -3.11. HR MALDI-TOF MS: m/z: calcd for $C_{44}H_{60}Cl_3NO_{14}Si$ $[M+Na]^+$: 982.2741; found: 982.2703.

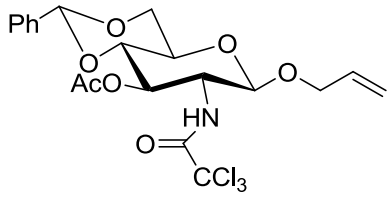


Thexyldimethylsilyl 2-azido-3-*O*-allyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**58**): Compound **57** (7 mg, 7.28 μmol) and DMAP (0.11 mg, 0.874 μmol) were dissolved in a mixture of DCM (200 μL) and pyridine (50 μL). The mixture was cooled to 0°C and trifluoromethanesulfonic anhydride (34 μL, 0.204 mmol) was added to the stirring mixture *via* dropwise addition. After stirring for 1 h at 0°C and 1 h at room temperature, TLC indicated product formation (25% EtOAc/Hexanes + 1 drop TEA, R_f = 0.25). The reaction mixture was diluted in DCM (20 mL) and transferred to a separatory funnel where it was washed with $NaHCO_3$ (sat, aq, 10mL) and brine (10 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a yellow solid. Without further purification, the residue was taken up in DMF (0.25 mL) along with sodium azide (5.6 mg, 0.088 mmol) and heated at 55°C for 18 h. The reaction mixture was concentrated under vacuum and the resulting crude was taken up in DCM (20 mL) and washed with water (2 x 20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (20% EtOAc/Hexanes) to give **58** (5 mg, 4.97 μmol, 68%) as a white solid. R_f = 0.5 (25% EtOAc/hexanes). H^1NMR (600 MHz, $CDCl_3$): δ 7.40-7.31 (m, 10H, aromatic), 6.79 (d, 1H, J = 9.39 Hz), 5.90-5.83 (m, 1H, =CH-), 5.35-5.25 (m, 2H, C*H*H, C*H*H^{I}), 5.18-5.11 (m, 3H, H-3^{I}, C*H*H, C*H*H^{I}), 4.73-4.71 (m, 2H, H-1^{I}, C*H*H''), 4.53-4.51 (m, 2H, H-1, C*H*H''), 4.31-4.28 (m, 1H, C*H*H'''), 4.09-4.06 (m, 1H, C*H*H'''), 3.99-3.95 (m, 3H, H-2^{I}, H-4^{I}, H-6a), 3.78-3.76 (m, 1H, H-6a^{I}), 3.71-3.67 (m, 3H, H-2, H-6e, H-6e^{I}), 3.60 (t, 1H, J = 9.39 Hz, H-4), 3.55-3.53 (m, 1H, H-5^{I}), 3.38-3.36 (m, 1H, H-3), 3.08-3.04 (m, 1H, H-5), 1.93 (s, 3H, OAc), 1.65-1.57 (m, 1H, CH), 1.52 (s, 3H, CH_3), 0.86-0.82 (m, 12H, 4xCH_3), 0.17 (s, 3H, Si-CH_3), 0.12 (s, 3H,

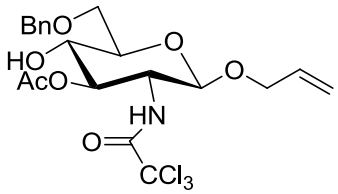
Si-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.95, 169.41, 161.98, 137.85, 135.18, 134.71, 128.80, 128.75, 128.56, 128.33, 128.25, 128.05, 117.02, 100.58, 99.42, 96.26, 92.58, 76.57, 76.32, 75.08, 74.65, 73.97, 72.41, 71.91, 68.67, 67.70, 66.99, 64.94, 63.62, 57.77, 34.02, 25.53, 24.90, 20.95, 20.14, 20.00, 18.69, 18.64, -1.74, -3.16. HR MALDI-TOF MS: m/z: calcd for $C_{44}H_{59}Cl_3N_4O_{13}Si$ $[M+Na]^+$: 1007.2811; found: 1007.2782.



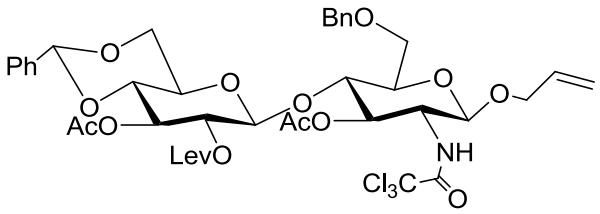
2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside: (**59**): Compound **36** (283 mg, 0.630 mmol) and 1,5-Cyclooctadiene-bis[methyldiphenylphosphine]-iridium Hexafluorophosphate (5.3 mg, 6.30 μmol) were dissolved in THF (8 mL) to give a pink solution. The solution was placed under H_2 for 5-10 seconds until the mixture turned yellow. The H_2 was flushed out with argon and the reaction was allowed to stir at room temperature until complete as indicated by NMR. The reaction mixture was concentrated and the residue was taken up in 5:1 (Acetone:Water). $HgCl_2$ (103 mg, 0.378 mmol) and HgO (82 mg, 0.378 mmol) were added to the mixture and the reaction was stirred until completion as indicated by TLC. The reaction mixture was filtered through celite and concentrated. The residue was taken up in DCM (50 mL), washed with sat KI (aq, 25 mL), dried with $MgSO_4$, filtered, and concentrated under vacuum to give an anomeric mixture of **59** (244 mg, 0.598 mmol, 95%) as an amorphous white solid. R_f = 0.2, (50% EtOAc/Hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.45-7.42 (m, 2H, aromatic), 7.37-7.32 (m, 3H, aromatic), 5.63 (t, J = 9.76 Hz), 5.52-5.49 (m), 5.41 (d, J = 3.42 Hz, H-1α), 5.37 (t, J = 9.27), 4.92-4.89 (m), 4.82 (d, H-1β), 4.37 (dd, J = 4.88, 10.74 Hz), 4.29 (dd, J = 4.88, 10.25 Hz), 4.20-4.15 (m), 3.99 (bs.s), 3.80 (t, 1H, J = 10.25 Hz), 3.74 (t, 1H, J= 10.25 Hz), 3.71-3.63 (m), 3.58-3.53 (m), 2.85-2.67 (m), 2.63-2.53 (m),2.19 (s), 2.18 (s), 2.10 (s), 2.08 (s). HR MALDI-TOF MS: m/z: calcd for $C_{20}H_{24}O_9$ $[M+Na]^+$: 431.1313; found: 431.1351.



1-O-allyl-2-trichloroacetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-Glucopyranoside: (**63**): **61** (1.76 g, 3.6 mmol) was dissolved in methanolic NaOMe (100 mL, 0.1M) and the reaction was stirred for 4 h. Afterwards, the reaction was neutralized with Dowex 50 W (H+) acidic resin, filtered, and concentrated under vacuum. The residue was coevaporated with dichloromethane and toluene and dried under high vacuum for 3 h to remove residual methanol. The residue was dissolved in acetonitrile (35 mL). Benzaldehyde dimethyl acetal (1.11 mL, 7.38 mmol) and CSA (171 mg, 0.738 mmol) were added. After stirring at room temperature for 12 h, EtOAc was added and the mixture was washed with saturated $NaHCO_3$ (aq). The organic phase was dried over MgSO4, filtered and concentrated under vacuum. The residue was passed through a small plug of silica gel using 5% EtOAc/Hexanes to remove residual benzaldehyde dimethyl acetal and 40% EtOAc/Hexanes to elute **62**. Without further purification, compound **62** was dissolved in a 1:1 mixture of pyridine and acetic anhydride (30 mL). The reaction was stirred at room temperature for 1 h and concentrated under vacuum. The residue was taken up in DCM (50 mL) and transferred to separatory funnel. The organic phase was washed with water (2 x 100 mL), dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (35% EtOAc/hexanes) to give **63** (1.39 g, 2.81 mmol, 78% over 3 steps) as an amorphous white solid. R_f = 0.7 (35% EtOAc/hexanes). H^1NMR (300 MHz, $CDCl_3$): δ 7.46-7.34 (m, 5H, aromatic), 5.86-5.73 (m, 1H, =CH-), 5.52-5.45 (m, 2H, H-3, CH), 5.24-5.13 (m, 2H, CH_2), 4.51 (d, 1H, J = 8.79 Hz, H-1), 4.24-4.10 (m, 3H, H-2, C-6a, C*H*H), 3.93-3.86 (m, 1H, C*H*H), 3.78-3.69 (m, 2H, H-4, C-6e), 3.59-3.51 (m, 1H, H-5), 2.08 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.77, 162.44, 137.14, 133.34, 129.23, 128.38, 126.06, 117.88, 101.32, 100.71, 92.73, 78.82, 71.59, 70.55, 68.55, 66.40, 56.02, 20.94. HR MALDI-TOF MS: m/z: calcd for $C_{20}H_{22}Cl_3NO_7$ $[M+Na]^+$: 516.0360; found: 516.0401.

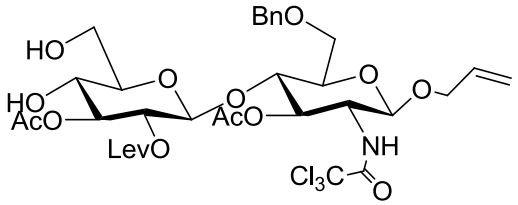


1-O-allyl-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**64**): Compound **63** (0.5g, 1.011 mmol) was taken up in DCM (13.8 mL) along with activated 4Å molecular sieves and stirred for 30 minutes under argon. The mixture was cooled to -78^{o}C and triethylsilane (0.323 mL, 2.021 mmol) was added followed by the dropwise addition of triflic acid (0.135mL, 1.516 mmol). After 15 mins, the reaction mixture was quenched by the addition of 1:1 triethylamine/MeOH (2 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (50 mL) and washed with water (50 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (30% EtOAc/hexanes) to give **64** (318 mg, 0.64 mmol, 63%) as an amorphous white solid. R_f = 0.2 (35% EtOAc/hexanes). H^{1}NMR (500 MHz, $CDCl_3$): δ 7.37-7.28 (m, 5H, aromatic), 6.83 (d, 1H, J = 8.78 Hz, NH), 5.87-5.79n(m, 1H, =C*H*-), 5.26 (d, 1H, J = 17.57 Hz, =C*H*H), 5.19-5.16 (m, 1H, H-3, =C*H*H), 4.63-4.56 (m, 3H, H-1, CH_2), 4.34 (dd, 1H, J = 4.88, 13.18 Hz, C*H*H'), 4.06 (dd, 1H, J = 5.86, 12.69 Hz, C*H*H'), 3.98 (dd, 1H, J = 8.78, 19.52 Hz, H-2), 3.84-3.75 (m, 3H, H-4, H-6), 3.59-3.55 (m, 1H, H-5), 3.09 (br.s, 1H, OH), 2.09 (s, 3H, OAc), 1.63 (br.s, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.95, 162.15, 137.54, 133.46, 128.70, 128.17, 127.94, 117.97, 99.87, 74.50, 74.06, 74.00, 70.99, 70.46, 70.17, 55.82, 21.00. HR MALDI-TOF MS: m/z: calcd for $C_{20}H_{24}Cl_3NO_7$ $[M+Na]_+$: 518.0516; found: 518.0574.

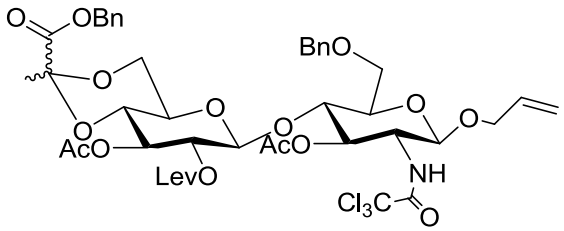


Allyl 2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**65**): To a solution of lactol

59 (135 mg, 0.331 mmol) in DCM (3 mL) was added Cs_2CO_3 (215 mg, 0.661 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.464mL, 3.31 mmol). The reaction was stirred at RT until the completion was verified by TLC. R_f = 0.75 (30% EtOAc/hexanes + 0.1% TEA). Triethylamine (100 μL) was added to the reaction mixture and the mixture was filtered through celite and concentrated. The crude was passed through a short silica gel column (5% to 25% Ea/hex + 0.1% TEA) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride and afford **60** (178 mg, 0.308 mmol, 93%) as an amorphous white solid. Donor compound **60** and acceptor **64** (198 mg, 0.4 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (3.5 mL) along with 4Å molecular sieves (400 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20°C and TfOH (6.7 μL, 0.037 mmol) was added. The mixture was allowed to warm to 0°C and stir for 1 h. The reaction was quenched by the addition of pyridine (100 μL) and diluted in DCM (20 mL). The organic layer was washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum to give a 1:1.3 (α:β) mixture of product. The residue was purified by preparatory TLC (15% Acetone/Toluene) to give the β-anomer **116** (66.7 mg, 0.075 mmol, 24%) as a white solid. R_f = 0.5 (α), 0.47 (β), (15% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 7.45-7.30 (m, 10H, aromatic), 6.82 (d, J = 9.38 Hz, NH), 5.91-5.78 (m, 1H, =CH-), 5.44 (s, 1H, CH), 5.27 (dd, 1H, J = 1.17, 17.00 Hz, =C*H*H), 5.19-5.11 (m, 3H, H-3, H-3^I, =C*H*H), 4.85 (d, 1H, J = 8.21 Hz, H-2), 4.78 (d, 1H, J = 12.31 Hz, C*H*H), 4.55-4.48 (m, 3H, H-1, H-1^I, C*H*H), 4.39-4.31 (m, 2H, C-6a, C*H*H^I), 4.12-3.96 (m, 3H, H-2^I, H-4^I, OC*H*H^I), 3.85-3.49 (m, 5H, H-4, H-5^I, H-6^{I}ae, H-6e), 3.32-3.24 (m, H-5), 2.77-2.57 (m, 2H, CH_2), 2.42 (t, 2H, J = 6.45 Hz, CH_2), 2.15 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.01 (2, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.95, 171.20, 170.76, 170.29, 162.06, 137.89, 136.83, 133.60, 129.31, 128.81, 128.39, 128.33, 128.29, 126.24, 117.73, 101.61, 100.83, 99.76, 92.57, 78.37, 75.30, 74.95, 73.85, 72.60, 72.40, 71.74, 69.93, 68.64, 67.45, 66.17, 55.75, 53.93, 37.66, 29.85, 27.77, 21.00, 20.89. HR MALDI-TOF MS: m/z: calcd for $C_{40}H_{46}Cl_3NO_{15}$ $[M+Na]^+$: 908.1825; found: 908.1869.

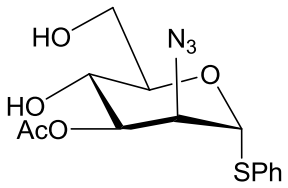


Allyl 2-*O*-levulinyl-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**66**): Compound **65** (58 mg, 0.065 mmol) was taken up in 80% HOAc(aq) (7 mL) and heated at 50°C for 16 h and then concentrated. The residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (20% Acetone/Toluene) to give **66** (41 mg, 0.051 mmol, 78%) as an amorphous white solid. R_f = 0.45 (40% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 7.43-7.31 (m, 5H aromatic), 6.88 (d, J = 8.21 Hz, NH), 5.90-5.77 (m, 1H, =CH-), 5.26 (dd, 1H, J = 1.76, 17.58 Hz, =C*H*H), 5.19-5.13 (m, 2H, H-3^{I}, =C*H*H), 4.87 (t, 1H, J = 9.38 Hz, H-3), 4.80-4.73 (m, 2H, H-2, C*H*H), 4.57-4.48 (m, 3H, H-1, H-1^{I}, C*H*H), 4.35 (dd, 1H, C*H*H^{I}), 4.10-3.94 (m, 3H, H-2^{I}, H-4^{I}, OC*H*H'), 3.91-3.66 (m, 4H, H-6ae, H-6^{I}ae), 3.63-3.53 (m, H-4, H-5^{I}), 3.30-3.24 (m, 1H, H-5), 3.01 (br.s, 1H, OH), 2.69-2.63 (m, 2H, CH_2), 2.42-2.38 (m, 2H, CH_2), 2.15 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.04 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.17, 172.00, 171.26, 162.11, 137.86, 133.52, 128.78, 128.29, 117.83, 101.16, 99.67, 92.51, 76.02, 75.60, 75.10, 74.98, 73.89, 72.40, 71.62, 70.03, 69.88, 67.68, 62.51, 55.79, 37.66, 29.86, 29.39, 27.77, 20.98, 20.89. HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{42}Cl_3NO_{15}$ $[M+Na]^+$: 820.1518; found: 820.1563.



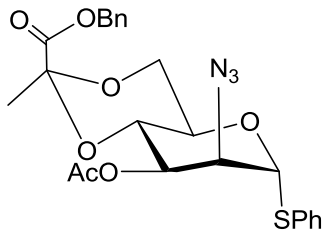
Allyl 2-*O*-levulinyl-3-*O*-acetyl-4,6-*O*-[(R/S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (**67**): Compound **66** (24 mg, 0.03 mmol) and benzyl pyruvate (10.5 mg, 0.059 mmol) were dissolved in dry acetonitrile (0.1

mL) under argon atmosphere. $BF_3(OEt)_2$ (7.3 uL, 0.059 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated undr vacuum. The residue was purified by preparatory TLC (15% Acetone/Toluene) to give an inseparable R/S mixture of **67** (8 mg, 8.44 μmol, 28%). R_f = 0.3 (15% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): Mix of R and S stereoisomers: δ 7.47-7.24 (aromatic), 6.59 (d, J = 9.27 Hz), 6.53-6.50 (m), 5.87-5.89 (m), 5.28-5.16 (m), 5.12-5.05 (m), 5.00 (t, J = 9.27 Hz), 4.80-4.76 (m), 4.72 (t, J = 4.70 Hz), 4.52-4.39 (m), 4.35 (dd, J = 4.39, 12.69 Hz), 4.08-4.02 (m), 3.99-3.92 (m), 3.86-3.81 (m), 3.78-3.67 (m), 3.56-3.48 (m), 3.35-3.28 (m), 3.17-3.12 (m), 2.7-2.69 (m), 2.65-2.59 (m), 2.41-2.37 (m), 2.16 (s), 2.03 (s), 1.98 (s), 1.95 (s), 1.89 (s), 1.88 (s), 1.62 (s), 1.51 (s). HR MALDI-TOF MS: m/z: calcd for $C_{43}H_{50}Cl_3NO_{17}$ $[M+Na]^+$: 980.2037; found: 980.2078.



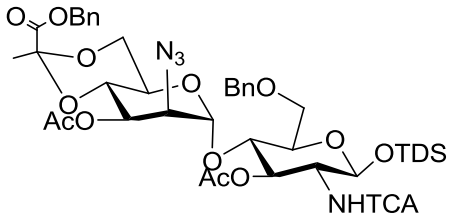
Phenyl 2-azido-3-*O*-Acetyl-2-deoxy-1-thio-α-D-mannopyranoside (**70**): To a solution of Phenyl 2-azido-2-deoxy-4,6-O-benzylidene-1-thio-α-D-mannopyranoside **68** (1g, 2.59 mmol) in pyridine (15 mL), was added acetic anhydride (15 mL). After stirring for 2 h, the reaction mixture was concentrated. The residue was taken up in toluene and concentrated under vacuum (3x) in order to remove any residual pyridine. The crude was taken up in ethyl acetate (50mL) and washed with water (50mL) and brine (50mL).The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum to yield **69** R_f = 0.6 (25% EtOAc/Hexanes): Without further purification, compound **69** was taken up in 80% HOAc (aq, 80 mL) and THF (20 mL) and heated at 50°C for 16 h. The mixture was concentrated and the resulting residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the

removal of residual acetic acid. The resulting crude was purified by silica gel column chromatography (20% Acetone/Toluene) to give **70** (800 mg, 2.36 mmol, 91%) as an amorphous white solid. R_f = 0.1 (15% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 7.50-7.48 (m, 2H, aromatic), 7.37-7.32 (m, 3H, aromatic), 5.47 (s, 1H, h-1), 5.22 (dd, 1H, *J= 4.1, 9.4* Hz, H-3), 4.31 (dd, 1H, *J* = 1.2, 4.1 Hz, H-2), 4.24-4.18 (m, 1H, H-4), 4.15-4.07 (m, 1H, H-5), 3.87 (dd, 1H, J = 4.1, 6.5, H-6), 2.20 (s, 3H, OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.05, 132.74, 132.47, 129.48, 128.44, 86.24 (C-1), 74.28 (C-3), 73.51 (C-4), 66.32 (C-5), 62.84 (C-2), 62.22 (C-6), 20.90. HR MALDI-TOF MS: m/z: calcd for $C_{14}H_{17}N_3O_5S$ $[M+Na]^+$: 362.0781; found: 362.0800.



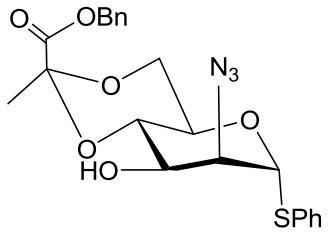
Phenyl 2-azido-3-O-acetyl-4,6-O-[(S)-1-benzyloxycarbonylethylidene]-2-deoxy-1-thio-α-D-mannopyranoside (71): Compound **70** (1.65g, 4.86 mmol) and benzyl pyruvate (1.73g, 9.72 mmol) were dissolved in dry acetonitrile (5.7 mL) under argon atmosphere. $BF_3(OEt)_2$ (1.2 mL, 9.72 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (50 mL) and washed with saturated $NaHCO_3$ (50 mL). The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/hexanes) to give **71** (1.79 g, 3.58 mmol, 74%) as an amorphous white solid. R_f = 0.35 (15% Acetone/Toluene). H^1NMR (300 MHz, $CDCl_3$): δ 7.44-7.31 (m, 10H, aromatic), (m, 3H, CH*H*Ph, H-1, H-3), 5.13 (d, 1H, J = 12.9Hz CH*H*Ph), 4.35-4.27 (m, 2H, H-2, H-5), 3.97-3.88 (m, 2H, H-4, H-6a), 3.77 (t, 1H, J = 10.5, H-6e), 2.07 (s, 3H, OAc), 1.56 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.21, 169.51, 135.28, 132.70, 132.21, 129.46, 128.81, 128.68, 128.44, 128.39, 99.78, 86.94 (C-1),

78.21 (C-4), 70.14 (C-3), 67.71, 64.86 (C-6), 64.81 (C-5), 63.47 (C-2), 25.47, 20.71. HR MALDI-TOF MS: m/z: calcd for $C_{24}H_{25}N_3O_7S$ [M+Na]+: 522.1305; found: 522.1323.

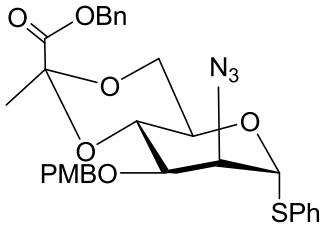


Thexyldimethylsilyl 2-azido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**72**) Compound **71** (28 mg, 0.055 mmol), 2,4,6 tri-tert-butylpyrimidine (34 mg, 0.14 mmol), and diphenylsulfoxide (15 mg, 0.072 mmol) were dissolved in toluene and concentrated under vacuum (3x). The dried mixture was taken up in DCM (1.1 mL) along with activated 4Å molecular sieves (100 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78°C and trifluoromethanesulfonic anhydride (12 uL, 0.072 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50°C in order to activate the donor. The mixture was cooled back to -78°C and compound **22** (47 mg, 0.078 mmol) was dissolved in a minimum amount of DCM and added by dropwise addition. The mixture was allowed to warm to 0°C before being quenched with TEA, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/Hexanes) to give **72** as an α product (48 mg, 0.049 mmol, 88%) as a colorless syrup. R_f = 0.7 (35% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** δ 7.43-7.26 (m, 10H, aromatic), 6.66 (d, 2H, J = 9.39 Hz, NHTCA), 5.41 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.22-5.18 (m, 2H, H-3, H-3^I), 5.11 (d, 1H, J = 12.32 Hz), 4.96 (brs, 1H, H-1^I), 4.75 (d, 1H, J = 7.63 Hz), 4.64-4.56 (m, 2H, CH_2), 4.06 (t, 1H, J = 9.39 Hz), 3.97-3.92 (m, 1H, H-2), 3.91-3.87 (m, 1H, H-2^I), 3.87-3.85 (m, 1H), 3.83-3.76 (m, 3H), 3.70-3.66 (m, 2H), 3.54-3.51 (m, 1H), 2.08 (s, 1H, OAc), 2.03 (s, 1H, OAc), 1.61 (m, 1H, CH), 1.53 (s, 3H, CH_3), 0.86-0.82 (m, 12H, 4 x CH_3), 0.18 (s, 3H, CH_3), 0.13 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.91, 169.91, 169.32, 161.70, 137.77,

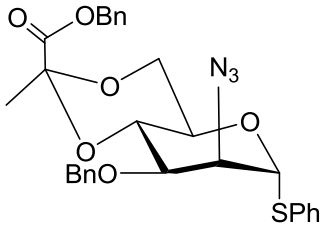
136.58, 135.10, 129.41, 128.65, 128.52, 128.42, 128.25, 127.68, 127.49, 99.57 (C-1^{I}), 99.37 (C-1), 95.88, 92.34, 74.44, 74.36 (C-3), 73.67, 71.92, 69.52 (C-3^{I}), 68.36, 67.54, 64.74, 64.38, 62.24 (C-2^{I}), 57.92 (C-2), 33.87, 25.31, 24.74, 20.76, 20.48, 19.98, 19.84, 18.53, 18.47, -1.84, -3.31. HRMS(ESI): calcd for $C_{43}H_{57}Cl_3N_4O_{14}Si$ $[M+H]^+$: 987.2779, found: 987.2822.



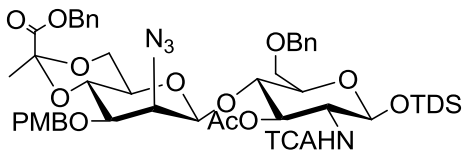
Phenyl 4,6-O-[(S)-1-methoxycarbonylethylidene]-2-azido-2-deoxy-1-thio-α-D-mannopyranoside (73): A clear solution of deacetylation reagent was prepared by dissolving guanidinium chloride (330 mg, 3.46 mmol) in MeOH (25mL) and DCM (3mL) along with 0.2M NaOMe (3.46 mL, 0.69 mmol). Compounds **71** (1.73 g, 3.46 mmol) was dissolved into the mixture and stirred at room temperature for 10 min. The mixture was neutralized by the addition of Dowex 50WX8-200 acidic resin, filtered, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (20% Ethyl Acetate/Hexanes) to give **73** (1.39 g, 3.05 mmol, 88%) as an amorphous white solid. R_f = 0.5 (25% EtOAc/Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** δ 7.44-7.30 (m, 10H, aromatic), 5.42-5.38 (m, 2H, CH*H*Ph, H-1), 5.17 (d, 1H, J = 11.72 Hz, CH*H*Ph), 4.23-4.14 (m, 3H, H-2, H-3, H-4), 3.94 (dd, 1H, J = 4.69 Hz, 10.55 Hz, H-6a), 3.76-3.64 (m, 2H, H-5, H-6e). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.54, 135.17, 133.00, 132.03, 129.43, 128.91, 128.84, 128.68, 128.30, 99.82, 86.99 (C-1), 75.37 (C-4), 69.33 (C-3), 67.85, 65.23 (C-6), 64.90 (C-5), 64.26 (C-2), 25.45. HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{23}N_3O_6S$ $[M+Na]_+$: 480.1200; found: 480.1218.



Phenyl 2-azido-3-*O*-*p*-methoxybenzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-1-thio-α-D-mannopyranoside: (**74**) Compound **73** (0.5 g, 1.1 mmol) was dissolved in DMF (9 mL) and cooled to 0°C. 60% NaH (120 mg, 3.0 mmol) was added and the mixture was stirred for 5 min. P-methoxybenzyl chloride (435 mg, 2.77 mmol) was added and the mixture was stirred at 0°C for 1.5 h. Afterwards, the reaction mixture was quenched by the dropwise addition of saturated ammonium chloride (aq) at 0°C. The reaction mixture was diluted with DCM (50 mL) and washed with water (50 mL, 3x) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (10% EtOAc/Hexanes) to give **12** (490 mg, 0.848 mmol, 78%) as a colorless syrup. R_f = 0.65 (25% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** δ 7.42-7.26 (m, 12H, aromatic), 6.90-6.86 (m, 2H, aromatic), 5.42-5.29 (m, 2H, H-1, CH*H*Ph), 5.18 (d, 1H, J = 12.31, CH*H*Ph), 4.90 (d, 1H, J = 11.72 Hz, CH*H*Ph'), 4.64 (d, 1H, J = 11.72 Hz, CH*H*Ph'), 4.24-4.16 (m, 1H, H-5), 4.09-4.07 (m, 1H, H-2), 4.01-3.91 (m, 3H, H-3, H-4, H-6a), 3.85-3.76 (m, 2H, H-6e, OMe), 1.60 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.66, 159.36, 135.22, 132.99, 131.95, 130.29, 129.45, 129.37, 128.75, 128.50, 128.26, 128.17, 114.48, 113.88, 99.56, 87.31 (H-1), 75.98 (H-4), 75.18 (H-3), 73.18, 67.65, 64.89 (C-6), 64.66 (H-5), 64.40 (H-2), 55.38, 25.65. HR MALDI-TOF MS: m/z: calcd for $C_{30}H_{31}N_3O_7S$ $[M+Na]^+$: 600.1775; found: 600.1788.

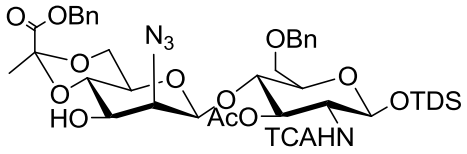


Phenyl 2-azido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-1-thio-α-D-mannopyranoside (**74a**): Compound **73** (0.25 g, 0.55 mmol) was dissolved in DMF (4.5 mL) and cooled to 0°C. 60% NaH (28 mg, 0.71 mmol) was added and the resulting mixture was stirred for 5 min. benzyl bromide (0.078 mL, 0.656 mmol) was added and the reaction mixture was stirred at 0°C for 1.5 h. Afterwards, the reaction mixture was quenched by addition of saturated ammonium chloride (aq) at 0^{o}C. The reaction mixture was diluted with DCM (50 mL) and washed with water (50 mL, 3x) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (10% EtOAc/Hexanes) to give **74a** (250 mg, 0.457 mmol, 84%) as a colorless syrup. R_f = 0.7 (25% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** δ 7.44-7.31 (m, 15H, aromatic), 5.44-5.38 (m, 2H, H-1, CH*H*Ph), 5.18 (d, 1H, J = 12.31, CH*H*Ph), 5.00 (d, 1H, J = 12.31 Hz, CH*H*Ph'), 4.74 (d, 1H, J = 12.31 Hz, CH*H*Ph'), 4.28-4.20 (m, 1H, H-5), 4.17-4.14 (m, 1H, H-2), 4.05-3.93 (m, 3H, H-3, H-4, H-6a), 3.8 (t, 1H, J = 10.6 Hz, H-6e), 1.62 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.62, 138.18, 135.19, 133.92, 132.96, 131.95, 129.37, 128.73, 128.50, 128.46, 128.26, 128.17, 127.78, 127.69, 99.56, 87.29 (H-1), 76.05 (H-4), 75.56 (H-3), 73.51, 67.67, 64.89 (C-6), 64.63 (H-5), 64.40 (H-2), 25.64. HR MALDI-TOF MS: m/z: calcd for $C_{29}H_{29}N_3O_6S$ $[M+Na]^+$: 570.1669; found: 570.1679.

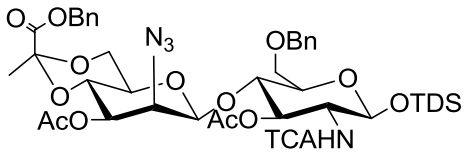


Thexyldimethylsilyl 2-azido-3-*O*-*p*-methoxybenzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**75**) Compound **74** (139 mg, 0.241 mmol), 2,4,6 tri-tert-butylpyrimidine (149 mg,

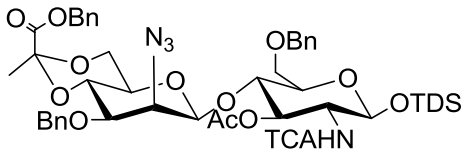
0.602 mmol), and diphenylsulfoxide (63 mg, 0.313 mmol) were dissolved in toluene and concentrated under vacuum (3x). The mixture was taken up in DCM (4.8 mL) along with activated 4Å molecular sieves (250 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78^{o}C and trifluoromethanesulfonic anhydride (53 uL, 0.313 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50^{o}C in order to activate the donor. The mixture was cooled back to -78^{o}C and compound **22** (202mg, 0.337 mmol) was dissolved in a minimum amount of DCM and added by dropwise addition. The mixture was warmed to 0^{o}C and quenched with TEA, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/Hexanes) to give **75** in 1.1/1 (β:α) ratio (183 mg, 0.172 mmol, 71%) as a colorless syrup. R_f = 0.45 (25% EtOAc/hexanes). H^{1} NMR (300 MHz, $CDCl_3$)**:** δ 7.40-7.23 (m, 12H, aromatic), 6.85 (d, 2H, J = 8.21 Hz, aromatic), 6.69 (d, 1H, J = 9.38 Hz, NHTCA), 5.32 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.19 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.12 (t, 1H, J = 9.38 Hz, H-3^{I}), 4.78 (d, 1H, J = 11.72 Hz, CH*H*Ph'), 4.70 (d, 1H, J = 7.63 Hz, H-1^{I}), 4.67 (d, 1H, J = 12.33 Hz, CH*H*Ph''), 4.57 (d, 1H, J = 11.15 Hz, CH*H*Ph'), 4.49-4.45 (m, 2H, H-1, CH*H*Ph''), 4.00-3.91 (m, 3H, H-2^{I}, H-4^{I}, H-6a), 3.79 (s, 3H, OMe), 3.74-3.61 (m, 5H,H-2, H-4, H-6e, H-6^{I}a,e), 3.51 (d, 1H, J = 8.8 Hz, H-5^{I}), 3.41 (dd, 1H, J = 3.52, 8.79 Hz, H-3), 3.09-3.00 (m, 1H, H-5), 1.92 (s, 1H, OAc), 1.63-1.56 (m, 4H, CH, CH_3), 0.86-0.82 (m, 12H, 4 x CH_3), 0.16 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.86, 169.45, 161.93, 159.40, 137.82, 135.18, 130.27, 129.43, 128.78, 128.73, 128.56, 128.35, 128.22, 128.00, 113.89, 100.51 (C-1), 99.44, 96.28 (C-1'), 92.55, 76.09 (C-4'), 75.94 (C-3), 75.20 (C-4), 74.65 (C-5'), 73.89, 72.57, 72.33 (C-3'), 68.57 (C-6'), 67.72, 67.00 (C-5), 64.97 (C-6), 63.83 (C-2), 57.78 (C-2'), 55.42, 34.01, 25.58, 24.90, 20.91, 20.13, 20.00, 18.68, 18.64, -1.73, -3.16. HRMS(ESI): calcd for $C_{49}H_{63}Cl_3N_4O_{14}Si$ $[M+H]^+$: 1065.3248, found: 1065.3266.



Thexyldimethylsilyl 2-azido-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**76**): To a solution of **75** (49 mg, 0.046 mmol) in DCM (0.7mL) and phosphate buffer (0.175 mL) was added DDQ (16mg, 0.069 mmol). The reaction was allowed to proceed at room temperature for 3 h. The reaction mixture was diluted with DCM (20 mL) and transferred to a separatory funnel. The organic layer was washed with sat $NaHCO_3$ (20 mL) followed by water (20 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (35% EtOAc/hexanes) to give **76** (36 mg, 0.038 mmol, 84%) as an amorphous white solid. R_f = 0.3 (35% EtOAc/hexanes). H^1 NMR (500 MHz, $CDCl_3$)**:** δ 7.41-7.33 (m, 10H, aromatic), 6.63 (d, 1H, J = 9.27 Hz, NHTCA), 5.36 (d, 1H, J = 12.20 Hz, CH*H*Ph), 5.15 (d, 1H, J = 11.71 Hz, CH*H*Ph), 5.10 (d, 1H, J = 10.74 Hz, H-3'), 4.73-4.70 (m, 2H, H-1^I, CH*H*Ph'), 4.54 (s, 1H, H-1), 4.49 (d, 1H, J = 11.71 Hz, CH*H*Ph'), 4.01-3.93 (m, 3H, H-2^I, H-4^I, H-6a), 3.75-3.60 (m, 4H, H-2, H-6e, H-6^Ia,e,), 3.52-3.44 (m, 2H, H-5^I, H-3), 3.26 (t, 1H, J = 9.27 Hz, H-4), 3.05-3.00 (m, 1H, H-5), 2.07 (d, 1H, J = 7.32 Hz, OH), 1.92 (s, 3H, OAc), 1.63-1.58 (m, 1H, CH), 1.54 (s, 3H, CH_3) 0.86-0.82 (m, 12H, 4 x CH_3), 0.18 (s, 3H, CH_3), 0.12 (s, 3H, CH_3).). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.81,169.27,161.92, 137.67, 135.25, 128.90, 128.82, 128.78, 128.39, 128.23, 100.74 (C-1), 99.66, 96.31 (C-1^I), 92.56, 76.17 (C-4^I), 74.63 (C-4, C-5^I), 74.00, 72.29 (C-3^I), 69.91 (C-3), 68.51 (C-6^I), 67.70, 66.66 (C-5), 64.89 (C-6), 64.72 (C-2), 57.82 (C-2^I), 34.05, 29.85, 25.33, 20.92, 20.14, 20.02, 18.69, 18.85, -1.70, -3.13. HRMS(ESI): calcd for $C_{41}H_{55}Cl_3N_4O_{13}Si$ $[M+H]^+$: 945.2673, found: 95.2687.

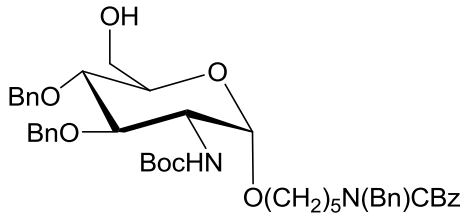


Thexyldimethylsilyl 2-azido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**77**): Compound **76** (36 mg, 0.038 mmol) was dissolved in a 1:1 mixture of pyridine and acetic anhydride (1mL) and the mixture was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated and the residue was taken up in DCM (20 mL) and transferred to separatory funnel. The organic layer was washed with water (20 mL), dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (30% EtOAc/hexanes) to give **77** (38 mg, 0.038 mmol) as an amorphous white solid. R_f = 0.7 (35% EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** δ 7.41-7.32 (m, 10H, aromatic), 6.63 (d, 1H, J = 9.39 Hz, NHTCA),5.34 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.14 (d, 1H, J = 11.74 Hz, CH*H*Ph), 5.12-5.10 (m, 1H, H-3^I), 4.79 (dd, 1H, J = 3.52, 9.39 Hz, H-3), 4.72-4.70 (m, 2H, H-1^I, CH*H*Ph'), 4.62 (s, 1H, H-1), 4.53 (d, 1H, J = 12.33 Hz, CH*H*Ph'), 4.03-3.93 (m, 3H, H-2^I, H-4^I, H-6a), 3.76-3.59 (m, 4H, H-4, H-6e, H-6^{I}a,e,), 3.50 (d, 2H, J = 9.39 Hz H-5^I), 3.13-3.09 (m, 1H, H-5), 2.06 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.63-1.58 (m, 1H, CH), 1.51 (s, 3H, CH_3), 0.86-0.82 (m, 12H, 4 x CH_3), 0.17 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.83, 170.40, 169.25, 161.89, 137.70, 135.21, 128.79, 128.78, 128.67, 128.43, 128.26, 128.09, 99.97 (C-1), 99.61, 96.29 (C-1^I), 92.54, 75.74 (C-4^I), 74.70 (C-5^I), 73.93, 72.22 (C-3^I), 71.71 (C-4), 70.82 (C-3), 68.39 (C-6^I), 67.69, 66.96 (C-5), 64.89 (C-6), 62.49 (C-2), 57.81 (C-2^I), 34.02, 25.36, 24.91, 20.90, 20.88, 20.13, 20.00, 18.68, 18.63, -1.71, -3.15. HRMS(ESI): calcd for $C_{43}H_{57}Cl_3N_4O_{14}Si$ $[M+H]^+$: 987.2779, found: 987.2798.



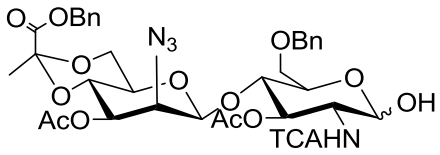
Thexyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside: (**77a**): Compound **74a** (180 mg, 0.329 mmol), 2,4,6 tri-tert-butylpyrimidine (204 mg, 0.822 mmol), and diphenylsulfoxide (86 mg, 0.427 mmol) were dissolved in toluene and concentrated under vacuum (3x). The mixture was taken up in DCM (6.5 mL) along with activated 4Å molecular sieves (250 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78^{o}C and trifluoromethanesulfonic anhydride (72 uL, 0.427 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50^{o}C in order to activate the donor. The mixture was cooled back to -78^{o}C and compound **22** (276mg, 0.460 mmol) was dissolved in a minimum amount of DCM and added by dropwise addition. The mixture was warmed to 0^{o}C and quenched with TEA, filtered, and concentrated. The residue was taken up in DCM (20 mL) and washed with water (20 mL), dried over MgSO4, filtered, and concentrated under vacuum to give **77a** as a separable 1.1/1 (β:α) mixture. The residue was purified by silica gel column chromatography (15% -20% EtOAc/Hexanes) to give **77a** (Beta, 133 mg, 0.127 mmol, 39%) as a colorless syrup and (alpha, 120 mg, 117 mmol, 35%). R_f = 0.45 (25% EtOAc/hexanes). H^{1} NMR (300 MHz, $CDCl_3$)**:** δ 7.40-7.26 (m, 15H, aromatic), 6.67 (d, 1H, J = 9.38 Hz, NHTCA), 5.32 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.19 (d, 1H, J = 12.31 Hz, CH*H*Ph),5.11 (t, 1H, J = 9.38 Hz, H-3^{I}), 4.85 (d, 1H, J = 12.31 Hz, CH*H*Ph'), 4.72-4.61 (m, 2H, H-1^{I}, CH*H*Ph', CH*H*Ph''), 4.49-4.45 (m, 2H, H-1, CH*H*Ph''), 3.99-3.90 (m, 3H, H-2^{I}, H-4^{I}, H-6a), 3.74-3.64 (m, 5H,H-2, H-4, H-6e, H-6^{I}a,e), 3.51 (d, 1H, J = 9.38 Hz, H-5^{I}), 3.41 (dd, 1H, J = 3.52, 8.79 Hz, H-3), 3.08-3.00 (m, 1H, H-5), 1.93 (s, 1H, OAc), 1.65-1.55 (m, 4H, CH, CH_3), 0.86-0.82 (m, 12H, 4 x CH_3), 0.17 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.87, 169.46, 161.94, 159.38, 138.27, 137.83, 135.19, 128.78, 128.59, 128.53, 128.39, 128.26, 128.04, 127.86, 127.75, 100.45

(C-1), 99.47, 96.33 (C-1^{I}), 92.57, 76.38 (C-3), 76.02 (C-4^{I}), 75.18 (C-4), 74.67 (C-5^{I}), 73.92, 72.90, 72.32 (C-3^{I}), 68.60 (C-6^{I}), 67.76, 67.00 (C-5), 64.98 (C-6), 63.82 (C-2), 57.82 (C-2^{I}), 34.05, 25.57, 24.93, 20.90, 20.14, 20.02, 18.69, 18.65, -1.71, -3.13. HRMS(ESI): calcd for $C_{48}H_{61}Cl_3N_4O_{13}Si$ $[M+H]^+$: 1035.3143, found: 1035.3159.



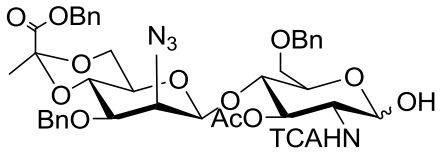
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-t-Butoxycaronylamino-3,4-*O*-benzyl-2-deoxy-*α*-D-glucopyranoside (78): To a stirring solution of **23** (730 mg, 1.05 mmol) in acetic acid (25 mL) was added a large excess of Zn-Cu couple (1.5 g). The mixture was allowed to stir for 1hr. Completion of the reaction was verified by MALDI. The reaction mixture was filtered and concentrated under vacuum. Afterwards, the crude was coevaporated with toluene (3x) and dried under high vacuum. The residue was dissolved in THF (45 mL) with Boc_2O (0.459g, 2.101 mmol) and triethylamine (0.295 mL, 2.101 mmol). The mixture was stirred at room temperature overnight and concentrated under vacuum. The residue was taken up in EtOAc (30 mL) and washed with water (30 mL). The organic phase was separated and dried over MgSO4, filtered, and concentrated under vacuum. The crude was chromatographed with 40%EtOAc/Hexanes to afford **78** (580 mg, 0.754 mol, 72 %) as a colorless solid. R_f = 0.25 (50% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** δ 7.36-7.15 (m, 20H, aromatic), 5.22-5.11 (m, 2H, C*H*$_2$Ph), 4.88-4.56 (m, 5H, H-1, 2 x C*H*$_2$Ph), 3.97-3.87 (m, 1H,H-2), 3.84-3.48 (m, 6H, H-3, H-4, H-5, H-6, OC*H*H), 3.39-3.10 (m, 3H, OC*H*H, NC*H*2), 1.63-1.38 (m, 13H, 2 x CH_2, 3 x CH_3), 1.38-1.10 (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.86, 156.38, 138.54, 138.13, 138.01, 136.89, 128.68, 128.64, 128.59, 128.44, 128.27, 128.12, 128.03, 127.98, 127.84, 127.72, 127.45, 127.34, 98.24 (C-1), 81.14 (C-3), 79.79

78.37 (C-4), 75.42, 75.31, 75.22, 71.53 (C-5), 67.87, 67.35, 62.10 (C-6), 54.53 (C-2), 50.65, 50.44, 47.19, 46.21, 29.18, 28.54, 27.99, 27.50, 23.51. HRMS(ESI): calcd for $C_{45}H_{56}N_2O_9$ $[M+Na]^+$: 791.3878, found: 791.3890.



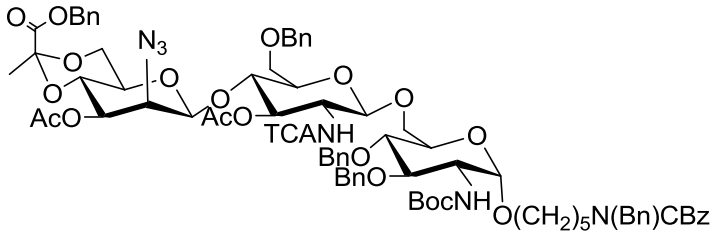
2-azido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α/β-D-Glucopyranose: (**79**): Compound **77** (48 mg, 0.049 mmol) was dissolved in THF (2.2mL) in a screw-cap plastic vial and cooled to 0°C. HF•Py (70:30, 0.4 mL) was added by dropwise addition and the reaction was allowed to warm to room temperature and stir for 12 h. After completion of the reaction, the mixture was cooled to 0°C and quenched *via* dropwise addition of aqueous $NaHCO_3$ (sat). The mixture was diluted in EtOAc (20 mL) and transferred to separatory funnel. The organic phase was washed with aqueous $NaHCO_3$ (sat, 20 mL), water (20 mL), dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (50% EtOAc/hexanes) to give an anomeric mixture (~9:1, α:β) of **79** (37 mg, 0.043 mmol, 90%) as an amorphous white solid. R_f = 0.25 (α), 0.2 (β) (35% EtOAc/hexanes). alpha anomer, H^1 NMR (600 MHz, $CDCl_3$)**:** δ 7.42-7.35 (m, 10H, aromatic), 7.00 (d, 1H, J = 8.80 Hz, NHTCA), 5.35-5.33 (m, 3H, CH*H*Ph, H-1^I, H-3^I), 5.14 (d, J = 11.74 Hz, CH*H*Ph), 4.73-4.71 (m, 2H, CH*H*Ph', H-3), 4.48-4.44 (m, 2H, CH*H*Ph', H-1), 4.15 (t, 1H, J = 9.98 Hz, H-2^I), 4.07 (d, 1H, J = 9.98 Hz, H-5^I), 4.00-3.97 (m, 2H, H-4^I, H-6a), 3.74-3.58 (m, 5H, H-2, H-4^I, H-4, H-6e, H-6^{I}ae), 3.06-3.03 (m, 1H, H-5), 2.06 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.51 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 211.07, 170.93, 170.37, 169.26, 162.16, 137.46, 135.18, 128.94, 128.91, 128.77, 128.67, 128.58, 128.51, 128.49, 128.43, 128.38, 99.70 (C-1), 99.60, 92.22, 91.16 (C-1^I), 75.46 (C-4^I), 73.83, 71.73 (C-4), 70.79 (C-3), 70.68 (C-3^I), 69.93 (C-5^I), 67.97 (C-6^I),

67.71, 66.89 (C-5), 64.85 (C-6), 62.46 (C-2), 54.27 (C-2^{I}), 25.36, 20.97, 20.86. HRMS(ESI): calcd for $C_{35}H_{39}Cl_3N_4O_{14}$ $[M+H]^+$: 845.1601, found: 845.1618.



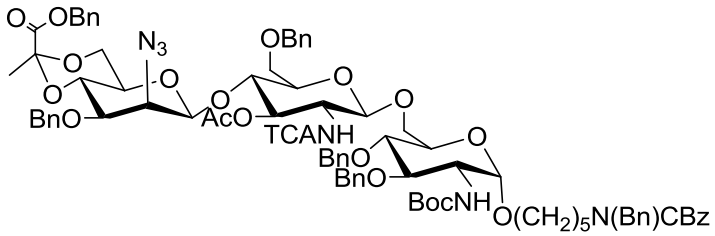
2-azido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α/β-D-Glucopyranose: (**79a**): Compound **77a** (93 mg, 0.090 mmol) was dissolved in THF (3.6 mL) in a screw-cap plastic vial and cooled to 0^{o}C. HF•Py (70:30; 0.821 mL, 9.11 mmol) was added by dropwise addition and the reaction was allowed to warm to room temperature and stir for 12 h. After completion of the reaction, the mixture was cooled to 0^{o}C and quenched *via* dropwise addition of aqueous $NaHCO_3$ (sat). The mixture was diluted in ethyl acetate (20 mL) and transferred to separatory funnel and washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic phase was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (50% EtOAc/hexanes) to give an anomeric mixture (~9:1, α:β) of **77a** (71 mg, 0.079 mmol, 88%) as an amorphous white solid. R_f = 0.25 (α), 0.2 (β) (35% EtOAc/Hexanes). alpha anomer, H^1 NMR (600 MHz, $CDCl_3$)**:** δ 7.38-7.23 (m, 15H, aromatic), 7.00 (d, 1H, J = 9.39 Hz), 5.37-5.31 (m, 3H, H-1^{I}, H-3^{I}, CH*H*Ph), 5.19 (d, 1H, J = 12.33Hz, CH*H*Ph), 4.86 (d, 1H, J = 12.33 Hz, CH*H*Ph'), 4.70 (d, 1H, J = 11.74 Hz, CH*H*Ph''), 4.63 (d, 1H, J = 11.74 Hz, CH*H*Ph'), 4.39 (d, 1H, J = 11.74 Hz, , CH*H*Ph''), 4.35 (s, 1H, H-1), 4.15 (t, 1H, J = 10.56, H-2^{I}), 4.08 (d, 1H, J = 9.98 Hz, H-5^{I}), 3.99-3.93 (m, 2H, H-4^{I}, H-6a), 3.72-3.61 (m, 4H, H-4, H-6e, H6^{I}ae), 3.54-3.51 (m, 1H, H-2), 3.35 (dd, 1H, J = 3.52, 9.39 Hz, H-3), 3.10 (s, 1H, OH), 3.02-2.97 (m, 1H, H-5), 1.93 (s, 3H, OAc), 1.56 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 170.94, 169.45, 164.52, 162.20, 138.26, 137.55, 135.14, 128.84, 128.81, 128.78, 128.59, 128.54, 128.51, 128.49, 128.46, 128.39, 128.35, 127.90, 127.86, 127.69, 100.12 (C-1), 99.46, 91.24 (C-1^{I}), 76.34 (H-3), 75.68 (C-4^{I}), 75.21 (C-4), 73.83, 72.84, 70.62 (C-3^{I}), 70.00 (C-5^{I}), 68.08 (C-6^{I}), 67.78, 66.92 (C-5),

64.97 (C-6), 63.72 (C-2), 54.33 (C-2^{I}), 25.59, 20.98. HRMS(ESI): calcd for $C_{40}H_{43}Cl_3N_4O_{13}$ $[M+H]^+$: 893.1965, found: 893.1979.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside: (**81**): To a solution of **79** (36 mg, 0.042 mmol) in DCM (2.4 mL) was added Cs_2CO_3 (14mg, 0.042 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.069mL, 0.423 mmol). The reaction was stirred at RT until complete as verified by TLC. R_f = 0.6 (35% EtOAc/hexanes + 0.1% TEA). Triethylamine (100 μL) was added to the reaction mixture and the mixture was filtered through celite and concentrated. The crude was passed through a short silica gel column (5% to 25% Ea/hex + 0.1% TEA) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride and afford **80** (78%, 0.033g, 0.033 mmol) as an amorphous white solid. Donor compound **80** (0.033g, 0.033 mmol) and acceptor **78** (0.050g, 0.066 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (1.6 mL) along with 4Å molecular sieves (100 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20^{o}C and TMSOTf (1.37 μL, 7.55 μmol) was added. The mixture was allowed to warm to 0^{o}C and stir for 1 h. The reaction was quenched by the addition of TEA (100 μL) and diluted in DCM (20 mL). The organic layer was washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% EtOAc/hexanes) to give **81** (35 mg, 0.022 mmol, 67%) as an amorphous white solid. R_f = 0.5 (40%

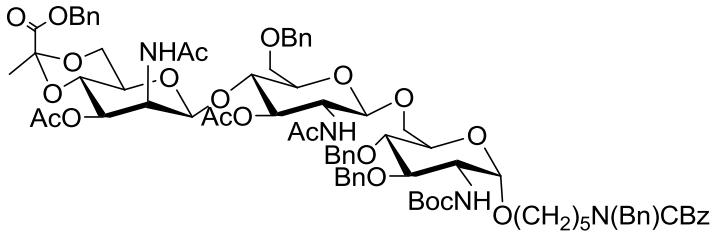
EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** δ 7.40-7.18 (m, 30H, aromatic), 6.67 (d,1H, 100.36 Hz, NHTCA), 5.34 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.20-5.12 (m, 4H, H-3^I, CH*H*Ph, CH_2Ph'), 4.8-4.35 (14H, H-1, H-1^I, H-1^{II}, H-3, 5 x CH_2Ph''), 4.05 (d, 1H, J = 10.56, C6a^I), 4.00-3.92 (m, 3H, H-2^I, H-4^I, C6a), 3.90-3.86 (m, 1H, H-2^{II}), 3.84-3.79 (m, 1H, H-2), 3.79-3.49 (m, 8H, H-3^{II}, H-4, H-4^{II},H-6e, H-6^Ie, H-6^{II}ae, Linker-OCH_2), 3.49-3.37 (m, 2H, H-5^I, H-5^{II}), 3.33-3.10 (m, 2H, Linker-NCH_2), 3.09-3.00 (m, 1H, H-5), 2.05 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.62-1.41 (m, 16H, 4xCH_3, 2xCH_2), 1.32-1.19 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 210.83, 170.43, 170.18, 169.10, 161.71, 156.71, 156.16, 155.21, 138.43, 138.17, 137.89, 137.45, 136.90, 136.71, 135.05, 128.66, 128.60, 128.52, 128.44, 128.26, 128.23, 128.17, 128.09, 128.01, 127.77, 127.48, 127.25, 127.19, 100.98-100.79 (C-1^I), 99.66-99.42 (C-1), 97.88-97.67 (C-1^{II}), 92.33, 81.31 (C-4^{II}), 79.52, 78.24 (C-5^{II}), 75.62 (C-4^I), 75.00, 74.75, 74.52 (C-5^I), 73.70, 71.89 (C-3^I), 71.53 (C-4), 70.62 (C-3), 70.07 (C-3^{II}), 68.48-68.36 (C-6^{II}), 68.05 (C-6^{II}), 67.89, 67.53, 67.15, 66.72 (C-5), 64.67 (C-6), 62.27 (C-2), 55.47 (C-2^I), 54.23 (C-2^{II}), 50.50, 50.21, 47.12, 46.09, 29.68, 29.05, 28.38, 27.94, 27.39, 25.20, 23.52, 23.33, 20.71. HRMS(ESI): calcd for $C_{80}H_{93}Cl_3N_6O_{22}$ $[M+H]^+$: 1595.5481, found: 1595.5498.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-benzy-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-trichloroacetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside: (**81a**) To a solution of **79a** (74 mg, 0.082 mmol) in DCM (4.8 mL) was added Cs_2CO_3 (26 mg, 0.082 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.134 mL, 0.824 mmol). The reaction was stirred at RT until the reaction was complete as verified by TLC. R_f = 0.75 (35% EtOAc/hexanes + 0.1% TEA). The imidate was stabilized by addition of triethylamine (100 μL) and the mixture was filtered

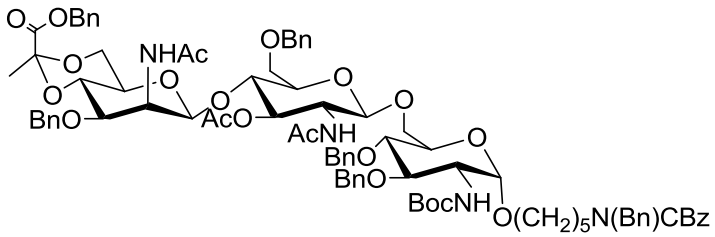
through celite and concentrated. The crude was passed through a short silica gel column (5% to 25% Ea/hex + 0.1% TEA) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl chloride and afford **80a** (0.072 g, 0.067 mmol, 82%) as an amorphous white solid. Donor compound **80a** (0.072g, 0.067 mmol) and acceptor **78** (0.103g, 0.134 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (3.2 mL) along with 4Å molecular sieves (210 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20^{o}C and TMSOTf (2.79 μL, 0.015 mmol) was added. The mixture was allowed to warm to 0^{o}C and stir for 1 h. The reaction was quenched by the addition of TEA (100 μL) and diluted in DCM (20 mL). The organic phase was washed with aqueous $NaHCO_3$ (sat, 20 mL), water (20 mL), dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% EtOAc/hexanes) to give **81a** (61 mg, 0.037 mmol, 56%) as an amorphous white solid. R_f = 0.7 (35% EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** δ 7.38-7.17 (m, 35H, aromatic), 6.73 (d, 1H, J = 85.10 Hz, NHTCA), 5.32 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.19-5.16 (m, 4H, H-3^{I}, CH*H*Ph, C*H*$_2$Ph'), 4.84 (d, 1H, J = 11.74 z, CH*H*Ph''), 4.82-4.70 (m, 3H, CH*H*Ph''', C*H*$_2$Ph^{IV}), 4.69-4.59 (m, 3H, H-1^{II}, CH*H*Ph^{II}, CH*H*Ph^{V}), 4.59-4.47 (m, 3H, CH*H*Ph''', C*H*$_2$Ph^{VI}), 4.44-4.35 (m, 3H, H-1, H-1^{I}, CH*H*Ph^{V}), 4.05 (d, 1H, J = 9.98 Hz, H-6^{I}a), 4.03-3.84 (m, 4H, H-2^{I}, H-2^{II}, H-4^{I}, H-6a), 3.75-3.43 (m, 11H, H-2, H-3^{II}, H-4, H-4^{II}, H-5^{I}, H-5^{II}, H-6e, H-6^{I}a, H-6^{II}ae, NCH_2), 3.36 (dd, 1H, J = 3.52, 8.80 Hz, H-3), 3.30-3.17 (m, 2H, OCH_2), 3.02-2.98 (m, 1H, H-5), 1.91 (s, 3H, OAc), 1.64-1.49 (m, 7H, 2xCH_2, CH_3), 1.41 (s, 9H, 3xCH_3), 1.30-1.21 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 214.01, 170.64, 169.43, 161.89, 156.88, 156.33, 155.38, 138.58, 138.34, 138.23, 138.06, 137.72, 137.05, 136.88, 135.14, 128.75, 128.74, 128.68, 128.57, 128.49, 128.37, 128.28, 128.17, 128.08, 127.92, 127.83, 127.68, 127.40, 127.35, 101.16-100.98 (C-1), 100.24 (C-1^{I}), 99.42, 98.05-97.81 (C-1^{II}), 92.49, 81.44 (C-5^{II}), 79.66, 78.39 (C-5^{I}), 76.32, 75.96 (C-3), 75.90 (C-4^{I}), 75.15, 74.92 (C-3^{II}), 74.63 (C-4^{II}), 73.83, 72.82, 72.10 (C-3^{I}), 70.25 (C-3^{II}), 68.68-68.51 (C-6^{I}), 68.38 (C-6^{II}), 68.08, 67.74, 67.60, 67.31, 66.90 (C-5), 64.93 (C-6), 63.69 (C-2), 55.62 (C-

2^{I}), 54.38 (C-2^{II}), 50.65, 50.36, 47.28, 46.25, 29.20, 28.54, 28.09, 27.56, 25.57, 23.67, 23.48, 20.88. HRMS(ESI): calcd for $C_{85}H_{97}Cl_3N_6O_{21}$ $[M+H]^+$: 1643.5845, found: 1643.5863.



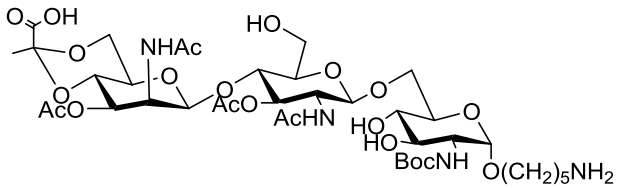
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside: (**82**) To a solution of **81** (88 mg, 0.055 mmol) in toluene (3 mL) were added tributylstannane (0.148 mL, 0.554 mmol) and 2,2'-azobisisobutyronitrile (12 mg, 0.073 mmol). After stirring at RT for 45 min, the reaction mixture was heated to 80°C and stirred for an additional 3.5 h. The reaction was then cooled to RT and concentrated. A 1:1 mixture of Ac_2O and Pyridine (2mL) was added to the crude amine and the mixture was allowed to 12 hr. The reaction mixture was concentrated and purified by preparatory-TLC (5% MeOH/DCM). Compound **82** (80 mg, 0.053 mmol, 96%) was obtained as an amorphous white solid. R_f = 0.45 (5% MeOH/DCM). H^1 NMR (600 MHz, $CDCl_3$)**:** 7.40-7.16 (m, 30H, aromatic), 5.66-5.46 (m, 2H, 2xNH), 5.39 (d, 1H, J = 12.33 Hz, CH*H*Ph 5.19-5.12 (m, 3H, H-3^{I}, CH*H*Ph, C*H*$_2$Ph'), 4.89-4.39 (m, 13H, H-1, H-1^{I}, H-1^{II}, H-2, H-3, 4x C*H*$_2$Ph), 4.05 (d, 1H, J = 9.39 Hz, H-6^{II}a), 3.97 (dd, 1H, J = 4.70, 9.98 Hz, H-6a), 3.92-3.87 (m, 2H, H-2^{I}, H-4^{I}), 3.76-3.47 (m, 10H, H-2^{II}, H-3^{II}, H-4^{II}, H-5^{II}, H-6e, H-6^{II}e, H-6^{I}ae, OCH_2), 3.46-3.15 (m, 4H, H-4, H-5^{I}, NCH_2), 3.04-3.00 (m, 1H, H-5), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.96 (s, 3H, NHAc), 1.88 (s, 3H, NHAc), 1.64-1.45 (m, 7H, 2xCH_2, CH_3), 1.42 (s, 9H, 3xCH_3), 1.36-1.19 (m, 2H, 1xCH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 175.19, 171.77, 171.28, 170.72, 170.17, 169.36, 156.86, 156.36, 155.44, 138.50, 138.21, 137.98, 137.80, 137.00, 136.79, 135.37, 128.79, 128.78, 128.68, 128.62, 128.42, 128.29, 128.19, 128.09, 128.02, 127.96, 127.72, 127.43, 127.33, 101.51 (C-1), 99.40, 98.58 (C-1^{I}),

98.14-98.04 (C-1^{II}), 81.38 (C-5^{II}), 79.70, 78.41 (C-4^{II}), 75.51 (C-4^{I}), 75.36, 74.87, 74.35 (C-5^{I}), 73.58, 73.26 (C-3^{I}), 72.28 (C-4), 70.88 (C-3), 70.36 (C-3^{II}), 67.86 (C-6^{I}), 67.75 (C-6^{II}), 67.40, 67.35, 66.80 (C-5), 64.80 (C-6), 54.58 (C-2^{II}), 54.37 (C-2^{I}), 53.90, 51.39 (C-2),50.66, 50.40, 47.26, 46.25, 29.19, 28.55, 28.07, 27.97, 27.51, 27.19, 25.45, 23.64, 23.49, 23.33, 21.51, 21.18. HRMS(ESI): calcd for $C_{82}H_{100}N_4O_{23}$ $[M+H]^+$: 1509.6851, found: 1509.6869.



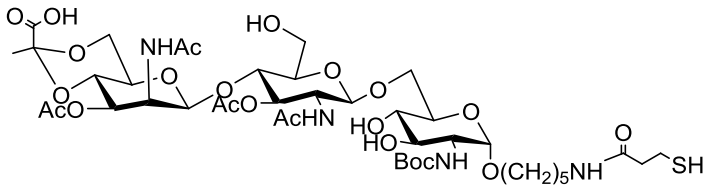
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-acetamido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside: (**82a**): To a solution of **81a** (43 mg, 0.026 mmol) in toluene (1.4 mL) were added tributylstannane (0.07 mL, 0.264 mmol) and 2,2'-azobisisobutyronitrile (6 mg, 0.037 mmol). After stirring at RT for 45 min, the reaction mixture was heated to 80 °C and stirred for an additional 3.5 h. The reaction was then cooled to RT and concentrated. A 1:1 mixture of Ac_2O and Pyridine (2mL) was added to the crude amine and the mixture was allowed to 12 hr. The reaction mixture was concentrated and purified by preparatory-TLC (5% MeOH/DCM). Compound **26** (31 mg, 0.02 mmol, 75%) was obtained as an amorphous white solid. R_f = 0.45 (5% MeOH/DCM). H^1 NMR (500 MHz, $CDCl_3$)**:** 7.37-7.15 (m, 35H, aromatic), 5.64-5.48 (m, 1H, NH), 5.34 (d, 1H, J = 12.69 Hz, CH*H*Ph), 5.23-5.03 (m, 4H, H-3^{I}, CH*H*Ph, C*H*$_2$Ph'), 4.84-4.30 (m, 14H, H-1, H-1^{I}, H-1^{II}, H-2, 5xC*H*$_2$Ph), 4.04 (d, 1H, J = 9.76 Hz, H-6^{II}a), 4.00-3.00 (m, 19H, H-2^{I}, H-2^{II},H-3, H-3^{II}, H-4, H-4^{I}, H-4^{II}, H-5, H-5^{I}, H-5^{II}, H-6ae, H-6^{I}ae, H-6^{II}e, OCH_2, NCH_2), 1.92 (s, 3H, OAc), 1.87 (s, 3H, NHAc), 1.82 (NHAc), 1.72-1.45 (m, 7H, 2xCH_2, CH_3), 1.42 (s, 9H, 3xCH_3), 1.38-1.17 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 171.11, 170.92, 170.02, 169.53, 156.87, 156.35, 155.41, 155.38,

138.54, 138.26, 138.54, 138.26, 138.01, 137.97, 136.83, 135.40, 128.72, 128.69, 128.65, 128.46, 128.42, 128.35, 128.12, 128.09, 128.02, 127.96, 127.70, 127.55, 127.43, 127.34, 100.98 (C-1), 100.09 (C-1^{I}), 99.23, 98.12 (C-1^{II}), 81.44 (C-5^{II}), 79.67, 78.36-78.28 (C-5^{I}), 76.06 (C-4, C-4^{I}), 75.51, 75.34, 75.22, 74.84 (C-3), 74.60 (C-4^{II}), 73.66, 73.59 (C-3^{I}), 71.55, 70.38 (C-3^{II}), 68.05 (C-6^{I}), 67.78 (C-6^{II}), 67.34, 66.18 (C-3), 66.11 (C-5), 65.31 (C-6), 54.36 (C-2^{II}), 53.92 (C-2^{I}), 50.66, 50.40, 47.26, 46.26, 29.21, 28.55, 27.99, 27.57, 25.61, 23.67, 23.45, 23.36, 21.30. HRMS(ESI): calcd for $C_{87}H_{104}N_4O_{22}$ $[M+H]^+$: 1557.7215, found: 1557.7234.



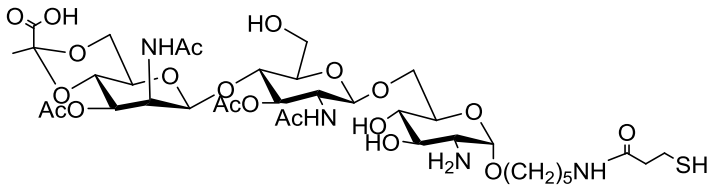
5-aminopentyl 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-2-deoxy-Glucopyanoside: (**83**): Compound **82** (9 mg, 5.96 μmol) was dissolved in a mixture of t-BuOH (1.8 mL) and H_2O (0.045 mL). $Pd(OH)_2$/C (18 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **83** (3.7 mg, 4 μmol, 67%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.08 (t, 1H, J = 8.80 Hz, H-3^1), 5.02-4.98 (m, 2H, H-1, H-3), 4.82 (br.s, 1H, H-1^{II}), 4.69-4.68 (m, 1H, H-2), 4.65 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.19 (d, 1H, J = 10.56 Hz, H-6^{II}a), 4.03 (dd, 1H, J = 5.28, 11.15 Hz, H-6a), 3.94 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.89 (t, 1H, J = 9.98 Hz, H-2^{I}), 3.86-3.81 (m, 2H, H-4, H-$6e^{I}$), 3.80-3.69 (m, 4H, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$), 3.69-3.53 (m, 4H, H-2^{II},H-3^{II},H- 5^{I}, OC*H*H), 3.53-3.43 (m, 2H, H-5,

OC*H*H), 3.40 (t, 1H, J = 9.39 Hz, H-4^{II}), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, NHAc), 1.97 (s, 3H, NHAc), 1.71-1.58 (m, 4H, 2xCH_2), 1.48-1.40 (m, 14H, 4xCH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 174.97, 174.05, 173.11, 173.10, 157.74, 101.81, 100.75 (C-1^{I}), 99.15 (C-1), 97.04 (C-1^{II}), 81.16, 76.07 (C-4^{I}), 74.25 (C-5^{I}), 73.66 (C-3^{I}), 71.28 (C-3^{II}), 71.14 (C-4), 70.84 (C-3), 70.46 (C-5^{II}), 69.95 (C-4^{II}), 68.86 (C-6^{II}), 67.53, 66.59 (C-5), 64.09 (C-6), 59.75 (C-6^{I}), 54.69 (C-2^{II}), 53.56 (C-2^{I}), 50.74 (C-2), 39.30, 27.85, 27.50, 26.37, 24.34, 22.30, 21.86, 21.63, 20.41, 20.21. HRMS(ESI): calcd for $C_{39}H_{64}N_4O_{21}$ $[M+H]^+$: 925.4136, found: 925.4123.



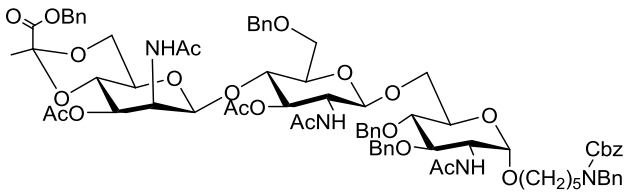
3-Mercapto-N-pentyl-propionamido 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-2-deoxy-Glucopyanoside: (**84**): To a stirring mixture of **83** (1mg, 1.08 μmol) in water (0.2 mL) was added DTSSP (1mg, 1.62 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (4.2 mg, 0.027 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a C18 silica gel column and purified using a solvent gradient of 100% H_2O, 5%, 10%, 20%, 40%, 60%, and 80% MeOH/H_2O. The fractions containing the product were concentrated under vacuum to yield **84** (800 μg, 0.8 μmol, 73%). H^1 NMR (600 MHz, D_2O)**:** 5.08 (t, 1H, J = 9.98 Hz, H-3^{I}), 5.02-4.98 (m, 2H, H-1, H-3), 4.82 (br.s, 1H, H-1^{II}), 4.69-4.68 (m, 1H, H-2), 4.65 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.17 (d, 1H, J = 10.56 Hz, H-6^{II}a), 4.03 (dd, 1H, J = 5.70, 10.56 Hz, H-6a), 3.94 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.90 (t, 1H, J = 8.80 Hz, H-2^{I}), 3.86-3.81 (m, 2H, H-4, H-6a^{I}), 3.79-3.68 (m, 4H, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}), 3.68-3.59 (m, 2H, H-3^{II}, OC*H*H), 3.59-3.42 (m, 4H, H-2^{II}, H-5, H-5^{I}, OC*H*H), 3.39 (t, 1H, J = 8.80 Hz,

H-4^{II}), 3.27-3.17 (m, 2H, CH_2), 2.78 (t, 2H, J = 6.46 Hz, NCH_2), 2.55 (t, 2H, J = 6.46 Hz, CH_2), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, NHAc), 1.97 (s, 3H, NHAc), 1.67-1.52 (m, 4H, 2xCH_2), 1.48-1.36 (m, 14H, 4xCH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 174.97, 174.02, 173.97, 173.14, 173.11, 157.74, 101.80, 100.73 (C-1^{I}), 99.16 (C-1), 96.99 (C-1^{II}), 81.16, 76.16 (C-4^{I}), 74.27 (C-5^{I}), 73.69 (C-3^{I}), 71.23 (C-3^{II}), 71.15 (C-4), 70.85 (C-3), 70.44 (C-5^{II}), 69.96 (C-4^{II}), 68.71 (C-6^{II}), 67.77, 66.59 (C-5), 64.10 (C-6), 59.80 (C-6^{I}), 54.74 (C-2^{II}), 53.57 (C-2^{I}), 50.78 (C-2), 39.34, 39.21, 27.92, 27.81, 27.52, 24.34, 22.68, 21.87, 21.63, 20.43, 20.21, 19.95. HRMS(ESI): calcd for $C_{42}H_{68}N_4O_{22}S$ $[M+H]^+$: 1013.4119, found: 1013.4133.



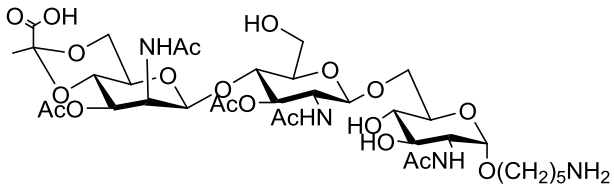
3-Mercapto-N-pentyl-propionamido 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-amino-2-deoxy-Glucopyanoside: (**1**): To a stirring solution of **84** (800 μg, 0.8 μmol) in DCM (0.7 mL) at 0^{o}C was added triethylsilane (0.07 mL, 0.442 mmol), followed by the dropwise addition of TFA (0.35 mL, 4.61 mmol). The mixture was allowed to stir for 15 min at 0^{o}C. Completion of the reaction was verified by LCMS. The mixture was concentrated under vacuum and taken up in PBS buffer (pH 7.4, 300μL). DTT (4.6 mg, 0.03 mmol) was added and the mixture was allowed to stir in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **1** (700 μg, 0.767 μmol, 97%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.07 (t, 1H, J = 9.98 Hz, H-3^{I}), 5.02-4.96 (m, 3H, H-1, H-1^{II}, H-3), 4.68 (d, 1H, J = 3.52 Hz, H-2), 4.64 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.17 (d, 1H, J = 9.98 Hz, H-6^{II}a), 4.03 (dd, 1H, J = 5.28, 11.15 Hz, H-6a), 3.93 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.89 (t, 1H, J = 8.80 Hz, H-2^{I}, H-2^{II}), 3.86-3.81 (m, 2H, H-4, H-6a^{I}), 3.79-3.68 (m, 6H, H-3^{II}, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II} OC*H*H,), 3.57-3.55 (m, 1H, H-5^{I}), 3.52-3.47 (m, 2H, H-5, OC*H*H),

3.38 (t, 1H, J = 9.39 Hz, H-4^{II}), 3.22 (t, 1H, J = 7.04 Hz, CH_2), 3.05 (d, J = 8.22 Hz, H-2^{II}), 2.78 (t, 2H, J = 6.46 Hz, NC*H*$_2$), 2.55 (t, 2H, J = 6.46 Hz, CH_2), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, NHAc), 1.96 (s, 3H, NHAc), 1.68-1.52 (m, 4H, 2xCH_2),1.45-1.36 (m, 14H, 4xCH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 174.99, 174.97, 174.04, 173.13, 173.12, 101.81, 100.68 (C-1^{I}), 99.16 (C-1), 96.29 (C-1^{II}), 76.15 (C-4^{I}), 74.28 (C-5^{I}), 73.68 (C-3^{I}), 71.58 (C-3^{II}), 71.15 (C-4), 70.85 (C-3), 70.67 (C-5^{II}), 69.56 (C-4^{II}), 68.43 (C-6^{II}), 67.96, 66.59 (C-5), 64.09 (C-6), 59.80 (C-6^{I}), 54.18 (C-2^{II}), 53.55 (C-2^{I}), 50.75 (C-2), 39.37, 39.14, 28.08, 27.92, 24.33, 22.75, 21.85, 21.63, 20.42, 20.21, 19.94. HRMS(ESI): calcd for $C_{37}H_{60}N_4O_{20}S$ $[M+H]^+$: 913.3594, found: 913.3608.



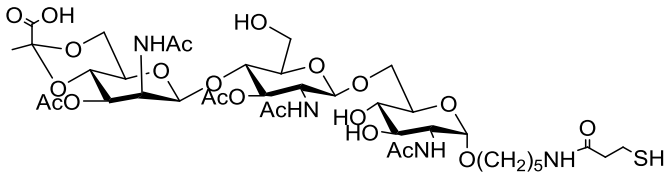
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside: (**85**): To a stirring solution of **82** (10 mg, 6.69 μmol) in DCM (0.75 mL) at 0^{o}C was added triethylsilane (0.075 mL, 0.468 mmol), followed by the dropwise addition of TFA (0.376 mL, 4.88 mmol). The mixture was allowed to stir for 15 min at 0^{o}C. Completion of the reaction was verified by LCMS. The mixture was concentrated and taken up in a 1:1 mixture of Ac_2O/Pyridine (0.5mL) and stirred for 2h at room temperature. The mixture was concentrated under vacuum and purified by preparatory TLC (5% MeOH/DCM). The product spot was identified by MALDI, excised, and eluted to afford **85** (8.8 mg, 6.06 μmol, 91%) as an amorphous white solid. H^1 NMR (600 MHz, $CDCl_3$)**:** 7.39-7.15 (m, 30H, aromatic), 5.67 (dd, 1H, J = 8.80, 66.91 Hz, NH), 5.54-5.43 (m, 1H, NH), 5.38 (d, 1H, J = 12.32 Hz, CH*H*Ph), 5.19-5.07 (m, 3H, H-3^{I}, CH*H*Ph, C*H*$_2$Ph'), 4.80 (t, 2H, J = 9.39 Hz, *CH*HPh''), 4.71-4.57 (m, 7H, H-1, H-1^{II},H-3, *CH*HPh'', C*H*$_2$Ph''', *CH*HPh^{IV}), 4.53-4.36 (m, 5H, H-1^{I}, H-2, *CH*HPh^{IV}, C*H*$_2$Ph^{V}, 4.26-4.17 (m, 1H, H-2^{I}), 4.06 (d, 1H, J =

9.98 Hz, H-6^{II}a), 3.97 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.88 (t, 1H, J = 8.80 Hz, H-4^{I}), 3.77-3.48 (m, 9H, H-2^{II}, H-3^{II}, H-4^{I}, H-5^{II}, H-6e, H-6ae^{I}, H-6e^{II}, OC*H*H), 3.45-3.35 (m, 2H, H-4, H-5^{I}), 3.30-3.15 (m, 3H, OC*H*H, NCH_2), 3.04-3.00 (m, 1H, H-5), 2.02 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.86-1.81 (m, 3H, Ac), 1.62-1.43 (m, 7H, 2xCH_2, CH_3), 1.36-1.17 (m, 2H, 1xCH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 171.67, 171.19, 170.70, 170.11, 169.82, 169.35, 156.86, 156.37, 138.56, 138.12, 137.90, 137.80, 136.98, 136.67, 135.38, 128.80, 128.78, 128.71, 128.66, 128.62, 128.60, 128.40, 128.33, 128.30, 128.18, 128.12, 128.06, 127.96, 127.92, 127.88, 127.82, 127.79, 127.49, 127.32, 100.47 (C-1), 99.40, 98.58 (C-1^{I}), 97.63 (C-1^{II}), 80.66 (C-5^{II}), 78.51 (C-4^{II}), 75.50 (C-4^{I}), 75.04, 74.98, 74.36 (C-5^{I}), 73.58, 73.21 (C-3^{I}), 72.29 (C-4), 70.91 (C-3), 70.33 (C-3^{II}), 68.02, 67.88 (C-6^{I}), 67.63 (C-6^{II}), 67.51, 67.35, 67.31, 66.81 (C-5), 64.80 (C-6), 54.59 (C-2^{II}), 52.76 (C-2^{I}), 51.38 (C-2), 50.58, 50.41, 47.31, 46.09, 29.13, 28.73, 27.98, 27.35, 25.45, 24.05, 23.50, 23.34, 21.51, 21.18. HRMS(ESI): calcd for $C_{79}H_{94}N_4O_{22}$ $[M+H]^+$: 1451.6432, found: 1451.6443.



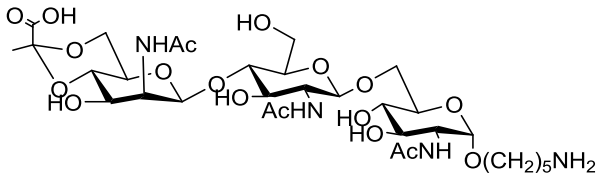
5-aminopentyl 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside: (**86**): Compound **85** (8.6 mg, 5.92 μmol) was dissolved in a mixture of t-BuOH (1.8 mL) and H_2O (0.045 mL). $Pd(OH)_2$/C (20 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **86** (3.2mg, 3.69 μmol, 62%) as an amorphous white solid. H^1 NMR (600 MHz,

D_2O)**:** 5.08 (t, 1H, J = 9.39 Hz, H-3^{I}), 5.02-4.98 (m, 2H, H-1, H-3), 4.83 (m, 1H, H-1^{II}), 4.69-4.68 (m, 1H, H-2), 4.65 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.20 (d, 1H, J = 10.56 Hz, H-6^{II}a), 4.03 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.94 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.91-3.82 (m, 4H, H-2^{I}, H-2^{II}, H-4, H-6a^{I}), 3.80-3.68 (m, 5H, H-3^{II},H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}), 3.68-3.60 (m, 1H, OC*H*H), 3.60-3.55 (m, 1H, H-5^{I}),3.54-3.43 (m, 2H, H-5, OC*H*H), 3.41 (t, 1H, J = 9.39 Hz, H-4^{II}), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.97 (s, 3H, NHAc), 1.71-1.58 (m, 4H, 2xCH_2), 1.48-1.39 (m, 5H, CH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 174.97, 174.22, 174.05, 173.12, 173.10, 101.81, 100.72 (C-1^{I}), 99.15 (C-1), 96.50 (C-1^{II}), 76.08 (C-4^{I}), 74.27 (C-5^{I}), 73.68 (C-3^{I}), 71.28 (C-5^{II}), 71.14 (C-4), 70.95 (C-3), 70.84 (C-3^{II}), 70.45 (C-5^{II}), 70.00 (C-4^{II}), 68.89 (C-6^{II}), 67.41, 66.59 (C-5), 64.09 (C-6), 59.76 (C-6^{I}), 54.56 (C-2^{II}), 53.52 (C-2^{I}), 50.75 (C-2), 39.29, 27.88, 26.38, 24.34, 22.30, 21.85, 21.72, 21.63, 20.40, 20.21. HRMS(ESI): calcd for $C_{36}H_{58}N_4O_{20}$ $[M+H]^+$: 867.3717, found: 867.3731.



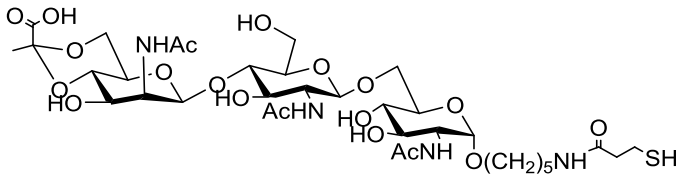
3-Mercapto-N-pentyl-propionamido 2-acetamido-3-*O*-acetyl-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside (**2**): To a stirring mixture of **86** (1.1mg, 1.27 μmol) in water (0.23 mL) was added DTSSP (1.5 mg, 2.54 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (4.9 mg, 0.032 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **2** (1.1 mg, 1.15 μmol, 91%) as an amorphour white solid. H^1 NMR (600 MHz, D_2O)**:** 5.07 (t, 1H, J = 10.56 Hz, H-3^{I}), 5.02-4.98 (m, 2H, H-1, H-

3), 3.84 (m, 1H, H-1^{II}), 4.69-4.68 (m, 1H, H-2), 4.65 (d, 1H, J = 8.22 Hz, H-1^{I}),4.18 (d, 1H, J = 11.15 Hz, H-6^{II}a), 4.03 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.94 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.91-3.68 (m, 10H, H-2^{I}, H-2^{II} H-3^{II}, H-4, H-5^{II}, H-6a^{II}, H-6e, H-6e^{I}, H-6e^{II}, OC*H*H), 3.66-3.62 (m, 1H, OC*H*H), 3.58-3.56 (m, 1H, H-5^{I}), 3.52-3.48 (m, 2H, H-5, OC*H*H), 3.46-3.38 (t, 1H, H-4^{II}, OC*H*H), 3.35-3.34 (m, 1H, OH), 3.25-3.16 (m, 1H, CH_2), 2.78 (t, 2H, J = 7.04 Hz, NC*H*$_2$), 2.55 (t, 2H, J = 7.04 Hz, CH_2), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.96 (s, 3H, NHAc), 1.67-1.52 (m, 4H, 2xCH_2), 1.42-1.37 (m, 5H, CH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.00, 174.97, 174.22, 174.00, 173.14, 173.10, 101.80, 100.70 (C-1^{I}), 99.16 (C-1), 96.49 (C-1^{II}), 76.17 (C-4^{I}), 74.27 (C-5^{I}), 73.71 (C-3^{I}), 71.15 (C-4), 70.98 (C-3), 70.85 (C-3^{II}), 70.44 (C-5^{II}), 70.00 (C-4^{II}), 68.74 (C-6^{II}), 67.68, 66.58 (C-5), 64.09 (C-6), 59.80 (C-6^{I}), 53.56 (C-2^{I}, C-2^{II}), 50.76 (C-2), 39.34, 39.18, 27.98, 27.87, 24.34, 22.71, 21.85, 21.75, 21.63, 20.43, 20.21, 19.95. HRMS(ESI): calcd for $C_{39}H_{62}N_4O_{21}S$ $[M+H]^+$: 955.3700, found: 955.3719.



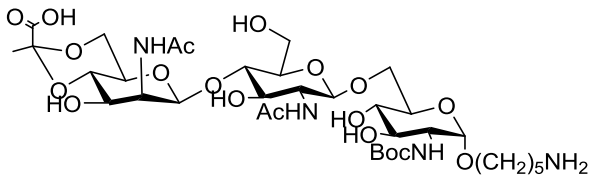
5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside: (**87**): Compound **85** (9.8 mg, 6.76 μmol) was taken up in a solution of THF (0.3 mL), water (0.015 mL), and 2M LiOH (0.066 mL) and stirred in a warm water bath (40^{o}C) for 2.5 h. Completion of the reaction was verified by LC/MS. The reaction mixture was made neutral by the dropwise addition of a 20% aqueous solution of HOAc. The reaction mixture was concentrated and the crude was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were identified by LCMS and concentrated to afford a mixture of deacetylated intermediate with and without benzyl ester. The intermediate was dissolved in a mixture of t-BuOH (2 mL) and H_2O (0.05 mL). $Pd(OH)_2$/C (20 mg, 20 wt.%, Degussa type)

was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **87** (2.8 mg, 3.58 μmol, 53% over 2 steps) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 4.94 (s, 1H, H-1), 4.83 (d, 1H, J = 3.52 Hz, H-1^{II}), 4.60 (d, 1H, J = 4.70 Hz, H-2), 4.49 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.18 (d, 1H, J = 11.15 Hz, H-$6a^{II}$), 4.03 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.98 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.89-3.85 (m, 2H, H-2^{II}, H-$6a^{I}$), 3.78-3.75 (m, 1H, H-3^{I}), 3.74-3.60 (m, 9H, H-2^{I}, H-3^{II}, H-4, H-4^{I}, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$, OC*H*H), 3.50-3.47 (m, 1H, H-5^{I}), 3.46-3.38 (m, 3H, H-4^{II}, H-5, OC*H*H), 3.00 (t, 2H, J = 8.22 Hz, NCH_2), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.71-1.59 (m, 4H, 2xCH_2), 1.49-1.40 (m, 5H, CH_2, CH_3). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.22, 101.73, 101.38 (C-1^{I}), 99.73 (C-1), 96.46 (C-1^{II}), 78.66 (C-4^{I}), 74.33 (C-5^{I}), 73.79 (C-4), 72.06 (C-5^{II}), 70.92 (C-3^{II}), 70.47 (C-3^{I}), 70.04 (C-4^{II}), 69.21 (C-3), 68.77 (C-6^{II}), 67.34, 66.71 (C-5), 63.95 (C-6), 59.96 (C-6^{I}), 55.18 (C-2^{I}), 53.52 (C-2^{II}), 53.38 (C-2), 39.28, 27.84, 26.38, 24.55, 22.27, 22.11, 21.91, 21.72. HRMS(ESI): calcd for $C_{32}H_{54}N_4O_{18}$ $[M+H]^+$: 783.3506, found: 783.3522.



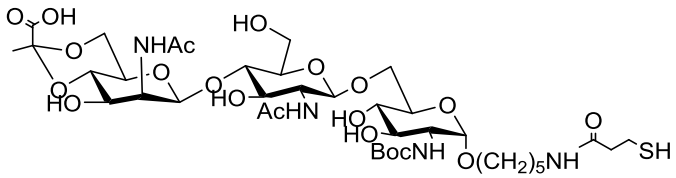
3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside: (**3**): To a stirring mixture of **87** (0.9 mg, 1.15 μmol) in water (0.22 mL) was added DTSSP (1.4 mg, 2.3 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT

(4.4 mg, 0.029 mmol) was added and the reaction mixture was stirred in a warm water bath (~40°C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **3** (1.1 mg, 1.15 μmol, 100%). H^1 NMR (600 MHz, D_2O)**:** 4.94 (s, 1H, H-1), 4.83 (d, 1H, J = 3.52 Hz, H-1^{II}),4.60 (d, 1H, J = 4.11 Hz, H-2), 4.49 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.15 (d, 1H, J = 10.56 Hz, H-$6a^{II}$), 4.03 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.98 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.87-3.85 (m, 2H, H-2^{II}, H-$6a^{I}$), 3.78-3.75 (m, 1H, H-3^{I}), 3.74-3.60 (m, 9H, H-2^{I}, H-3^{II}, H-4, H-4^{I}, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$, OC*H*H), 3.50-3.47 (m, 1H, H-5^{I}), 3.46-3.38 (m, 3H, H-4^{II}, H-5, OC*H*H), 3.24-3.19 (m, 2H, J = 8.22 Hz, NCH_2), 2.78 (t, 2H, J = 7.04 Hz, CH_2), 2.54 (t, 2H, J = 7.04 Hz, CH_2), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.66-1.52 (m, 4H, 2xCH_2), 1.46 (s, 3H, CH_3), 1.43-1.36 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.22, 174.02, 101.73, 101.38 (C-1^{I}), 99.74 (C-1), 96.48 (C-1^{II}), 78.71 (C-4^{I}), 74.34 (C-5^{I}), 73.79 (C-4), 72.06 (C-5^{II}), 70.95 (C-3^{II}), 70.44 (C-3^{I}), 70.00 (C-4^{II}), 69.23 (C-3), 68.55 (C-6^{II}), 67.67, 66.72 (C-5), 63.95 (C-6), 59.99 (C-6^{I}), 55.19 (C-2^{I}), 53.56 (C-2^{II}), 53.40 (C-2), 39.32, 39.18, 27.96, 27.91, 24.55, 22.71, 22.12, 21.91, 21.75, 19.94. HRMS(ESI): calcd for $C_{35}H_{58}N_4O_{19}S$ $[M+H]^+$: 871.3489, found: 871.3501.



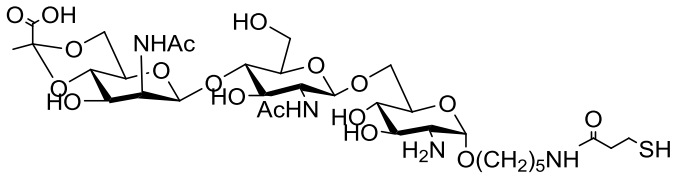
5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-2-deoxy-Glucopyanoside (**88**): **82** (10 mg, 6.62 μmol) was taken up in a solution of THF (0.3 mL), water (0.015 mL), and 2M LiOH (0.066 mL) and stirred in a warm water bath (40°C) for 2.5 h. completion of the reaction was verified by LC/MS. The reaction mixture was made neutral by the dropwise addition of a 20% aqueous solution of HOAc and concentrated. The crude was purified with a C18 silica column using an eluent gradient starting with

100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were identified by LCMS and concentrated to afford a mixture of deacetylated intermediate with and without benzyl ester. The intermediate was dissolved in a mixture of t-BuOH (2 mL) and H_2O (0.05 mL). $Pd(OH)_2/C$ (20 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **88** (3 mg, 3.57 μmol, 61%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 4.94 (s, 1H, H-1), 4.83 (br.s, 1H, H-1^{II}), 4.60 (d, 1H, J = 4.70 Hz, H-2), 4.49 (d, 1H, J = 8.22 Hz, H-1^{I}), 4.16 (d, 1H, J = 10.56 Hz, H-$6a^{II}$), 4.03 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.98 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.87-3.85 (m, 1H, H-$6a^{I}$), 3.75-3.60 (m, 10H, H-2^{II}, H-3^{I}, H-3^{II}, H-4, H-4^{I}, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$, OC*H*H), 3.58-3.53 (m, 1H, H-2^{I}), 3.50-3.38 (m, 4H, H-4^{II}, H-5, H-5^{I}, OC*H*H), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.72-1.58 (m, 4H, 2xCH_2), 1.48-1.43 (m, 14H, CH_2, 4xCH_3). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.21, 157.74, 101.73, 101.41 (C-1^{I}), 99.73 (C-1), 97.00 (C-1^{II}), 81.16, 78.67 (C-4^{I}), 74.33 (C-5^{I}), 73.79 (C-4), 72.04 (C-5^{II}), 71.24 (C-3^{II}), 70.49 (C-3^{I}), 69.99 (C-4^{II}), 69.22 (C-3), 68.73 (C-6^{II}), 67.46, 66.72 (C-5), 63.95 (C-6), 59.96 (C-6^{I}), 55.19 (C-2^{I}), 54.69 (C-2^{II}), 53.38 (C-2), 39.29, 27.81, 27.51, 26.37, 24.55, 22.27, 22.13, 21.91. HRMS(ESI): calcd for $C_{35}H_{60}N_4O_{19}$ $[M+H]^+$: 841.3925, found: 841.3943.



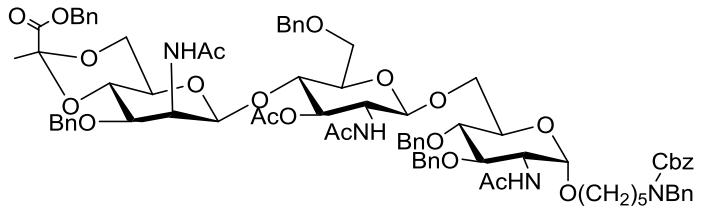
3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-t-Butoxycaronylamino-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-

deoxy-Glucopyanoside: (**89**): To a stirring mixture of **88** (1.1 mg, 1.31 μmol) in water (0.24 mL) was added DTSSP (1.2 mg, 1.96 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (5 mg, 0.033 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **89** (1.2 mg, 1.29 μmol, 99%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 4.94 (s, 1H, H-1), 4.83 (br.s, 1H, H-1^{II}), 4.60 (d, 1H, J = 4.70 Hz, H-2), 4.49 (d, 1H, J = 8.22 Hz, H-1^{I}), 4.14 (d, 1H, J = 10.56 Hz, H-6a^{II}), 4.03 (dd, 1H, J = 5.28, 11.15 Hz, H-6a), 3.98 (dd, 1H, J = 4.11, 9.98 Hz, H-3), 3.87-3.85 (m, 1H, H-6a^{I}), 3.75-3.60 (m, 10H, H-2^{II}, H-3^{I}, H-3^{II}, H-4, H-4^{I}, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}, OC*H*H), 3.58-3.53 (m, 1H, H-2^{I}), 3.50-3.38 (m, 4H, H-4^{II}, H-5, H-5^{I}, OC*H*H), 3.24-3.19 (m, 2H, NCH_2), 2.78 (t, 2H, J = 7.04 Hz, CH_2), 2.52 (t, 2H, J = 6.46 Hz, CH_2), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.65-1.53 (m, 4H, 2xCH_2), 1.46-1.38 (m, 14H, CH_2, 4xCH_3). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.22, 173.99, 157.75, 101.73, 101.40 (C-1^{I}), 99.74 (C-1), 96.96 (C-1^{II}), 81.15, 78.70 (C-4^{I}),74.33 (C-5^{I}), 73.79 (C-4), 72.04 (C-5^{II}), 71.19 (C-3^{II}), 70.45 (C-3^{I}), 69.95 (C-4^{II}), 69.24 (C-3), 68.51 (C-6^{II}), 67.74, 66.72 (C-5), 63.95 (C-6), 59.99 (C-6^{I}), 55.20 (C-2^{I}), 54.75 (C-2^{II}), 53.40 (C-2), 39.31, 39.21, 27.89, 27.83, 27.53, 24.55, 22.67, 22.14, 21.91, 19.94. HRMS(ESI): calcd for $C_{38}H_{64}N_4O_{20}$ $[M+H]^+$: 929.3907, found: 929.3916.



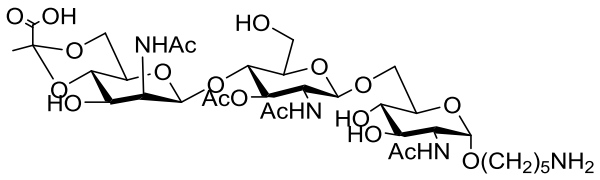
3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-amino-2-deoxy-Glucopyanoside: (**4**): To a stirring solution of **89** (1.2 mg, 1.29 μmol) in DCM (0.75 mL) at 0^{o}C was added

triethylsilane (0.075 mL, 0.468 mmol), followed by the dropwise addition of TFA (0.376 mL, 4.88 mmol). The mixture was allowed to stir for 15 min at 0°C. Completion of the reaction was verified by LCMS. The crude was dissolved in PBS (pH 7.4, 1 mL) and DTT (5 mg, 0.032 mmol) was added and the reaction mixture was stirred in a warm water bath (~40°C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **4** (900 μg, 1.09 μmol, 84%). H^1 NMR (600 MHz, D_2O)**:** 5.07 (d, 1H, J = 3.52 Hz, H-1^{II}), 4.93 (s, 1H, H-1), 4.61 (d, 1H, J = 4.70 Hz, H-2), 4.49 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.15 (d, 1H, J = 10.56 Hz, H-6a^{II}), 4.03 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.98 (dd, 1H, J = 4.11, 9.98 Hz, H-3), 3.87-3.82 (m, 2H, H-3^{I}, H-6a^{I}), 3.79-3.77 (m, 1H, H-3^{II}), 3.75-3.60 (m, 8H, H-2^{I}, H-4, H-4^{I}, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}, OC*H*H), 3.53-3.47 (m, 2H, H-5^{I}, OC*H*H), 3.46-3.42 (m, 2H, H-4^{II}, H-5), 3.28 (dd, 1H, J = 3.52, 10.56 Hz, H-2^{II}), 3.22 (t, 2H, J = 7.04Hz, NCH_2), 2.78 (t, 2H, J = 7.04 Hz, CH_2), 2.54 (t, 2H, J = 6.46 Hz, CH_2), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.70-1.60 (m, 2H, CH_2), 1.57-1.52 (m, 2H, CH_2), 1.46 (s, 3H, CH_3), 1.44-1.37 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.26, 174.05, 101.74, 101.33 (C-1^{I}), 99.74 (C-1), 94.91 (C-1^{II}), 78.70 (C-4^{I}),74.34 (C-5^{I}), 73.79 (C-4), 72.02 (C-5^{II}), 70.66 (C-3^{II}), 70.03 (C-3^{I}), 69.34 (C-4^{II}), 69.21 (C-3), 68.03 (C-6^{II}), 66.72 (C-5), 63.95 (C-6), 59.99 (C-6^{I}), 55.17 (C-2^{I}), 53.80 (C-2^{II}), 53.39 (C-2), 39.31, 39.12, 28.05, 27.97, 24.55, 22.71, 22.12, 21.91, 19.92. HRMS(ESI): calcd for $C_{33}H_{56}N_4O_{18}S$ $[M+H]^+$: 829.3383, found: 829.3400.

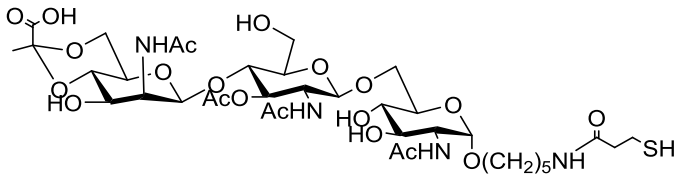


N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-acetamido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-3,4-di-O-Benzyl-2-deoxy-Glucopyanoside:

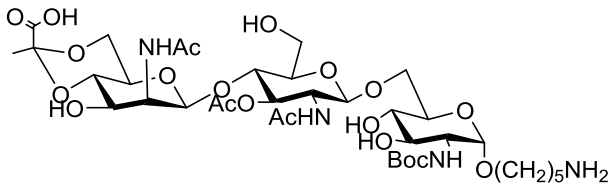
(**90**): To a stirring solution of **82a** (8.6 mg, 5.52 μmol) in DCM (0.62 mL) at 0°C was added triethylsilane (0.062 mL, 0.386 mmol), followed by the dropwise addition of TFA (0.310 mL, 4.03 mmol). The mixture was allowed to stir for 15 min at 0°C. Completion of the reaction was verified by LCMS. The mixture was concentrated and taken up in a 1:1 mixture of Ac_2O/Pyridine (0.5mL) and stirred for 2h at room temperature. The mixture was concentrated under vacuum and purified by preparatory TLC (5% MeOH/DCM). The product spot was identified by MALDI, excised, and eluted to afford **90** (8 mg, 5.33 μmol, 97%) as an amorphous white solid. H^1 NMR (600 MHz, $CDCl_3$)**:** 7.39-7.12 (m, 35H, aromatic), 5.73 (d, 1H, J = 8.80 Hz, NH), 5.55 (d, 1H, J = 8.22 Hz, NH), 5.34 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.18-5.07 (m, 3H, H-3^{I}, CH*H*Ph, CH_2Ph'), 4.82-4.79 (m, 2H, C*H*HPh'', C*H*HPh'''), 4.68-4.57 (m, 7H, H-1^{I}, H-1^{II}, *CH*HPh'', CH*H*Ph''', C*H*HPh^{IV} , CH_2Ph^{V}), 4.50-4.48 (m, 3H, H-2, CH_2Ph^{VI}) 4.46-4.40 (m, 1H, H-1), 4.37 (d, 1H, J = 11.74 Hz, *CH*HPh^{IV}), 4.26-4.16 (m, 1H, H-2^{II}), 4.05 (d, 1H, J = 9.39 Hz, H-$6a^{II}$), 3.94 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.84-3.79 (m, 2H, H-2^{I}, H-4^{I}), 3.79-3.67 (m, 3H, H-3^{II}, H-5^{II}, H-$6e^{II}$), 3.67-3.50 (m, 6H, H-3, H-4^{II}, H-6e, H-$6ae^{I}$, OC*H*H), 3.44-3.35 (m, 2H, H-4, H-5^{I}), 3.30-3.15 (m, 3H, OC*H*H, NCH_2), 3.13-3.03 (m, 1H, H-5), 2.02-1.83 (m, 12H, 4xAc), 1.60-1.42 (m, 7H, 2xCH_2, CH_3), 1.35-1.18 (m, 2H, 1xCH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 171.09, 170.90, 170.06, 169.78, 169.51, 156.85, 156.36, 138.53, 138.16, 137.97, 137.91, 137.01, 136.69, 135.39, 128.71, 128.65, 128.59, 128.45, 128.34, 128.13, 128.11, 128.07, 127.92, 127.79, 127.70, 127.54, 127.47, 127.32, 100.90 (C-1^{I}), 100.08 (C-1), 99.22 (C-1^{II}), 97.60, 80.69 (C-5^{II}), 78.47 (C-4^{II}), 76.04 (C-4^{I}), 75.54, 75.20, 75.00, 74.94, 74.60 (C-4, C-5^{I}), 73.66, 73.53 (C-3^{I}), 71.47, 70.37 (C-3^{II}), 68.05, 67.98 (C-6^{I}), 67.62 (C-6^{II}), 67.52, 67.33, 67.30, 66.22 (C-5), 65.34 (C-6), 54.34 (C-2^{I}), 52.74 (C-2^{II}), 50.59 (C-2), 50.40, 47.31, 46.09, 29.84, 29.41, 28.75, 27.98, 27.38, 25.59, 24.05, 23.46, 23.36, 21.29. MALDI MS: calcd for $C_{84}H_{98}N_4O_{21}$ $[M+Na]^+$: 1521.6616, found: 1521.6639.



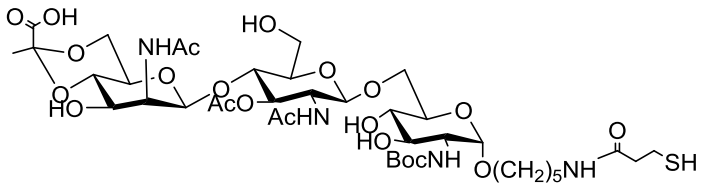
5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside: (**91**): Compound **90** (5.5 mg, 3.9 μmol) was dissolved in a mixture of t-BuOH (1.2 mL) and H_2O (0.03 mL). $Pd(OH)_2$/C (12 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **91** (2.4 mg, 2.9 μmol, 74%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.08 (t, 1H, J = 10.56 Hz, H-3'), 4.87 (s, 1H, H-1),4.83 (d, 1H, J = 3.52 Hz, H-1''), 4.64 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.54 (d, 1H, J = 4.11 Hz, H-2^{I}), 4.19 (d, 1H, J = 10.56 Hz, H-$6a^{II}$), 3.99 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.95 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.93-3.86 (m, 4H, H-2^{I}, H-2^{II}, H-4^{I}, H-$6a^{I}$), 3.79-3.76 (m, 1H, H-3^{II}), 3.74-3.69 (m, 4H, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$), 3.66-3.62 (m, 1H, OC*H*H), 3.60-3.55 (m, 2H, H-4, H-5^{I}), 3.47-3.43 (m, 1H, OC*H*H), 3.42-3.34 (m, 2H, H-4^{II}, H-5), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.70-1.58 (m, 4H, 2xCH_2), 1.49-1.39 (m, 5H, CH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.22, 174.04, 172.99, 101.72, 100.71 (C-1^{I}), 99.77 (C-1), 96.49 (C-1^{II}), 75.73 (C-4^{I}), 74.34 (C-5^{I}), 73.81 (C-3^{I}), 73.75 (C-4), 70.95 (C-5^{II}), 70.44 (C-3^{II}), 69.99 (C-4^{II}), 68.92 (C-6^{II}), 68.86 (C-3), 67.41, 66.75 (C-5), 64.06 (C-6), 59.76 (C-6^{I}), 53.59 (C-2^{II}), 53.51 (C-2^{I}), 53.42 (C-2), 39.28, 27.87, 26.38, 24.54, 22.30, 21.91, 21.85, 21.72, 20.38. HRMS(ESI): calcd for $C_{34}H_{56}N_4O_{19}$ $[M+H]^+$: 825.3612, found: 825.3623.



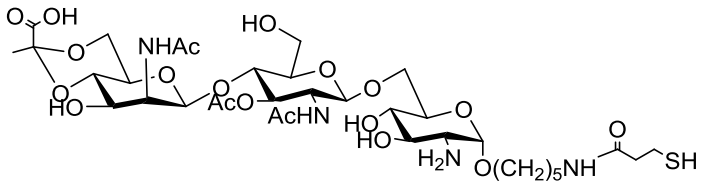
3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido-2-deoxy-Glucopyanoside: (**5**): To a stirring mixture of **91** (1.1 mg, 1.33 μmol) in water (0.25 mL) was added DTSSP (1.2 mg, 2.0 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (5.1 mg, 0.033 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **5** (1 mg, 1.1 μmol, 82%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.07 (t, 1H, J = 9.98 Hz, H-3^{I}), 4.87 (s, 1H, H-1), 4.83 (m, 1H, H-1^{II}), 4.65 (d, 1H, J = 8.22 Hz, H-1^{I}), 4.54 (d, 1H, J = 4.11 Hz, H-2^{I}), 4.18 (d, 1H, J = 11.15 Hz, H-6a^{II}), 4.00 (dd, 1H, J = 5.28, 11.15 Hz, H-6a), 3.95 (dd, 1H, J = 4.11, 9.98 Hz, H-3), 3.93-3.86 (m, 4H, H-2^{I}, H-2^{II}, H-4^{I}, H-6a^{I}), 3.79-3.77 (m, 1H, H-3^{II}), 3.77-3.70 (m, 4H, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}), 3.66-3.62 (m, 1H, OC*H*H), 3.60-3.54 (m, 2H, H-4, H-5^{I}), 3.46-3.43 (m, 1H, OC*H*H), 3.42-3.34 (m, 2H, H-4^{II}, H-5), 3.24-3.19 (m, 2H, NCH_2), 2.78 (t, 2H, J = 6.46 Hz, CH_2), 2.54 (t, 2H, J = 7.04 Hz, CH_2), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.66-1.52 (m, 4H, 2xCH_2), 1.45 (s, 3H, CH_3), 1.43-1.37 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.39, 175.36, 174.22, 174.00, 173.04, 101.71, 100.69 (C-1^{I}), 99.80 (C-1), 96.50 (C-1^{II}), 75.84 (C-4^{I}), 74.35 (C-5^{I}), 73.81 (C-3^{I}, C-4), 70.98 (C-5^{II}), 70.44 (C-3^{II}), 69.99 (C-4^{II}), 68.94 (C-6^{II}), 68.71 (C-3), 67.69, 66.75 (C-5), 64.06 (C-6), 59.81 (C-6^{I}), 53.60 (C-2^{II}), 53.55 (C-2^{I}), 53.45 (C-2), 39.48, 39.17, 27.98, 27.87, 24.54, 22.72, 21.92, 21.85, 21.75, 20.40, 19.99. HRMS(ESI): calcd for $C_{37}H_{60}N_4O_{20}S$ $[M+H]^+$: 913.3594, found: 913.3609.



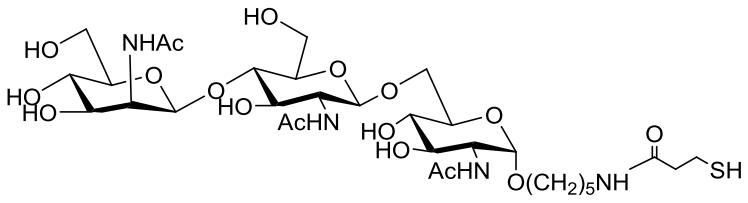
5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-2-deoxy-Glucopyanoside: (**92**): **82a** (8.6 mg, 5.5 μmol) was dissolved in a mixture of t-BuOH (1.7 mL) and H_2O (0.04 mL). $Pd(OH)_2/C$ (20 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **92** (2.8 mg, 3.2 μmol, 57%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.06 (t, 1H, J = 10.56 Hz, H-3^{I}), 4.87 (s, 1H, H-1), 4.83 (s, 1H, H-1^{II}), 4.64 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.54-4.53 (m, 1H, H-2^{I}), 4.19 (d, 1H, J = 10.56 Hz, H-6a^{II}), 3.99 (dd, 1H, J = 4.70, 10.56 Hz, H-6a), 3.95 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.93-3.86 (m, 3H, H-2^{I}, H-4^{I}, H-6a^{I}), 3.77-3.70 (m, 4H, H-3^{II}, H-6e, H-6e^{I}, H-6e^{II}), 3.67-3.55 (m, 5H, H-2^{II}, H-4, H-5, H-5^{II}, OC*H*H), 3.48-3.44 (m, 1H, OC*H*H), 3.41-3.34 (m, 2H, H-4^{II}, H-5), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.71-1.59 (m, 4H, 2xCH_2), 1.47-1.38 (m, 14H, 4xCH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.04, 172.99, 157.74, 101.72, 100.73 (C-1^{I}), 99.78 (C-1), 97.04 (C-1^{II}), 81.16, 75.74 (C-4^{I}),74.33 (C-5^{I}), 73.81 (C-3^{I}), 73.73 (C-4), 71.27 (C-5^{II}), 70.45 (C-3^{II}), 69.93 (C-4^{II}), 68.93 (C-6^{II}), 68.83 (C-3), 67.53, 66.75 (C-5), 64.06 (C-6), 59.76 (C-6^{I}), 54.68 (C-2^{II}), 53.59 (C-2^{I}), 53.42 (C-2), 39.30, 27.85, 27.50, 26.37, 24.54, 22.30, 21.92, 21.86, 20.39. HRMS(ESI): calcd for $C_{37}H_{62}N_4O_{20}$ $[M+H]^+$: 883.4030, found: 883.4043.



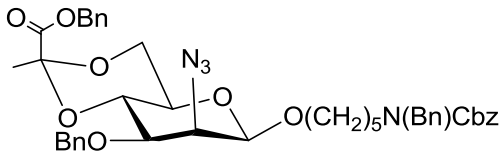
3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-t-Butoxycaronylamino-2-deoxy-Glucopyanoside: (**93**): To a stirring mixture of **92** (1.1 mg, 1.25 μmol) in water (0.23 mL) was added DTSSP (1.1 mg, 1.87 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (4.8 mg, 0.031 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **93** (1.1 mg, 1.13 μmol, 91%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** 5.07 (t, 1H, J = 10.56 Hz, H-3^{I}), 4.87 (s, 1H, H-1), 4.82 (s, 1H, H-1^{II}), 4.64 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.54-4.53 (m, 1H, H-2^{I}), 4.17 (d, 1H, J = 9.98 Hz, H-6a^{II}), 4.00 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.95 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.93-3.86 (m, 3H, H-2^{I}, H-4^{I}, H-6a^{I}), 3.77-3.70 (m, 4H, H-3^{II}, H-6e, H-6e^{I}, H-6e^{II}), 3.67-3.53 (m, 5H, H-2^{II}, H-4, H-5, H-5^{II}, OC*H*H), 3.47-3.44 (m, 1H, OC*H*H), 3.41-3.34 (m, 2H, H-4^{II}, H-5), 3.25-3.18 (m, 2H, NCH_2), 2.78 (t, 2H, J = 7.04 Hz, CH_2), 2.54 (t, 2H, J = 7.04 Hz, CH_2), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.65-1.52 (m, 4H, 2xCH_2), 1.48-1.35 (m, 14H, 4xCH_3, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.36, 174.00, 173.04, 157.75, 101.71, 100.72 (C-1^{I}), 99.80 (C-1), 96.98 (C-1^{II}), 81.15, 75.83 (C-4^{I}), 74.34 (C-5^{I}), 73.81 (C-3^{I}), 73.76 (C-4), 71.24 (C-5^{II}), 70.44 (C-3^{II}), 69.95 (C-4^{II}), 68.94 (C-6^{II}), 68.69 (C-3), 67.76, 66.75 (C-5), 64.08 (C-6), 59.81 (C-6^{I}), 54.74 (C-2^{II}), 53.60 (C-2^{I}), 53.44 (C-2), 39.35, 39.21, 27.92, 27.80, 27.52, 24.54, 22.67, 21.92, 21.86, 20.40, 19.95. HRMS(ESI): calcd for $C_{37}H_{62}N_4O_{20}$ $[M+H]^+$: 883.4030, found: 883.4043.



3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-amino-2-deoxy-Glucopyanoside: (**6**): To a stirring solution of **93** (1.1 mg, 1.13 μmol) in DCM (0.75 mL) at 0°C was added triethylsilane (0.075 mL, 0.468 mmol), followed by the dropwise addition of TFA (0.376 mL, 4.88 mmol). The mixture was allowed to stir for 15 min at 0°C. Completion of the reaction was verified by LCMS. The crude was dissolved in PBS (pH 7.4, 1mL) and DTT (4.4 mg, 0.028 mmol) was added and the reaction mixture was stirred in a warm water bath (~40°C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **6** (900 μg, 1.03 μmol, 91%). H^1 NMR (600 MHz, D_2O)**:** 5.08 (t, 1H, J = 10.56 Hz, H-3^{I}), 4.96 (d, 1H, J = 3.52 Hz, H-1), 4.86 (s, 1H, H-1^{II}), 4.63 (d, 1H, J = 8.80 Hz, H-1^{I}), 4.54-4.53 (m, 1H, H-2^{I}), 4.17 (d, 1H, J = 11.15 Hz, H-6a^{II}), 4.00 (dd, 1H, J = 5.28, 11.15 Hz, H-6a), 3.95 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.92-3.84 (m, 3H, H-2^{I}, H-4^{I}, H-6a^{I}), 3.79-3.77 (m, 1H, H-3^{II}), 3.75-3.67 (m, 5H, H-5^{II}, H-6e, H-6e^{I}, H-6e^{II}, OC*H*H), 3.60-3.54 (m, 2H, H-4, H-5^{II}), 3.51-3.48 (m, 3H, H-4^{II}, H-5, OC*H*H), 3.22 (t, 2H, J = 7.04 Hz, NCH_2), 3.04 (br.d, 1H, J = 8.80 Hz, H-2^{II}), 2.78 (t, 2H, J = 7.04 Hz, CH_2), 2.54 (t, 2H, J = 7.04 Hz, CH_2), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.68-1.52 (m, 4H, 2xCH_2), 1.45 (s, 3H, CH_3), 1.43-1.36 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.38, 174.02, 173.03, 101.72, 100.67 (C-1^{I}), 99.79 (C-1), 96.42 (C-1^{II}), 75.81 (C-4^{I}),74.36 (C-5^{I}), 73.81 (C-3^{I}), 73.74 (C-4), 71.61 (C-5^{II}), 70.67 (C-3^{II}), 69.54 (C-4^{II}), 68.93 (C-6^{II}), 68.42 (C-3), 67.96, 66.75 (C-5), 64.07 (C-6), 59.81 (C-6^{I}), 54.20 (C-2^{II}), 53.58 (C-2^{I}), 53.42 (C-2), 39.37, 39.13, 28.08, 27.92, 24.54, 22.75, 21.92, 21.85, 20.40, 19.95. HRMS(ESI): calcd for $C_{35}H_{59}N_4O_{19}S$ $[M+H]^+$: 871.3489, found: 871.3502.

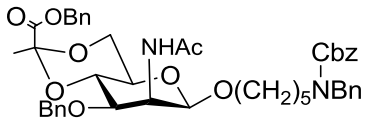


3-Mercapto-N-pentyl-propionamido 2-acetamido-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido -2-deoxy-β-D-Glucopyranosyl-(1→6) -2-acetamido-2-deoxy-Glucopyanoside (**7**): To a stirring mixture of 5-aminopentyl 2-acetamido-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-acetamido -2-deoxy-Glucopyanoside (1 mg, 1.23 μmol) in water (0.23 mL) was added DTSSP (1.1 mg, 1.84 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (4.7 mg, 0.031 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **7** (800 μg, 0.89 μmol, 72%) as an amorphous white solid. HRMS(ESI): calcd for $C_{37}H_{64}N_4O_{19}S$ $[M+H]^+$: 801.3434, found: 801.3451.



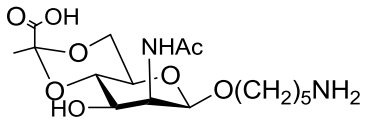
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranoside: (**95**): Compound **74a** (100mg, 0.183 mmol), 2,4,6 tri-tert-butylpyrimidine (113 mg, 0.457 mmol), and Diphenylsulfoxide (48 mg, 0.237 mmol) were dissolved in toluene and concentrated under vacuum (3x). The mixture was taken up in DCM (3.65 mL) along with activated 4Å molecular sieves (250 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78^{o}C and Trifluoromethanesulfonic anhydride (40

μL, 0.237 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50°C in order to activate the donor. The mixture was cooled back to -78°C and compound **94** (87 mg, 0.256 mmol) was dissolved in a minimal amount of DCM and added by dropwise addition. The mixture was allowed to warm to 0°C and before being quenched with TEA, filtered, and concentrated under vacuum to give **95** in 1.1/1 (β:α) ratio. The residue was taken up in DCM (20 mL) and transferred to separatory funnel and washed with aqueous $NaHCO_3$ (sat, 20 mL) and water (20 mL). The organic phase was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel column chromatography (15-20% EtOAc/Hexanes) to give **95** (Beta, 65 mg, 0.083 mmol, 46%) as a colorless syrup and (alpha, 59 mg, 0.076 mmol, 41%) side product. R_f = 0.7 (α), 0.5 (β) (25% EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** 7.41-7.16 (m, 20H, aromatic), 5.34 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.20-5.14 (m, 3H, CH*H*Ph, C*H*$_2$Ph'), 4.91 (d, 1H, J = 12.33 Hz, CH*H*Ph''), 4.72 (d, 1H, J = 12.33 Hz, CH*H*Ph''), 4.49-4.24 (m, 3H, H-1, C*H*$_2$Ph'''), 4.06-4.00 (m, 1H, H-6a), 3.97-3.73 (m, 4H, H-2, H-4, H-6e, OC*H*H), 3.65-3.58 (m, 1H, H-3), 3.46-3.16 (m, 4H, H-5, OC*H*H, NC*H*$_2$), 1.62-1.45 (m, 5H, 2xCH_2, CH_3), 1.36-1.19 (m 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 169.52, 156.86, 156.34, 138.19, 138.00, 136.99, 136.85, 135.16, 128.78, 128.77, 128.75, 128.66, 128.59, 128.55, 128.51, 128.50, 128.38, 128.35, 128.29, 128.24, 128.05, 127.94, 127.89, 127.83, 127.74, 127.62, 127.57, 127.45, 127.38, 127.33, 100.25 (C-1), 99.50, 76.37-76.36 (C-3), 75.41 (C-4), 72.87, 70.04, 69.94, 67.74, 67.29, 67.00 (C-5), 65.00 (C-6), 63.80 (C-2), 50.65, 50.34, 47.17, 46.25, 29.23, 27.94, 27.49, 25.60, 23.21. HRMS(ESI): calcd for $C_{43}H_{48}N_4O_9$ $[M+H]^+$: 765.3494, found: 765.3508.



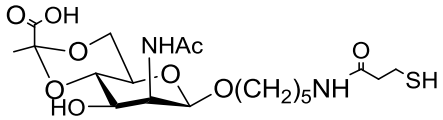
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-acetamido-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranoside (**96**): To a stirring solution of **95** (22 mg, 0.029 mmol) in acetic acid (1.26 mL) was added a large excess of Zn-Cu couple (130 mg). The mixture

was allowed to stir for 1hr. Completion of the reaction was verified by MALDI. The reaction mixture was filtered and concentrated under vacuum. The crude was taken up in a 1:1 mixture of pyridine/Ac_2O (2mL) and allowed to stir for 3 h. The reaction mixture was concentrated and the residue was taken up in ethyl acetate (20 mL) and washed with water (20 mL). The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude was chromatographed with 40%EtOAc/Hexanes to afford **96** (17.1 mg, 0.022 mol, 76%) as a colorless solid. R_f = 0.5 (40% EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** 7.40-7.16 (m, 20H, aromatic), 5.58 (Br dd, J = 8.22, 59.87 Hz, NH), 5.34 (d, 1H, J = 12.33 Hz, CH*H*Ph), 5.20-5.13 (m, 3H, CH*H*Ph, C*H*$_2$Ph'), 4.80-4.68 (m, 3H, H-2, C*H*$_2$Ph''), 4.52-4.43 (m, 3H, H-1, C*H*$_2$Ph'''), 4.06-4.00 (m, 1H, C-6a), 3.74 (d, 1H, J = C-6e), 3.70-3.55 (m, 3H, H-3, H-4, OC*H*H), 3.45-3.27 (m, H-5, OC*H*H), 3.26-3.14 (m, 2H,NC*H*$_2$), 1.95 (s, 3H, NHAc), 1.58 (s, 3H, CH_3), 1.56-1.40 (m, 4H, 2xCH_2), 1.30-1.15 (m 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): 170.67, 169.52, 156.81, 156.29, 138.34, 138.01, 137.02, 136.84, 135.35, 128.71, 128.65, 128.55, 128.44, 128.35, 128.05, 127.94, 127.79, 127.55, 127.44, 127.37, 127.30, 100.06 (C-1), 99.33, 75.47 (C-3), 75.14 (C-4), 71.33, 69.42, 67.26, 66.54 (C-5), 65.31 (C-6), 50.62, 50.33, 50.03 (C-2), 47.22, 46.21, 29.20, 27.93, 27.45, 25.57, 23.61, 23.37, 23.26. HRMS(ESI): calcd for $C_{45}H_{52}N_2O_{10}$ $[M+H]^+$: 781.3695, found: 781.3705.

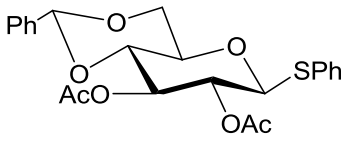


5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranoside (**97**): **96** (16.4 mg, 0.021 mmol) was dissolved in a mixture of t-BuOH (3.1 mL) and H_2O (0.075 mL). $Pd(OH)_2/C$ (30 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified by C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80%

MeOH/H_2O washes. The fractions containing product were concentrated to afford **97** (6.1 mg, 0.016 mmol, 77%) as a colorless solid. H^1 NMR (600 MHz, D_2O)**:** 4.84 (s, 1H, H-1), 4.54 (d, 1H, J = 4.70 Hz), 4.02 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.97 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 3.83-3.79 (m, 1H, OCH*H*), 3.73 (t, 1H, J = 10.56, C-6e), 3.33-3.61 (m, 2H, H-4, OCH*H*), 3.43 (td, 1H, J = 5.28, 9.98 Hz, H-5), 2.98 (t, 2H, J = 7.63 Hz), 2.07 (s, 3H, NHAc), 1.65 (p, 2H, J = 7.63 Hz, CH_2), 1.62-1.55 (m, 2H, CH_2), 1.46 (s, 3H, CH_3), 1.38 (p, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.34, 101.70 (C-1), 99.68, 73.94 (C-4), 69.73, 69.11 (C-3), 66.74 (C-5), 64.00 (C-6), 53.29 (C-2), 39.25, 27.89, 26.26, 24.57, 22.05, 21.94. HRMS(ESI): calcd for $C_{16}H_{28}N_2O_8$ $[M+H]^+$: 377.1918, found: 377.1930.

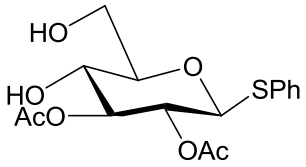


3-Mercapto-N-pentyl-propionamido 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranoside (**8**) TGT-8 SH To a stirring mixture of **97** (1.2 mg, 3.19 μmol) in water (0.25 mL) was added DTSSP (2.91 mg, 4.78 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (12mg, 0.080 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^oC) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **8** (1.2 mg, 2.58 μmol, 81%). HRMS(ESI): calcd for $C_{19}H_{32}N_2O_9S$ $[M+H]^+$: 465.1901, found: 465.1917.



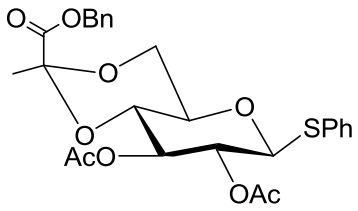
Phenyl 2,3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: (**99**): To a solution of **98** (1.32g, 3.65 mmol) in pyridine (14 mL), was added acetic anhydride (14 mL). After stirring for 2 h, the reaction

mixture was concentrated. The residue was taken up in toluene and concentrated under vacuum (3x) in order to remove any residual pyridine. The crude was taken up in ethyl acetate (50mL) and washed with water (50mL) and brine (50mL).The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (35% EtOAc/Hexanes) to give **99** (1.62 g, 3.6 mmol, 99%) as a colorless syrup. R_f = 0.9 (50% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.50-7.29 (m, 10H, aromatic), 5.50 (s, 1H, CH), 5.34 (t, 1H, J = 9.38 Hz, H-3), 5.01 (t, 1H, J = 9.96 Hz), 4.81 (d, 1H, J = 9.96 Hz, H-1), 4.39 (dd, 1H, J = 5.27, 10.55 Hz, H-6a), 3.80 (t, 1H, J = 9.96 Hz, H-6e), 3.67 (t, 1H, J = 9.38 Hz, H-4), 3.32-3.54 (m, 1H, H-5), 2.10 (s, 3H, OAc), 2.03 (s, 3H, OAc). ^{13}C NMR (75 MHz, $CDCl_3$): 170.23, 169.66, 136.88, 133.14, 131.89, 129.31, 129.20, 128.58, 128.39, 126.29, 101.67, 86.76 (C-1), 78.26 (C-4), 73.07 (C-3), 70.95 (C-2), 70.81 (C-5), 68.61 (C-6), 20.94. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{24}O_7S$ $[M+Na]_+$: 467.1135; found: 467.1155.

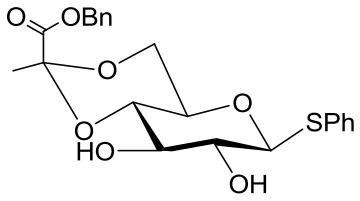


Phenyl 2,3-O-acetyl-1-thio-β-D-glucopyranoside (**100**): Compound **99** (1.05g, 2.36 mmol) was taken up in 80% HOAc(aq) (50 mL) and THF (9 mL) and heated at 50°C for 16 h. The mixture was concentrated and the residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (20% Acetone/Toluene) to give **100** (721 mg, 2.02 mmol, 86%) as an amorphous white solid. R_f = 0.10 (50% EtOAc /Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.47-7.43 (m, 2H, aromatic), 7.35-7.29 (m, 3H, aromatic), 5.05 (t, 1H, J = 9.38 Hz, H-3), 4.94 (t, 1H, J = 9.38 Hz, H-2), 4.75 (d, 1H, J = 9.96 Hz, H-1), 3.98-3.89 (m, 1H, H-6a), 3.85-3.77 (m, 1H, H-6e), 3.76-3.69 (dd, 1H, J = 4.69, 9.96 Hz, H-4), 3.49-3.3 (m, 1H, H-5), 2.86-2.76 (m, 1H, OH), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc). ^{13}C NMR (75 MHz, $CDCl_3$): 171.77, 169.65,

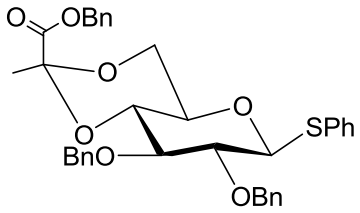
132.68, 132.19, 129.23, 128.42, 85.92 (C-1), 79.79 (C-5), 77.33 (C-3), 70.11 (C-2), 69.45 (C-4), 62.43 (C-6), 21.00, 20.95. HR MALDI-TOF MS: m/z: calcd for $C_{16}H_{20}O_7S$ $[M+Na]^+$: 379.0822; found: 379.0845.



Phenyl 2,3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-β-D-glucopyranoside: (**101**): Compound **100** (232 mg, 0.650 mmol) and benzyl pyruvate (232 mg, 1.30 mmol) were dissolved in dry acetonitrile (0.76 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.160 mL, 1.30 mmol) was added dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/hexanes) to give **101** (231 mg, 0.447 mmol, 69%) as an amorphous white solid. R_f = 0.45 (25% EtOAc/Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.43-7.28 (m, 10H, aromatic), 5.50 (s, 1H, CH), 5.29 (d, 1H, J = 12.20 Hz, CH*H*Ph), 5.21-5.17 (m, 2H, CH*H*Ph), 4.88 (t, 1H, J = 9.76 Hz, H-3), 4.73 (d, 1H, J = 9.76 Hz, H-1), 4.12-4.09 (m, 1H, C-6a), 3.72-3.67 (m, 1H, C-6e), 3.48-3.43 (m, 2H, H-4, H-5), 2.08 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.51 (s, 1H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 170.39, 169.54, 169.28, 135.19, 133.05, 131.86, 129.20, 128.84, 128.55, 128.39, 99.35, 86.73 (C-1), 74.40 (C-5), 72.95 (C-3), 70.73 (C-2), 70.35 (C-4), 67.70, 64.94 (C-6), 25.25, 20.92, 20.83. HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{28}O_9S$ $[M+Na]^+$: 539.1346; found: 539.1365.

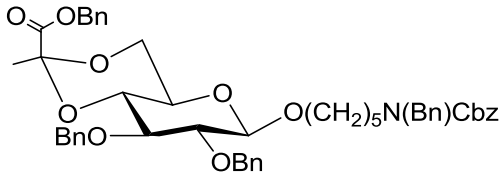


Phenyl 4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-β-D-glucopyranoside: (**102**): A clear solution of deacetylation reagent was prepared by dissolving Guanidinium Chloride (139 mg, 1.456 mmol) in MeOH (5.4 mL) and DCM (0.6 mL) along with 0.2M NaOMe (1.46 mL, 0.291 mmol). Compound **101** was dissolved into the mixture and stirred at room temperature for 10 min. The mixture was neutralized by the addition of Dowex 50WX8-200 acidic resin, filtered, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (40% EtOAc /Hexanes) to give **102** (267 mg, 0.617 mmol, 85%) as an amorphous white solid. R_f = 0.5 (50% EtOAc/Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.50-7.45 (m, 2H, aromatic), 7.41-7.29 (m, 8H, aromatic), 5.25 (dd, 2H, J = 11.14, 11.72, CH_2), 4.56 (d, 1H, J = 9.96 Hz, H-1), 4.08 (dd, 1H, J = 4.69, 10.55 Hz, H-6a), 3.76-3.63 (m, 2H, H-3, H-6e), 3.43-3.28 (m, 3H, H-2, H-4, H-5), 1.54 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 169.63, 135.09, 132.82, 132.06, 131.83, 131.77, 129.17, 129.13, 128.73, 128.65, 128.50, 128.46, 128.27, 99.26, 88.61 (C-1), 76.23 (C-4), 74.72 (C-3), 72.78 (C-5), 70.03 (C-2), 67.71, 65.03 (C-6), 25.27. HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{24}O_7S$ $[M+Na]^+$: 455.1135; found: 455.1156.



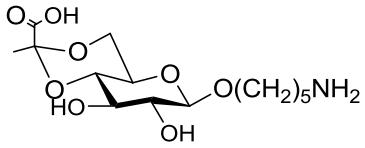
Phenyl 2,3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-β-D-mannopyranoside: (**103**): Compound **102** (0.125 g, 0.289 mmol) was dissolved in DMF (2.2 mL) and cooled to 0°C. 60% NaH (30 mg, 0.751 mmol) was added and the resulting mixture was stirred for 5 min. Benzyl Bromide (119 mg, 0.694 mmol) was added and the reaction mixture was stirred at 0°C for 1.5 h. Afterwards, the reaction

mixture was quenched by the dropwise addition of saturated ammonium chloride (aq) at 0°C. The reaction mixture was diluted with DCM (30 mL) and washed with water (50 mL, 3x) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (10% EtOAc/Hexanes) to give **103** (106 mg, 0.172 mmol, 60%) as a colorless syrup. R_f = 0.8 (35% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.50-7.26 (m, 20H, aromatic), 5.35-5.25 (dd, 2H, J = 8.21, 12.31 Hz, C*H*$_2$Ph), 5.00 (d, 1H, J = 11.14 Hz, CH*H*Ph'), 4.86-4.69 (m, 4H, H-1, CH*H*Ph', C*H*$_2$Ph''), 4.12 (dd, 1H, J = 5.28, 10.55 Hz, C-6a), 3.82-3.71 (m, 2H, C-3, C-6e), 3.55 (t, 1H, J = 9.96 Hz, H-4), 3.45-3.33 (m, H-2, H-5), 1.59 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 169.60, 138.62, 138.16, 135.29, 133.19, 132.36, 132.04, 129.10, 128.76, 128.68, 128.58, 128.50, 128.47, 128.35, 128.27, 128.15, 128.00, 127.95, 127.92, 127.69, 127.51, 99.03, 88.32 (C-1), 82.78 (C-3), 80.27 (C-5), 77.88 (C-4), 76.00, 74.76, 69.83 (C-2), 67.56, 65.18 (C-6), 25.53. HR MALDI-TOF MS: m/z: calcd for $C_{36}H_{36}O_7S$ $[M+Na]^+$: 635.2074; found: 635.2085.



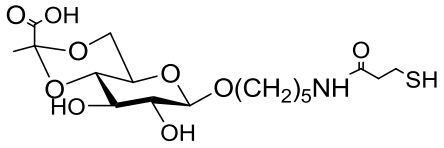
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-β-D-Glucopyranoside: (**104**): Compound **103** (25mg, 0.041 mmol), 2,4,6 tri-tert-butylpyrimidine (25 mg, 0.102 mmol), and diphenylsulfoxide (11 mg, 0.053 mmol) were dissolved in toluene and concentrated under vacuum (3x). The mixture was taken up in DCM (0.9 mL) along with activated 4Å molecular sieves (50 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78°C and trifluoromethanesulfonic anhydride (9 μL, 0.053 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50°C in order to activate the donor. The mixture was cooled back to -78°C and compound **94** (19mg, 0.057 mmol) was dissolved in a minimal amount of DCM and added by dropwise addition. The mixture was warmed to 0°C and quenched with

TEA, filtered, and concentrated under vacuum. The residue was taken up in DCM (30 mL) and washed with water (50 mL, 3x) and brine (30 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude was purified by silica gel column chromatography (25% EtOAc/Hexanes) to give an anomeric mixture 1/1 (β:α) of **104** (30 mg, 0.036 mmol, 88%) as a colorless syrup. Afterwards, preparatory TLC (2% Et_2O/DCM) was used to obtain the Beta anomer. R_f = 0.4 (α:β mix) (25% EtOAc/Hexanes) and R_f = 0.5 (β), (2% Et_2O/DCM). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.38-7.11 (m, 25H, aromatic), 5.25 (dd, 2H, J = 12.31, 2.93 Hz, C*H*$_2$Ph), 5.16 (br.s, 2H, C*H*$_2$Ph), 4.93 (d, 1H, J = 11.72 Hz, CH*H*Ph'), 4.82-4.69 (m, 3H, CH*H*Ph', C*H*$_2$Ph''), 4.47 (br.s, 2H, C*H*$_2$Ph'''), 4.42-4.34 (m, 1H, H-1), 4.05 (dd, 1H, J = 4.69, 10.55 Hz), 3.85-3.67 (m, 2H, C-6e, OC*H*H, 3.61 (t, 1H, J = 8.79, H-3), 3.54-3.37 (m, 2H, H-4, OC*H*H), 3.37-3.10 (m, 4H, H-2, H-5, NC*H*$_2$), 1.71-1.41 (m, 7H, 2xCH_2, CH_3), 1.40-1.19 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 169.69, 138.92, 138.57, 138.05, 136.99, 135.32, 128.78, 128.67, 128.58, 128.42, 128.29, 128.15, 127.98, 127.78, 127.54, 127.45, 104.04 (H-1), 99.11, 81.88 (C-5), 80.84 (C-3), 77.98 (C-4), 75.47, 74.52, 70.28, 67.55, 67.30, 65.64 (C-2), 65.32 (C-6), 50.67, 50.42, 47.25, 46.32, 29.59, 28.03, 27.65, 25.59, 23.44. HRMS(ESI): calcd for $C_{50}H_{55}NO_{10}$ $[M+Na]^+$: 830.3899, found: 830.3914.

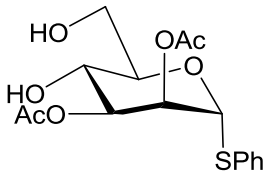


5-aminopentyl 4,6-*O*-[(S)-1-Carboxylethylidene]-β-D-Glucopyranoside (**105**): **104** (29 mg, 0.035 mmol) was dissolved in a mixture of t-BuOH (3 mL) and H_2O (0.075 mL). $Pd(OH)_2$/C (60 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes.

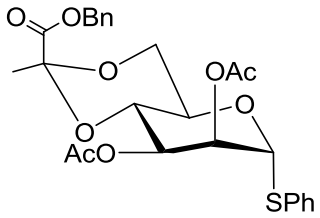
The fractions containing product were concentrated to afford **105** (11 mg, 0.032 mmol, 92%) as a crystalline white solid. H^1 NMR (600 MHz, D_2O)**:** 7.38-7.11 (m, 25H, aromatic), 5.25 (dd, 2H, J = 12.31, 2.93 Hz, C*H*$_2$Ph), 4.56 (d, 1H, J = 8.21 Hz, H-1), 4.14 (dd, 1H, J = 4.69, 10.55 Hz, H-6a), 3.93-3.86 (m, 1H, OC*H*H), 3.73-3.77 (m, 3H, H-3, H-6e, OC*H*H), 3.55 (1H, dd, J = 4.69, 9.38 Hz, H-5), 3.44 (t, 1H, J = 9.38 Hz, H-4), 3.32 (t, 1H, J = 8.79 Hz), 3.01 (t, 1H, J = 7.62 Hz, NC*H*$_2$), 1.74-1.62 (m, 4H, 2xCH_2), 1.57 (s, 3H, CH_3). ^{13}C NMR (150 MHz, D_2O): 173.06, 102.79 (C-1), 99.66, 76.05 (C-4), 73.82 (C-2), 72.68 (C-3), 70.36, 65.34 (C-5), 64.44 (C-6), 39.27, 28.07, 26.31, 24.38, 21.97. HRMS(ESI): calcd for $C_{14}H_{25}NO_8$ $[M+H]^+$: 336.1653, found: 336.1662.



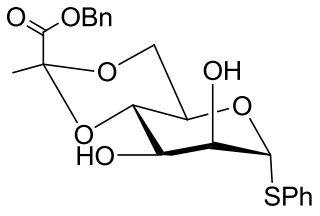
3-Mercapto-N-pentyl-propionamido 4,6-*O*-[(S)-1-Carboxylethylidene]-β-D-Glucopyranoside (**9**) To a stirring mixture of **105** (1.0 mg, 2.98 μmol) in water (0.25 mL) was added DTSSP (2.72 mg, 4.27 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (11 mg, 0.075 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **9** (1.2 mg, 2.83 μmol, 95%) as a white solid. HRMS(ESI): calcd for $C_{17}H_{29}N_4O_9S$ $[M+H]^+$: 424.1636, found: 424.1649.



Phenyl 2,3-O-acetyl-1-thio-α-D-mannopyranoside (**108**): To a solution of **106** (1.86g, 5.16 mmol) in pyridine (20 mL), was added acetic anhydride (20 mL). After stirring for 2 h, the reaction mixture was concentrated and the residue was taken up in toluene and concentrated under vacuum (3x) in order to remove any residual pyridine. The crude was taken up in ethyl acetate (50mL) and washed with water (50mL) and brine (50mL).The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum (R_f= 0.5; 35% EtOAc/Hexanes). The crude material was used in the proceeding reaction without further purification. Crude compound **107** was taken up in 80% HOAc (aq) (80 mL) and THF (20 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated. The residue was taken up in toluene and concentrated under vacuum (3x) in order to facilitate the removal of residual acetic acid. The resulting residue was purified by silica gel column chromatography (20% Acetone/Toluene) to give **108** (1.2 g, 3.44mmol, 66% over two steps) as an amorphous white solid. R_f = 0.15 (50% EtOAc /Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.49-7.46 (m, 2H, aromatic), 7.35-7.26 (m, 3H, aromatic), 5.50-5.49 (m, 1H, H-2), 5.44 (s, 1H, H-1), 5.18 (dd, 1H, J = 2.93, 9.38 Hz, H-3), 4.26-4.21 (m, 1H, H-5), 4.13-4.05 (m, 1H, H-4), 3.89 (d, 1H, J = 3.52 Hz, H-6), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc). ^{13}C NMR (75 MHz, $CDCl_3$): 171.02, 170.04, 132.99, 132.47, 131.86, 129.37, 128.26, 86.18 (C-1), 73.51 (C-5), 72.41 (C-3), 71.36 (C-2), 66.43 (C-4), 62.26 (C-6), 21.01, 20.97. HR MALDI-TOF MS: m/z: calcd for $C_{16}H_{20}O_7S$ $[M+Na]^+$: 379.0822; found: 379.0845.

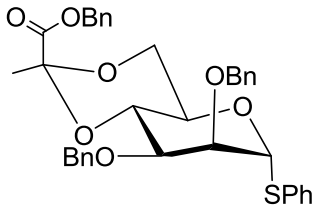


Phenyl 2,3-*O*-acetyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α-D-mannopyranoside: (**109**): Compound **108** (300 mg, 0.842 mmol) and Benzyl pyruvate (300 mg, 1.684 mmol) were dissolved in dry acetonitrile (0.98 mL) under argon atmosphere. $BF_3(OEt)_2$ (0.207 mL, 1.684 mmol) was added by dropwise to the stirring mixture and the reaction was allowed to proceed at room temperature for 16 h. The reaction mixture was quenched *via* dropwise addition of $NaHCO_3$ and filtered. The mixture was diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (30 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc/hexanes) to give **109** (282 mg, 0.545 mmol, 65%) as an amorphous white solid. R_f = 0.45 (25% EtOAc/Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.47-7.26 (m, 10 H, aromatic), 5.54-5.52 (m, 1H, H-2), 5.40 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.36 (s, 1H, H-1), 5.31 (dd, 1H, J = 3.52, 9.96 Hz, C-3), 5.16 (d, 1H, J = 12.31 Hz, CH*H*Ph), 4.36 (td, 1H, J = 5.27Hz), 4.01-3.92 (m, 2H, H-4, H-6a), 3.80 (t, 1H, J = 10.55 Hz, H-6e), 2.04 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.57 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.06, 169.86, 169.65, 135.41, 132.93, 132.32, 129.37, 128.75, 128.59, 128.26, 128.03, 99.72, 86.97 (C-1), 72.60 (C-4), 71.65 (C-2), 68.43 (C-3), 67.47, 64.90 (C-5, C-6), 25.56, 20.98, 20.92. HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{28}O_9S$ $[M+Na]^+$: 539.1346; found: 539.1360.



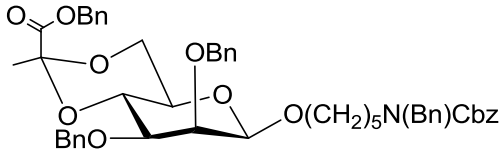
Phenyl 4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α-D-mannopyranoside: (**110**): A clear solution of deacetylation reagent was prepared by dissolving guanidinium chloride (100 mg, 1.038

mmol) in MeOH (3.8mL) and DCM (0.42mL) along with 0.2M NaOMe (1.038 mL, 0.208 mmol). Compound **109** was dissolved into the mixture and stirred at room temperature for 10 min. The mixture was neutralized by the addition of Dowex 50WX8-200 acidic resin, filtered, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (40% EtOAc /Hexanes) to give **110** (156 mg, 0.361 mmol, 70%) as an amorphous white solid. R_f = 0.5 (50% EtOAc/Hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.45-7.26 (m, 10 H, aromatic), 5.52 (s, 1H, H-1), 5.34 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.17 (d, 1H, J = 11.72 Hz, CH*H*Ph), 4.25-4.16 (m, H-2, H-5), 4.01-3.92 (m, 2H, H-3, H-6a), 3.73 (t, 2H, J = 9.96, H-4, H-6e), 1.56 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 169.75, 135.23, 133.46, 131.74, 129.29, 128.87, 128.67, 128.84, 128.67, 127.86, 99.76, 87.95 (C-1), 75.22 (C-4), 72.26 (C-5), 69.15 (C-3), 67.76, 65.10 (C-6), 63.79 (C-2), 25.50. HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{24}O_7S$ $[M+Na]^+$: 455.1135; found: 455.1149.



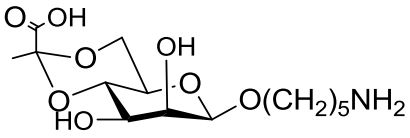
Phenyl 2,3-O-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-1-thio-α-D-mannopyranoside (**111**): Compound **110** (0.148 g, 0.342 mmol) was dissolved in DMF (2.6 mL) and cooled to 0°C. 60% NaH (36 mg, 0.888 mmol) was added and the resulting mixture was stirred for 5 min. Benzyl Bromide (140 mg, 0.820 mmol) was added and the reaction mixture was stirred at 0°C for 1.5 h. Afterwards, the reaction mixture was quenched by the dropwise addition of saturated ammonium chloride (aq) at 0°C. The reaction mixture was diluted with DCM (30 mL) and washed with water (50 mL, 3x) and brine (30 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (10% EtOAc/Hexanes) to give **110** (136 mg, 0.222 mmol, 65%) as a colorless syrup. R_f = 0.75 (25% EtOAc/hexanes). H^1 NMR (300 MHz,

$CDCl_3$)**:** 7.45-7.25 (m, 15 H, aromatic), 5.45 (s, 1H, H-1), 5.29 (dd, 1H, J = 12.31, 21.69 Hz, C*H*$_2$Ph), 4.94 (d, 1H, 12.31 Hz, CH*H*Ph'), 4.76-4.66 (m, 3H, CH*H*Ph', CH*H*Ph''), 4.27-4.16 (m, 2H, H-3, H-5), 4.04 (s, 1H, H-2), 4.01-3.86 (m, 3H, H-4, H-6), 1.63 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 169.94, 138.85, 138.07, 135.34, 133.92, 131.58, 129.23, 128.67, 128.44, 128.35, 128.09, 128.03, 127.83, 127.69, 127.52, 99.52, 87.55 (C-1), 78.52 (C-2), 76.44 (C-4), 75.95 (C-3), 73.33, 73.15, 67.53, 65.09 (C-6), 65.04 (C-5), 25.76. HR MALDI-TOF MS: m/z: calcd for $C_{36}H_{36}O_7S$ $[M+Na]^+$: 635.2074; found: 635.2092.

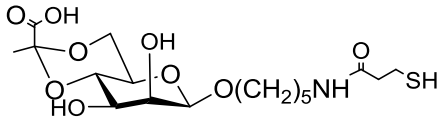


N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene] -β-D-Mannopyranoside: (**112**): Compound **111** (85mg, 0.139 mmol), 2,4,6 tri-tert-butylpyrimidine (86 mg, 0.347 mmol), and diphenylsulfoxide (36 mg, 0.180 mmol) were dissolved in toluene and concentrated under vacuum (3x). The dried mixture was taken up in DCM (2.8 mL) along with activated 4Å molecular sieves (150 mg) under argon atmosphere and stirred at room temperature for 30 mins. The mixture was cooled to -78^{o}C and trifluoromethanesulfonic anhydride (30 μL, 0.180 mmol) was added *via* dropwise addition. The mixture was allowed to warm to -50^{o}C in order to activate the donor. The mixture was cooled back to -78^{o}C and compound **94** (64mg, 0.194 mmol) was dissolved in a minimal amount of DCM and added by dropwise addition. The mixture was allowed to warm to 0^{o}C and before being quenched with TEA, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15-20% EtOAc/Hexanes) to give **112** in 2/1 (β:α) ratio (75 mg, 0.091 mmol, 66%) as a colorless syrup. R_f = 0.45 (α), 0.4 (β) (25% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.39-7.17 (m, 25 H, aromatic), 5.28 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.2-5.09 (m 3H, H-1, CH*H*Ph, C*H*$_2$Ph'), 4.89-4.74 (m, 3H, C*H*$_2$Ph'', CH*H*Ph'''), 4.58 (d, 1H, J = 12.31 Hz, CH*H*Ph'''), 4.47 (br.s, 2H, C*H*$_2$Ph^{IV}), 4.33 (d, 1H, J = 9.96 Hz, H-1), 4.09-4.00 (m, 2H, H-4, H-6a), 3.90 (t, 1H, J = 10.55, H-6e),

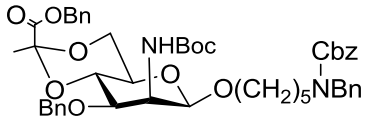
3.87-3.73 (m, 2H, H-2, OCH*H*), 3.48 (dd, 1H, J = 2.93, 9.38 Hz), 3.37-3.10 (m, 4H, H-5, OCH*H*, NHCH_2), 1.59-1.4 (m, 5H, CH_3, 2xCH_2), 1.34-1.2 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): (Rotomers) 169.72, 156.70, 156.22, 138.72, 138.57, 137.77, 136.75, 135.13, 129.01, 128.54, 128.51, 128.42, 128.33, 128.20, 128.16, 127.97, 127.88, 127.79, 127.49, 127.30, 101.91 (C-1), 99.33, 78.24 (C-3), 76.30 (C-2), 75.47 (C-4), 74.56, 72.42, 69.74, 67.45, 67.13, 67.06 (C-5), 65.05 (C-6), 50.51, 50.23, 47.07, 46.14, 29.26, 27.87, 27.44, 25.55, 23.28. HRMS(ESI): calcd for $C_{50}H_{55}NO_{10}$ $[M+Na]^+$: 830.3899, found: 830.3914.



5-aminopentyl 4,6-*O*-[(S)-1-Carboxylethylidene]-β-D-Mannopyranoside (**113**): Compound **112** (40 mg, 0.048 mmol) was dissolved in a mixture of t-BuOH (3.1 mL) and H_2O (0.075 mL). $Pd(OH)_2$/C (60 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified by C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O. The fractions containing product were concentrated to afford an anomeric mixture (2.3 : 1, β:α) of **113** (6.1 mg, 0.016 mmol, 77%) as a colorless solid. The anomers were separated by LCMS using a ZIC-HILIC semi-prep column with ammonium bicarbonate/Acetonitrile eluent. The alpha peaks was first to elute. H^1 NMR (600 MHz, D_2O)**:** 4.72 (s, 1H, H-1), 4.03-4.00 (m, 2H, H-2, H-6a), 3.85-3.78 (m, 2H, H-3, OC*H*H), 3.72-3.64 (m, 3H, H-4, H-6e, OC*H*H), 3.37 (td, 1H, J = 4.70, 10.06Hz, H-5), 2.98 (t, 2H, J = 7.63 Hz), NHCH_2), 1.69-1.60 (m, 4H, 2xCH_2), 1.45 (s, 3H, CH_3), 1.45-1.39 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.20, 170.43, 101.63, 100.39 (C-1), 73.69 (C-4), 70.85 (C-2), 70.36 (C-3), 69.69, 66.38 (C-5), 64.12 (C-6), 39.22, 28.00, 26.27, 24.63, 22.02. HRMS(ESI): calcd for $C_{14}H_{25}NO_8$ $[M+H]^+$: 336.1653, found: 336.1666.

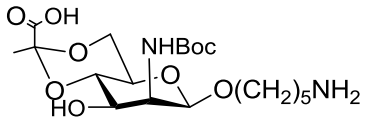


3-Mercapto-N-pentyl-propionamido 4,6-*O*-[(S)-1-Carboxylethylidene]-β-D-Mannopyranoside (**10**) To a stirring mixture of **113** (1.0 mg, 2.98 μmol) in water (0.25 mL) was added DTSSP (2.72 mg, 4.47 μmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (11 mg, 0.075 mmol) was added and the reaction mixture was stirred in a warm water bath (~40^{o}C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **10** (1.2 mg, 2.83 μmol, 95%). HRMS(ESI): calcd for $C_{17}H_{29}NO_9S$ $[M+H]^+$: 424.1636, found: 424.1650.



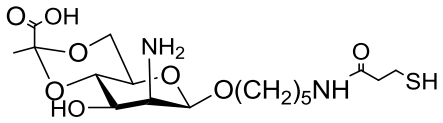
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-t-Butoxycaronylamino-3-*O*-benzyl-4,6-*O*-[(S)-1-Benzyloxycarbonylethylidene]-2-deoxy-β-D-Mannopyranoside (114): To a stirring solution of **95** (55 mg, 0.072 mmol) in acetic acid (3.15 mL) was added a large excess of Zn-Cu couple (325 mg). The mixture was allowed to stir for 1hr. Completion of the reaction was verified by MALDI. The reaction mixture was filtered and concentrated under vacuum. The crude was taken up in toluene and concentrated under vacuum 3x. The crude was dissolved in THF (3.2 mL) along with triethylamine (20 μL, 0.14 mmol) and Boc_2O (31 mg, 0.14 mmol). The reaction mixture was stirred overnight at RT. Completion of the reaction was verified by TLC (25% Ea/Hex) and MALDI. The reaction mixture was concentrated under vacuum and the crude was taken up in Ethyl acetate (20 mL), washed with water (20 mL), dried over MgSO4, and concentrated under vacuum. The crude was chromatographed with

20%EtOAc/Hexanes to afford **114** (49 mg, 0.058 mmol, 81%) as a colorless solid. R_f = 0.25 (25% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.40-7.14 (m, 20H, aromatic), 5.32 (d, 1H, J = 12.31 Hz, CH*H*Ph), 5.20-5.12 (m, 3H, CH*H*Ph, C*H*$_2$Ph'), 4.73 (dd, 1H, J = 29.31, 12.31 Hz, C*H*$_2$Ph'), 4.50-4.35 (m, 4H, H-1, H-2, CH*H*Ph''), 4.01-3.96 (m, 1H, H-6a), 3.72-3.45 (m, 4H, H-3, H-4, H-6e, OC*H*H), 3.44-3.11 (m, H-5, C*H*$_2$N, OC*H*H), 1.57-1.37 (m, 16H, 4xCH_3, 2xCH_2), 1.35-1.15 (m 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 169.59, 156.37, 138.50, 138.05, 128.78, 128.67, 128.58, 128.50, 128.38, 128.29, 128.23, 128.04, 127.97, 127.77, 127.46, 127.42, 100.45 (C-1), 99.34, 79.60, 75.48 (C-3), 75.18 (C-4), 71.02, 69.33, 67.45, 67.29, 66.68 (C-5), 65.29 (C-6), 51.73 (C-2), 50.68, 50.38, 47.25, 46.32, 29.85, 29.28, 28.49, 25.59, 23.29. HRMS(ESI): calcd for $C_{48}H_{58}N_2O_{11}$ $[M+H]^+$: 839.4113, found: 839.4122.



5-aminopentyl 2-t-Butoxycaronylamino-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranoside (**115**): Compound **114** (15.9 mg, 0.019 mmol) was dissolved in a mixture of t-BuOH (3.1 mL) and H_2O (0.077 mL). $Pd(OH)_2$/C (23 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O). The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated to afford **115** (6.1 mg, 0.014 mmol, 74%) as a colorless solid. H^1 NMR (600 MHz, D_2O)**:** . H^1 NMR (600 MHz, $CDCl_3$)**:** 4.82 (s, 1H, H-1), 4.19 (brs, 1H, H-2), 4.00 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.92 (dd, 1H, J = 4.11, 9.98 Hz, H-3), 3.83-3.79 (m, 1H, OCH*H*), 3.72-3.62 (m, 2H, C-6e, OCH*H*), 3.51 (t, 1H, J = 9.98 Hz, H-4), 3.41-3.37 (m, 1H, H-5), 2.97 (t, 2H, J = 8.22 Hz, NC*H*$_2$), 1.70-1.52 (m, 4H, 2xCH_2), 1.45-1.39 (m, 14H, 4xCH_3, CH_2), ^{13}C NMR (150 MHz, D_2O): 175.38, 158.49, 101.68 (C-1), 99.94, 80.92, 74.14 (C-4),

69.54, 69.07 (C-3), 66.64 (C-5), 64.03 (C-6), 54.71 (C-2), 39.25, 27.98, 27.49, 26.27, 24.57, 22.04. HRMS(ESI): calcd for $C_{14}H_{26}N_2O_7$ $[M+H]^+$: 335.1813, found: 335.1826.



3-Mercapto-N-pentyl-propionamido 2-amino-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranoside (**11**): To a stirring mixture of **115** (6.0 mg, 0.014 mmol) in water (0.25 mL) was added DTSSP (13 mg, 0.021 mmol). 0.1M NaOH was added to the mixture *via* dropwise addition to adjust the pH to ~8. The reaction was complete within 1 h and product formation was verified by LC/MS. Afterwards, DTT (10.6 mg, 0.069 mmol) was added and the reaction mixture was stirred in a warm water bath (~40°C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized. The intermediate was taken up in DCM (1.2 mL) at 0°C was added triethylsilane (0.123 mL, 0.772 mmol), followed by the dropwise addition of TFA (0.6 mL, 8.05 mmol). The mixture was allowed to stir for 15 min at 0°C. Completion of the reaction was verified by LCMS. The crude was dissolved in PBS (pH 7.4, 250 μL) and DTT (10.6 mg, 0.069 mmol) was added and the reaction mixture was stirred in a warm water bath (~40°C) for 30 mins. Completion of the reaction was verified by LC/MS. The reaction mixture was loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **11** (3.9 mg, 9.23 μmol, 67%). H^1 NMR (600 MHz, D_2O)**:** 5.01 (s, 1H, H-1), 4.17 (dd, 1H, J = 4.70, 9.98 Hz, H-3), 4.06 (dd, 1H, J = 5.28, 10.56 Hz, H-6a), 3.87-3.83 (m, 1H, OCH*H*), 3.82-3.80 (m, 1H, H-2), 3.74-3.66 (m, 2H, C-6e, OCH*H*), 3.60 (t, 1H, J = 9.98 Hz, H-4), 3.50-3.46 (m, 1H, H-5), 3.20 (t, 2H, J = 7.63 Hz, NCH_2), 2.77 (t, 2H, J = 6.46 Hz, CH_2), 2.54 (t, 2H, J = 6.46 Hz, CH_2), 1.63-1.59 (m, 2H, CH_2), 1.55-1.50 (m, 2H, CH_2), 1.47 (s, 1H, CH_3), 1.39-1.34 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): 175.24, 174.09, 101.79, 97.11 (C-1), 73.13 (C-4), 70.13, 66.79 (C-3), 66.07 (C-5), 63.91 (C-6), 54.77 (C-2), 39.31,

39.12, 28.14, 27.87, 24.57, 22.35, 19.96. HRMS(ESI): calcd for $C_{17}H_{30}N_2O_8S$ $[M+H]^+$: 423.1796, found: 423.1812.

REFERENCES

1) a) Koch, R. Untersuchungen über Bakterien: V. Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des *Bacillus anthracis*, *Beitr. Biol. Pflanz*., **1876**, *2*, 277–310. (1876). b) Mock M. & Fouet A. Anthrax. *Annu. Rev. Microbiol*. **2001,** *55*, 647-671.
2) a) Engelhardt, H. Crystalline Surface Layers on Bacteria. *J. Struct. Biol.* **2007**, *160*, 115-124. b) U.B. Sleytr *et al*. Crystalline surface layers on bacteria. *Annu Rev Microbiol.***1983**, *37*, 311-339. C) Sara M. & Sleytr U.B. S-Layer proteins*. J. Bacteriol.* **2000**, *182*(4), 859-868., d) Albers S.V. & Meyer B.H. The archaeal cell envelope. *Nat. Rev. Microbiol*. **2011**, *9*(6), 414-426. e) Ilk N., Egelseer E.M. & Sleytr U.B. S-layer fusion proteins-construction principles and applications. *Curr. Opin. Biotechnol*. **2011**, *22*(6), 824-831.
3) Fairweather, N. F., Fagan, R. P. Biogenesis and functions of bacterial S‐layers. *Nature Reviews. Microbiology* **2014**, *12*(3), 211-22.
4) Kern V.J., Kern J.W., Theriot J.A., Schneewind O. & Missiakas D. Surface-layer (S-layer) proteins Sap and EA1 govern the binding of the S-layer-associated protein BslO at the cell septa of *Bacillus anthracis*. *J. Bacteriol.* **2012**, *194*(15), 3833-3840.
5) Nguyen-Mau, S. M., Oh, S. Y., Kern, V. J., Missiakas, D. M. & Schneewind, O. Secretion genes as determinants of Bacillus anthracis chain length*. J. Bacteriol*. **2012**, *194*, 3841–3850.
6) Etienne-Toumelin , Sirard J.C., Duflot E., Mock M., Fouet A. Characterization of the Bacillus anthracis S-layer: cloning and sequencing of the structural gene. *J Bacteriol*. **1995**, *177*(3), 614-20.
7) Kern J.W. & Schneewind O. BslA, a pXO1-encoded adhesin of *Bacillus anthracis*. *Mol. Microbiol.* **2008**, *68*(2), 504-515.

8) Tarlovsky Y., Fabian M., Solomaha E., Honsa E., Olson J.S. & Maresso A.W. A *Bacillus anthracis* S-layer homology protein that binds heme and mediates heme delivery to IsdC. *J. Bacteriol.* **2010**, *192*(13), 3503-3511.

9) Anderson V.J., Kern J.W., McCool J.W., Schneewind O. & Missiakas D. The SLH-domain protein BslO is a determinant of *Bacillus anthracis* chain length. *Mol. Microbiol.* **2011**, *81*(1), 192-205.

10) Forsberg L.S., Choudhury B., Leoff C., Marston C.K., Hoffmaster A.R., Saile E., Quinn C.P., Kannenberg E.L., Carlson R.W. Secondary cell wall polysaccharides from *Bacillus cereus* strains G9241, 03BB87 and 03BB102 causing fatal pneumonia share similar glycosyl structures with the polysaccharides from *Bacillus anthracis*. Glycobiology. **2011**, *21*(7), 934-48.

11) Mesnage S., Fontaine T., Mignot T., Delepierre M., Mock M., Fouet A. Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *EMBO J*. **2000**, *19*(17), 4473-4484.

12) Lunderberg JM, Liszewski Zilla M, Missiakas D, Schneewind O, Bacillus anthracis tagO Is Required for Vegetative Growth and Secondary Cell Wall Polysaccharide Synthesis. *J Bacteriol*. **2015**, *197*(22), 3511-20.

13) Lunderberg J.M., Nguyen-Mau S.-M., Richter G.S., Wang Y.-T., Dworkin J., Missiakas, D.M., and Schneewind O.; *Bacillus anthracis* Acetyltransferases PatA1 and PatA2 Modify the Secondary Cell Wall Polysaccharide and Affect the Assembly of S-Layer Proteins*, J Bacteriol*. **2013**, *195*(5), 977–989.

14) Forsberg L.S., Abshire T.G., Friedlander A., Quinn C.P., Kannenberg E.L., Carlson R.W. Localization and structural analysis of a conserved pyruvylated epitope in *Bacillus anthracis* secondary cell wall polysaccharides and characterization of the galactose-deficient wall polysaccharide from avirulent B. anthracis CDC 684. Glycobiology. **2012**, *22*(8), 1103-17.

15) Etienne-Toumelin, Sirard J.C., Duflot E., Mock M., Fouet A. Characterization of the *Bacillus anthracis* S-layer: cloning and sequencing of the structural gene. *J. Bacteriol*. **1995**, *177*(3), 614-20.

16) Mesnage, S., Tosicouture E., Mock M., Gounon P., and Fouet A. Molecular characterization of the Bacillus anthracis main S-layer component: evidence that it is the major cell-associated antigen. *Mol. Microbiol*. **1997**, *23***,** 1147-1155.

17) Mignot T., Mesnage S., Couture-Tosi E., Mock M., Fouet A. Developmental switch of S-layer protein synthesis in *Bacillus anthracis*. *Mol. Microbiol*. **2002**, *43*, 1615–1627.

18) Couture-Tosi E., et al. Structural analysis and evidence for dynamic emergence of *Bacillus anthracis* S-layer networks. *J. Bacteriol*. **2002**, *184*, 6448–6456.

19) Kern J.W., Schneewind O. BslA, a pXO1-encoded adhesin of Bacillus anthracis. *Mol. Microbiol*. **2008**, *68*, 504-515.

20) Tarlovsky Y., Fabian M., Solomaha E., Honsa E., Olson J.S., and Maresso A.W. A Bacillus anthracis S-Layer Homology Protein That Binds Heme and Mediates Heme Delivery to IsdC. *J Bacteriol*. **2010**, *192*, 3503-3511.

21) Kern J., Schneewind O. BslA, the S-layer adhesin of *B. anthracis*, is a virulence factor for anthrax pathogenesis. *Mol. Microbiol*. **2010**, *75*, 324-332.

22) Anderson V.J., Kern J.W., McCool J.W., Schneewind O., Missiakas D. The SLH-domain protein BslO is a determinant of Bacillus anthracis chain length. *Mol. Microbiol*. **2011**, *81*, 192-205.

23) Liszewski Zilla M., Chan Y.G., Lunderberg J.M., Schneewind O., Missiakas D. LytR-CpsA-Psr enzymes as determinants of Bacillus anthracis secondary cell wall polysaccharide assembly. *J Bacteriol.* **2015**, *197*(2), 343-53.

24) Liszewski Zilla M., Lunderberg J.M., Schneewind O., Missiakas D. *Bacillus anthracis* lcp Genes Support Vegetative Growth, Envelope Assembly, and Spore Formation. *J Bacteriol*. **2015**, *197*(23), 3731-41.

25) Mesnage S., Fontaine T., Mignot T., Delepierre M., Mock M., Fouet A. Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *EMBO J*. **2000**, *19*(17), 4473-4484.

26) Kern J., Ryan C., Faull K., Schneewind O. Bacillus anthracis surface-layer proteins assemble by binding to the secondary cell wall polysaccharide in a manner that requires csaB and tagO. *J Mol Biol*. **2010**, *401*(5), 757-775.

27) Kern J.,1, Wilton R., Zhang R., Binkowski T.A., Joachimiak A. and Schneewind O. Structure of Surface Layer Homology (SLH) Domains from Bacillus anthracis Surface Array Protein. *J Biol Chem*. **2011**, *286*, 26042-26049.

28) Candela T., Mignot T., Hagnerelle X., Haustant M., Fouet A. Genetic analysis of Bacillus anthracis Sap S-layer protein crystallization domain. *Microbiology* **2005**, *151*, 1485–1490.

29) Houwink A. L., Le Poole J. B. Eine Struktur in der Zellmembran einer Bakterie. Physikalische Verhandlungen **1952**, *3*, 98.

30) Choudhury B., Leoff C., Saile E., Wilkins P., Quinn C.P., Kannenberg E., and Carlson R.W. The Structure of the Major Cell Wall Polysaccharide of Bacillus anthracisIs Species-specific. *J Biol Chem* **2006**, *281*, 27932-27941.

31) a) Kojima N., Araki Y., Ito E. Structure of the linkage units between ribitol teichoic acids and peptidoglycan. *J Bacteriol*. **1985**, *161*(1), 299-306. b) Soufiane B., Côté J.C. *Bacillus weihenstephanensis* characteristics are present in *Bacillus cereus* and *Bacillus mycoides* strains. *FEMS Microbiol Lett*. **2013**, *341*(2), 127-37.

32) Wang Y.T., Oh S.-Y., Hendrickx A.P.A., Lunderberg J.M., and Schneewind O. *Bacillus cereus* G9241 S-Layer Assembly Contributes to the Pathogenesis of Anthrax-Like Disease in Mice. *J Bacteriol*. **2013**, *195*(3), 596–605.

33) Sara M. & Sleytr U.B. S-Layer proteins. *J. Bacteriol*. **2000**, *182*(4), 859-868.

34) Ruthel G., Ribot W.J., Bavari S., Hoover T.A. Time-lapse confocal imaging of development of Bacillus anthracis in macrophages. *J. Infect. Dis*. **2004**, *189*(7), 1313-6.

35) Laaberki M.H., Pfeffer J., Clarke A.J., Dworkin J. O-Acetylation of peptidoglycan is required for proper cell separation and S-layer anchoring in Bacillus anthracis. *J Biol Chem*. **2011**, *286*(7), 5278-88.

36) Leoff C., Choudhury B., Saile E., Quinn C.P., Carlson R.W., and Kannenberg E.L. Structural Elucidation of the Nonclassical Secondary Cell Wall Polysaccharide from Bacillus cereus ATCC 10987. *J Biol Chem*. **2008**, *283*(44), 29812-29821.

37) (a) Ziegler T., Eckhardt E., Birault V. Synthetic studies toward pyruvate acetal containing saccharides. Synthesis of the carbohydrate part of the Mycobacterium smegmatis pentasaccharide glycolipid and fragments thereof for the preparation of neoantigens. *J. Org. Chem*. **1993**, *58*, 1090-1099. (b) Ziegler T., Eckhardt E., Birault V. Synthetic studies toward pyruvate acetal containing saccharides. Synthesis of the carbohydrate part of the Mycobacterium smegmatis pentasaccharide glycolipid and fragments thereof for the preparation of neoantigens. *J. Org. Chem.* **1993**, *58*(5), 1090-1099.

38) a) Paulsen H., *Angew. Chem*. **1982**, *94*, 184 – 201.; b) Advances in Selective Chemical Syntheses of Complex Oligosaccharides *Angew. Chem. Int. Ed. Engl*. **1982**, *21*, 155 – 173.; c) Barresi F., Hindsgaul O. Modern Methods in Carbohydrate Synthesis (Eds.: S. H. Khan, R. A. O'Neill), Harwood , Amsterdam, pp. 251 – 276 (1996); d) Pozsgay V. in Carbohydrates in Chemistry and Biology, Vol.1 (Eds.: B. Ernst, G. W. Hart, P. Sinay), Wiley-VCH, Weinheim, pp. 319 – 343 (2000).;

e) Gridley J.J., Osborn H.M.I. Recent advances in the construction of β-D-mannose and β-D-mannosamine linkages. *J. Chem. Soc. Perkin Trans. 1*: **2000**, 1471 – 1491.; f) Demchenko A.V., *Synlett*. **2003**, 1225 – 124.

39) Bongat A.F.G. & Demchenko A.V. Recent trends in the synthesis of O-glycosides of 2-amino-2-deoxysugars. *Carbohydr. Res.* **2007**, *342*(3-4), 374-406.

40) Blatter G., Beau J.M. & Jacquinet J.C. The use of 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives in syntheses of oligosaccharides. *Carbohydr. Res.* **1994**, *260*(2), 189-202.

41) Liu R., Xu Y., Chen M., Weiwer M., Zhou X., Bridges A.S., DeAngelis P.L., Zhang Q., Linhardt R.J. & Liu J. Chemoenzymatic design of heparan sulfate oligosaccharides. *J. Biol. Chem.* **2010**, *285*(44), 34240-34249.

42) Classon B., Garegg P.J., Oscarson S. & Tiden A.K. Synthesis of an artificial antigen that corresponds to a disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* Type-D - a facile synthesis of methyl 2-acetamido-2-deoxy-beta-D-mannopyranoside. *Carbohydr. Res.* **1991**, *216*, 187-196.

43) Mo K.F., Li X., Li H., Low L.Y., Quinn C.P. & Boons G.J. Endolysins of *Bacillus anthracis* bacteriophages recognize unique carbohydrate epitopes of vegetative cell wall polysaccharides with high affinity and selectivity. *J. Am. Chem. Soc.* **2012**, *134*(37), 15556-15562.

44) Prabhu A., Venot A. & Boons G.J. New set of orthogonal protecting groups for the modular synthesis of heparan sulfate fragments. *Org. Lett.* **2003**, *5*(26), 4975-4978.

45) Zhu X.M. & Schmidt R.R. New principles for glycoside-bond formation. *Angew. Chem., Int. Ed.* **2009**, *48*(11), 1900-1934.

46) Vibert A., Lopin-Bon C. & Jacquinet J.-C. Efficient alternative for the reduction of *N*-trichloroacetyl groups in synthetic chondroitin oligosaccharide intermediates. *Tetrahedron Lett.* **2010**, *51*(14), 1867-1869.

47) Fan Y.-S., Jiang Y.J., An D., Sha D., Antilla J.C., and Zhang S. H8-BINOL Chiral Imidodiphosphoric Acids Catalyzed Enantioselective Synthesis of Dihydroindolo-/-pyrrolo[1,2-a]quinoxalines *Organic Letters* **2014**, *16*(23), 6112-6115.

48) Grindley T.B., Thangara R., The structures and reactions of stannylene acetals from carbohydrate-derived trans-diols. Part I. In the absence of added nucleophiles, *Can. J. Chem*. **1990**, *68*, 1007-1019.

49) (a) Ziegler T., Eckhardt E., Herold H. Lewis Acid-Catalyzed Anomerisation and Rearrangement of Alkyl D-Glycopyranosides During Acetalisation with Methyl Pyruvate: How to Utilise of it for the Preparation of 1-(Carboxyethylidene)glycopyranosyl Donors. *Liebigs Ann Chem*. **1992**, *5*, 441-451. (b) Bailey W.F., Eliel E.L. Conformational-Analysis .29. 2-Substituted And 2,2-Disubstituted 1,3-Dioxanes - Generalized And Reverse Anomeric Effects . *J. Am. Chem. Soc*. **1974**, *96*(6), 1798.

50) (a) Ziegler T., Eckhardt E., Herold G. Diastereoselective Formation of Pyruvylated Glycosides from Partly Protected Sugars and Methyl Pyruvate. *Tetrahedron Lett.* **1992**, *33*, 4413. (b) Ziegler T., Herold G. Preparation of Diastereomeric 3,4-O-Pyruvate Acetal-Containing D-Galactopyranose Derivatives, Structural and Use for Oligosaccharide Synthesis. *Liebigs Ann Chem*.**1994**, *9*, 859-866. (c) Ziegler T. Rhizobial Saccharides 2. Selective Synthesis of Both Diastereomers of 4,6-O-Pyruvylated D-Glycopyranosides. *Tetrahedron Lett.* **1994**, *35*, 6857-6860. (d) Ziegler T., Eckhardt E., Strayle, Herzog H. Synthesis of 5-Aminopentyl 4,6-O-[(R)-1-Carboxyethylidene]-b-D-galactopyranoside and its Use as a Ligand for the Affinity Chromatography of Human Serum Amyloid P Protein. *Carbohydr Res.* **1994**, *253*, 167.

51) Schfile G. (**1996**) PhD Dissertation, University of Stuttgart.

52) Ziegler T., Eckhardt E., and Herold G. Diastereoselective Formation of Pyruvylated Glycosides from Partly Protected Sugars and Methyl Pyruvate, *Tetrahedron Letters*. **1992**, *33*(31), 4413-4416.

53) Garegg, P. J.; Lindberg, B.; Kvarnstrom, I. Preparation and N.M.R.Studies of pyruvic acid and related acetals of pyranosides: Configuration at the acetal carbon atom. *Carbohydr. Res*. **1979**, *77*, 71-78.

54) Gorin, P. A. J.; Mazurek, M.; Duarte, H. S.; Iacomini, M.; Duarte, J. H. Properties of ^{13}C-N.M.R. spectra of O-(1-carboxyethylidene) derivatives of methyl β-D-galactopyranoside: Models for determination of pyruvic acid acetal structures in polysaccharides. *Carbohydr. Res*. **1982**, *100*, 1-15.

55) Macchione G., de Paz J.L., Nieto P.M., Synthesis of hyaluronic acid oligosaccharides and exploration of a fluorous-assisted approach, *Carbohydrate Research.* **2014**, *394*, 17–25.

56) Sychantha D., Little D.J., Chapman R.N., Boons G.J., Howell P.L., and Clarke A.J. Structural and mechanistic basis for the O-acetylation of SCWP, an essential activity for the proper assembly of cell walls in the Bacillus cereus group of pathogens. Submitted to *Nat. Chem. Biol*.

57) (a) Code'e J. D. C., Van den Bos, L. J., Litjens, R. E. J. N., Overkleeft, H. S., Van Boom, J. H., Van der Maerl, G. A. Ph2SO/Tf2O: a Powerful Promotor System in Chemoselective Glycosylations Using Thioglycosides. *Org. Lett*. **5**: 1519-1522 (2003). (b) Code'e, J. D. C., Litjens, R. E. J. N., Van den Bos, L. J., Overkleeft, H. S., Van Boom, J. H., Van der Marel, G. A. Chemoselective glycosylations using sulfonium triflate activator systems. *Tetrahedron* **2004**, *60*, 1057.

58) Dinkelaar, J., Witte, M. D., Van den Bos, L. J., Overkleeft, H. S., Van der Marel, G. A. NIS/TFA: a general method for hydrolyzing thioglycosides. *Carb. Res.*, **2006**, *341*, 1723-1729.

59) Dessolin M., Guillerez M.-G., Thieriet N., Guibé F. & Loffet A. New allyl group acceptors for palladium catalyzed removal of allylic protections and transacylation of allyl carbamates. *Tetrahedron Lett.* **1995**, *36*(32), 5741-5744.

60) Werz D.B., Castagner B., and Seeberger P.H. Automated Synthesis of the Tumor-Associated Carbohydrate Antigens Gb-3 and Globo-H: Incorporation of α-Galactosidic Linkages. J. Am. Chem. Soc. **2007**, *129*(10), 2770-2771.

61) Arungundram S., Al-Mafraji K., Asong J., Leach F.E., Amster I.J., Venot A., Turnbull J.E., Boons G.J. Modular synthesis of heparan sulfate oligosaccharides for structure-activity relationship studies. *J Am Chem Soc*. **2009**, *131*(47), 17394-405.

62) Grathwohl, M. and Schmidt, R.R. Solid phase syntheses of oligomannosides and of a lactosamine containing milk trisaccharide using a benzoate linker. *Synthesis* **2001,** *15*, 2263-2272.

63) Schmidt M., Chatterjee S.K., Dobner B., Nuhn P. New modified single chained glycolipids. Part 1: synthesis of deoxy and partially O-methylated glycolipids with or without a sulfur containing spacer. *Chemistry and Physics of Lipids*. **2002**, *114*(2): 139–147.

64) Oltvoort, J. J.; Van Boeckel, C. A. A.; De Koning, J. H.; Van Boom, J. H.: Use of the Cationic Iridium Complex 1,5-Cyclooctadiene-bis[methyldiphenylphosphine]- iridium Hexafluorophosphate in Carbohydrate Chemistry: Smooth Isomerization of Allyl Ethers to 1-Propenyl Ethers. *Synthesis*, **1981**, *305*, 308.

65) van Boom J.H. & Burgers P.M.J. Use of levulinic acid in protection of oligonucleotides via modified phosphotriester method - Synthesis of decaribonucleotide U-A-U-A-U-A-U-A-U-A. *Tetrahedron Lett.* **1976**, *52*, 4875-4878.

66) Ziegler T., Eckhardt E., Herold G., Lewis Acid-Catalyzed Anomerisation and Rearrangement of Alkyl D-Glycopyranosides During Acetalisation with Methyl Pyruvate: How to Utilise of it for the Preparation of 1-(Carboxyethylidene)glycopyranosyl Donors. *Liebigs Ann. Chem*. **1992**, *5*, 441-451.

67) Ziegler, T. Pyruvated Saccharides- Novel Strategies for Oligosaccharide Synthesis. *Topics in Current Chemistry*, Springer Verlag, Heidelberg, **1997**, *186*, 203-229.

68) Bhaduri S. and Pohl N.L.B., Fluorous-Tag Assisted Syntheses of Sulfated Keratan Sulfate Oligosaccharide Fragments. *Org. Lett*. **2016**, *18*(6), 1414–1417.

69) Sakagami, M.; Hamana, H.: A selective ring opening reaction of 4,6-O-benzylidene acetals in carbohydrates using trialkylsilane derivatives. *Tetrahedron Lett*. **2000**, *41*, 5547-5551.

70) Crich, D.; Sun, S. Direct chemical synthesis of beta-mannopyranosides and other glycosides via glycosyl triflates. *Tetrahedron* **1998**, *54*(29), 8321–8348.

71) Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. Equatorial Anomeric Triflates from Mannuronic Acid Esters. *J. Am. Chem. Soc*. **2009**, *131*(34): 12080–12081.

72) Walvoort, M. T. C.; Witte, W. De; Dijk, J. Van; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; Der, G. A. Van; Cod, J. D. C.; de Witte, W.; van Dijk, J.; van der Marel, G. A. Mannopyranosyl Uronic Acid Donor Reactivity. *Org. Lett*. **2011**, *13*, 4360–4363.

73) Gagarinov I.A., Fang T., Liu L., Srivastava A.D., and Boons G.J., Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization, *Org. Lett.* **2015**, *17*, 928−931.

74) Crich, D.; Cai, W.; Dai, Z. Highly Diastereoselctive R-Mannosylation in the Absence of Participating Protecting Groups. *J. Org. Chem*. **2000**, *65*, 1291–1297.

75) Ulf Ellervik and Gtran Magnusson, Guanidine/Guanidinium Nitrate; a Mild and Selective O-Deacetylation Reagent that Leaves the N-Troc Group Intact. *Tetrahedron Letters*, **1997**, *38*(9), 1627-1628.

76) (a) Moon M.S., Jun S.J., Lee S.H., Cheong C.S., Kim K.S., Lee B.S. Synthesis of the Selectively Protected Garamine Derivatives as Aminoglycoside Intermediates. *Bull. Korean Chem. Soc.* **2003**, *24*, 163. (b) LeBel N.A., Caprathe B.W. Nitrones by heterolytic fragmentation of .gamma.-N-hydroxyamino sulfonates. Conversion of a decahydroquinoline to a perhydroazaazulene. *J. Org. Chem*. **1985**, *50*, 3940.

77) (a) Anderson J.A., Lin B.K., Williams H.J., Scott A.I. Deoxygenation of phenolic natural products. Enzymic conversion of emodin to chrysophanol. *J. Am. Chem. Soc*. **1988**, *110*, 1624. (b) Widmer U. A Convenient Benzylation Procedure for ß-Hydroxy Esters. *Synthesis*. **1987**, *6*, 568-570.

78) Trost B.M., Chung J.Y.L. An unusual substituent effect on a Pd mediated cyclization: A total synthesis of (+) Sterepolide. *J. Am. Chem. Soc*. **1985**, *107*, 4586.

79) Gololobov, Yu. G.; Kasukhin, L. F. Recent advances in the Staudinger reaction. *Tetrahedron* **1992**, *48*(8), 1353-406.

80) Gololobov Y.G., Zhmurova I.N., Kasukhin L.F., Sixty years of Staudinger reaction. *Tetrahedron* **1981**, *37*, 437-472.

81) Chea E.K., Fernández-Tejada A., Damani P., Adams M.M., Gardner J.R., Livingston P.O., Ragupathi G., Gin D.Y., Synthesis and Preclinical Evaluation of QS-21 Variants Leading to Simplified Vaccine Adjuvants and Mechanistic Probes. *J. Am. Chem. Soc*. **2012**, *134*(32): 13448-13457.

82) a) Bartra M., Romea P., Urpí F., Vilarrasa J. A fast procedure for the reduction of azides and nitro compounds based on the reducing ability of $Sn(SR)_3$-species. *Tetrahedron* **1990**, *46*, 587-594. b) Lee J.W., Fuchs P.L. Reduction of Azides to Primary Amines in Substrates Bearing Labile Ester Functionality. Synthesis of a PEG-Solubilized, "Y"-Shaped Iminodiacetic Acid Reagent for Preparation of Folate-Tethered Drugs. *Org. Lett*. **1999**, *1*, 179-182.

83) Periasamy M., Thirumalaikumar M. Methods of enhancement of reactivity and selectivity of sodium borohydride for applications in organic synthesis. *J. Organomet. Chem*. **2000**, *609*, 137-151.

84) Ashok M. Salunkhe, P. Veeraraghavan Ramachandran, Herbert C. Brown, Selective reductions. Part 60: Chemoselective reduction of organyl azides with dichloroborane–dimethyl sulfide, *Tetrahedron*, **2002**, *58*(50), 10059–10064.

85) Shangguan N., Katukojvala S., Greenberg R., Williams L.J., The Reaction of Thio Acids with Azides: A New Mechanism and New Synthetic Applications. *J. Am. Chem. Soc*. **2003**, *125*, 7754-7755.

86) Corey E.J., Nicolaou K.C., Balanson R.D., Machida Y. A Useful Method for the Conversion of Azides to Amines. *Synthesis*. **1975**, *9*, 590-591.

87) Neves Filho R.A.W., Stark S., Westermann B. and Wessjohann L.A. The multicomponent approach to N-methyl peptides: total synthesis of antibacterial (–)-viridic acid and analogues. *Beilstein J Org Chem*. **2012**, *8*, 2085–2090.

88) Baek J.Y., Kwona H.W., Myung S.J., Park J.J., Kim M.Y., Rathwell D.C.K., Jeon H.B., Seeberger P.H., Kim K.S. Directing effect by remote electron-withdrawing protecting groups at O-3 or O-4 position of donors in glucosylations and galactosylations. *Tetrahedron* **2015**, *71*(33), 5315–5320.

89) Crich D., Sun S. Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates. *Tetrahedron*. **1998**, *54*, 8321-8348.

90) Smith P.K., Krohn R.I., Hermanson G.T., Mallia A.K., Gartner F.H., Provenzano M.D., Fujimoto E.K., Goeke N.M., Olson B.J., Klenk D.C. Measurement of protein using bicinchoninic acid. *Anal Biochem* **1985**, *150*, 76-85.

91) Angela N. Koehler, Paul J. Hergenrother, Gavin MacBeath, David Barnes-Seeman, Seung Bum Park, Kristopher M. Depew, and Stuart L. Schreiber. Protocols for Manufacturing and Screening Small Molecule Microarrays, Harvard Institute for Chemistry and Cell Biology, 1-15 (**2003**).

92) Psylinakis E., Boneca I.G., Mavromatis K., Deli A., Hayhurst E., Foster S.J., Vårum K.M., Bouriotis V. Peptidoglycan N-acetylglucosamine deacetylases from Bacillus cereus, highly conserved proteins in Bacillus anthracis. *J Biol Chem*. **2005**, *280*(35), 30856-63.

93) Patel, M. K. *et al*. Analysis of the dispersity in carbohydrate loading of synthetic glycoproteins using MALDI-TOF mass spectrometry. *Chem. Commun*. **2010**, *46*, 9119–9121.

94) Nguyen-Mau S.M., Oh S.Y., Kern V.J., Missiakas D.M., Schneewind O. Secretion genes as determinants of Bacillus anthracis chain length. *J Bacteriol*. **2012**, *194*(15), 3841-50. Fagan, R. P. & Fairweather, N. F. Clostridium difficile has two parallel and essential Sec secretion systems. J. *Biol. Chem*. **2011**, *286*, 27483–27493.

95) Kern V.J., Kern J.W., Theriot J.A., Schneewind O., Missiakas D.M. Surface (S)-layer proteins Sap and EA1 govern the binding of the S-layer associated protein BslO at the cell septa of Bacillus anthracis. *J. Bacteriol*. **2012**, *194*, 3833–3840.

CHAPTER 3

PROBING THE ACTIVITY AND SPECIFICITY OF PATB1 O-ACETYLTRANSFERASE USING SYNTHETIC OLIGOSACCHARIDES DERIVED FROM THE BACILLUS ANTHRACIS SECONDARY CELL WALL POLYSACCHARIDE

Chapman, R.N., Sychantha D., Liu, L., Clarke, A., Boons, G.J.
To be submitted to Org. Biomol. Chem.

ABSTRACT

The envelope of *Bacillus anthracis*, which is the causative agent of anthrax, is composed of a complex matrix of peptidoglycan covalently decorated with secondary cell wall polysaccharides (SCWP) and a poly-D-γ-glutamic acid capsule. The SCWP of *B. anthracis* plays a key role in the organization of the envelope of vegetative cells and is intimately involved in host-guest interactions making it an attractive target of study. PatB1 is a putative O-acetyltransferase and is believed to play a role in the 3-*O*-acetylation of the penultimate β-GlcNAc residue on *B. anthracis* SCWP. O-acetylation of SCWP has been proposed as a means to enable the deposition of certain S-Layer and S-Layer associated proteins at specific sites on the cell envelope where they can play roles in virulence or act to regulate downstream cellular processes. PatB1 was previously shown to acetylate a synthetic compound derived from the *B. anthracis* SCWP repeating unit with site specificity and specific activity superior to that of chitooligosaccharide derivatives. However, the previous synthetic compound did not take into account other important functional groups on the natural structure. In order to characterize PatB1 specificity and activity in the context of its natural environment, a library of polysaccharides based on the *B. anthracis* SCWP internal repeating unit were synthesized. The library was used to determine if the presence of galactose in certain positions can enhance or deter PatB1 specific activity and alter the positional specificity of PatB1-catalyzed acetylation. In addition, a trisaccharide based on the terminal (non-reducing) unit was synthesized to see if the presence of the 4,6-*O*-pyruvyl ketal and free amine seen on the natural structure can affect PatB1 activity and the site specificity. It was found that the presence of galactosylation on the trisaccharide repeating unit did not alter specificity, but led to a decrease in specific activity relative to the non-galactosylated trisaccharide repeat. Furthermore, it was found that the trisaccharide bearing the 4,6-*O*-pyruvyl ketal and free amine showed a significant increase in specific activity demonstrating that these features are likely utilized *in vivo* as PatB1 binding epitopes and help to facilitate O-3 acetylation of the penultimate β-GlcNAc.

INTRODUCTION

The *Bacillus cereus* group of pathogens includes *B. cereus*, *B. anthracis*, and *B. thuringiensis* differing only by the types of plasmids present in the cell.[1] The cell envelope of these Gram-positive cells is composed of a complex matrix of peptidoglycan with covalently attached secondary cell wall polysaccharides (SCWP). The SCWP plays a key role in the organization of the envelope of vegetative cells and is intimately involved in host-guest interactions.[2] The SCWP serves as a scaffold for a variety of surface associated proteins including S-Layer (SLP) and S-layer associated (BSL) proteins (Figure 3.1).[3-5]

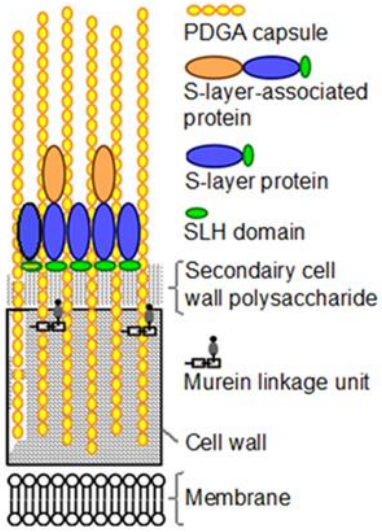


Figure 3.1 The Cell Wall of *B. anthracis*

The structures of secondary cell wall polysaccharides from *B. anthracis Ames*, *Sterne* and *Pasteur* and several phylogenetically related *B. cereus* strains have been elucidated and it was found they have unique and shared structural features (Figure 3.2).[6-9] *B. cereus* G9241 and 03BB87 (human pathogens), *B. cereus* Cameroon and Ivory Coast (great ape pathogens) and *B. cereus* ATCC 10987 (non-pathogenic) have the same trisaccharide backbone repeat as *B. anthracis*, but differ in the pattern of galactose substitution. In addition, galactosylation is non-stoichiometric along the SCWP polymer creating

considerable heterogeneity. Furthermore, an avirulent *B. anthracis* CDC-684 strain produces a polysaccharide with the same trisaccharide backbone but lacking galactosylation.[6]

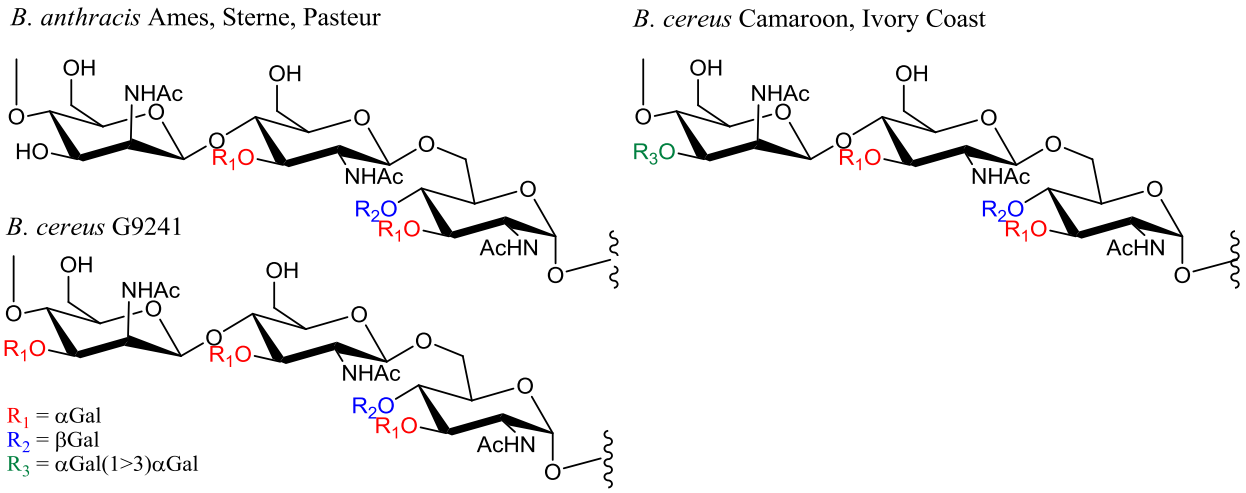


Figure 3.2: SCWP repeating units from various Bacilli.

The secondary cell wall polysaccharide of *B. anthracis* has a →4)-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→6)-α-D-GlcNAc-(1→) repeating backbone that can be substituted at C-3 and C-4 of the α-D-GlcNAc moiety with α-D-Gal and β-D-Gal, respectively and at C-3 of the β-GlcNAc residue with a α-Gal (Figure 3.3). Galactose substitution is non-stoichiometric resulting in considerable micro-heterogeneity along the polymer.[10] In addition, a single trisaccharide repeating unit located at the non-reducing end of *B. anthracis* and pathogenic *B. cereus* SCWP is structurally further modified with a 4,6-*O*-pyruvyl ketal on β-D-ManNAc, a 3-*O*-acetyl ester on β-D-GlcNAc, and an N-deacetylated α-D-GlcNAc residue. The α-GlcNH$_2$ moiety may also be modified with an α-Gal at C-3 and β-Gal at C-4 positions.[6] However, NMR studies could not confirm this modification.

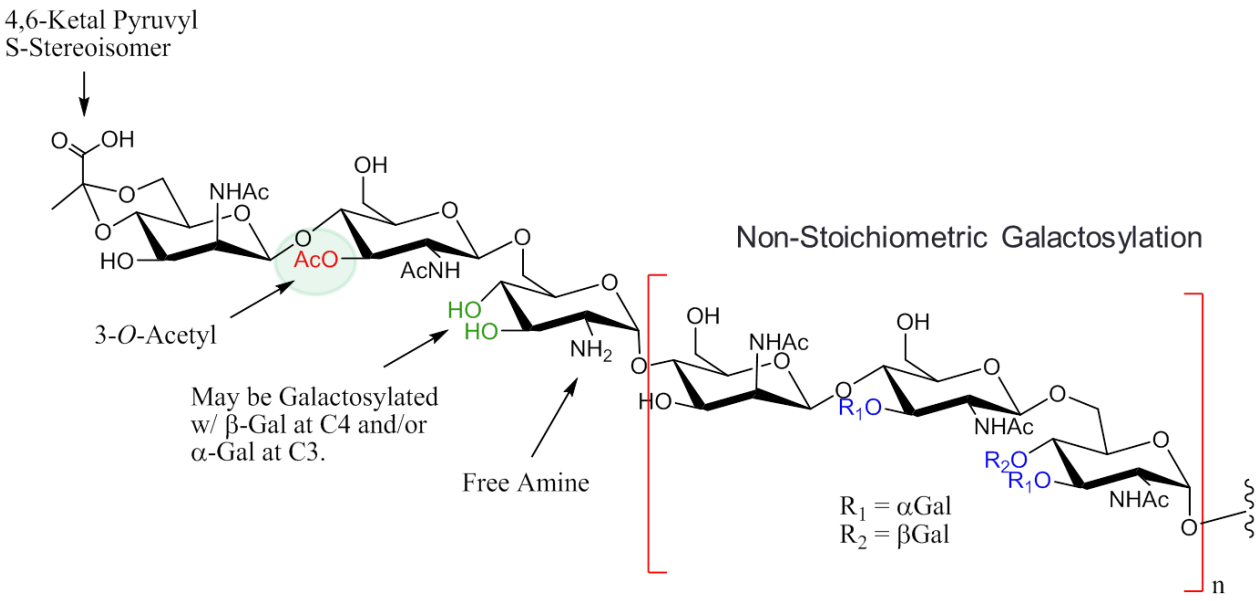


Figure 3.3: The Terminal unit of the secondary cell wall polysaccharide of *Bacillus anthracis*.

PatB1 was recently identified as an SCWP O-acetyltransferase and is believed to play a role in the 3-*O*-acetylation of penultimate β-GlcNAc on the *B. anthracis* and *B. cereus* SCWP terminal unit (Unpublished).[11,12] S-layer and BSL proteins are both known to bind SCWP, but their deposition onto the cell surface is not random. Previous genetic studies have shown that O-acetylation of SCWP may be a means to direct the deposition of certain SLP and BSL proteins to specific sites on the cell envelope where they can act to regulate various downstream biological processes such as controlling the chain length of vegetative cells.[11-14,47] Two putative acetyltransferase systems have been identified in *B. anthracis* and include the PatA1/PatB1 and PatA2/PatB2 systems. The location of the *patA1-patB1-patA2-patB2* gene cluster on the *B. anthracis* chromosome is adjacent to genes encoding Sap (*sap*) and EA1 (*eag*) S-layer proteins as well as the putative pyruvyl transferase (csaB) responsible for modifying the non-reducing SCWP unit.[11,16] The proximity of these genes to one another has been used to suggest that the PatA1/PatA2 gene products play a role in S-Layer assembly. In previous genetic studies, it was suggested that PatA1 and PatA2 are two membrane bound O-acetyltransferases that play complementary roles in modifying SCWP and possibly peptidoglycan.[11,17,18] The *patA1*, *patA2*, and

patA1patA2 deletion mutant strains produced elongated chains of vegetative cells with SCWP quantities comparable to those of WT cells, but with fewer O-acetyl modifications. Phenotypic studies of these *patA1* and *patA2* mutant strains provided evidence suggesting that O-acetylation of SCWP was necessary for the proper binding of EA1, BslO, and BslA to the cell surface. *B. anthracis* is known to evade host immune responses by elongating its chain-length to exceed the size of macrophages and it is beleived that O-acetylation may play a role in this process.[15] In wild type cells, BslO murein hydrolase is found to localize around cell division septa along with EA1 in order to promote the separation of vegetative cells.[19] The deposition of BslO and EA1 was completely abolished in *patA1patA2* deletion mutants. It was suggested that the absence of BslO at the cell speta was the cause of the elongated phenotype seen in the PatA1/PatA2 mutants. BslA was found to act as a surface adhesin in *B. anthracis* Ames enabling the binding of vegetative forms to host cells. Mutants lacking BslA were less efficient at infecting animal models suggesting a role for this protein in the Anthrax infection process.[20] The single and double deletion *patA1/patA2* mutants failed to incorporate any BslA into the S-layer suggesting O-acetylation of SCWP is a prerequisite for binding. The results in chapter 2 of this thesis has raised some questions regarding the exact role of O-acetylation and how it may act to regulate S-layer and BSL protein binding, but its presence on SCWP appears to be important for cell wall growth and maintenance functions as evidenced by the phenotypes observed in the *patA1/patA2* deletion mutants. It was initially assumed that PatA1 and PatA2 were directly responsible for SCWP O-acetylation, but recent biochemical evidence has shown that PatB1 is likely responsible for this modification. A model for the O-acetylation of SCWP was proposed whereby PatA1 and PatA2 act by transporting the acetyl-donor (presumably from acetyl-CoA) across the cytoplasmic membrane to the membrane bound PatB1 for transfer to the O-3 position of the penultimate GlcNAc (Figure 3.4; Unpublished).[12] The function and role of PatB2 still needs further study. Additionally, viable *B. anthracis* and *B. cereus* mutants lacking a functional PatB1 could not be generated further hinting at the importance of the enzyme and the O-

acetyl modification.[11] Evidence seems to indicate that O-acetylation of SCWP may play a role in the binding of proteins and could be necessary for the proper growth and maintenance of the cell envelope. Better understanding of PatB1 binding and activity could aid in the development of compounds that act to inhibit the interaction of PatB1 with SCWP thereby affecting these processes.[21]

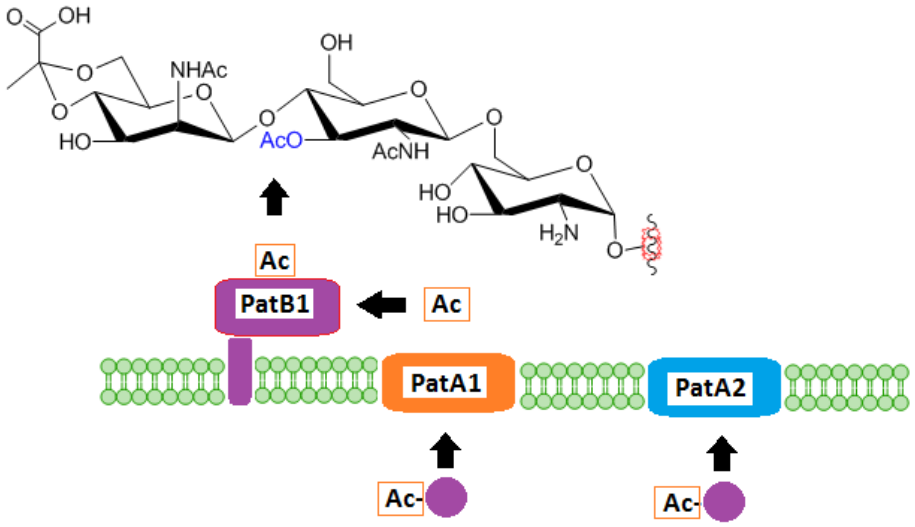


Figure 3.4: Model for PatB1 mediated O-Acetylation of *Bacillus anthracis* SCWP.

Previous *in vitro* experiments have shown that PatB1 can specifically acetylate the O-3 position of β-GlcNAc on a synthetic oligosaccharide derived from the *B. anthracis* SCWP repeating unit (Compound **1**). The site of O-acetylation on compound **1** was similar to that seen on the natural substrate. In addition, the specific activity and selectivity of PatB1 for Compound **1** was superior in comparison to other GlcNAc based substrates including a Chitosan pentamer and various chitooligomers that were O-acetylated at multiple positions (Unpublished).[12] The previous study, however, did not take into account other important features present on the natural structure such as branching galactose residues, a free amine, and a 4,6-*O*-pyruvyl ketal. The structural heterogeneity of isolated polysaccharides has made it difficult to perform detailed binding studies and has prevented the determination of saccharide motifs that are important for PatB1 selectivity and activity. Herein, we

report the synthesis of a library of novel oligosaccharides (**1**-**5**) derived from the *B. anthracis* SCWP (Figure 3.5). Compound **1** is representative of the non-galactosylated trisaccharide backbone. Tetrasaccharides **2**, **3**, and **4** have a galactose substitution at each of the biologically relevant sites found on the *B. anthracis* SCWP and will be used to determine if galactose substitution can affect PatB1 activity or specificity. Compound **5** is derived from the terminal non-reducing unit of *B. anthracis* and will help to determine if the 4,6-*O*-pyruvyl ketal on the terminal ManNAc and/or free amine on the β-GlcNAc can affect PatB1 mediated 3-*O*- acetylation of SCWP.

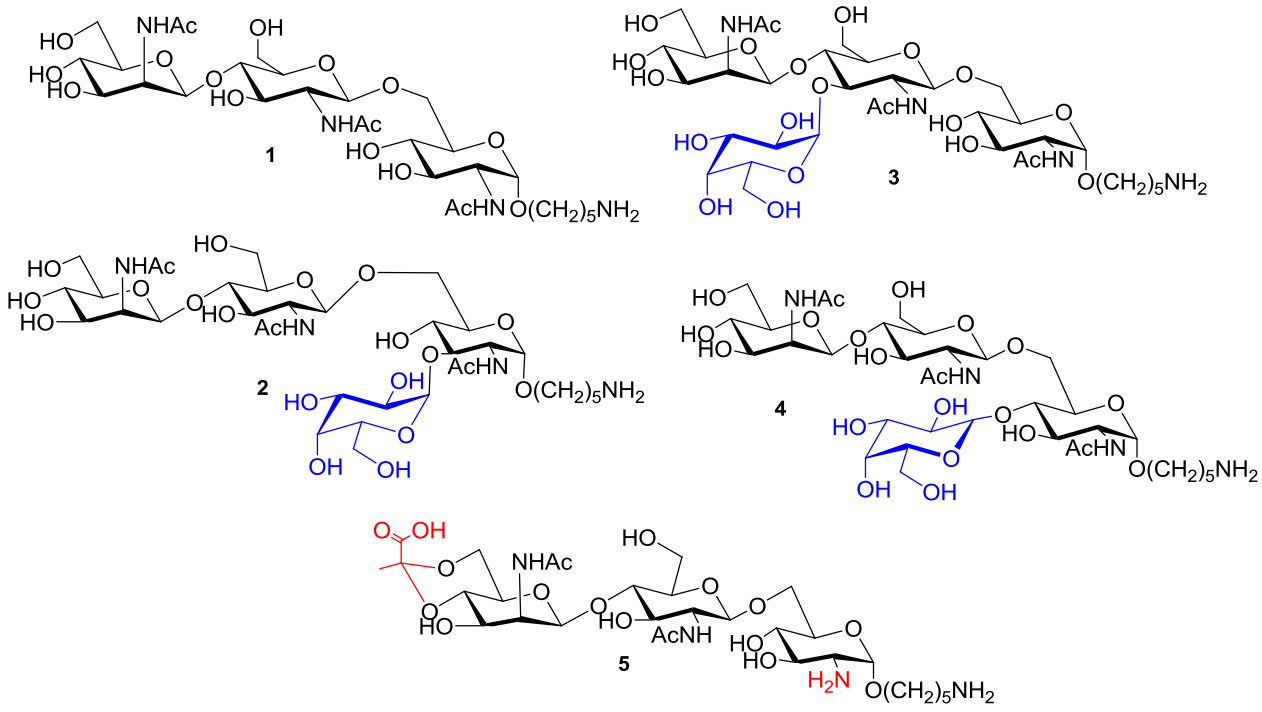


Figure 3.5: Target Compounds Derived from the Secondary Cell Wall Polysaccharide of *B. anthracis.*

RESULTS AND DISCUSSION

Synthesis: *General Synthetic Strategy:* Target **1-4** compounds were assembled using monosaccharide building blocks **6**-**10** (Figure 3.6). Target **5** was synthesized utilizing a previously described strategy.[22] It was envisaged that compounds **1**–**4** would be prepared from a common trisaccharide **16**, which at key

positions is modified by the orthogonal protecting groups N^{α}-9-fluorenylmethyloxycarbonate (Fmoc),[23] 2-methylnaphthyl ether (Nap),[23] and levulinoyl ester (Lev).[24]

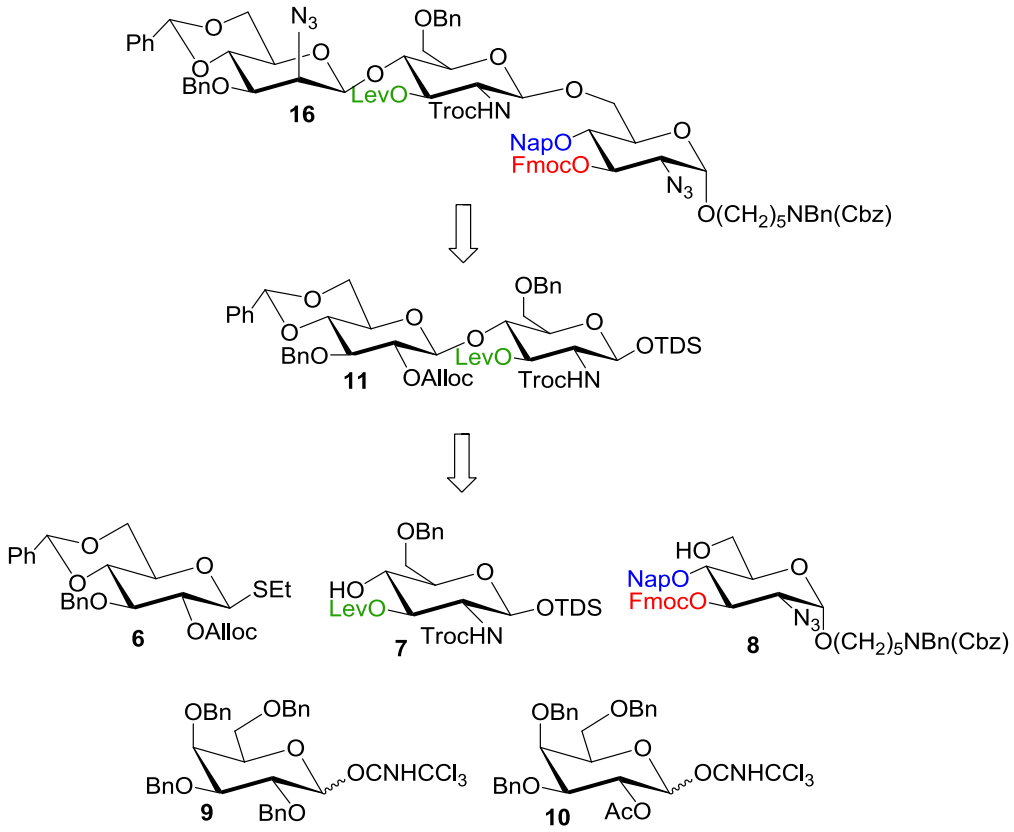
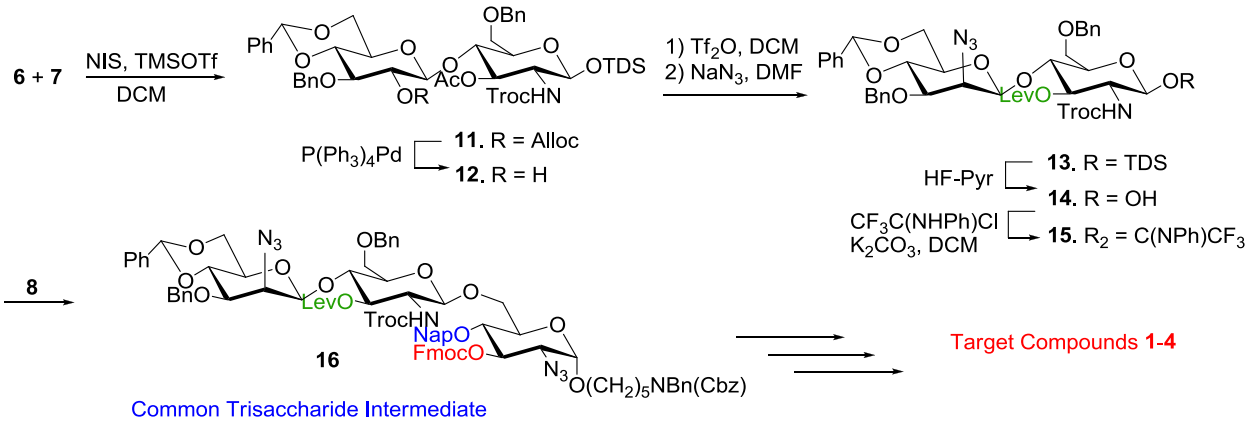


Figure 3.6: Retrosynthetic Analysis of Common Trisaccharide Intermediate

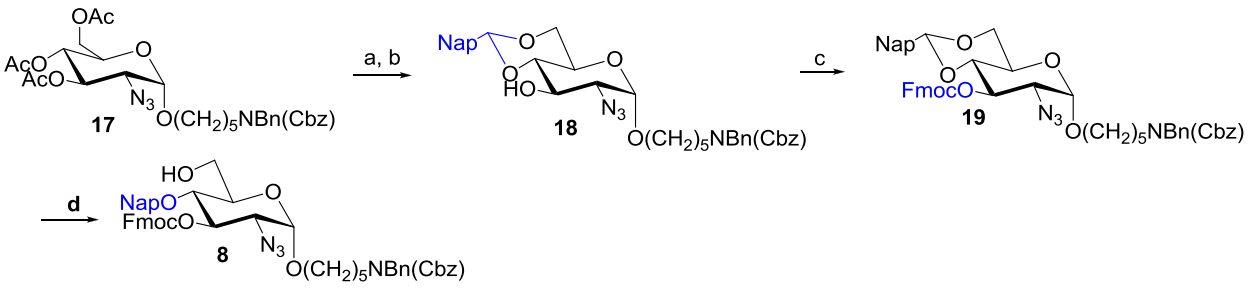
Planned Synthetic Procedure: The challenging β-mannosamine linkage is introduced by a strategy whereby initially a β-glucoside is formed by TMSOTf catalyzed glycosylation of glucosyl donor **6** with acceptor **7**. Donor **6** has a participating allylxoycarbonyl (Alloc) protecting group at C-2 to control beta-anomeric selectivity to give disaccharide **11** (Scheme 3.1).[25] Next, the C-2 Alloc protecting group of **11** can be removed using $Pd(PPh_3)_4$ [26] and the resulting hydroxy of **12** can be converted to a triflate, which can be subsequently displaced by NaN_3 [27] to give 2-azido-β-D-mannoside (**13**). Next, the anomeric thexyldimethylsilyl (TDS) can be cleaved using HF-pyridine complex[28] to afford compound **14.** Lactol **14** can then be converted to an imidate donor (**15**) with K_2CO_3 and *N*-phenyltrifluoroacetimidoyl chloride.[29]

A TMSOTf catalyzed glycosylation of **15** with acceptor **8** should provide trisaccharide **16** as only the β-glucoside due to neighboring group participation of the 2,2,2-Trichlorethoxycarbonyl (Troc).[30]



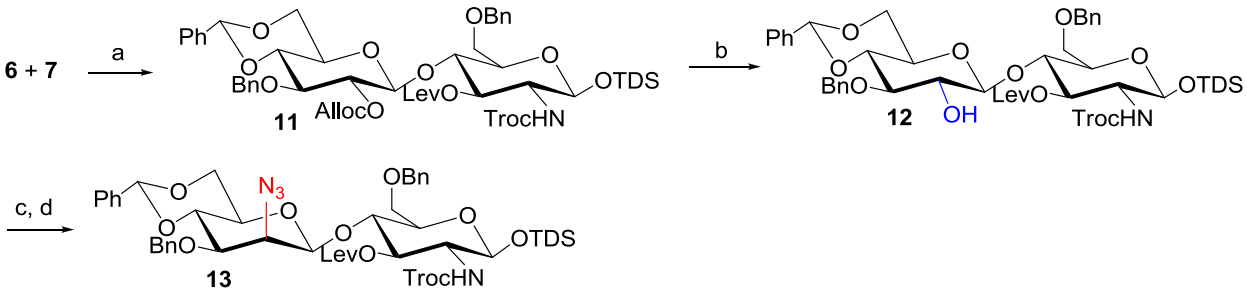
Scheme 3.1: General Synthetic Scheme for Trisaccharide Common Intermediate

The orthogonal protecting groups on the trisaccharide intermediate (**16**) can be selectively removed and subsequently coupled with imidate donor **9** or **10** to afford target compounds **2**-**4**. Diethyl ether can also be employed in the solvent system to promote selectivity for the α-anomer.[31] The Troc and azide functions can then be converted into acetamido groups using Zinc-Copper couple[32] followed by treatment with Ac_2O.[33] Following the removal of aromatic protecting groups with catalytic hydrogenation, the resulting free amine on the anomeric spacer can be used as a handle for conjugation to a carrier protein or surface.



Scheme 3.2: Synthesis of Compound **8**. a) MeOH, $NaOCH_3$; b) CSA, CH_3CN, 2-(dimethoxymethyl)-naphthalene (63% 2-steps); c) Fmoc-Cl, DCM, Pyridine (84%) d) $PhBCl_2$, TES, DCM (63%).

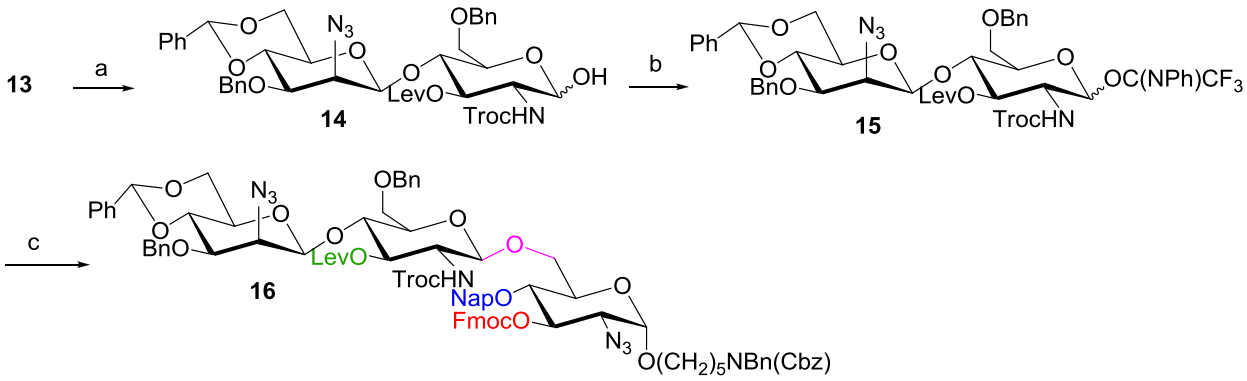
Building block compounds **6**, **7**, **9**, and **10** were synthesized according to previously published procedures.[27,34,35] Synthesis of the third building block compound (**8**) was accomplished by first treating **17** with methanolic sodium methoxide (Scheme 3.2). This was followed by installation of a 4,6-*O*-(2-naphthyl)methylene acetal using 2-Naphthaldehyde dimethyl acetal in the presence of CSA to obtain compound **18** in 63% yield over two steps. Afterwards, Fmoc was installed at the 2 position with Fmoc-Cl to obtain compound **19** in 84% yield. The 4,6-*O*-(2-naphthyl)methylene acetal ring was selectively opened at the C-6 hydroxy position with $PhBCl_2$ and triethylsilane[36] at -78°C to afford compound **8** in 63% yield.



Scheme 3.3: Synthesis of Disaccharide **11** and conversion to a 1,2-Cis glycoside. a) TMSOTf, NIS, DCM (87%); b) $P(Ph_3)_4Pd$, THF, Morpholine (77%); c) DMAP, TfOTf, DCM, Pyr d) DMF, NaN_3 (78% 2-steps).

The next step of the synthesis involved making the key disaccharide intermediate with a 1,2-cis-β glycosidic linkage (Scheme 3.3). Neighboring group participation from the C-2 Alloc function on **6** was exploited to first form a 1,2-trans-glycoside. Donor **6** and acceptor **7** were reacted under NIS (1.5eq) and TMSOTf (0.26eq) activation conditions at -20°C to provide **11** in 87% yield. Next, the C-2 Alloc protecting group of **11** was removed using $Pd(PPh_3)_4$ to afford **12** in 77% yield.[26] The resulting hydroxy on compound **12** was converted into a triflate using triflic anhydride. After workup, the crude was concentrated and heated at 55°C in DMF along with NaN_3 to invert the C-2 position and give the 2-azido-β-D-mannoside (**13**) in 78% over two steps.[27] Next, the anomeric TDS ether was cleaved using

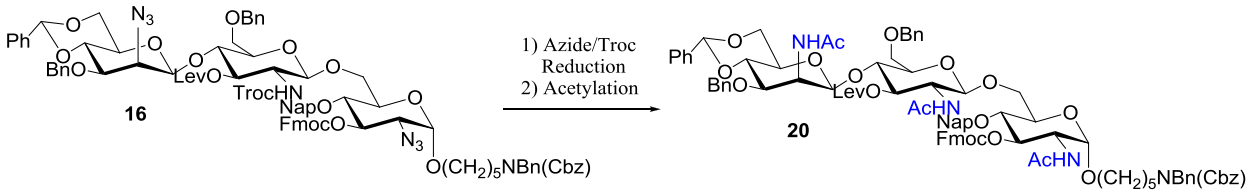
HF-pyridine complex to give lactol **14** in 88% yield (Scheme 3.4).[28] Compound **14** was then converted to an N-phenyltrifluoroacetimidate donor with *N*-phenyltrifluoroacetimidoyl chloride and K_2CO_3 to afford compound **15**. Donor **15** and acceptor **8** were used in a 2+1 TMSOTf catalyzed glycosylation to provide trisaccharide **16** as the β-glucoside in 85% over two steps.



Scheme 3.4: Synthesis of Common Trisaccharide Intermediate. a) HF-Py, THF (88%) b) 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride, K_2CO_3, DCM c) **8**, TMSOTf, DCM (85%).

The next major hurdle was the reduction of Troc and azide and their subsequent conversion to acetamide (Table 3.1). For the first condition attempted, compound **16** was reacted with Zn, Ac_2O, Cu(II)SO_4, and HOAc.[37] High resolution MALDI-TOF mass spectrometry indicated the presence of an [M+1]+Na^+ byproduct. This side product could be separated, but the purification of **20** was difficult and only feasible on a small scale using preparatory TLC. The second condition attempted involved reduction of compound **19** with Zn-Cu couple in acetic acid at 50°C.[32] High resolution MALDI-TOF mass spectrometry indicated the presence of an [M+1]+Na^+ byproduct as well as a product lacking benzylidene. The heated acidic conditions led to the partial loss of the benzylidene. Although, deprotection of the target compounds ultimately involves removal of benzylidene, the mixture of compounds in the crude would make purification and characterization more difficult. The third condition attempted involved reaction of compound **16** with Zn-Cu couple in acetic acid at room temperature.

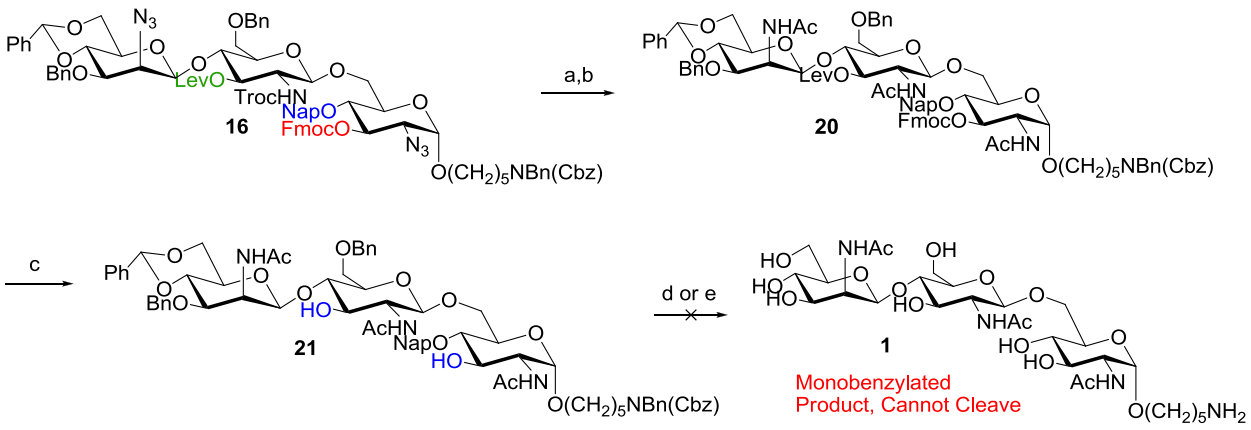
Table 3.1: Troc and Azide Reduction Conditions.



Conditions:	Results:
Zn, Ac_2O, Cu(II)SO_4, HOAc, THF	Mixture w/ (+1) prod
Zn/Cu, HOAc, 50°C	Mixture w/ (+1) prod and loss Benzylidene, lev
1) Zn/Cu, HOAc, RT 2) Ac_2O, Pyridine	Product formation

Following the reduction of Troc to acetamide and azide to amine, the amine was converted to acetamide with acetic anhydride and pyridine to afford compound **20**. The reaction was attempted on a small scale initially and it was found that reaction times in excess of 1 h resulted in the formation of the $[M+1]+Na^+$ by-product seen in the previous high resolution MALDI-TOF mass spectra. The proceeding reactions used large excesses of Zn-Cu couple and were quickly filtered and concentrated by the 1 h mark. While concentrating the filtered reaction mixture, a non-heated water bath was used to prevent cleavage of the benzylidene.

Synthesis of Compound 1: Following reduction of the Troc and azide functionalities on **16** and their subsequent conversion to acetamide, the Fmoc and Lev on **20** were removed using NaOMe in methanol. The final deprotection of the aromatic groups of **21** proved problematic (Scheme 3.5). The first attempt at deprotection involved use of catalytic $Pd(OH)_2$ under hydrogen atmosphere After stirring for ~14 h, the partially deprotected compound could be detected *via* MALDI-TOF mass spectrometry. The reaction mixture was filtered and concentrated. Fresh solvent and $Pd(OH)_2$ was used and after an additional 8 h, MALDI-TOF MS detected a $[M+Bn]+Na^+$ product.

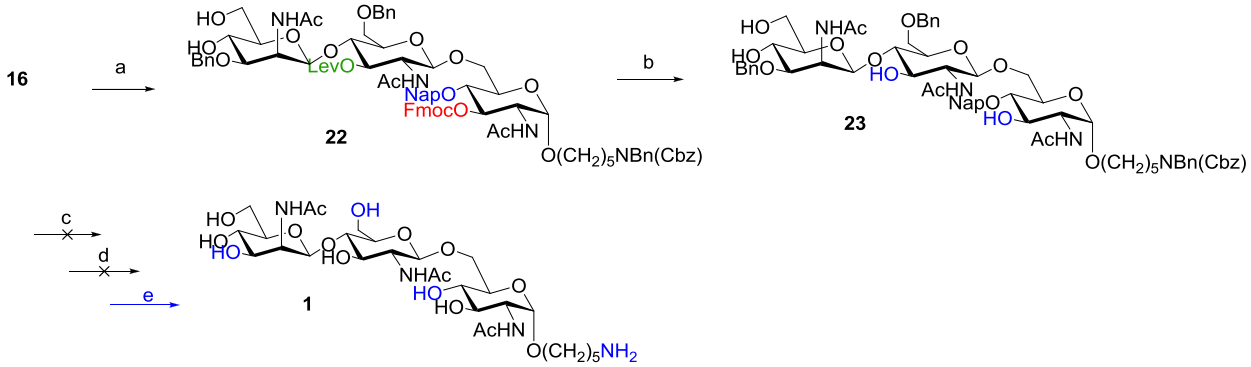


Scheme 3.5: Compound **1** Deprotection: a) Zn-Cu couple, HOAc; b) Ac_2O, Pyridine; c) MeOH, NaOMe; d) $Pd(OH)_2$, *t*-BuOH, H_2O, HOAc, H_2 (1 Atm); e) Na, NH_3, THF, tBuOH.

Further reduction with fresh solvent and catalyst was not successful at removing the remaining benzyl. The second condition attempted for removal of the aromatic protecting groups was Birch reduction.[38] Compound **21** was taken up in NH_3 and THF along with a small piece of solid sodium. The reaction produced the same $[M+Bn]+Na^+$ product as the $Pd(OH)_2$ conditions. It was speculated that the benzylidene may be the problem so a decision was made to remove the group prior to the final aromatic deprotection.

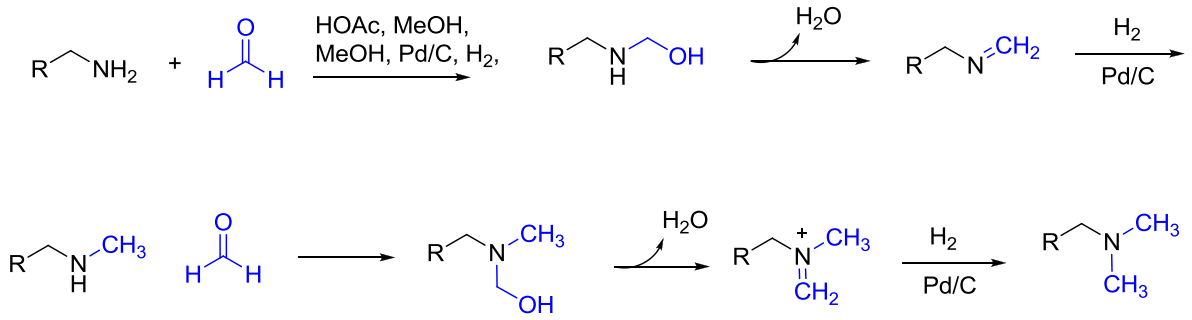
In the modified method (Scheme 3.6) the Troc and azide of **16** were first reduced to free amine with Zinc-Copper couple and then converted to acetamido functions with Ac_2O in pyridine.[32,33] Afterwards, compound **16** was heated in 80% HOAc (aq) in order to remove benzylidene and afford compound **22**. Compound **22** was taken up in LiOH, THF, and water and stirred in a warm water bath to give **23**. Sodium methoxide in methanol can also be used, but the reaction time was found to be significantly longer (~16 h). The intermediate was purified at this point in order to exploit the non-polar nature of the aromatic groups for separation and the UV activity of the aromatic groups for the purpose of detecting the product. Compound **23** was obtained in 47% over 4 steps. Several conditions were attempted for the removal of the aromatic groups. Compounds with unshielded basic nitrogen can act

as inhibitors, however use of protic solvents such as methanol or ethanol during hydrogenation along with an organic acid such as acetic acid can help to mitigate these inhibitory effects.[39]



Scheme 3.6: Compound **1** Deprotection Continued: a) 80% HOAc, 50°C; b) LiOH, THF, H_2O; (47% over 4 steps) c) $Pd(OH)_2$, MeOH, H_2O, HOAc, H_2 (1 Atm); d) $Pd(OH)_2$, EtOH, H_2O, HOAc, H_2 (1 Atm). e) $Pd(OH)_2$, *t*-BuOH, H_2O, HOAc, H_2 (1 Atm) (78%) .

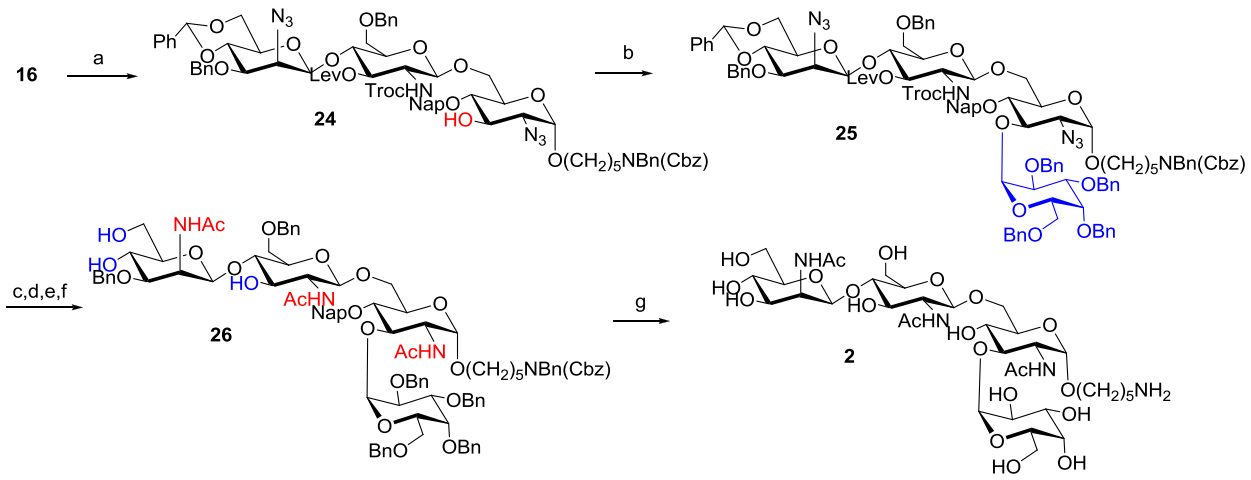
In the first trial reaction (Scheme 3.6), it was thought that the less polar *t*-Butanol solvent was slowing the removal of the aromatic protecting groups by precipitating the increasingly polar deprotected intermediates so a decision was made to change solvents. The first condition involved using $Pd(OH)_2$, MeOH, water, and acetic acid to remove the remaining protecting groups. Unfortunately, MALDI-TOF MS detected significant methyl and dimethylamine product. Catalysts have the ability to add hydrogens, but can also take them away in some cases. This can lead to the formation of formaldehyde which can react with available amines and lead to reductive N-methylation (Scheme 3.7).



Scheme 3.7: Reductive N-Methylation under Palladium Reduction Conditions.

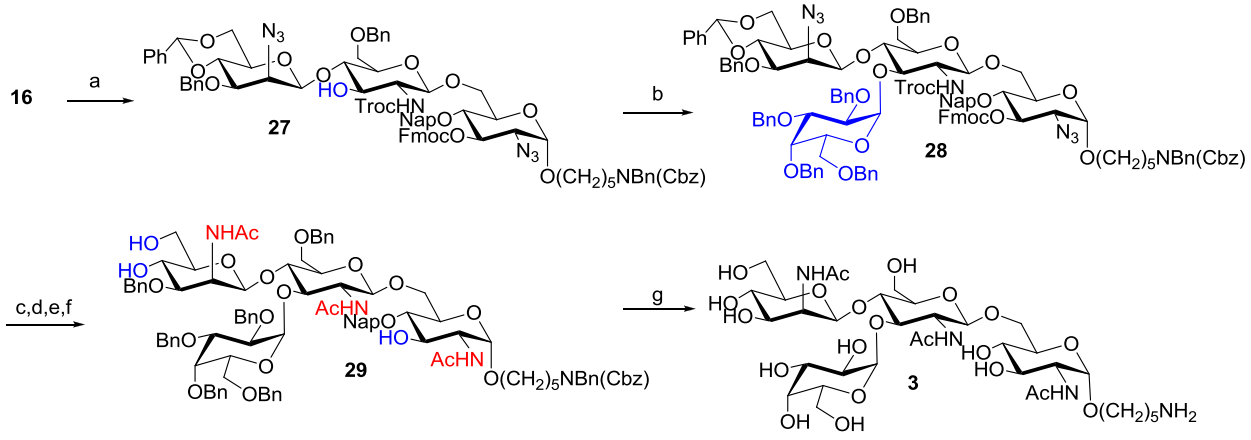
The reaction pathway involves the addition of amine with formaldehyde, dehydration to form the imine followed by hydrogenation.[40] The second condition attempted involved using ethanol as a substitute for methanol. Previous experience has shown that ethanol is much less likely to form N-alkyl side products. Unfortunately, MALDI-TOF MS detected significant N-ethyl and diethylamine product. The N-ethyl side product was confirmed by NMR. A decision was made to switch back to *t*-butanol. *Tert*-butanol is a tertiaty alcohol and is unable to form an aldehyde intermediate making N-alkylation impossible. In order to speed up the reaction and help improve solubility of partially deprotected intermediate compounds, additional water was added to the reaction mixture. Compound **23** was taken up in $Pd(OH)_2$, *t*-butanol, water, and acetic acid. MALDI-TOF MS indicated that target compound **1** was formed in 78% yield without the N-alkyl impurity. Following the reaction, care was also taken during filtration and rinsing of the catalyst to avoid methanol and ethanol. It was found that simply washing the catalyst with methanol would lead to N-methylation. A series of washes with *t*-butanol and water were used ranging in concentration from 90% t-BuOH/H_2O to 20% t-BuOH/H_2O.

Synthesis of Compound 2: The Fmoc protecting group was selectively removed from compound **16** using Diethylamine (DEA) in DCM to afford **24** in 99% yield (Scheme 3.8). Donor compound **9** and acceptor compound **24** were dissolved in DCM and Et_2O and cooled to -20^oC for a 3+1 TMSOTf catalyzed glycosylation reaction. Compound **25** was formed in 84% yield as the desired alpha (1$\rightarrow$3) galactosylted product. The azide and Troc functionalities on compound **25** were then converted to acetamido groups using Zinc-Copper couple in acetic acid followed by reaction with acetic anhydride and pyridine.[32,33] Next, the benzylidene was removed by heating in an 80% HOAc (aq) solution. Afterwards, the Lev group was removed by stirring with LiOH in THF and water at 40^oC. At this point, the intermediate compound was purified in order to provide **26** in 43% yield over 4 steps. Compound **26** was taken up in $Pd(OH)_2$, *t*-butanol, water, and acetic acid in order to remove the remaining aromatic protecting groups affording target compound **2** in 72% yield.



Scheme 3.8: Synthesis of Target Compound **2.** a) DCM, DEA, (99%); b) **9**, DCM, Et_2O, TMSOTf, -20°C, (84%); c) Zn-Cu couple, HOAc; d) Ac_2O, Pyridine; e) 80% HOAc, 50°C; f) LiOH, THF, H_2O, (43% over 4 steps); g) $Pd(OH)_2$, *t*-BuOH, H_2O, HOAc, H_2 (1 Atm) (72%).

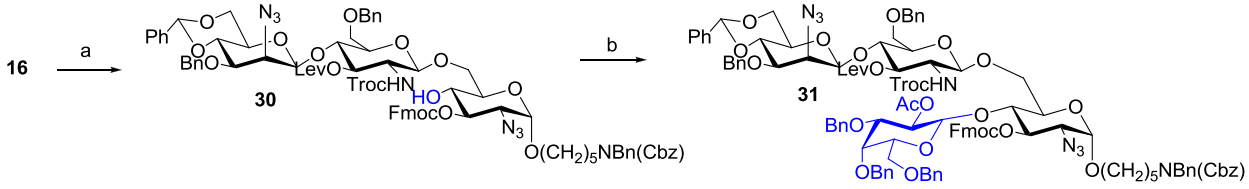
Synthesis of Compound 3: The levulinoyl ester protecting group was selectively removed from compound **16** using hydrazine acetate to give **27** in 64% yield (Scheme 3.9).[41] Donor compound **9** and acceptor compound **27** were dissolved in DCM and Et_2O and cooled to -20°C for a 3+1 TMSOTf catalyzed glycosylation reaction. Compound **28** was formed in 63% yield as the desired alpha (1→3) galactosylated product.



Scheme 3.9: Synthesis of Target Compound **3**. a) Hydrazine acetate, MeOH, DCM, (64%); b) **9**, DCM, Et_2O, TMSOTf, -20°C, (63%); c) Zn-Cu couple, HOAc; d) Ac_2O, Pyridine; e) 80% HOAc, 50°C; f) DCM, DEA, (40% over 4 steps); g) $Pd(OH)_2$, *t*-BuOH, H_2O, HOAc, H_2 (1 Atm) (89%).

The azide and Troc functionalities on compound **28** were then converted to acetamido functions using Zn-Cu couple in acetic acid followed by reaction with acetic anhydride and pyridine.[32,33] Next, the benzylidene was removed by heating in an 80% HOAc (aq) solution. Afterwards, the Fmoc group was removed by stirring with Diethylamine (DEA) in DCM. At this point, the intermediate compound was purified in order to provide **29** in 40% yield over 4 steps. Compound **29** was taken up in $Pd(OH)_2$, t-butanol, water, and acetic acid in order to remove the remaining aromatic protecting groups affording target compound **3** in 89% yield.

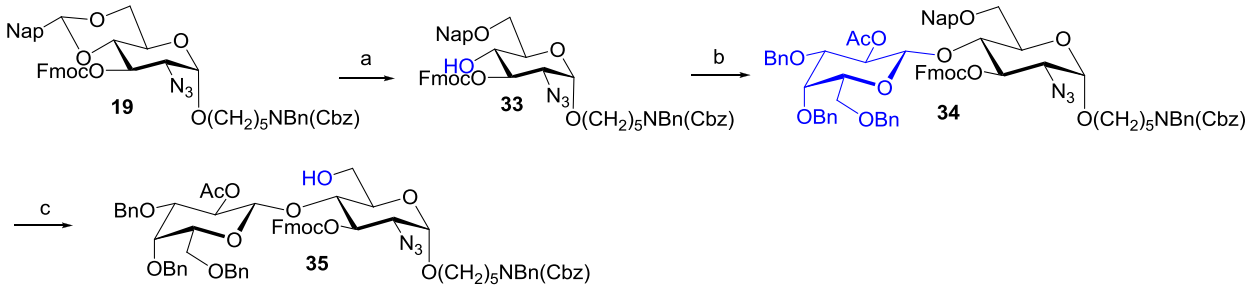
Synthesis of Target Compound 4: Target compound **4** was synthesized by first selectively removing the Nap protecting group from **16** using recrystallized DDQ, DCM, and PBS buffer (pH 7.4) to give **30** in 84% yield (Scheme 3.10).[24] Earlier attempts at this reaction involved use of water instead of PBS buffer. In those reactions, side products missing one or more benzyl protecting groups were detected by MALDI-TOF MS and the yield was adversely affected. The use of PBS buffer (pH 7.4) helped to prevent side product formation and significantly improve yield. In addition, recrystallized DDQ helped to improve the reaction yield.



Scheme 3.10: Synthesis of Target Compound **4**. a) DDQ, DCM, PBS (pH 7.4), (84%); b) **10**, DCM, TMSOTf, -20°C, (19%).

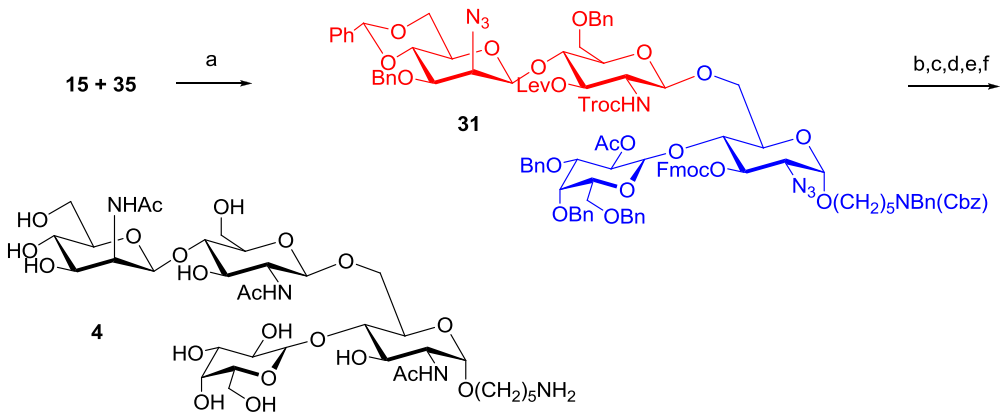
Donor compound **10** and acceptor compound **30** were dissolved in DCM and cooled to -20°C for a 3+1 TMSOTf catalyzed glycosylation reaction. Compound **31** was formed in 19% yield as the desired beta (1→3) galactosylted product. The reaction was repeated, but the yield remained low (~15% average). It was thought that steric crowding around the C4-hydroxy of the 2-azido glucoside and the bulky Fmoc at

the O-3 position might be contributing to the low yield. Two options were available at this point. The first option involved using a smaller protecting group on the O-3 position of the 2-azido glucoside. The second option involved using a 2+2 beta (1→6) glycosylation reaction. A decision was made to attempt the 2+2 glycosylation due to the fact that the C-6-hydroxy is a flexible primary alcohol and should more easily form a glycosidic linkage compared to a reaction involving a secondary alcohol (Scheme 3.11).



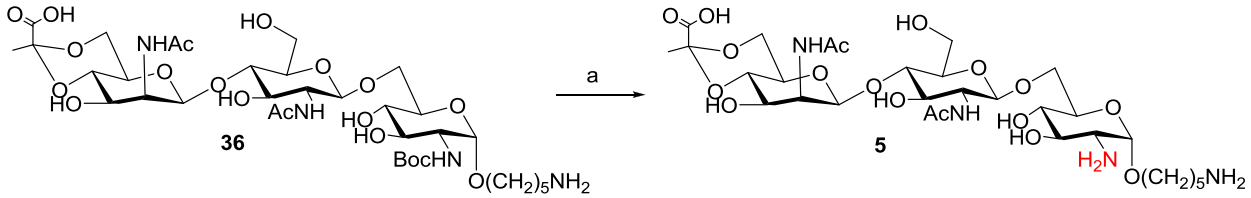
Scheme 3.11: Synthesis of Acceptor for Compound **4** for (2+2) Glycosylation. a) Triflic acid, Triethylsilane, DCM -78°C, (58%); b) **10**, DCM, TMSOTf, -20°C, (65%); c) DDQ, DCM, PBS (pH 7.4) (78%).

Compound **19** was reacted with triflic acid, triethylsilane, and DCM at -78°C in order to open up the 4,6-*O*-(2-naphthyl)methylene acetal ring[42] and afford **33** with the free hydroxyl at the C-4 position in 58% yield. Donor compound **10** and acceptor compound **34** were dissolved in DCM and cooled to -20°C for a 1+1 TMSOTf catalyzed glycosylation reaction. Compound **34** was formed in 65% yield as the desired beta (1→3) galactosylated product. Next the Nap protecting group was removed using freshly recrystallized DDQ, DCM, and PBS buffer (pH 7.4) to give **35** in 78% yield.[24] Donor compound **15** and acceptor compound **35** were dissolved in DCM and cooled to -20°C for a 2+2 TMSOTf catalyzed glycosylation reaction (Scheme 3.12). Compound **31** was formed in 48% yield as the desired beta (1→3) galactosylted product. Utilization of the more flexible C-6 primary alcohol for a glycosyl acceptor helped to improve the reaction yield. The azide and Troc functionalities on compound **31** were then converted to acetamido groups using Zn-Cu couple in acetic acid followed by reaction with acetic anhydride and pyridine.[32,33] Next, the benzylidene was removed by heating in an 80% HOAc (aq) solution.



Scheme 3.12: Modified (2+2) synthesis of Compound **4**. a) DCM, TMSOTf, -20°C, (48%); b) Zn-Cu couple, HOAc; c) Ac_2O, Pyridine; d) 80% HOAc, 50°C; e) LiOH, THF, H_2O,(37% over 4 steps); f) $Pd(OH)_2$, *t*-BuOH, H_2O, HOAc, H_2 (1 Atm), (quant.).

Afterwards, the Fmoc and acetyl groups were removed by stirring with LiOH, THF, and water in a warm water bath. At this point, the intermediate compound was purified give **32** in 37% yield over 4 steps. Compound **32** was taken up in $Pd(OH)_2$, t-butanol, water, and acetic acid in order to remove the remaining aromatic protecting groups affording target compound **4** in quantitative yield.



Scheme 3.13: Synthesis of Compound **5**: a) 1:4 TFA/DCM, 0°C, (85%).

Compound **36** was synthesized according to a previously described procedure described in Chapter 2 of this thesis. The Boc protecting group on **36** was removed using a 1:4 mixture of TFA:DCM at 0°C to afford target compound **5** in 85% yield (Scheme 3.13).

PatB1 Biological Studies: Previous work has shown the PatB1 acetyltransferase to be capable of specifically acetylating the O-3 β-GlcNAc position of a synthetic oligosaccharide based on the

trisaccharide backbone of *B. anthracis* SCWP (**1**).[12] In nature, the terminal trisaccharide unit of *B. anthracis* has some distinct differences from the internal trisaccharide repeat including a 4,6 ketal puryvyl on the non-reducing ManNAc, a 3-*O*-Acetyl on the β-GlcNAc, and a free amine on the reducing end portion of the unit. In addition, the non-reducing α-glucosamine of the terminal trisaccharide unit may be further modified with an α-galactose at C-3 and a β-galactose at C-4, but these modifications have yet to be confirmed.[6] To see if galactosylation found on the natural substrate could play a role in enhancing or deterring 3-*O*-acetylation activity relative to non-galactosylated structures, PatB1 reaction assays were performed using target compounds **1**-**4** as acceptor substrates. In addition, experiments were performed using compound **5** in order to determine if the 4,6-*O*-pyruvyl ketal and/or free amine could play a modulating role in PatB1 specific activity or site specificity.

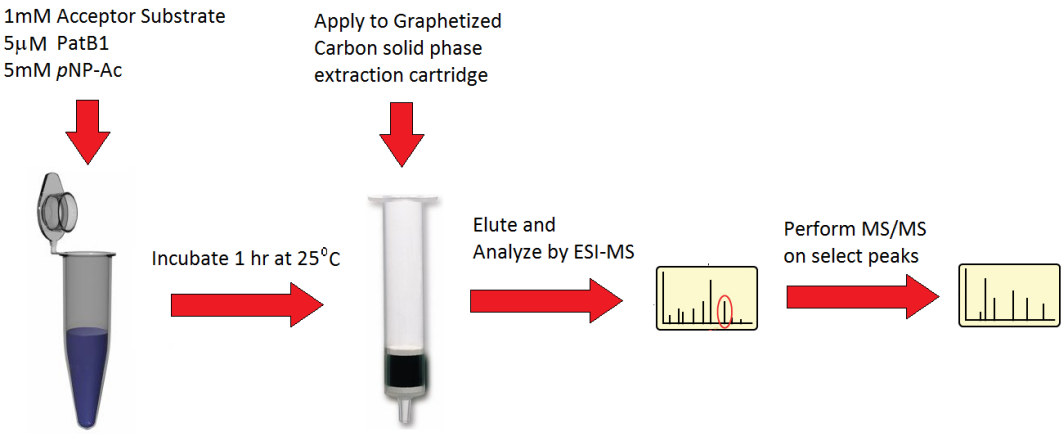


Figure 3.7: PatB1 Reaction Assay Using Synthetic Targets As Substrates

A qualitative endpoint experiment was performed in order to determine if compounds **1**-**5** could serve as acceptor substrates for PatB1 (Figure 3.7). In each experiment, 1mM of each acceptor compound was incubated in 50 mM sodium phosphate at pH 7 with 100 mM NaCl, 5μM PatB1, and 5mM pNP-Ac (Acetyl donor). The reactions were allowed to proceed for 1 h at 25°C and were analyzed by ESI-MS to determine the amount of acceptor converted to product. In addition, an MS/MS analysis

was performed on the product peaks to narrow down the site of O-acetylation to either the O-3 or O-6 position on β-GlcNAc. Compound **3** has an α-(1→3) linked galactose on β-GlcNAc and was not expected to be acetylated due to the O-3 position being blocked. This was confirmed when PatB1 was unable to use **3** as a substrate (Figure 3.8). The absence of O-acetylation served to demonstrate the site specificity of PatB1 as the enzyme was unable to acetylate any of the remaining hydroxyls. The fact that PatB1 has been previously confirmed by NMR to selectively install 3-*O*-acetyl on the β-GlcNAc of compound **1** along with the lack of a reaction between PatB1 and compound **3** suggest that site specificity is most likely retained for all the synthetic substrates in the panel.

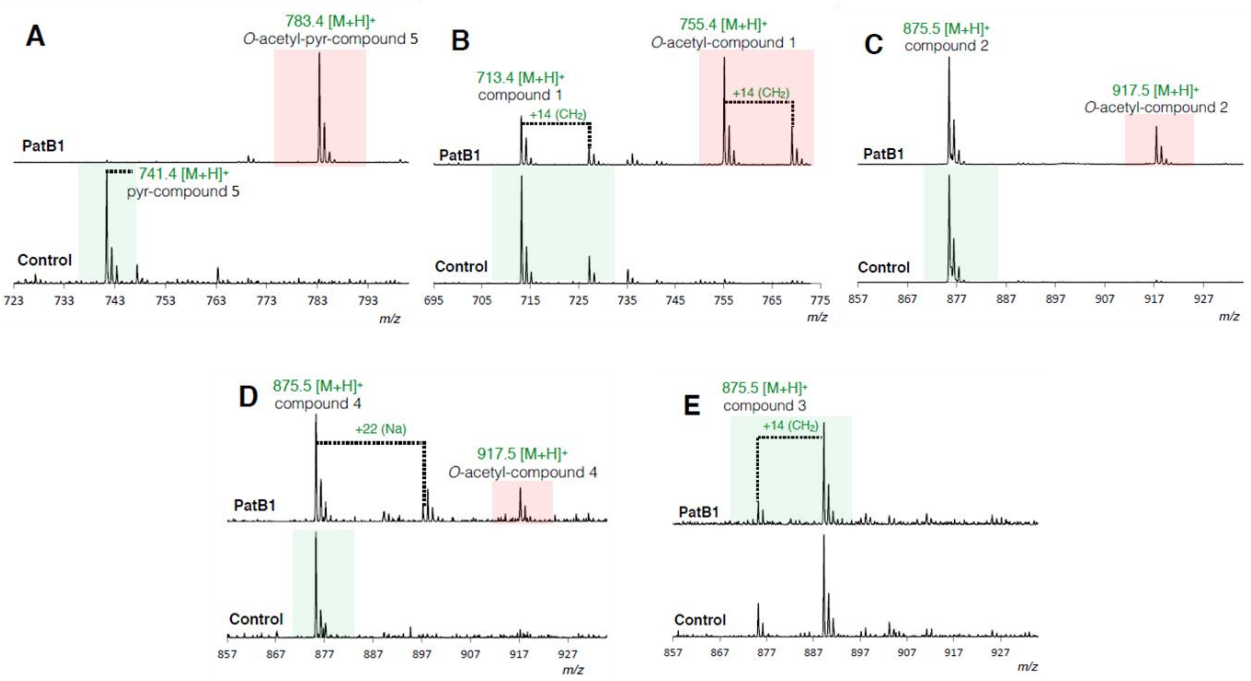


Figure 3.8: Conversion of Acceptor Substrate to O-acetylated product after 1 h at 25°C with PatB1. Monitored by ESI-MS.

Compound **2** has an α-(1→3) linked galactose on the reducing end α-GlcNAc and compound **4** has an β-(1→4) linked galactose on the reducing end α-GlcNAc. Acceptor compounds **2** and **4** both showed a modest conversion to the O-acetylated product. By comparison, the non-galactosylated compound **1**

appeared to be a much better substrate indicating that galactose substitution on SCWP has a negative effect on PatB1 mediated O-acetylation. The different positions of the galacose residues on **2** and **4** as well as their anomeric configuration did not provide for any significant differences in product formation between the two compounds. Surprisingly, compound **5** was found to be a significantly better substrate than compound **1** and was fully converted to the 3-*O*-acetylated form (Figure 3.8). The presence of unique structural features on the terminal unit appears to enhance the activity of the PatB1 enzyme.

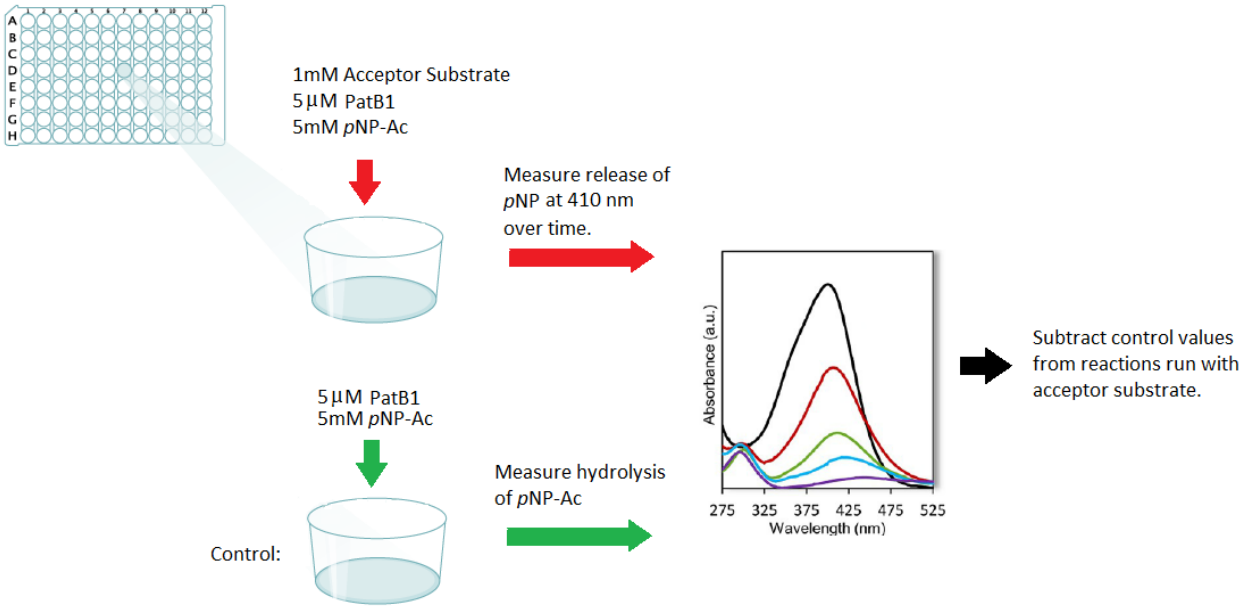
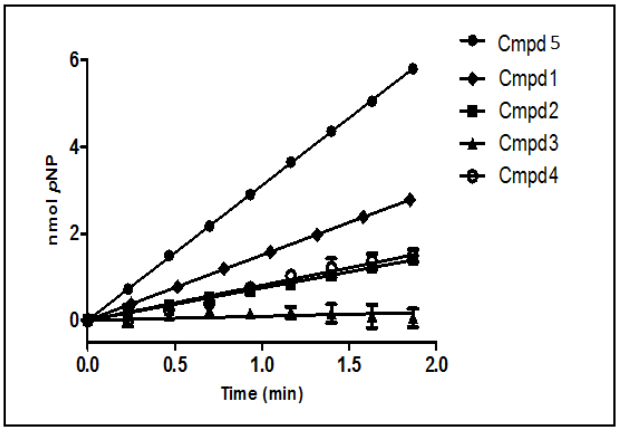


Figure 3.9: Biochemical assay To Determine PatB1 Specific Activity.

The quantitative chromogenic assay of Moynihan and Clarke[43] was adapted to determine the specific activity of $PatB1_{32\text{-}396}$ with the synthetic oligosaccharide substrates (Figure 3.9). The biochemical assay exploits the chromogenic properties of the liberated p-nitrophenol (pNP) co-product as the acetyl group is transferred from pNP-Ac to an acceptor co-substrate. The experiments were performed in 50 mM sodium phosphate at pH 7 with 100 mM NaCl, 5µM PatB1, 5mM pNP-Ac and 1 mM of each acceptor. It was found that the PatB1 acetyltransferase was capable of installing the 3-*O*-acetyl onto the β-GlcNAc of compound **1** with a specific activity of 47.20 ± 0.26 (nmol/min/mg). Compound **2** yielded a

specific activity of of 24.15 ± 0.23 (nmol/min/mg) which is almost half of that of compound **1**. Compound **4** yielded a similar result with a specific activity of 22.61 ± 0.26 (nmol/min/mg). Compound **3** did not show any activity due to an α-linked galactose at the O-3 position of the β-GlcNAc preventing O-3 acetylation (Figure 3.10). The results indicate that the presence of galactosylation on the *B. anthracis* SCWP can reduce the specific activity of the enzyme.



	Sp. Activity (nmol/min/mg)	SD	Relative activity (%)	SD
C5	96.321	0.538	100.000	0.789
C1	47.201	0.265	49.004	0.388
C2	24.151	0.172	25.073	0.227
C3	0.000	0.000	0.000	0.000
C4	22.610	0.264	23.474	0.304

Figure 3.10: Specific Activity of PatB1 for Substrates **1**-**5.**

Target compound **5**, based on the non-reducing terminal unit of *B. anthracis*, was also tested and yielded a specific activity of 96.32 ± 0.54 (nmol/min/mg). The specific activity of PatB1 on compound **5** was double that of compound **1** indicating that features of the terminal unit significantly enhance PatB1 binding and subsequent O-3 acetylation. The 4,6-*O*-pyruvyl ketal and/or free amine may help to confer selectivity for PatB1 O-acetylation and provide an explanation as to why 3-*O*-acetylation is only seen on the penultimate GlcNAc as opposed to the internal repeating units. Furthermore, the findings indicate that the 4,6-*O*-pyruvyl ketal function may play multiple roles *in vivo*. In addition to serving as a binding epitope for the SLH domain of S-layer and S-Layer associated proteins, it may also serve to enhance PatB1 mediated 3-*O*-acetylation of the penultimate β-GlcNAc on the *B. anthracis* SCWP.

CONCLUSIONS

In summary, a chemical synthesis methodology was developed for the purpose of synthesizing a library of complex oligosaccharides derived from the internal repeating unit of *B. anthracis* SCWP. A number of synthetic challenges were addressed, including the choice of appropriate orthogonal protecting groups, the selection of proper glycosylation conditions, the selection of method for the installation of the 1,2-cis-glycosidic linkage with beta configuration, and the selection of appropriate conditions for the reduction of the Troc and azide functions. A key part of the strategy for compounds **1**-**4** involves the use of neighboring group participation *via* an O-2 alloxycarbonyl on the donor to first install the beta linked glucoside. Following disaccharide formation, deprotection of the O-2 protecting group was performed and a triflate was installed at the position. Subsequent S_N2 inversion of the chiral center with the azide nucleophile afforded the β-linked mannoside. The C-2 Troc function was then utilized as a participation function in the proceeding glycosylation to afford a 1,2-trans linkage. The resulting trisaccharide was orthogonally protected with Nap, Fmoc, and Lev functions that could be selectively removed to introduce α-D-Gal and β-D-Gal branching points at desired positions. Compounds **1**-**3** were obtained in good yield using this strategy, but steric crowding led to low yields of compound **4**. To circumvent the problem, 2+2 glycosylation strategy was employed for the synthesis of target compound **4**. The strategy relied on the flexible C6 primary hydroxy of a disaccharide acceptor instead of the sterically crowded secondary alcohol at the C-4 position of α-GlcNAc on the trisaccharide acceptor. Furthermore, a carefully controlled reduction of the Troc and azide functions using Zinc-Copper couple was found to be a key step as excessive reaction times could lead to side product formation. A trisaccharide based on the terminal repeat (**5**) was also synthesized in order to determine if the presence of 4,6-*O*-pyruvyl ketal and/or free amine seen on the natural structure can affect PatB1 enzymatic activity and specificity. It was found that the presence of galactosylation on the trisaccharide repeating unit led to a decrease in specific activity relative to the non-galactosylated trisaccharide repeat. However, no loss of site

specificity was seen. In addition, it was found that the trisaccharide bearing the 4,6-*O*-pyruvyl ketal and free amine showed a significant increase in specific activity demonstrating that one or both of that these features are likely utilized in the natural environment to facilitate PatB1 mediated O-acetylation of SCWP.

EXPERIMENTAL

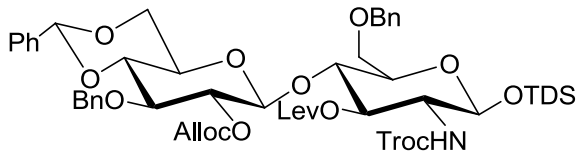
Cloning, Engineering, and Production of *B. cereus* PatB1: (Performed by David Sychantha/Anthony Clarke Group/ University of Guelph) *B. cereus* 10987 of BCE_0974 encoding PatB1 lacking its predicted N-terminal transmembrane helix (*viz*., amino acids 32-396) was PCR-amplified and cloned in E. coli DH5α (Invitrogen, Burlington, ON) using the Champion pET-SUMO expression vector in frame with an N-terminal His_6-SUMO tag. Mutant constructs of B. cereus patB1 (involving the replacement of Asp200, His202, Ser337, and Arg359) were produced using PCR-based site-directed mutagenesis involving the appropriate primers as described previously.[12,44] The residues of *B. cereus* PatB1 chosen for surface entropy reduction were determined using the SERp Server v1.20.[45] For general protein production, constructs were transformed into the expression strain E. coli T7 SHuffle (New England Biolabs, Mississauga, ON). Cells were grown in Luria-Bertani (LB) medium supplemented with 50 µg/mL kanamycin. To over-produce the proteins,1 L broth cultures were grown with shaking at 37 °C until early exponential phase (OD_{600} ~ 0.4) before being transferred to 15 °C. Cultures were left to grow for approximately one additional hour until a final OD_{600} of 0.6 - 0.8 was reached, at which point expression of the respective patB1s were induced by the addition of IPTG to a final concentration of 1 mM. Cells were left to incubate overnight (~ 14 h) at 15 °C and then they were harvested by centrifugation (8,000 × g, 15 min, 4 °C) and frozen at -20 °C until required. For the production of seleno-methione-labelled B. cereus PatB1, constructs were transformed into E. coli B834 and grown in M9 minimal media supplemented with 1 $mg \bullet mL^{-1}$ seleno-methionine as previously described.[46]

Determination of PatB1 activity as an O-acetyltransferase: (Performed by David Sychantha/Anthony Clarke Group/ University of Guelph) PatB1 catalyzed acetyltransfer to various carbohydrate acceptors was determined spectrophotometrically[43] using 1 mM of the respective oligosaccharide and pNP-Ac (5mM) as acetyl donor in assay buffer (50 mM sodium phosphate pH 7, 100 mM NaCl) and 5μM PatB1. To confirm PatB1 acetyltransferase activity, reaction products were analyzed by mass spectrometry. Reaction mixtures containing 1 mM of various acetyl-acceptors and 5 mM pNP-Ac were incubated for 1 h at 25 ºC. Products were reduced with sodium borohydride (10 $mg \cdot mL^{-1}$) and separated by adsorption chromatography on porous graphitized carbon (PGC), using either solid phase extraction cartridges (Carbograph SPE; Grace Altech; Columbia, MD) or a Thermo Hypercarb HPLC column (4.6 mm x 250 mm; Thermo Scientific, Rockford, IL) as described previously[48] with modifications. The cartridges were pre-equilibrated with one column volume of acetonitrile (ACN) and three column volumes of water and after sample adsorption, the cartridges were washed with five column volumes of water prior to desorption of the reaction products in a column volume of 50 % ACN. PGC-HPLC was performed at ambient temperature and samples were applied to the column in water at 1 $mL \cdot min^{-1}$. Elution was achieved with a linear gradient from 0 – 50 % ACN over 50 min with UV absorbance detection (λ =210 nm).

The eluted fractions were analyzed by electrospray ionization mass spectrometry (ESI-MS) by direct infusion using an Amazon SL ion-trap mass spectrometer (Bruker) at a flow rate of 5 $\mu L \cdot min^{-1}$ with a spray voltage of 4.5 kV. The ion-trap was operated in positive ion mode with and MS scans ranging from 200-2200 m/z. MS/MS scans were made on the the major ions with a fragmentation amplitude of 1.0. Mass spectra were analyzed using Bruker Compass tool (Bruker) and mMass (http//mmass.org).

General Synthetic Methods: Reactions were performed using flame-dried glassware under an atmosphere of argon using anhydrous solvents unless otherwise noted. Ambient temperature in the laboratory was usually 20°C. CH_2Cl_2 and CH_3CN were distilled freshly from CaH_2. Other commercially available reagents were obtained from Aldrich, Fisher or TCI and used as received. Thin layer

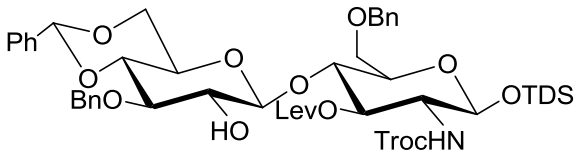
chromatography (TLC) was performed using aluminium backed Silica Gel 60 TLC plates w/UV254 from Merck KGaA. Visualization of TLC plates was performed by UV, 5% sulfuric acid/ethanol, or cerium molybdate stain. Silica gel flash chromatography was performed using silica gel (60 Å, 40-63 μm) from Silicycle, Canada. NMR spectra were recorded in the NMR facility of Complex Carbohydrate Research Center, UGA, on a Varian Mercury 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C), Varian Inova 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C), Varian Inova 600 with cryoprobe (600 MHz for ^{1}H, 150 MHz for ^{13}C), Varian VNMRS 600 with cryoprobe (600 MHz for ^{1}H, 150 MHz for ^{13}C) or Varian Inova 800 with cryoprobe (800 MHz for ^{1}H, 200 MHz for ^{13}C). Chemical shifts are reported in parts per million (ppm) on the δ scale. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ was referenced the solvent peak at 7.260 ppm (^{1}H) and 77.16 ppm (^{13}C), HOD (4.79). The assignments of ^{1}H NMR peaks were made from 2D ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY spectra. High resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF system (ESI) or an AB SCIEX TOF/TOF™ 5800 System (MALDI). Protein mass spectrometry data were obtained using a Bruker Microflex MALDI-TOF spectrometer



Thexyldimethylsilyl 2-*O*-allyloxycarbonyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-Glucopyranosyl-(1→4)-2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (11):

Glucosyl donor **6** (449 mg, 0.924 mmol) and glucosyl acceptor **7** (487 mg, 0.712 mmol) were taken up in toluene (15 mL) and concentrated under vacuum (3x). The mixture was taken up in DCM (16.2 mL) along with activated 4Å molecular sieves (900 mg) and stirred under argon for 30 min. The mixture was cooled to -20^{o}C and N-iodosuccinimide (248 mg, 1.102 mmol) was added followed by dropwise addition of TMSOTf (33.4 uL, 0.185 mmol). After stirring for 1 h, the reaction was quenched by the addition of Et_3N (0.5 mL), filtered, and concentrated. The residue was dissolved in DCM (50 mL) and washed with NaS_2O_3

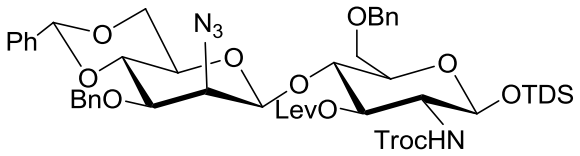
(sat, aq, 50 mL) and brine (50 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% EtOAc/hexanes) to give **11** (690 mg, 0.622 mmol, 87%) as an amorphous white solid. R_f = 0.35 (25% EtOAc/hexanes). H^1NMR (500 *MHz*, $CDCl_3$): δ 7.50-7.26 (m, 15H, aromatic), 6.04 (d, 1H, J = 6.65 Hz, NH), 5.97-5.83 (m, 1H, -CH=), 5.56 (s, 1H, CH), 5.38-5.24 (m, 2H, $=CH_2$), 5.14-5.05 (m, 1H, H-3^I), 4.92-4.78 (m, 2H, CHH, CHH^I), 4.91-4.52 (m, 9H, H-1, H-1^I, H-2, 3 x CH_2), 4.35-4.30 (m, 1H, H-6a), 3.93 (t, 1H, J = 9.45 Hz, H-4^I), 3.81-3.61 (m, 5H, H-3, H-4, H-6e, H-6^{I}ae), 3.61-3.53 (m, 1H, H-2^I), 3.46-3.39 (m, 1H, H-5^I), 3.31-3.23 (m, 1H, H-5), 2.73 (t, 2H, J = 6.30 Hz, CH_2), 2.53 (t, 2H, J = 6.30 Hz, CH_2), 2.18 (s, 3H, CH_3), 1.66-1.57 (m, 1H, CH), 0.87-0.84 (m, 12H, 4xCH_3), 0.18 (s, 3H, CH_3), 0.12 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.22, 172.38, 154.21, 154.13, 138.27, 137.24, 131.54, 129.16, 128.64, 128.55, 128.40, 128.37, 127.84, 127.74, 126.14, 119.23, 101.32, 101.15 (C-1), 96.22 (C-1^I), 95.65, 81.27 (C-4), 78.90 (C-3), 77.34 (C-2), 75.91 (C-4^I), 74.80 (C-5^I), 74.38, 73.44, 72.75 (C-3^I), 68.89, 68.64 (C-6), 67.91 (C-6^I), 66.21 (C-5), 58.46 (C-2^I), 38.00, 34.12, 30.04, 29.82, 28.12, 24.94, 20.11, 18.64, -1.78, -3.24. HR MALDI-TOF MS: m/z: calcd for $C_{53}H_{68}Cl_3NO_{16}Si$ $[M+Na]_+$: 1130.3265; found: 1130.3277.



Thexyldimethylsilyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-Glucopyranosyl-(1→4)-2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (12):

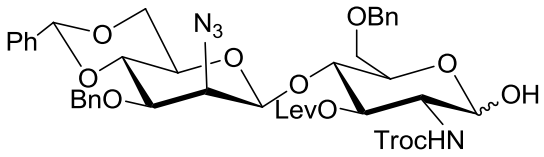
Compound **11** (1 g, 0.901 mmol) was dissolved in a mixture of THF (17.6 mL), water (1.76 mL), and morpholine (0.492 mL, 5.56 mmol). Tetrakis(triphenylphosphine)palladium(0) (521 mg, 0.451 mmol) was added and the reaction was stirred an atmosphere of argon for 3 h. After the reaction reached completion, the reaction mixture was filtered through a short pad of silica gel and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography

over silica gel (25% → 30%, EtOAc/hexanes) to give **12** (708 mg, 0.690 mmol, 77%) as an amorphous white solid: R_f = 0.15 (30% EtOAc/hexanes). H^1NMR (600 *MHz*, $CDCl_3$): δ 7.48-7.46 (m, 2H, aromatic), 7.39-7.25 (m, 13H, aromatic), 5.54 (s, 1H, CH), 5.38 (d, 1H, J = 9.39 Hz, NH), 5.18 (t, 1H, J = 9.98 Hz, H-3^{I}), 4.93 (d, 1H, J = 11.74 Hz, C*H*H), 4.77-4.74 (m, 2H, C*H*H, C*H*H^{I}), 4.71-4.65 (m, 3H, H-1^{I}, C*H*H', C*H*H^{II}), 4.58 (d, 1H, J = 12.32 Hz, C*H*H^{II}), 4.38 (d, 1H, J = 7.04 Hz, H-1), 4.31-4.28 (m, 1H, H-6a), 3.87-3.84 (m, 3H, H-4^{I}, C-6^{I}ae), 3.75 (t, 1H, J = 9.98 Hz, C-6e), 3.65-3.60 (m, 3H, H-2, H-2^{I}, H-4), 3.55 (t, 1H, J = 9.39 Hz, H-3), 3.43-3.40 (m, 1H, H-5^{I}), 3.34-3.30 (m, 1H, H-5), 2.71 (t, 2H, J = 6.30 Hz, CH_2), 2.57-2.51 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.66-1.59 (m, 1H, CH), 0.87-0.83 (m, 12H, 4xCH_3), 0.18 (s, 3H, CH_3), 0.13 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.03, 172.79, 154.35, 138.65, 137.81, 137.35, 132.21, 129.12, 128.58, 128.45, 128.38, 128.00, 127.98, 127.96, 127.76, 126.15, 103.91 (C-1), 101.30, 96.25 (C-1^{I}), 95.68, 81.29 (C-4), 80.57 (C-3), 76.83 (C-4^{I}), 74.82 (C-2), 74.71, 76.64, 74.52 (C-5^{I}), 73.64, 73.46 (C-3^{I}), 68.79 (C-6), 66.38 (C-6^{I}), 66.50 (C-5), 58.34 (C-2^{I}), 37.94, 34.13, 30.04, 28.16, 24.92, 20.16, 20.13, 18.68, 18.66, -1.88, -3.10. HR MALDI-TOF MS: m/z: calcd for $C_{49}H_{64}Cl_3NO_{14}Si$ $[M+Na]^+$: 1046.3054; found: 1046.3071.



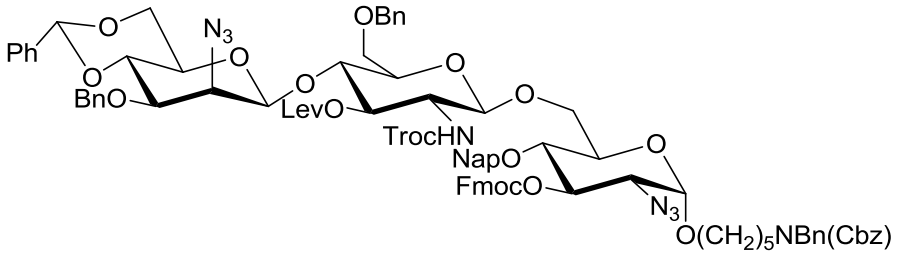
Thexyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranoside (13): To a stirred and cooled (0^{o}C) solution of **12** (666 mg, 0.65 mmol) and 4-dimethylaminopyridine (9.7 mg, 0.078 mmol) in DCM (8.8 mL) and pyridine (2.2 mL) under an atmosphere of argon was added triflic anhydride (0.769 mL, 4.55 mmol). The reaction mixture was stirred for 1 h at 0^{o}C and 1 h at room temperature. Completion of the reaction was verified by TLC (25% EtOAc/Hexanes + 1 drop Et_3N). The reaction mixture was diluted with DCM (80 mL) and washed with saturated NaHCO3 (2 × 80 mL), and brine (80 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to

afford an amorphous yellow solid. The crude was used in the proceeding reaction without further purification. To a stirred solution of the crude intermediate in DMF (11.7 mL) was added sodium azide (254 mg, 3.9 mmol). After stirring at 55 ^{o}C for 18 h, the reaction mixture was diluted with DCM (80 mL) and was washed with water (80 mL) and brine (80 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude was purified by flash chromatography over silica gel (20% EtOAc/hexanes) to give **13** (533 mg, 0.507 mmol, 78% over 2 steps) as an amorphous white solid: R_f = 0.25 (25% EtOAc/hexanes). H^{1}NMR (600 MHz, $CDCl_3$): δ 7.48-7.46 (m, 2H, aromatic), 7.40-7.25 (m, 13H, aromatic), 5.56 (s, 1H, CH), 5.17 (t, 1H, J = 9.98 Hz, H-3^{I}), 5.02 (d, 1H, J = 8.80 Hz, NH), 4.80 (d, 1H, J = 12.33 Hz, C*H*H), 4.75-4.66 (m, 4H, H-1^{I}, C*H*H, C*H*H^{I}, CH_2), 4.58 (s, 1H, H-1), 4.49 (d, 1H, J = 12.33 Hz, C*H*H^{I}), 4.28-4.26 (m, 1H, H-6a), 3.99 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.89 (t, 1H, J = 9.39 Hz, H-4), 3.81-3.68 (m, 4H, H-2, H-6e, H-6^{I}ae), 3.63-3.52 (m, 3H, H-2^{I}, H-3, H-5^{I}), 3.20-3.16 (m, 1H, H-5), 2.81-2.68 (m, 2H, CH_2), 2.60-2.51 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.64-1.59 (m, 1H, CH), 0.87-0.84 (m, 12H, 4xCH_3), 0.18 (s, 3H, CH_3), 0.13 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.30, 172.47, 154.25, 138.08, 137.97, 137.45, 129.14, 128.72, 128.63, 128.38, 128.17, 128.00, 127.98, 127.73, 126.21, 101.75, 99.83 (C-1), 96.46 (C-1^{I}), 95.61, 78.54 (C-4), 76.72 (C-3), 75.56 (C-4^{I}), 74.91, 74.54 (C-5^{I}), 73.93, 73.01, 72.59 (C-3^{I}), 68.86 (C-6^{I}), 68.57 (C-6), 67.49 (C-5), 63.66 (C-2), 58.50 (C-2^{I}), 38.13, 34.20, 30.01, 28.27, 25.03, 20.19, 20.16, 18.68, -1.73, -3.15. HR MALDI-TOF MS: m/z: calcd for $C_{49}H_{63}Cl_3N_4O_{13}Si$ $[M+Na]^+$: 1071.3119; found: 1071.3131.



2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranose (14): Compound **13** (529 mg, 0.504 mmol) was dissolved in THF (23 mL) and cooled to 0^{o}C under argon. HF·Py (2.8 mL)

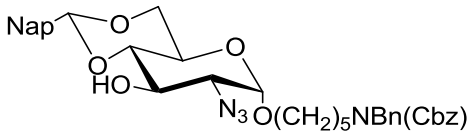
was slowly added and the reaction was allowed to warm to room temperature and stir for 12 h. The reaction mixture was cooled to 0°C and quenched *via* dropwise addition of $NaHCO_3$ (aq) to the stirring mixture. The quenched mixture was diluted with DCM (100 mL) and washed with saturated NaHCO3 (2 × 100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and, concentrated under vacuum. The crude was purified by flash chromatography over silica gel (30%→35%, EtOAc/hexanes) to give an α /β mixture of **14** (404 mg, 0.444 mmol, 88%) as an amorphous white solid: R_f = 0.8-0.9 (50 % EtOAc/hexanes). H^1NMR (600 MHz, $CDCl_3$): δ 7.49-7.47 (m, 2H, aromatic), 7.41-7.22 (m, 13H, aromatic), 5.56 (s, 1H, CH), 5.48 (d, 1H, J = 9.98 Hz, NH), 5.35 (t, 1H, J = 9.98 Hz, H-3^I), 5.30 (s, 1H, H-1^I), 4.81-4.67 (m, 5H, 2 x CH_2, C*H*H), 4.41 (s, 1H, H-1), 4.49 (d, 1H, J = 11.74 Hz, C*H*H), 4.29-4.27 (m, 1H, H-6a), 4.14-4.10 (m, 1H, H-5^I), 4.00-3.96 (m, 2H, H-2^I, H-4^I), 3.88 (t, 1H, J = 9.39 Hz, H-4), 3.78 (t, 1H, J = 9.98 Hz, H-6e), 3.72-3.64 (m, 4H, H-2, H-6e, H-6^{I}ae), 3.52-3.50 (m, 1H, H-3), 3.38 (br-s, 1H, OH), 3.16-3.11 (m, 1H, H-5), 2.81-2.75 (m, 1H, C*H*H^I), 2.72-2.67 (m, 1H, C*H*H), 2.59-2.51 (m, 2H, CH_2), 2.17 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.47, 172.73, 154.47, 149.80, 138.03, 137.64, 137.38, 129.16, 128.78, 128.62, 128.39, 128.35, 128.27, 127.99, 127.65, 126.17, 101.69, 99.40 (C-1), 95.62, 92.05 (C-1^I), 78.46 (C-4), 76.64 (C-3), 75.27 (C-4^I), 74.81, 73.80, 72.88, 70.94 (C-3^I), 69.95 (C-5^I), 68.51 (C-6), 68.38 (C-6^I), 67.33 (C-5), 63.49 (C-2), 54.41 (C-2^I), 37.98, 30.07, 28.22. HR MALDI-TOF MS: m/z: calcd for $C_{41}H_{45}Cl_3N_4O_{13}$ $[M+Na]^+$: 929.1941; found: 929.1955.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-4-*O*-(2-methylnaphthyl)-2-deoxy-

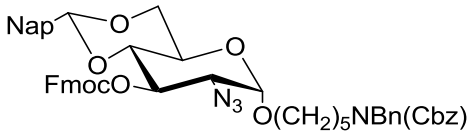
Glucopyanoside (16): To a solution of **14** (99.8 mg, 0.110 mmol) in DCM (6.4 mL) was added K_2CO_3 (152 mg, 1.098 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride (0.154mL, 1.1 mmol). The reaction was stirred at RT until completion was verified by TLC. R_f = 0.8 (35% EtOAc/hexanes + 0.1% TEA). Triethylamine (100 uL) was added to the reaction mixture and the mixture was filtered through celite and concentrated. The crude was passed through a short silica gel column (5% → 25% EtOAc/hexanes + 0.1% Et_3N) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl chloride and afford **15** (98%, 0.116g, 0.108 mmol) as an amorphous white solid. Donor compound **15** (0.116g, 0.107 mmol) and acceptor **8** (0.189g, 0.215 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (5.2 mL) along with 4Åmolecular sieves (300 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20^{o}C and TMSOTf (4.5 μL, 0.025 mmol) was added *via* dropwise addition. The mixture was allowed to warm to 0^{o}C and stir for 1 h. The reaction was quenched by the addition of pyridine (100 μL) and diluted in DCM (50 mL). The organic phase was washed with aqueous $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (20% → 40% EtOAc/hexanes) to afford a partially purified product. Afterwards, preparative TLC was used (50% EtOAc/hexanes) was used to remove residual acceptor and afford **16** (162 mg, 0.092 mmol, 85% over two steps) as an amorphous white solid. R_f = 0.3 (40% EtOAc/hexanes). H^1NMR (600 MHz, $CDCl_3$): δ 7.84-7.16 (m, 40H, aromatic), 5.55 (s, 1H, CH), 5.40 (t, 1H, J = 9.98 Hz, H-3^{II}), 5.17 (d, 2H, J = 20.54 Hz, CH_2), 4.09-4.97 (m, 1H, NH), 4.97-4.84 (m, 2H, H-1^{II}, H-3^{I}), 4.82-4.64 (m, 6H, 3 x CH_2), 4.59 (d, 1H, C*H*H), 4.50-4.44 (m, 4H, H-1, CH_2, C*H*H^{I}), 4.38-4.31 (m, 2H, C*H*H, C*H*H^{I}), 4.27-4.24 (m, 1H, H-6^{II}a), 4.20-4.14 (m, 1H, CH), 4.06-3.97 (m, 2H, H-1^{I}, H-6^{I}a), 3.89-3.79 (m, 3H, H-3, H-4, H-4^{I}), 3.79-3.60 (m, 7H, H-2, H-2^{I}, H-4^{II}, H-6e, H-6^{I}e, H-6^{II}ae), 3.51-3.49 (m, 1H, H-5^{II}), 3.47-3.32 (m, 2H, OCH_2), 3.30-3.10 (m, 5H, H-2^{II}, H-5, H-5^{I}, NCH_2), 2.81-2.68 (m, 2H, CH_2), 2.58-2.47 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 1.66-1.46 (m, 4H, 2 x CH_2), 1.40-1.24 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.37, 172.27, 156.81, 156.28, 154.74,

154.18, 143.34, 143.25, 141.35, 137.99, 137.93, 137.67, 137.28, 136.96, 136.85, 135.51, 133.24, 133.01, 131.03, 129.65, 129.12, 128.93, 128.65, 128.61, 128.57, 128.49, 128.34, 128.17, 127.94, 127.64, 127.33, 127.25, 126.65, 126.55, 126.43, 126.11, 125.85, 125.20, 120.08, 101.63, 100.98 (C-1^{I}), 99.72 (C-1), 98.12 (C-1^{II}), 95.53, 78.36 (C-4), 77.20 (C-3^{II}), 76.36 (C-5^{II}), 75.89 (C-4^{II}), 75.40 (C-4^{I}), 74.72, 74.33 (C-5^{I}), 73.64, 72.86, 72.32 (C-3^{I}), 70.19, 69.81 (C-3), 68.48, 68.43 (C-6^{II}), 68.31 (C-6^{I}), 67.28 (C-5), 66.97 (C-6), 63.50 (C-2), 61.72, 61.01 (C-2^{II}), 55.97 (C-2^{I}), 50.66, 50.35, 47.17, 46.79, 46.24, 38.03, 29.97, 29.14, 28.11, 27.99, 27.53, 23.48. HR MALDI-TOF MS: m/z: calcd for $C_{93}H_{95}Cl_3N_8O_{21}$ $[M+Na]^+$: 1787.5570; found: 1787.5589.



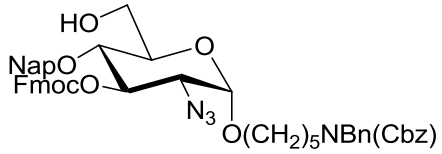
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-Glucopyanoside (18): Compound **17** (10.2 g, 15.92 mmol) was dissolved in methanol (30 mL) and NaOMe (30 mL, 0.2 M) and the reaction was stirred for 2 h. Afterwards, the reaction was neutralized with Dowex 50 W (H+) acidic resin, filtered, and concentrated under vacuum. The residue was co-evaporated with toluene and dried under high vacuum for 3 h. The crude was dissolved in acetonitrile (176 mL) with 2-naphthaldehyde dimethyl acetal (4.1 g, 20.22 mmol) and CSA (2.35 mg, 10.11 mmol). After stirring at room temperature for 8 h, the mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ (sat. aq.) solution. The organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography (20 →35% EtOAc/Hexanes) to give **18** (6.25 g, 9.96 mmol, 63 % over 2 steps) as an amorphous white solid. R_f = 0.3 (25% EtOAc/hexanes). H^1 NMR (300 MHz, $CDCl_3$)**:** 7.95 (s, 1H, aromatic), 7.83-7.79 (m, 3H, aromatic), 7.59-7.57 (m, 1H, aromatic), 7.47-7.46 (m, 2H, aromatic), 7.37-7.12 (m, 10H, aromatic), 5.62 (s, 1H, CH), 5.16 (d, 2H, J = 17.57 Hz, CH_2), 4.81 (d, 1H, J = 10.74 Hz, H-1), 4.47 (d, 2H, J = 13.67 Hz, CH_2), 4.30-4.23

(m, 1H, C-6a), 4.20 (t, 1H, J = 9.76 Hz, H-3), 3.88-3.79 (m, 1H, H-5), 3.72 (t, 1H, J = 10.25 Hz, H-6e), 3.68-3.56 (m, 1H, OC*H*H), 3.48 (t, 1H, J = 9.27 Hz, H-4), 3.41-3.12 (m, 4H, H-2, OCHH, NCH_2), 2.33 (s, 1H, OH), 1.64-1.43 (m, 4H, 2xCH_2), 1.39-1.20 (m 2H, CH_2).^{13}C NMR (75 MHz, $CDCl_3$): 156.79, 156.28, 137.92, 136.84, 134.36, 133.79, 132.92, 129.08, 128.57, 128.47, 128.29, 127.89, 127.77, 127.33, 126.59, 126.31, 125.88, 125.35, 123.76, 102.11, 98.64 (C-1), 82.04 (C-4), 68.92 (C-6), 68.60 (C-3), 68.43, 67.25,63.06 (C-2), 62.54 (C-5), 50.59, 50.32, 47.12, 46.18, 29.07, 27.87, 27.46, 23.29. HR MALDI-TOF MS: m/z: calcd for $C_{37}H_{40}N_4O_7$ $[M+Na]^+$: 675.2789; found: 675.2806.



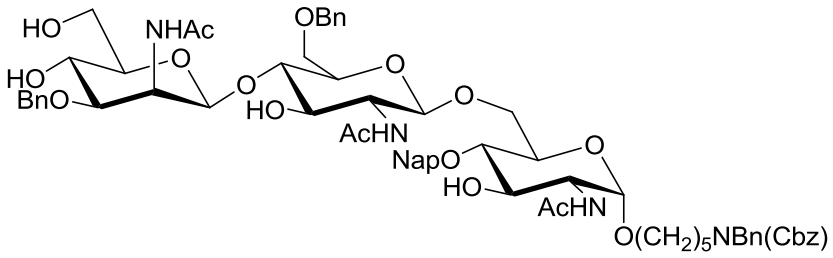
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-Fluorenylmethyloxycarbonyl- 4,6-*O*-benzylidene-2-deoxy-α-D-Glucopyanoside (19): 18 (6.25 g, 9.58 mmol) was dissolved in DCM (91 mL) and pyridine (45.3 mL). Fluorenylmethyloxycarbonyl chloride was dissolved in DCM (30 mL) and added to the stirring reaction mixture *via* cannula. After stirring at room temperature for 12 h, the reaction mixture was diluted with DCM (100 mL) and extracted with water (100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by recrystallization in ethyl acetate. The crystals were collected by filtration, rinsed with cold ethyl acetate, and dried under vacuum to afford **19** (7.07 g, 8.08 mmol, 84%) as a crystalline white solid. R_f = 0.35 (30% EtOAc/hexanes). H^1 NMR (500 MHz, $CDCl_3$)**:** 7.91 (s, 1H, aromatic), 7.80-7.70 (m, 4H, aromatic), 7.60-7.54 (m, 2H, aromatic), 7.47-7.14 (m, 18H, aromatic), 5.68 (s, 1H, CH), 5.46 (t, 1H, J = 9.98 Hz, H-3), 5.17 (d, 2H, J = 24.06 Hz, CH_2), 4.99-4.95 (m, 1H, H-1), 4.49 (d, 2H, J = 14.67 Hz, CH_2), 4.45-4.44 (m, 2H, CH_2), 4.37-4.30 (m, 1H, H-6a), 4.26 (t, 1H, J = 7.63 Hz, CH), 4.04-3.95 (m, 1H, H-5), 3.81 (t, 1H, J = 10.56 Hz, H-6e), 3.78 (t, 1H, J = 9.39 Hz, H-4), 3.74-3.65 (m, 1H, OC*H*H), 3.52-3.37 (m, 1-H, OC*H*H), 3.31-3.18 (m, 3H, H-2, NCH_2), 1.71-1.47 (m, 4H, 2xCH_2), 1.44-1.24 (m 2H, CH_2).^{13}C NMR (75 MHz,

$CDCl_3$): 156.84, 156.28, 154.59, 143.37, 143.25, 141.35, 138.02, 136.95, 134.24, 133.78, 132.92, 128.63, 128.50, 128.20, 127.95, 127.74, 127.37, 127.25, 126.56, 126.23, 125.87, 125.23, 123.82, 120.09, 102.07, 99.02 (C-1), 79.54 (C-4), 73.46 (C-3), 70.45, 69.98 (C-6), 68.74, 67.26, 62.86 (C-5), 61.62 (C-2), 50.68, 50.40, 47.14, 46.73, 46.27, 29.16, 27.98, 27.56, 23.42. HR MALDI-TOF MS: m/z: calcd for $C_{52}H_{50}N_4O_9$ $[M+Na]^+$: 897.3470; found: 897.3489.



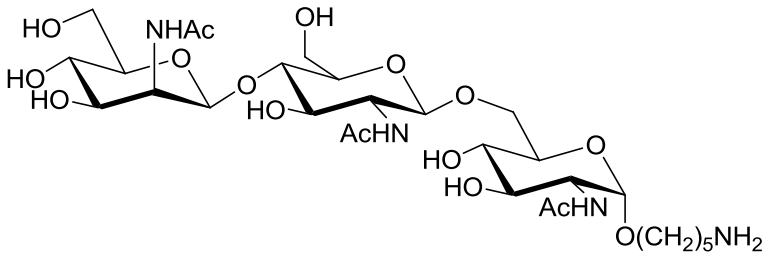
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-Fluorenylmethyloxycarbonyl-4-*O*-(2-methylnaphthyl)-2-deoxy-α-D-Glucopyanoside (8): Compound **19** (1.02 g, 1.17 mmol) was dissolved in DCM (45.2 mL) along with activated 4Å molecular sieves (1g) and cooled to -78°C under argon. Triethylsilane (0.560 mL, 3.51 mmol) was added to the stirring reaction mixture followed by dichlorophenyl borane (0.455 mL, 3.51 mmol). After stirring at -78°C for 2 h, the reaction mixture was quenched with pyridine, filtered over celite, and concentrated. The residue was taken up in DCM (100 mL) and extracted with water (100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel chromatography (30% → 35% EtOAc/Hexanes) to afford **8** (0.64 g, 0.728 mmol, 63%) as a colorless solid. R_f = 0.2 (35% EtOAc/hexanes). H^1 NMR (600 MHz, $CDCl_3$)**:** 7.80-7.16 (m, 25H, aromatic), 5.42 (t, 1H, J = 9.39 Hz, H-3), 5.16 (d, 2H, J = 25.82 Hz, CH_2), 4.92-4.92 (m, 1H, H-1), 4.83-4.76 (m, 2H, CH_2), 4.54-4.43 (m, 3H, C*H*H, CH_2), 4.34-4.25 (m, 2H, C*H*H), 4.20-4.12 (m, 1H, CH), 3.91-3.60 (m, 5H, H-4, H-5, H-6ae, OC*H*H), 3.51-3.34 (m, 1H, OC*H*H), 3.33-3.13 (m, 3H, H-2, NCH_2), 1.68-1.50 (m, 4H, 2xCH_2), 1.42-1.25 (m 2H, CH_2).^{13}C NMR (150 MHz, $CDCl_3$): 156.87, 156.38, 154.73, 143.38, 143.29, 141.41, 138.03, 137.02, 136.85, 135.00, 133.66, 133.30, 133.13, 131.32, 128.66, 128.60, 128.55, 128.40, 128.09, 127.99, 127.81, 127.44, 127.38, 127.30, 127.27, 126.87, 126.32, 126.19, 125.79, 125.24, 125.17, 120.14, 98.24 (C-1), 76.95 (C-3), 76.08-

75.89 (C-4), 75.14, 71.19-71.07 (C-5) 70.24, 68.48 (C-6), 67.31, 61.70, 61.59, 61.34 (C-2), 50.72, 50.41, 47.18, 46.82, 46.28, 29.12, 28.02, 27.42, 23.44. HR MALDI-TOF MS: m/z: calcd for $C_{52}H_{52}N_4O_9$ $[M+Na]^+$: 899.3627; found: 899.3645.



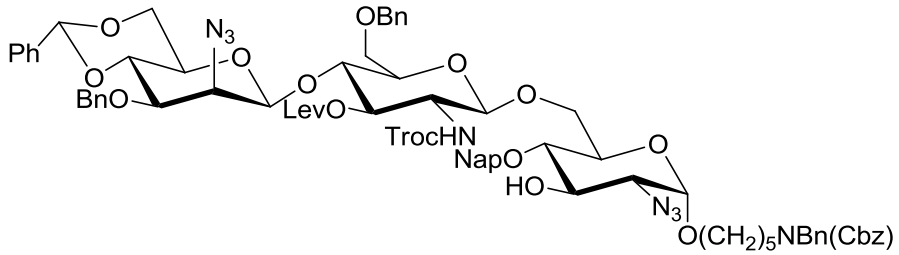
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl *O*-2-acetamido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl-(1→4)-*O*-2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D- glucopyranosyl-(1→6)-2-acetamido-4-*O*-(2-methylnaphthyl)-2-deoxy-*α*-D-glucopyranoside (23): Compound **16** (30 mg, 0.017 mmol) was dissolved in HOAc (0.97 mL) and Zinc-Copper couple (219 mg, 1.69 mmol) was added. The reaction was allowed to proceed until MALDI-TOF MS indicated disappearance of starting material (~1 h). It was found that reaction times in excess of 1 h led to the formation of an [M+1] side product. The reaction mixture was filtered and concentrated under vacuum. The crude was taken up in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude residue was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL) and stirred for 3hrs. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction mixture was concentrated under vacuum and the residue was co-evaporated three times with toluene in order to remove any residual pyridine. The residue was taken up in ethyl acetate (50 mL) and washed with $NaHCO_3$ (sat., aq., 50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude was taken up in 80% HOAc (aq, 2.8 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. Completion of the reaction was verified by MALDI-TOF MS analysis. The residue was taken up in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL)

and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude was taken up in a solution of THF (0.3 mL), water (0.015 mL), and 2M LiOH (0.066 mL) and stirred in a warm water bath (40°C) for 2.5 h. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction was neutralized with Dowex 50 W (H+) resin, filtered, and concentrated. The resulting crude was taken up in DCM (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL), and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum to afford an amorphous pale yellow solid. The crude material was purified by preparatory TLC (10% MeOH/DCM) to give **23** (10 mg, 7.95 μmol, 47 % over 4 steps) as an amorphous white solid. R_f = 0.25 (10% MeOH/DCM). HRMS(ESI): calcd for $C_{69}H_{84}N_4O_{18}$ $[M+Na]^+$: 1279.5673, found: 1279.5688.



5-Aminopentyl *O*-2-acetamido-2-deoxy- β-D-mannopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy -β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-α-D-glucopyranoside (1): Compound **23** (8.1 mg, 6.44 μmol) was dissolved in a mixture of t-BuOH (2 mL) and H_2O (0.05 mL). $Pd(OH)_2$/C (15 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated giving compound **1** (3.6 mg, 5.05 μmol, 78%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** δ 4.90 (bs, 1H, H-1), 4.84 (d, 1H, *J* = 3.52 Hz, H-1^{II}), 4.56

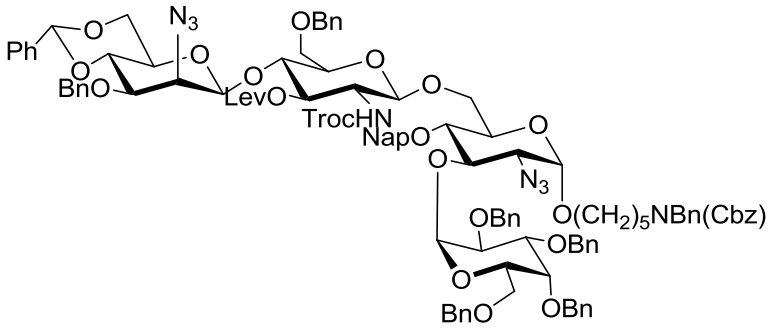
(d, 1H, *J* = 4.11 Hz, H-2), 4.52 (d, 1H, *J* = 8.22 Hz, H-1^{I}), 4.19 (d, 1H, *J* = 10.56 Hz, H-6a^{II}), 3.97-3.87 (m, 3H, H-2^{II}, H-6a^{I}, H-6a), 3.83-3.65 (m, 10H, H-2^{I}, H-3^{II}, H-3^{I}, H-3, H-4^{I}, H-5^{II}, H-6e^{II}, H-6e^{I}, H-6e, OC*H*H), 3.54-3.38 (m, 5H, H-4^{II}, H-4, H-5^{I}, H-5, OC*H*H), 3.01 (t, 2H, *J* = 7.63 Hz, NCH_2), 2.07 (1s, 3H), 2.04 (s, 6H), 1.72-1.58 (m, 4H, 2 x CH_2)), 1.48-1.43 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): δ 181.26, 175.28, 174.21, 101.39 (C-1^{I}), 99.27 (C-1), 96.46 (C-1^{II}), 78.71 (C-4^{I}), 76.41 (C-5), 74.31 (C-5^{I}), 72.20 (C-5^{II}), 71.80 (C-3), 70.90 (C-3^{I}), 70.44 (C-3^{II}), 70.00 (C-4^{II}), 68.71 (C-6^{II}), 67.35, 66.44 (C-4), 60.21 (C-6), 59.99 (C-6^{I}), 55.02 (C-2^{I}), 53.53 (C-2^{II}), 53.10 (C-2), 39.28, 27.84, 26.38, 22.27, 22.12, 21.87, 21.72. HRMS(ESI): calcd for $C_{29}H_{52}N_4O_{16}$ [M+H]+: 713.3451; found: 713.3475.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-azido-4-*O*-(2-methylnaphthyl)-2-deoxy-α-D-Glucopyanoside (24):

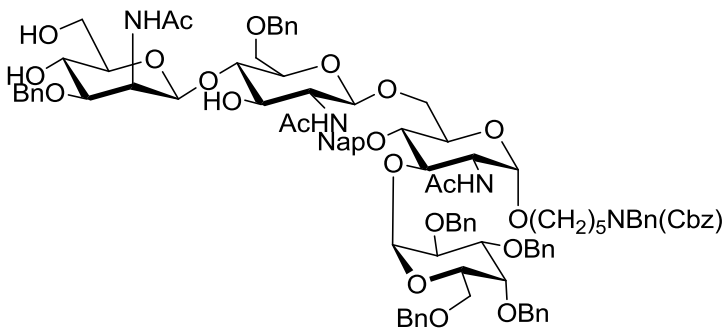
Compound **19** (78 mg, 0.044 mmol) was dissolved in DCM (4.3 mL) along with diethylamine (1 mL) and stirred for 2 h. The reaction mixture was concentrated and the resulting crude was purified by flash chromatography over silica gel (20% → 35% EtOAc/hexanes) to give **24** (67.5 mg, 0.044 mmol, 99%) as an amorphous white solid: R_f = 0.2 (35 % EtOAc/hexanes).H^{1}NMR (300 MHz, $CDCl_3$): δ 7.86-7.82 (m, 4H, aromatic), 7.51-7.13 (m, 28H, aromatic), 5.55 (s, 1H, CH), 5.22-5.12 (m, 2H, CH_2), 5.50-4.92 (m, 2H, H-3^{I}, C*H*H), 4.91-4.71 (m, 3H, H-1^{II}, C*H*H', C*H*H''), 4.70-4.54 (m, 4H, C*H*H, C*H*H', C*H*H'', C*H*H'''), 4.54-4.44 (m, 3H, H-1, CH_2), 4.38 (d, 1H, J = 11.72 Hz, C*H*H'''), 4.29-4.24 (m, 2H, H-1^{I}, H-6^{II}a), 4.16-4.06 (m, 2H, H-3^{II}, H-6a), 3.93-3.85 (m, 2H, H-4, H-4^{I}), 3.81-3.49 (m, 9H, H-2, H-2^{I}, H-3, H-4^{II}, H-5^{II}, H-6e, H-6ae^{I}, H-6^{II}e), 3.45-3.31 (m, 3H, H-5^{I}, OCH_2), 3.30-3.10 (m, 4H, H-2^{II}, H-5, NCH_2), 2.81-2.68 (m, 2H, CH_2), 2.58-2.47 (m, 2H,

CH_2), 2.16 (s, 3H, CH_3), 1.65-1.43 (m, 4H, 2 x CH_2), 1.40-1.24 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.49, 172.32, 156.90, 156.32, 154.23, 138.02, 137.98, 137.75, 137.34, 136.99, 135.84, 133.38, 133.16, 129.14, 128.67, 128.59, 128.36, 128.20, 128.00, 127.94, 127.68, 127.40, 126.85, 126.65, 126.44, 126.15, 126.01, 101.69, 101.00 (C-1^{I}), 99.70 (C-1), 97.79 (C-1^{II}), 95.50, 78.41 (C-4), 78.18 (C-5^{II}), 77.36 (C-4^{II}), 76.46 (C-4^{II}), 75.44 (C-4^{I}), 74.96, 74.73, 74.43 (C-5^{I}), 72.90, 72.31 (C-3^{I}, C-3^{II}), 69.96 (C-3), 68.63 (C-6^{I}), 68.46 (C-6^{II}), 68.29, 67.33 (C-6), 67.31 (C-5), 67.30, 63.53 (C-2), 63.04 (C-2^{II}), 55.98 (C-2^{I}), 50.63, 50.39, 47.23, 46.27, 38.06, 29.99, 29.21, 28.12, 27.59, 23.51. HR MALDI-TOF MS: m/z: calcd for $C_{78}H_{85}Cl_3N_8O_{19}$ $[M+Na]^+$: 1565.4889; found: 1565.4901.



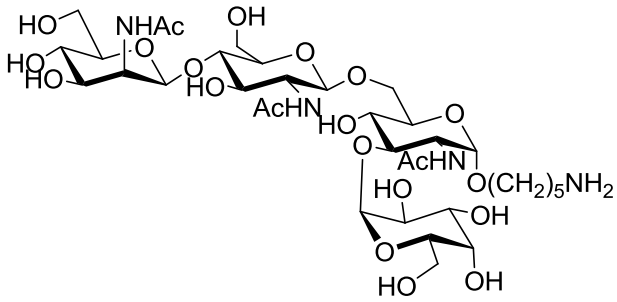
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-[*O*-(2, 3, 4, 6-tetra-*O*-benzyl-α-D-Galactopyanosyl -(1→3)]-2-azido-4-*O*-(2-methylnaphthyl)-2-deoxy-α-D-Glucopyanoside (25): Glucosyl acceptor **24** (39 mg, 0.025 mmol) was taken up in toluene (15 mL) and concentrated under vacuum (3x). The acceptor was then taken up in a mixture of DCM (0.14 mL) and diethyl ether (0.7 mL) along with activated 4Å molecular sieves (100 mg) and stirred under argon for 30 min. The mixture was cooled to -20^{o}C and TMSOTf (1.1 μL, 5.8 μmol) was added *via* dropwise addition. Donor **9** (69 mg, 0.101 mmol) was taken up in a minimal volume of DCM and added to the stirring reaction mixture via dropwise addition. The reaction mixture was allowed to warm to -10^{o}C and stir for 1 h. Afterwards, the reaction was quenched by the addition of pyridine (0.3 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (50 mL) and washed

with $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic phase was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% →35% EtOAc/hexanes) to afford a mixture of **24** and hydrolyzed donor. The partially purified product was taken up in Ac_2O (0.5 mL) and Pyridine (0.5 mL) in order to acetylate the hydrolyzed donor and change its R_f value. Afterwards, the mixture was concentrated and preparative TLC was used (10% Acetone/toluene) to remove residual acceptor and afford **24** (44.2 mg, 0.021 mmol, 84%) as an amorphous white solid. R_f = 0.35 (35% EtOAc/hexanes)/ R_f = 0.2 (10% Acetone/toluene). H^1NMR (600 MHz, $CDCl_3$): δ 7.86-7.02 (m, 52H, aromatic), 5.55 (s, 1H, CH), 5.48 (s, 1H, H-1^{III}), 5.26-4.36 (m, 21H, H-1, H-1^{II}, H-4^{III}, H-3^{I}, 8xCH_2, C*H*H,), 4.32-4.20 (m, 4H, H-3^{II}, H-$6a^{II}$, C*H*H), 4.17-4.04 (m, 4H, H-1^{I}, H-3^{III}, H-2^{III}, H-5^{III}), 3.97 (d, 1H, J = 11.15 Hz, H-6), 3.92-3.83 (m, 2H, H-4, H-4^{I}), 3.83-3.52 (m, 10H, H-2, H-2^{I}, H-3, H-5^{II}, H-6e, H-$6e^{II}$, H-$6ae^{I}$, H-$6ae^{II}$, 3.51-3.45 (m, 1H, H-4^{II}), 3.46-3.30 (m, 2H, OCH_2), 3.30-3.16 (m, 3H, NCH_2, H-5^{I}), 3.15-3.07 (m, 2H, H-2^{II}, H-5), 2.81-2.68 (m, 2H, CH_2), 2.57-2.48 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.66-1.45 (m, 4H, 2 x CH_2), 1.40-1.22 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$)(Rotomers): δ 206.43, 172.30, 156.82, 156.28, 154.14, 138.90, 138.67, 138.26, 138.20, 138.02, 137.95, 137.71, 137.32, 137.01, 136.86, 136.06, 133.33, 133.30, 132.98, 132.86, 129.14, 128.65, 128.59, 128.52, 128.47, 128.44, 128.36, 128.28, 128.21, 128.19, 128.16, 128.12, 128.07, 127.97, 127.94, 127.79, 127.75, 127.66, 127.65, 127.55, 127.50, 127.43, 127.33, 126.69, 126.42, 126.22, 126.13, 125.72, 125.70, 125.40, 101.64, 100.91 (C-1^{I}), 100.63, 99.73 (C-1), 99.60 (C-1^{III}), 98.11-97.94 (C-1^{II}), 95.65, 95.51, 79.57 (C-5^{III}), 78.98-78.89 (C-5^{II}), 78.37 (C-4), 76.44 (C-3^{II}), 76.38 (C-4^{II}), 75.42 (C-4^{I}), 75.34 (C-3^{III}), 75.06, 74.86, 74.73 (C-2^{III}), 74.53, 74.31, 74.28, 74.15, 74.09 (C-5^{I}), 73.62, 73.58, 73.50, 73.36, 72.85, 72.58, 72.30-72.25 (C-3^{I}), 69.90 (C-4^{III}), 69.79 (C-3), 68.59, 68.53 (C-6^{I}), 68.48 (C-6^{III}), 68.44 (C-6^{II}), 68.24, 68.02, 67.28, 67.08 (C-5, C-6), 64.49, 63.46 (C-2), 62.00 (C-2^{II}), 55.96 (C-2^{I}), 50.66, 50.36, 47.24, 46.25, 38.05, 30.02, 29.19, 29.12, 28.11, 28.02, 27.55, 23.50. HR MALDI-TOF MS: m/z: calcd for $C_{112}H_{119}Cl_3N_8O_{24}$ $[M+Na]^+$: 2087.7295; found: 2087.7311.



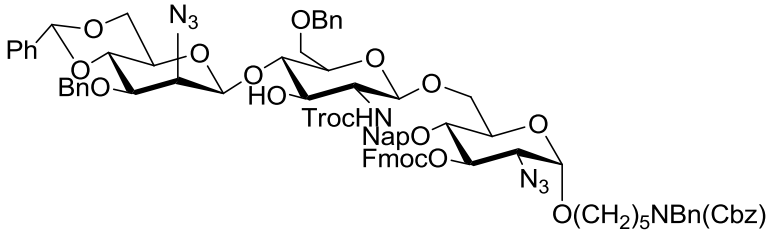
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl *O*-2-acetamido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl-(1→4)-*O*-2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D- glucopyranosyl-(1→6)-[*O*-(2, 3, 4, 6-tetra-*O*-benzyl-α-D-Galactopyanosyl -(1→3)]-2-acetamido-4-*O*-(2-methylnaphthyl)-2-deoxy-*α*-D-glucopyranoside (26). Compound **25** (35 mg, 0.017 mmol) was dissolved in HOAc (0.74 mL) and Zinc-Copper couple (218 mg, 1.69 mmol) was added. The reaction was allowed to proceed until MALDI-TOF MS indicated disappearance of starting material (~1 h). It was found that reaction times in excess of 1 h led to the formation of an [M+1] side product. The reaction mixture was filtered and concentrated under vacuum. The crude was taken up in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude residue was dissolved in Pyridine (0.5 mL) and acetic anhydride (0.5 mL) and stirred for 3hrs. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction mixture was concentrated under vacuum and the residue was co-evaporated three times with toluene in order to remove any residual pyridine. The residue was taken up in ethyl acetate (50 mL) and washed with $NaHCO_3$ (sat., aq., 50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum. Without further purification, the crude was taken up in 80% HOAc (aq, 2.8 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. Completion of the reaction was verified by MALDI-TOF MS analysis. The residue was taken up in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum. Without further

purification, the crude was taken up in a solution of THF (0.3 mL), water (0.015 mL), and 2M LiOH (0.066 mL) and stirred in a warm water bath (40°C) for 2.5 h. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction was neutralized with Dowex 50 W (H+) resin, filtered, and concentrated. The resulting crude was taken up in DCM (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL), and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to afford an amorphous pale yellow solid. The crude material was purified by preparatory TLC (5% MeOH/DCM) to give **26** (13 mg, 7.3 μmol, 43 % over 4 steps) as an amorphous white solid. R_f = 0.25 (5% MeOH/DCM). HRMS(ESI): calcd for $C_{103}H_{118}N_4O_{23}$ $[M+Na]^+$: 1801.8079, found: 1801.8102.



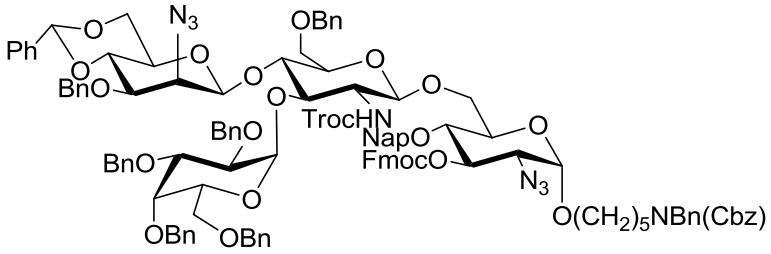
5-Aminopentyl O-2-acetamido-2-deoxy- β-D-mannopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[O-(α-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranoside (2): Compound **27** (12.1 mg, 6.80 μmol) was dissolved in a mixture of t-BuOH (2.1 mL) and H_2O (0.05 mL). $Pd(OH)_2$/C (20 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated giving compound **2** (4.3 mg, 4.91 μmol, 72%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** δ 5.32 (s, 1H, H-1^{III}),

4.86 (s, 1H, H-1), 4.78 (s, 1H, H-1^{II}), 4.51 (s, 1H, H-2), 4.47 (d, 1H, *J* = 7.63 Hz, H-1^{I}), 4.13 (d, 1H, *J* = 11.15 Hz, H-6^{II}a), 3.99-3.97 (m, 1H, H-2^{II}), 3.93 (brs, 1H, H-3^{III}), 3.88-3.62 (m, 18H, H-2^{I}, H-2^{III}, H-3^{II}, H-3^{I}, H-3, H-4^{II}, H-4^{I}, H-4^{III}, H-5^{II}, H-5^{III}, H-6^{II}e, H-6ae^{I}, H-6ae, H-6ae^{III}, OC*H*H), 3.47-3.34 (m, 4H, H-4, H-5^{I}, H-5, OC*H*H), 2.97-2.95 (m, 2H, NCH_2), 2.02 (s, 3H, CH_3), 2.00 (s, 6H, 2xCH_3), 1.69-1.55 (m, 4H, 2xCH_2), 1.47-1.36 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): δ 175.26, 174.18, 173.96, 101.44 (C-1^{I}), 99.39 (C-1^{III}), 96.24 (C-1), 99.67 (C-1^{II}), 78.67 (C-4^{I}), 77.86 (C-4^{III}), 76.37 (C-5), 74.27 (C-5^{I}), 72.12 (C-5^{II}), 71.76 (C-3), 70.84 (C-3^{II}), 70.49 (C-5^{III}), 70.19 (C-3^{I}), 69.14 (C-4^{II}), 68.84 (C-3^{III}), 68.46 (C-2^{III}), 68.40 (C-6^{II}), 67.30, 66.39 (C-4), 60.49 (C-6), 60.15 (C-6^{III}), 59.92 (C-6^{I}), 54.99 (C-2^{I}), 53.06 (C-2), 51.85 (C-2^{II}), 39.22, 27.87, 26.38, 22.29, 22.11, 21.89, 21.81. HRMS(ESI): calcd for $C_{35}H_{62}N_4O_{21}$ [M+H]+: 875.3979; found: 875.3991.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-4-*O*-(2-methylnaphthyl)-2-deoxy-Glucopyanoside (27): Compound **19** (23.2 mg, 0.013 mmol) was dissolved in a mixture of MeOH (0.09 mL) and DCM (0.9 mL) along with hydrazine acetate (7.25 mg, 0.079 mmol) and stirred at room temperature for 3 h. Completion of the reaction was verified by TLC (35% Ea/hex). The reaction mixture was concentrated and the crude was purified by flash chromatography over silica gel (35% EtOAc/hexanes) to give **27** (14.1 mg, 64 %) as an amorphous white solid: R_f = 0.35 (35% EtOAc/hexanes). H^{1}NMR (600 MHz, $CDCl_3$): δ 7.82-7.70 (m, 5H, aromatic), 7.61-7.59 (m, 2H, aromatic), 7.50-7.14 (m, 33H, aromatic), 5.55 (s, 1H, CH), 5.42-5.38 (m, 1H, H-3^{I}), 5.17 (d, 2H, J = 15.62 Hz, CH_2), 4.94 (d, 1H, J = 10.74 Hz, H-1^{II}), 4.84-4.33 (m, 13H, H-1, 6 x CH_2), 4.29-4.26 (m, 1H, H-6^{II}a), 4.21-4.18 (m,

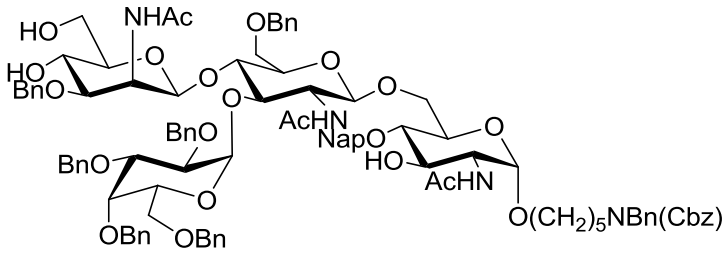
1H, CH), 4.05-4.03 (m, 1H, H-6a), 3.93 (t, 1H, J = 9.27 Hz, H-4^{I}), 3.85-3.50 (m, 9H, H-2, H-3, H-4, H-4^{II}, H-5^{I}, H-6^{I}e, H-6^{II}e, H-6^{I}ae), 3.47-3.32 (m, 3H, H-2^{I}, OCH_2), 3.30-3.12 (m, 6H, H-2^{II}, H-3^{I}, H-5, H-5^{II}, NCH_2), 1.70-1.47 (m, 4H, 2xCH_2), 1.40-1.24 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.84, 156.34, 154.78, 154.22, 143.42, 143.25, 141.44, 141.40, 138.03, 137.82, 137.79, 137.06, 136.88, 135.69, 133.20, 133.07, 129.25, 128.70, 128.66, 128.61, 128.54, 128.41, 128.39, 128.28, 128.17, 128.11, 127.98, 127.92, 127.70, 126.84, 126.77, 126.60, 126.21, 126.12, 125.25, 125.22, 120.16, 120.13, 101.76, 101.02 (C-1), 100.79 (C-1^{I}), 98.13 (C-1^{II}), 95.46, 81.47 (C-4), 78.21 (C-4^{I}), 77.57 (C-3^{II}), 76.25 (C-5^{I}), 74.82 (C-4^{II}), 74.39, 73.65, 73.58 (C-5^{II}), 73.25, 72.04 (C-3^{I}), 70.18, 69.79 (C-3), 68.51, 68.39 (C-6^{I}), 68.07 (C-6^{II}), 67.42 (C-5), 66.80 (C-6), 63.55 (C-2), 61.02 (C-2^{II}), 56.92 (C-2^{I}), 50.70, 50.39, 47.19, 46.88, 46.27, 29.16, 28.02, 27.54, 23.52. HR MALDI-TOF MS: m/z: calcd for $C_{88}H_{89}Cl_3N_8O_{19}$ $[M+Na]^+$: 1689.5202; found: 1689.5232.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)-[O-(2, 3, 4, 6-tetra-*O*-benzyl-α-D-Galactopyanosyl -(1→3)]- 2-(2,2,2-trichloroethoxy)carbonylamino-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-azido-4-*O*-(2-methylnaphthyl)-2-deoxy-α-D-Glucopyanoside (26): Glucosyl acceptor **27** (39 mg, 0.023 mmol) was taken up in toluene (15 mL) and concentrated under vacuum (3x). The acceptor was then taken up in a mixture of DCM (0.13 mL) and diethyl ether (0.64 mL) along with activated 4Å molecular sieves (100 mg) and stirred under argon for 30 min. The mixture was cooled to -30^{o}C and TMSOTf (0.96 μL, 5.32 μmol) was added *via* dropwise addition. Donor **9** (63 mg, 0.093 mmol) was taken up in a minimal volume of DCM and added to the stirring reaction mixture *via* dropwise addition. The reaction mixture was allowed to warm to -10^{o}C and stir for 1 h. Afterwards, the reaction was quenched by the addition of

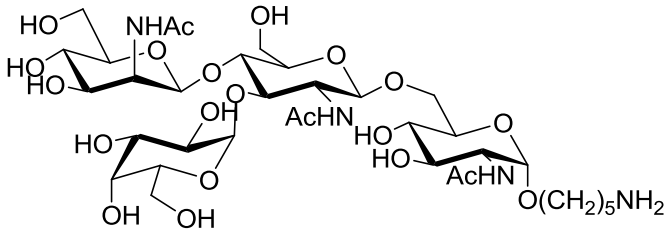
pyridine (0.3 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (50 mL) and washed with $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% → 35% EtOAc/hexanes) to afford an impure mixture of **24**. The partially purified product was taken up in Ac_2O (0.5 mL) and Pyridine (0.5 mL) in order to acetylate the hydrolyzed donor and change its R_f value. Afterwards, the mixture was concentrated and preparative TLC was used (35% EtOAc/hexanes) to remove residual acceptor and afford **24** (31.9 mg, 0.015 mmol, 63%) as an amorphous white solid. R_f = 0.65 (35% EtOAc/hexanes. H^1NMR (600 MHz, $CDCl_3$): δ 7.78-7.68 (m, 5H, aromatic), 7.51-7.14 (m, 55H, aromatic), 6.42 (d, 1H, J = 9.39 Hz, NH), 5.48 (bs, 1H, H-1^{III}), 5.39-5.35 (m, 2H, H-3^{I}, CH), 5.17 (d, 2H, J = 21.72 Hz, CH_2), 4.87-4.78 (m, 3H, H-1^{II}, C*H*H, C*H*H'), 4.75-4.4 (m, 18H, H-1, C*H*H, C*H*H', C*H*H'', 7xCH_2), 4.38-4.32 (m, 1H, C*H*H'''), 4.32-4.25 (m, 1H, H-4^{III}), 4.18 (d, 1H, J = 11.74 Hz, C*H*H''), 4.13-3.94 (m, 8H, H-2, H-2^{III}, H-3^{I}, H-3^{III}, H-6a, H-6^{II}a, C*H*H''', CH), 3.90-3.79 (m, 6H, H-3, H-4, H-4^{I}, H-4^{II}, H-5^{III}, H-$6a^{III}$), 3.72-3.69 (m, 1H, H-$6e^{III}$), 3.64-3.58 (m, 5H, H-5^{I}, H-6e, H-6e, H-$6a^{I}$, OC*H*H), 3.51 (t, 1H, J = 9.98 Hz, H-6^{II}e), 3.42-3.40 (m, 1H, H-5^{II}), 3.36-3.13 (m, 5H, H-2^{II}, H-6^{I}e, OC*H*H, NCH_2), 3.08-3.04 (m, 1H, H-5), 1.74-1.44 (m, 4H, 2xCH_2), 1.39-1.22 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.84, 156.28, 154.77, 154.15, 143.38, 143.29, 141.33, 141.28, 138.76, 138.50, 138.23, 138.01, 137.97, 137.95, 137.85, 137.41, 136.99, 136.87, 135.57, 135.54, 133.31, 133.02, 129.08, 128.70, 128.64, 128.58, 128.54, 128.52, 128.43, 128.36, 128.31, 128.25, 128.06, 128.02, 127.95, 127.92, 127.90, 127.83, 127.76, 127.71, 127.67, 127.57, 127.48, 127.36, 127.25, 127.19, 126.31, 126.15, 126.10, 126.06, 125.43, 125.26, 125.23, 120.06, 101.93 (C-1^{I}), 101.50, 98.15 (C-1), 98.07 (C-1^{II}), 97.88 (C-1^{III}), 95.89, 79.11 (C-4), 78.32 (C-4^{I}), 77.20 (C-5^{II}), 77.03 (C-3^{II}), 76.51 (C-5^{II}), 75.70 (C-3^{III}, C-4^{II}), 75.62 (C-2^{III}, C-5^{I}), 75.11, 74.93 (C-3^{I}), 74.29, 74.19, 74.09 (C-3), 73.96, 73.63, 73.14, 72.89, 72.83, 70.51 (C-6^{I}), 70.29, 69.88 (C-4^{III}), 69.82 (C-6^{III}), 68.38, 68.22 (C-6^{II}), 67.36 (C-5), 67.27, 67.02 (C-6), 63.25 (C-2), 61.13 (C-2^{II}), 55.15 (C-2^{I}), 50.69, 50.38, 47.18, 46.68, 46.29, 29.19, 28.05,

27.61, 23.51. HR MALDI-TOF MS: m/z: calcd for $C_{122}H_{123}Cl_3N_8O_{24}$ $[M+Na]^+$: 2211.7608; found: 2211.7635.



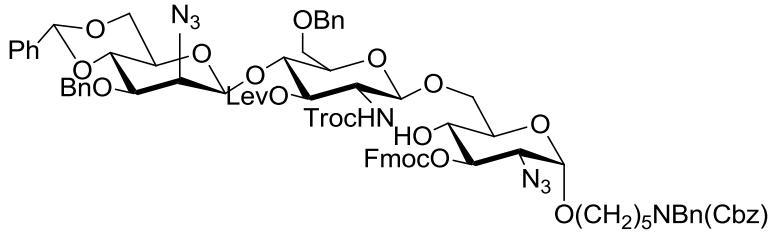
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl *O*-2-acetamido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl-(1→4)-[*O*-(2, 3, 4, 6-tetra-*O*-benzyl-α-D-Galactopyanosyl -(1→3)]-*O*-2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D- glucopyranosyl-(1→6)-2-acetamido-4-*O*-(2-methylnaphthyl)-2-deoxy-*α*-D-glucopyranoside (29): Compound **28** (35 mg, 0.016 mmol) was dissolved in HOAc (0.7 mL) and Zinc-Copper couple (206 mg, 1.6 mmol) was added. The reaction was allowed to proceed until MALDI-TOF MS indicated disappearance of starting material (~1 h). It was found that reaction times in excess of 1 h led to the formation of an [M+1] side product. The reaction mixture was filtered and concentrated under vacuum. The crude was taken up in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude residue was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL) and stirred for 3hrs. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction mixture was concentrated under vacuum and the residue was co-evaporated three times with toluene in order to remove any residual pyridine. The residue was taken up in ethyl acetate (50 mL) and washed with $NaHCO_3$ (sat., aq., 50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum. Without further purification, the crude was taken up in 80% HOAc(aq) (1.7 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. Completion of the reaction was verified by MALDI-TOF MS analysis. The residue was taken up in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL) and brine (50 mL). The

organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude was taken up in a solution of DCM (0.5 mL) and diethylamine (0.1 mL) and stirred for 2.5 h. Following completion of the reaction as verified by MALDI-TOF MS analysis, the reaction mixture was concentrated. The resulting crude was taken up in DCM (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL), and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum to afford an amorphous pale yellow solid. The crude material was purified by preparatory TLC (40% Acetone/toluene) to give **29** (12 mg, 6.7 μmol, 40 % over 4 steps) as an amorphous white solid. R_f = 0.25 (5% MeOH/DCM). HRMS(ESI): calcd for $C_{103}H_{118}N_4O_{23}$ $[M+Na]^+$: 1801.8079, found: 1801.8102.



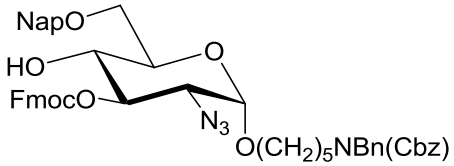
5-Aminopentyl O-2-acetamido-2-deoxy- β-D-mannopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[O-(α-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranoside (3): Compound **29** (8 mg, 4.49 μmol) was dissolved in a mixture of t-BuOH (1.3 mL) and H_2O (0.03 mL). $Pd(OH)_2/C$ (15 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated giving compound **3** (3.5 mg, 4 μmol, 89%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** δ 5.59 (d, 1H, J = 4.11 Hz, H-1^{III}), 4.90 (s, 1H, H-1), 4.84 (d, 1H, J = 3.52 Hz, H-1^{II}), 4.57 (d, 1H, J = 8.22 Hz, H-1^{I}), 4.54 (d, 1H, *J* = 4.11

Hz, H-2), 4.19 (d, 1H, J = 11.15 Hz, H-6^{II}a), 4.08 (t, 1H, J = 9.39 Hz, H-4^{I}), 3.99 (m, 1H, H-4^{III}), 3.95-3.70 (m, 15H, H-2^{I}, H-2^{II}, H-2^{III},H-3, H-3^{I}, H-3^{II}, H-3^{III}, H-4, H-6ae, H-6^{I}ae, H-6e^{II}, H-6^{III}ae), 3.67-3.63 (m, 1H, OC*H*H), 3.57-3.50 (m, 2H, H-4, H-5^{I}), 3.48-3.38 (m, 4H, H-5, H-5^{II}, H-5^{III}, OC*H*H), 3.00 (t, 2H, J = 7.63 Hz, NCH_2), 2.08 (s, 3H, CH_3), 2.03 (s, 6H, 2xCH_3), 1.71-1.59 (m, 4H, 2xCH_2), 1.48-1.40 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): δ 175.41, 174.23, 174.00, 100.99 (C-1^{I}), 98.50 (C-1), 97.51 (C-1^{III}), 96.49 (C-1^{II}), 78.55 (C-4^{I}, C-5^{II}), 75.26 (C-4), 74.46 (C-5^{I}), 71.61 (C-3^{III}), 70.95 (C-3), 70.79 (C-3^{I}), 70.39 (C-2^{III}), 70.01 (C-5, C-5^{III}), 69.21 (C-6^{II}), 68.83 (C-4^{III}), 68.70 (C-3^{II}), 68.50, 67.40, 66.42 (C-4), 60.55 (C-6^{III}), 60.04 (C-6^{I}), 59.80 (C-6), 54.38 (C-2^{I}), 53.53 (C-2^{II}), 53.03 (C-2), 39.28, 27.88, 26.38, 24.37, 22.31, 22.22, 21.96, 21.72. HRMS(ESI): calcd for $C_{35}H_{62}N_4O_{21}$ [M+H]+: 875.3979; found: 875.3996.



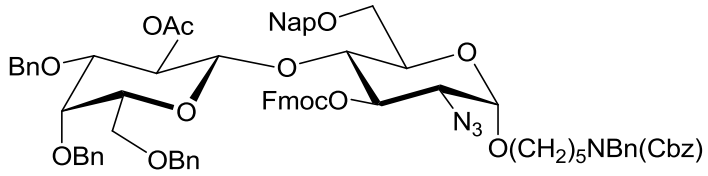
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-2-deoxy-Glucopyanoside (30): Compound **19** (54.2 mg,0.031 mmol) and DDQ (10.4 mg, 0.046 mmol) were dissolved in a mixture of DCM (0.5 mL) and PBS buffer (pH 7.4, 0.125 mL). The reaction was stirred until completion was verified by TLC (35% EtOAc/Hexanes). The reaction mixture was concentrated and the resulting crude was taken up in DCM (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL), and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude material was purified by silica gel chromatography (35% EtOAc/hexanes) to give **30** (42 mg, 0.026 mmol, 84%) as an amorphous white solid. R_f = 0.25 (35% EtOAc/hexanes). H^{1}NMR (600 MHz, $CDCl_3$): δ 7.77-7.75 (d, 2H, J = 7.04 Hz, aromatic), 7.64-7.59 (m, 2H, aromatic), 7.48-7.47 (m, 2H, aromatic), 7.42-7.17 (m, 27H,

aromatic), 5.55 (s, 1H, CH), 5.54-5.51 (m, 1H, NH), 5.23-5.20 (m, 3H, H-3^{I}, H-3^{II}, C*H*H), 5.14-5.11 (m, 1H, C*H*H), 4.93-4.91 (m, 1H, H-1^{II}), 4.79 (d, 1H, J = 12.33 Hz, C*H*H'), 4.77-4.69 (m, 2H, CH_2), 4.69-4.62 (m, 2H, C*H*H', CHH''), 4.51-4.25 (m, 7H, H-6^{II}a, CH, C*H*H'', 2xCH_2), 4.12-4.04 (m, 1H, H-6a), 3.96 (t, 1H, J = 9.39 Hz, H-3^{II}), 3.93-3.84 (m, 2H, H-4^{I}, H-6e), 3.85-3.55 (m, 8H, H-2, H-2^{I}, H-3, H-4, H-4^{II}, H-5^{I}, H-6^{II}e, H-6^{I}ae), 3.51-3.46 (m, 2H, H-5^{II}, OC*H*H), 3.46-3.24 (m, 2H, OC*H*H, NC*H*H), 3.24-3.16 (m, 2H, H-2^{II}, NC*H*H), 3.15-3.08 (m, 1H, H-5^{II}), 2.82-2.69 (m, 2H, CH_2), 2.58-2.50 (m, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.71-1.47 (m, 4H, 2 x CH_2), 1.44-1.26 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.62, 172.43, 156.91, 156.48, 155.32, 154.71, 143.48, 143.35, 141.42, 141.39, 137.98, 137.51, 137.32, 136.75, 129.18, 128.78, 128.68, 128.63, 128.51, 128.40, 128.34, 128.02, 127.89, 127.65, 127.37, 127.33, 126.15, 125.43, 125.40, 120.16, 101.70, 101.32-101.06 (C-1^{I}), 99.62 (C-1), 98.27 (C-1^{II}), 95.51, 78.42 (C-4), 76.92 (C-3^{II}), 76.45 (C-5^{II}), 75.14-74.95 (C-4^{I}), 74.83, 74.43 (C-5^{I}), 73.79, 72.90, 72.32 (C-3^{I}), 70.94 (C-4^{II}), 70.49, 69.21 (C-3), 68.87, 68.47 (C-6^{II}), 68.35-68.16 (C-6^{I}), 67.57 (C-6), 67.40 (C-5), 67.35, 63.46 (C-2), 61.10 (C-2^{II}), 55.85 (C-2^{I}), 50.62, 47.59, 46.82, 46.23, 38.16, 30.01, 29.42, 29.12, 28.99, 28.17, 27.99, 27.70, 24.13, 23.42. HR MALDI-TOF MS: m/z: calcd for $C_{82}H_{87}Cl_3N_8O_{21}$ $[M+Na]^+$: 1647.4944; found: 1647.4979.



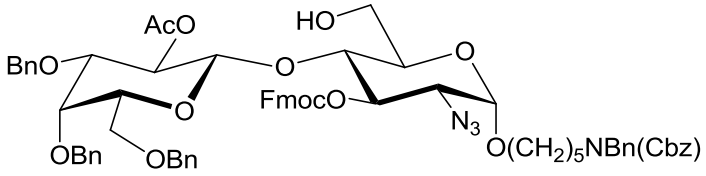
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-6-*O*-(2-methylnaphthyl)-2-deoxy-β-D-glucopyranoside (33): Compound **13** (0.25 g, 0.286 mmol) was dissolved in DCM (3.9 mL) along with activated 4Å molecular sieves (0.25 g) and cooled to -78^{o}C under argon. Triethylsilane (0.091 mL, 0.571 mmol) was added to the stirring reaction mixture followed by triflic acid (0.038 mL, 0.429 mmol). After stirring at -78^{o}C for 1 h, the reaction mixture was quenched with 1:1 MeOH/pyridine, filtered over celite, and concentrated. The residue was taken up in DCM (50 mL) and extracted with water (50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and

concentrated under vacuum. The residue was purified by silica gel chromatography (30% EtOAc/Hexanes) to afford **33** (0.145 g, 0.166 mmol, 58 %) as an amorphous white solid. R_f = 0.8 (35% EtOAc/hexanes). H^1NMR (500 MHz, $CDCl_3$): δ 7.84-7.76 (m, 6H, aromatic), 7.64-7.59 (m, 2H, aromatic), 7.42-7.15 (m, 17H, aromatic), 5.25-5.15 (m 1H, H-3), 5.24-5.20 (m 2H, H-2, C*H*H), 5.09 (bs, 1H, C*H*H), 4.98-4.94 (m, 1H, H-1), 4.80-4.71 (m, 2H, CH_2), 4.56-4.34 (m, 4H, 2xCH_2), 4.33-4.26 (m, 1H, CH), 3.95-3.62 (m, 5H, H-4, H-5, H-6ae, OC*H*H), 3.54-3.35 (m, 1H, OC*H*H), 3.33-3.10 (m, 3H, H-2, NCH_2), 1.74-1.46 (m, 4H, 2 x CH_2), 1.37-1.20 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.51, 149.38, 143.41, 141.46, 137.98, 133.38, 128.68, 128.53, 128.04, 127.89, 127.37, 126.72, 126.36, 126.18, 125.74, 125.40, 120.18, 98.18 (C-1), 77.37 (C-3), 73.99, 70.92, 70.72 (C-4), 70.55 (C-5), 69.38 (C-6), 68.84, 68.45, 67.42, 61.04 (C-2), 50.68, 47.77, 46.87, 46.21, 27.93, 27.73, 24.31. HR MALDI-TOF MS: m/z: calcd for $C_{52}H_{52}N_4O_9$ $[M+Na]^+$: 899.3627; found: 899.3650.



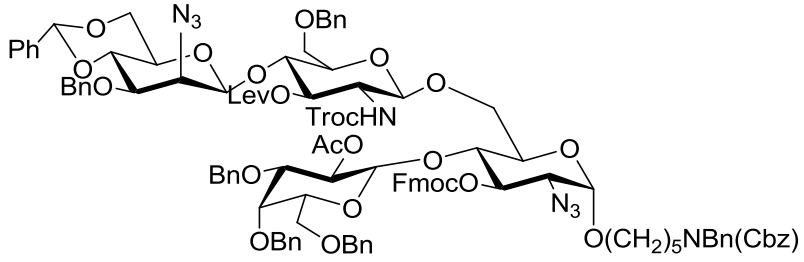
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-[O-(2, 3, 4, 6-tetra-*O*-benzyl-β-D-Galactopyanosyl-(1→4)]-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-6-*O*-(2-methylnaphthyl)-2-deoxy-β-D-glucopyranoside (34): Glucosyl acceptor **33** (145 mg, 0.166 mmol) was taken up in toluene (15 mL) and concentrated under vacuum (3x). The acceptor was then taken up in DCM (8 mL) along with activated 4Å molecular sieves (400 mg) and stirred under argon for 30 min. The mixture was cooled to -30°C and TMSOTf (6.9 μL, 0.038 mmol) was added *via* dropwise addition. Donor **10** (385 mg, 0.579 mmol) was taken up in a minimal volume of DCM and added to the stirring reaction mixture *via* dropwise addition. The reaction mixture was allowed to warm to -10°C while stirring. Afterwards, the reaction was quenched by the addition of pyridine (0.3 mL), filtered, and concentrated under vacuum. The residue was dissolved in DCM (50 mL) and washed with $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic

layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% EtOAc/hexanes) to afford **34** (145 mg, 0.107 mmol, 65%) as an amorphous white solid. R_f = 0.4 (30% EtOAc/hexanes). H^1NMR (500 MHz, $CDCl_3$): δ 7.82-7.73 (m, 6H, aromatic), 7.55-7.09 (m, 34H, aromatic), 5.29-5.25 (m 1H, H-3), 5.24-5.20 (m 1H, H-2^I), 5.16 (d, 2H, J = 13.18 Hz, CH_2), 4.95-4.91 (m, 1H, H-1), 4.87 (d, 1H, J = 12.69 Hz, C*H*H), 4.78 (d, 1H, J = 11.71 Hz, C*H*H'), 4.60 (d, 1H, J = 12.20 Hz, C*H*H), 4.56-4.53 (m, 1H, C*H*H''), 4.50-4.45 (m, 3H, C*H*H''', CH_2), 4.41 (d, 1H, J = 11.22 Hz, C*H*H'), 4.29 (d, 1H, J = 11.71 Hz, C*H*H^{IV}), 4.23 (d, 1H, J = 12.20 Hz, C*H*H'''), 4.20-4.14 (m, 1H, CH, C*H*H^{IV}), 3.97-3.91 (m, 2H, C*H*H'', H-4), 3.85-3.76 (m, 3H, H-4^I, H-5, C-6a), 3.69-3.51 (m, 3H, C-6e, C-6^{I}ae), 3.44-3.34 (m, 3H, H-5^I, OCH_2), 3.30-3.16 (m, 3H, H-2, NCH_2), 3.09 (d, 1H, J = 10.25 Hz, H-3^I), 1.84 (s, 3H, CH_3), 1.67-1.45 (m, 4H, 2 x CH_2), 1.39-1.21 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.04, 154.00, 143.62, 143.27, 141.28, 141.21, 138.44, 137.96, 137.82, 135.38, 133.22, 133.00, 128.49, 128.42, 128.36, 128.29, 128.25, 128.07, 127.85, 127.75, 127.65, 127.59, 127.33, 127.24, 127.10, 126.86, 126.34, 126.13, 126.07, 125.55, 125.29, 119.89, 119.84, 100.96 (C-1^I), 98.00 (C-1), 80.62 (C-3^I), 75.46 (C-4), 75.21 (C-3), 74.33, 73.75, 73.37, 73.28 (C-5^I), 72.15 (C-4^I), 71.75, 71.60 (C-2^I), 69.99 (C-5), 69.72, 68.41, 67.70 (C-6^I), 67.41 (C-6), 67.11, 61.05 (C-2), 50.52, 50.23, 46.75, 28.96, 23.26, 20.94. HR MALDI-TOF MS: m/z: calcd for $C_{81}H_{82}N_4O_{15}$ $[M+Na]^+$: 1373.5669; found: 1373.5686.



N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-[O-(2, 3, 4, 6-tetra-*O*-benzyl-β-D-Galactopyanosyl-(1→4)]-2-azido-3- *O*-Fluorenylmethyloxycarbonyl-2-deoxy-β-D-glucopyranoside (35): Compound **34** (139 mg, 0.103 mmol) and DDQ (35 mg, 0.154 mmol) were dissolved in a mixture of DCM (1 mL) and PBS buffer (pH 7.4, 0.25 mL). The reaction was stirred until completion was verified by TLC (35% EtOAc/Hexanes). The reaction mixture was concentrated and the resulting crude was taken up in DCM

(30 mL) and washed with saturated $NaHCO_3$ (sat., aq., 30 mL), and brine (30 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude material was purified by silica gel chromatography (35% EtOAc/hexanes) to give **35** (98 mg, 0.081 mmol, 78%) as an amorphous white solid. R_f = 0.15 (35% EtOAc/hexanes). H^1NMR (500 MHz, $CDCl_3$): δ 7.74 (d, 2H, J = 7.32 Hz, aromatic), 7.57 (d, 1H, J = 7.32 Hz, aromatic), 7.51 (d, 1H, J = 7.81 Hz, aromatic), 7.41-7.15 (m, 27H, aromatic), 7.08-7.06 (m, 2H, aromatic), 5.36-5.30 (m 2H, H-2^{I}, H-3), 5.17 (d, 2H, J = 16.59 Hz, CH_2), 4.93-4.86 (m, 2H, H-1, C*H*H), 4.66-4.58 (m, 2H, C*H*H', C*H*H''), 4.56 (d, 1H, J = 7.81 Hz, H-1^{I}), 4.52-4.45 (m, 4H, C*H*H, C*H*H', CH_2), 4.26-4.18 (m, 3H, CH_2, CH), 4.03-3.99 (m, 1H, C*H*H''), 3.93-3.91 (m, 1H, H-4^{I}), 3.90-3.82 (m, 1H, H-4), 3.78-3.59 (m, 5H, H-5, H-6ae, H-6^{I}a, OC*H*H), 3.51-3.46 (m, 3H, H-3^{I}, H-5^{I}, H-6^{I}e), 3.44-3.30 (m, 1H, OC*H*H), 3.30-3.15 (m, 2H, NCH_2), 3.07-3.01 (m, 1H, H-2), 2.05 (s, 3H, CH_3), 1.71-1.46 (m, 4H, 2 x CH_2), 1.39-1.25 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.71, 154.11, 143.66, 143.29, 141.46, 141.40, 138.56, 138.04, 137.99, 137.78, 128.65, 128.55, 128.44, 128.24, 128.03, 127.97, 127.88, 127.83, 127.54, 127.39, 127.28, 127.17, 125.60, 125.33, 120.11, 120.08, 101.59 (C-1^{I}), 98.21 (C-1), 80.30 (C-3^{I}), 75.65 (C-4), 75.63 (C-3), 74.58, 73.51, 73.47 (C-5^{I}), 72.15 (C-4^{I}), 71.85, 71.80 (C-2^{I}), 70.49 (C-5), 69.88, 68.51, 67.70 (C-6^{I}), 67.27, 61.30 (C-2), 60.81 (C-6), 50.73, 50.41, 47.16, 46.90, 46.30, 29.05, 27.97, 27.45, 23.36, 21.10. HR MALDI-TOF MS: m/z: calcd for $C_{70}H_{74}N_4O_{15}$ $[M+Na]^+$: 1233.5043; found: 1233.5076.

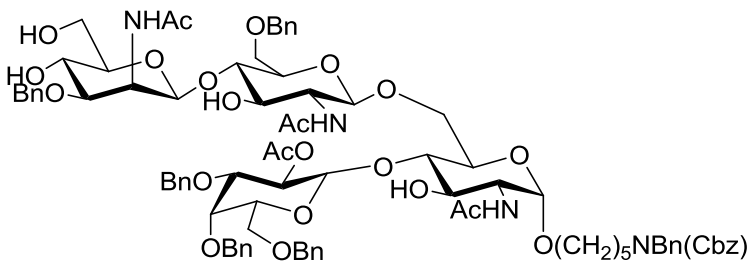


N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl-2-azido-3-*O*-benzy-4,6-*O*-benzylidene-2-deoxy-β-D-Mannopyranosyl-(1→4)- 2-(2,2,2-trichloroethoxy)carbonylamino-3-*O*-levulinyl-6-*O*-benzyl-2-deoxy-β-D-Glucopyranosyl-(1→6)- [O-(2, 3, 4, 6-tetra-*O*-benzyl-β-D-Galactopyanosyl -(1→4)]- 2-azido-3-*O*-Fluorenylmethyloxycarbonyl-2-deoxy-α-D-Glucopyanoside (31): ***Method 1*** (3+1): Glucosyl acceptor **30**

(151 mg, 0.093 mmol) was taken up in toluene (15 mL) and concentrated under vacuum (3x). The acceptor was then taken up in a mixture of DCM (4.5 mL) and along with activated 4Å molecular sieves (150 mg) and stirred under argon for 30 min. The mixture was cooled to -30^{o}C and TMSOTf (3.9 μL, 0.021 mmol) was added *via* dropwise addition. Donor **10** (247 mg, 0.372 mmol) was taken up in a minimal volume of DCM and added to the stirring reaction mixture *via* dropwise addition. The reaction mixture was allowed to warm to -0^{o}C and stir for 1 h. Afterwards, the reaction was quenched by the addition of pyridine (0.3 mL), filtered, and concentrated. The residue was dissolved in DCM (50 mL) and washed with $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic layer was separated, dried over MgSO4, filtered, and concentrated under vacuum. The crude was purified by silica gel chromatography (25% → 35% EtOAc/hexanes) to afford an impure mixture of **31**. The partially purified product was taken up in Ac_2O (0.5 mL) and Pyridine (0.5 mL) in order to acetylate the hydrolyzed donor and change its R_f value. Afterwards, the mixture was concentrated and preparative TLC was used (15% Acetone/toluene) to remove residual impurities and afford **31** (37.6 mg, 0.018 mmol, 19%) as an amorphous white solid. R_f = 0.4 (15% Acetone/toluene).

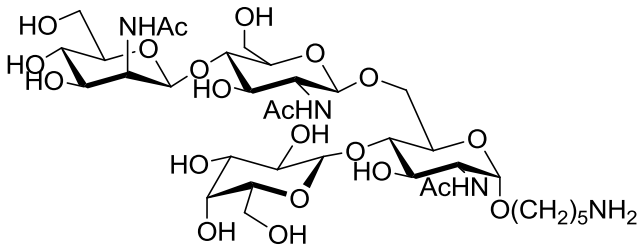
Method 2 (2+2): To a solution of **17** (62.9 mg, 0.069 mmol) in DCM (4 mL) was added K_2CO_3 (96 mg, 0.693 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride (0.097 mL, 0.693 mmol). The reaction was stirred at RT until completion was verified by TLC. R_f = 0.8 (35% EtOAc/hexanes + 0.1% TEA). Triethylamine (100 μL) was added to the reaction mixture and the mixture was filtered through celite and concentrated. The crude was passed through a short silica gel column (5% → 25% EtOAc/hexanes + 0.1% Et_3N) in order to remove residual 2,2,2-Trifluoro-N-phenylacetimidoyl chloride and afford **18** (95%, 0.071g, 0.066 mmol) as an amorphous white solid. Donor compound **18** (0.0.071g, 0.066 mmol) and acceptor **35** (0.095g, 0.079 mmol) were co-evaporated with toluene and dried under vacuum for 30 min. The mixture was taken up in DCM (3.2 mL) along with 4Å molecular sieves (160 mg) and stirred at room temperature for 30 mins. Afterwards, the mixture was cooled to -20^{o}C and TMSOTf (2.7 μL, 0.015 mmol)

was added *via* dropwise addition. The mixture was allowed to warm to -15°C and stir for 1 h. The reaction was quenched by the addition of pyridine (100 μL) and diluted in DCM (50 mL). The organic layer was washed with aqueous $NaHCO_3$ (sat, aq, 50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The crude was purified by silica gel chromatography (30% EtOAc/hexanes) to afford **31** (66.2 mg, 0.032 mmol, 48%) as an amorphous white solid. R_f = 0.45 (40% EtOAc/hexanes) / R_f = 0.4 (15% Acetone/toluene). H^1NMR (600 MHz, $CDCl_3$): δ 7.77-7.03 (m, 48H, J = 7.04 Hz, aromatic), 5.55 (s, 1H, CH), 5.30-5.27 (m, 1H, H-3^{II}), 5.26-5.20 (m, 1H, H-3^{I}), 5.16 (d, 2H, J = 19.37 Hz, CH_2), 4.88-4.78 (m, 3H, H-1^{II}, C*H*H, C*H*H'), 4.67-4.39 (m, 13H, H-1, H-1^{I}, H-1^{III}, C*H*H, C*H*H', C*H*H'', 2xCH_2), 4.28-4.23 (m, 2H, C-6^{II}a, C*H*H'''), 4.19-4.09 (m, 2H, CH, CHH'''), 4.05-3.86 (m, 6H, H-2^{III}, H-3^{III}, H-4^{I}, H-4^{III}, H-6a, C*H*H''), 3.84-3.48 (m, 14H, H-2, H-2^{I}, H-3, H-4, H-4^{II}, H-5^{I}, H-5^{II}, H-5^{III} C-6e, C-6^{II}e, C-6^{I}ae, C-6^{III}ae), 3.44-3.24 (m, 2H, OCH_2), 3.24-3.07 (m, 4H, H-2^{II}, H-5, NCH_2), 2.80-2.67 (m, 2H, CH_2), 2.59-2.50 (m, 2H, CH_2), 2.16 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.62-1.43 (m, 4H, 2 x CH_2), 1.36-1.22 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.50, 172.28, 169.83, 156.82, 156.32, 154.29, 154.19, 143.65, 143.29, 141.62, 141.41, 141.35, 138.62, 138.03, 137.96, 137.71, 137.33, 137.02, 136.89, 129.15, 128.79, 128.72, 128.64, 128.61, 128.58, 128.50, 128.42, 128.37, 128.25, 128.08, 127.99, 127.96, 127.89, 127.87, 127.83, 127.80, 127.69, 127.66, 127.51, 127.42, 127.37, 127.30, 127.22, 127.20, 126.15, 125.64, 125.40, 124.84, 120.18, 120.07, 120.04, 101.67, 101.40 (C-1^{I}), 101.28 (C-1^{III}), 99.52 (C-1), 97.98 (C-1^{II}), 95.70, 80.02 (C-5^{II}), 78.44 (C-3^{III}), 78.42 (C-4^{I}), 76.60 (C-4), 76.22 (C-4^{II}), 75.35 (C-2^{III}), 75.14 (C-3^{II}), 74.62, 74.56, 74.51 (C-5^{III}), 73.70, 73.43, 73.26 (C-5^{I}), 72.93, 72.39 (C-3^{I}), 72.29 (C-4^{III}), 71.82, 69.94, 69.75 (C-3), 68.63 (C-6^{II}), 68.45, 68.34 (C-6^{I}), 67.67 (C-6^{III}), 67.48 (C-6), 67.36 (C-5), 67.26, 63.50 (C-2), 61.19 (C-2^{II}), 56.27 (C-2^{I}), 50.69, 50.39, 47.23, 46.86, 46.29, 38.05, 30.03, 29.09, 28.15, 28.00, 27.50, 23.41, 21.37. HR MALDI-TOF MS: m/z: calcd for $C_{111}H_{117}Cl_3N_8O_{27}$ $[M+Na]^+$: 2121.6986; found: 2121.7003.



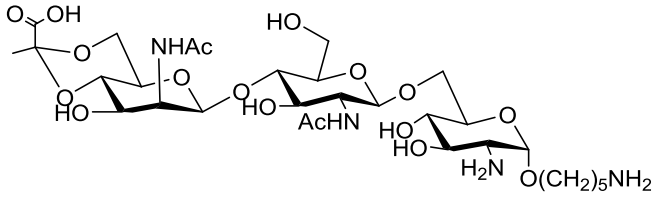
N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl *O*-2-acetamido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl-(1→4)-*O*-2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D- glucopyranosyl-(1→6)-[*O*-(2, 3, 4, 6-tetra-*O*-benzyl-β-D-Galactopyanosyl -(1→3)]-2-acetamido-4-*O*-(2-methylnaphthyl)-2-deoxy-*α*-D-glucopyranoside (32). Compound **31** (57 mg, 0.027 mmol) was dissolved in HOAc (1.2 mL) and Zinc-Copper couple (349 mg, 2.7 mmol) was added. The reaction was allowed to proceed until MALDI-TOF MS indicated disappearance of starting material (~1 h). It was found that reaction times in excess of 1 h led to the formation of an [M+1] side product. The reaction mixture was filtered and concentrated. The crude was taken up in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude residue was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL) and stirred for 3hrs. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction mixture was concentrated under vacuum and the residue was co-evaporated three times with toluene in order to remove any residual pyridine. The residue was taken up in ethyl acetate (50 mL) and washed with $NaHCO_3$ (sat, aq, 50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. Without further purification, the crude was taken up in 80% HOAc (aq, 2.9 mL). The reaction mixture was heated at 50°C for 16 h and then concentrated under vacuum. Completion of the reaction was verified by MALDI-TOF MS analysis. The residue was taken up in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under vacuum. Without further purification, the crude was taken up in a solution of THF (0.3 mL), water (0.015 mL), and 2M LiOH (0.066 mL) and stirred in a warm water

bath (40°C) for 2.5 h. and stirred for 2.5 h. Completion of the reaction was verified by MALDI-TOF MS analysis. The reaction was neutralized with Dowex 50 W (H+) resin, filtered, and concentrated. The resulting crude was taken up in DCM (50 mL) and washed with saturated $NaHCO_3$ (sat., aq., 50 mL), and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to afford an amorphous pale yellow solid. The crude material was purified by preparatory TLC (8% Methanol/DCM) to give **32** (17.5 mg, 0.011 mmol, 37 % over 4 steps) as an amorphous white solid. R_f = 0.5 (10% MeOH/DCM). HRMS(ESI): calcd for $C_{87}H_{106}N_4O_{24}$ $[M+Na]^+$: 1613.7089, found: 1613.7106.



5-Aminopentyl-*O*-2-acetamido-2-deoxy- β-D-mannopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[*O*-(β-D-galactopyranosyl)-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (4): 32 (16.5 mg, 10.65 μmol) was dissolved in a mixture of t-BuOH (3.2 mL) and H_2O (0.08 mL). $Pd(OH)_2$/C (20 mg, 20 wt.%, Degussa type) was added and the resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 16 h, the catalyst was filtered off and washed thoroughly with a gradient of gently warmed t-BuOH/H_2O. (100% tBuOH, 50% tBuOH/H_2O, 30% tBuOH/H_2O) The combined filtrates were concentrated under reduced pressure. The product was purified with a C18 silica column using an eluent gradient starting with 100% H_2O followed by 1%, 5%, 10%, 20%, 40%, 60% and 80% MeOH/H_2O washes. The fractions containing product were concentrated giving compound (9.3 mg, 10.65 μmol, 100%) as an amorphous white solid. H^1 NMR (600 MHz, D_2O)**:** δ 4.92 (s, 1H, H-1), 4.88 (d, 1H, J = 2.93 Hz, H-1^{II}), 4.58-4.54 (m, 2H, H-1^{I}, H-2), 4.38 (d, 1H, *J* = 7.81 Hz, H-1^{III}), 4.25 (d, 1H, *J* = 9.76 Hz, H-6^{II}a), 3.96-3.87 (m, 6H, H-2^{II}, H-3^{I}, H-3^{II}, H-4^{III}, H-6, H-6^{III}), 3.86-3.67 (m, 13H, H-2^{I}, H-3, H-3^{III}, H-4^{I}, H-5^{II}, H-5^{III}, H-6e, H-6^{II}e, H-6^{I}ae, H-6^{III}ae, OC*H*H), 3.63-3.60 (m, 1H, H-4^{II}), 3.59-3.30 (m, 3H, H-2^{III}, H-4, H-5^{I}),

3.50-3.44 (m, 2H, H-5, OC*H*H), 3.02 (t, 2H, J = 7.81 Hz, NCH_2), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.74-1.63 (m, 4H, 2xCH_2), 1.51-1.44 (m, 2H, CH_2). ^{13}C NMR (150 MHz, D_2O): δ 175.30, 174.15, 174.02, 103.24 (C-1^{III}), 101.00 (C-1^{I}), 99.27 (C-1), 96.15 (C-1^{II}), 79.65 (C-4^{II}), 78.69 (C-4^{I}), 76.42 (C-5), 75.33 (C-5^{III}), 74.30 (C-5^{I}), 72.38 (C-5^{II}), 72.20 (C-3^{III}), 71.81 (C-3), 70.77 (C-2^{III}), 69.47 (C-3^{II}), 69.12 (C-3^{I}), 68.29 (C-4^{III}), 67.62 (C-6^{II}), 67.56, 66.45 (C-4), 60.80 (C-6), 60.23 (C-6^{III}), 59.99 (C-6^{I}), 55.05 (C-2^{I}), 53.12 (C-2), 53.07 (C-2^{II}), 39.28, 27.84, 26.41, 22.31, 22.27, 21.89, 21.75. HRMS (ESI): calcd for $C_{35}H_{62}N_4O_{21}$ [M+H]+: 875.3979; found: 875.3987.



5-aminopentyl 2-acetamido-4,6-*O*-[(S)-1-Carboxylethylidene]-2-deoxy-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-Glucopyranosyl-(1→6)-2-amino-2-deoxy-Glucopyanoside (5): To a stirring solution of **36** (1.2 mg, 1.43 μmol) in DCM (0.16 mL) at 0°C was added triethylsilane (0.016 mL, 0.1 mmol), followed by the dropwise addition of TFA (0.08 mL, 1.04 mmol). The mixture was allowed to stir for 15 min at 0°C. Completion of the reaction was verified by LCMS. The mixture was concentrated and the crude was taken up in a small volume of 5% n-Butanol/H_2O and loaded directly onto a P2 size exclusion column and eluted with 5% n-Butanol/H_2O. The fractions containing the product were combined and lyophilized to afford **4** (900 μg, 1.22 μmol, 85%). H^1 NMR (600 MHz, D_2O)**:** 4.92 (s, 1H, H-1), 4.81 (s, 1H, H-1^{II}), 4.59-4.58 (m, 1H, H-2), 4.46 (d, 1H, J = 8.22 Hz, H-1^{I}), 4.14 (d, 1H, J = 11.15 Hz, H-$6a^{II}$), 4.02-4.00 (m, 1H, H-6a), 3.98-3.95 (m, 1H, H-3), 3.87-3.82 (m, 2H, H-3^{I}, H-$6a^{I}$), 3.76-3.56 (m, 9H, H-2^{I}, H-3, H-4, H-4^{I}, H-5^{II}, H-6e, H-$6e^{I}$, H-$6e^{II}$, OC*H*H), 3.51-3.39 (m, 4H, H-3^{II}, H-5, H-5^{I}, OC*H*H), 3.33-3.27 (m, 1H, H-4^{II}), 2.96 (t, 2H, J = 7.04Hz, NCH_2), 2.66-2.64 (m, 1H, H-2^{II}), 2.05 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.70-1.60 (m, 4H, 2xCH_2), 1.47-1.42 (m, 5H, CH_2, CH_3). ^{13}C NMR (150 MHz, D_2O): 175.37, 174.22, 161.20, 101.72, 101.38 (C-1^{I}), 99.72 (C-1), 98.69 (C-1^{II}), 78.66 (C-4^{I}), 74.30 (C-5^{I}), 74.28 (C-3^{II}),73.78 (C-4), 72.03

(C-3^{I}), 70.67 (C-5^{II}), 69.79 (C-4^{II}), 69.21 (C-3), 68.72 (C-6^{II}), 67.51, 66.69 (C-5), 63.93 (C-6), 59.94 (C-6^{I}), 55.16 (C-2^{I}), 54.80 (C-2^{II}), 53.37 (C-2), 39.33, 27.93, 26.65, 24.54, 22.41, 22.09, 21.90. HRMS (ESI): calcd for $C_{30}H_{52}N_4O_{17}$ $[M+H]^+$: 741.3400, found: 741.3417.

REFERENCES

1) Fouet A., Moya M. Virulence megaplasmids in *Bacillus anthracis* and their relatives in the *Bacillus cereus* groups. In: Microbial Megaplasmids (E. Schwartz, ed.) Springer, Munster, 187-206 (**2008**).
2) Fouet A. The surface of *Bacillus anthracis*. *Mol Aspects Med* **2009**, *30*, 374–385.
3) Mesnage S., Fontaine T., Mignot T., Delepierre M., Mock M. & Fouet A. Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *EMBO J*. **2000**, *19*(17), 4473-4484.
4) Schaffer C. & Messner P. The structure of secondary cell wall polymers: how Gram-positive bacteria stick their cell walls together. *Microbiology* **2005**, *151*(Pt 3), 643-651.
5) Kern J., Ryan C., Faull K. & Schneewind O. Bacillus anthracis surface-layer proteins assemble by binding to the secondary cell wall polysaccharide in a manner that requires csaB and tagO. *J. Mol. Biol.* **2010**, *401*(5), 757-775.
6) Forsberg L.S., Abshire T.G., Friedlander A., Quinn C.P., Kannenberg E.L. & Carlson R.W. Localization and structural analysis of a conserved pyruvylated epitope in Bacillus anthracis secondary cell wall polysaccharides and characterization of the galactose-deficient wall polysaccharide from avirulent B. anthracis CDC 684. *Glycobiology*. **2012**, *22*(8), 1103-1117.
7) Leoff C., Choudhury B., Saile E., Quinn C.P., Carlson R.W. & Kannenberg E.L. Structural elucidation of the nonclassical secondary cell wall polysaccharide from Bacillus cereus ATCC 10987. Comparison with the polysaccharides from Bacillus anthracis and B. cereus type strain ATCC 14579 reveals both unique and common structural features. *J. Biol. Chem.* **2008**, *283*(44), 29812-29821.

8) Leoff C., Saile E., Rauvolfova J., Quinn C.P., Hoffmaster A.R., Zhong W., Mehta A.S., Boons G.J., Carlson R.W. & Kannenberg E.L. Secondary cell wall polysaccharides of Bacillus anthracis are antigens that contain specific epitopes which cross-react with three pathogenic Bacillus cereus strains that caused severe disease, and other epitopes common to all the Bacillus cereus strains tested. *Glycobiology* **2009**, *19*(6), 665-673.

9) Forsberg L.S., Choudhury B., Leoff C., Marston C.K., Hoffmaster A.R., Saile E., Quinn C.P., Kannenberg E.L. & Carlson R.W. Secondary cell wall polysaccharides from Bacillus cereus strains G9241, 03BB87 and 03BB102 causing fatal pneumonia share similar glycosyl structures with the polysaccharides from Bacillus anthracis. *Glycobiology* **2011**, *21*(7), 934-948.

10) Choudhury B., Leoff C., Saile E., Wilkins P., Quinn C.P., Kannenberg E.L. & Carlson R.W. The structure of the major cell wall polysaccharide of Bacillus anthracis is species-specific. *J. Biol. Chem.* **2006**, *281*(38), 27932-27941.

11) Lunderberg J.M., *et al*. *Bacillus anthracis* acetyltransferases PatA1 and PatA2 modify the secondary cell wall polysaccharide and affect the assembly of S-layer proteins. *J Bacteriol* **2013**, *195*, 977-989.

12) Sychantha D., Little D.J., Chapman R.N., Boons G.J., Howell P.L., and Clarke A.J. Structural and mechanistic basis for the O-acetylation of SCWP, an essential activity for the proper assembly of cell walls in the Bacillus cereus group of pathogens. Submitted to *Nat. Chem. Biol*.

13) Kern V.J., Kern J.W., Theriot J.A., Schneewind O., Missiakas D.M. Surface (S)-layer proteins Sap and EA1 govern the binding of the S-layer associated protein BslO at the cell septa of *Bacillus anthracis*. *J. Bacteriol.* **2012**, *194*, 3833-3840.

14) Nguyen-Mau S.-M., Oh S.Y., Kern V., Missiakas D., Schneewind O. Secretion genes as determinants of *Bacillus anthracis* chain length. *J. Bacteriol.* **2012**, *194*, 3841-3850.

15) Ruthel G., Ribot W.J., Bavari S., Hoover T. Time-lapse confocal imaging of development of *Bacillus anthracis* in macrophages. *J. Infect. Dis.* **2004**, *189*, 1313-1316.

16) Mesnage S.,Fontaine T., Mignot T., Delepierre M., Mock M., and Fouet A., Bacterial SLH domain proteins are non-covalently anchored to the cell surface via a conserved mechanism involving wall polysaccharide pyruvylation. *EMBO J*. **2000**, *19*(17), 4473-4484.

17) Hofmann K. A superfamily of membrane-bound *O*-acyltransferases with implications for Wnt signaling. *Trends Biochem. Sci.* **2000**, *25*, 111-112.

18) Leoff C., Choudhury B., Saile E., Quinn C.P., Carlson R.W., and Kannenberg E.L. Structural Elucidation of the Nonclassical Secondary Cell Wall Polysaccharide from Bacillus cereus ATCC 10987. *J. Biol. Chem*. **2008**, *283*(44), 29812-29821.

19) Anderson V.J., Kern J.W., McCool J.W., Schneewind O. & Missiakas D. The SLH-domain protein BslO is a determinant of *Bacillus anthracis* chain length. *Mol. Microbiol.* **2011**, *81*(1), 192-205.

20) Kern J., Schneewind O. BslA, the S-layer adhesin of *B. anthracis*, is a virulence factor for anthrax pathogenesis. *Mol. Microbiol*. **2010**, *75*, 324-332.

21) Sara M. & Sleytr U.B. S-Layer proteins. *J. Bacteriol*. **2000**, *182*(4), 859-868.

22) Chapman R.N., Liu L., Lunderberg J., Schneewind O., and Boons, G.J. Probing Binding Interactions Between Synthetic Oligosaccharides Derived From The Non-Reducing Terminus Of *Bacillus Anthracis* SCWP And The SLH Domains Of S-Layer And S-Layer Associated Proteins. To be Submitted.

23) Zhu T. & Boons G.J. A new set of orthogonal-protecting groups for oligosaccharide synthesis on a polymeric support. *Tetrahedron: Asymmetry* **2000**, *11*(1), 199-205.

24) Gaunt M.J., Yu J.Q. & Spencer J.B. Rational design of benzyl-type protecting groups allows sequential deprotection of hydroxyl groups by catalytic hydrogenolysis. *J. Org. Chem.* **1998**, *63*(13), 4172-4173.

25) Classon B., Garegg P.J., Oscarson S. & Tiden A.K. Synthesis of an artificial antigen that corresponds to a disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* Type-D - a facile synthesis of methyl 2-acetamido-2-deoxy-beta-D-mannopyranoside. *Carbohydr. Res.* **1991**, *216*, 187-196.

26) Dessolin M., Guillerez M.-G., Thieriet N., Guibé F. & Loffet A. New allyl group acceptors for palladium catalyzed removal of allylic protections and transacylation of allyl carbamates. *Tetrahedron Lett.* **1995**, *36*(32), 5741-5744.

27) Mo K.F., Li X., Li H., Low L.Y., Quinn C.P. & Boons G.J. Endolysins of *Bacillus anthracis* bacteriophages recognize unique carbohydrate epitopes of vegetative cell wall polysaccharides with high affinity and selectivity. *J. Am. Chem. Soc.* **2012**, *134*(37), 15556-15562.

28) Prabhu A., Venot A. & Boons G.J. New set of orthogonal protecting groups for the modular synthesis of heparan sulfate fragments. *Org. Lett.* **2003**, *5*(26), 4975-4978.

29) Zhu X.M. & Schmidt R.R. New principles for glycoside-bond formation. *Angew. Chem., Int. Ed.* **2009**, *48*(11), 1900-1934.

30) Bongat A.F.G. & Demchenko A.V. Recent trends in the synthesis of O-glycosides of 2-amino-2-deoxysugars. *Carbohydr. Res.* **2007**, *342*(3-4), 374-406.

31) Lemieux, R. U. Effects of unshared pairs of electrons and their solvation on conformational equilibria. *Pure. Appl. Chem*. **1971**, *25*, 527.

32) Blatter G., Beau J.M. & Jacquinet J.C. The use of 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives in syntheses of oligosaccharides. *Carbohydr. Res.* **1994**, *260*(2), 189-202.

33) Vibert A., Lopin-Bon C. & Jacquinet J.-C. Efficient alternative for the reduction of N-trichloroacetyl groups in synthetic chondroitin oligosaccharide intermediates. *Tetrahedron Lett*. **2010**, *51*(14), 1867-1869.

34) Fernández-Herrera M.A., López-Muñoz H., Hernández-Vázquez J.M., López-Dávila M., Mohan S., Escobar-Sánchez M.L., Sánchez-Sánchez L., Pinto B.M., Sandoval-Ramírez J., Synthesis and biological evaluation of the glycoside (25R)-3β,16β-diacetoxy-22-oxocholest-5-en-26-yl β-d-glucopyranoside: a selective anticancer agent in cervicouterine cell lines. *Eur J Med Chem*. **2011**, *46*(9), 3877-86.

35) Wang, Zhen, Total synthesis of complex oligosaccharides by iterative one-pot glycosylation based on pre-activation of thioglycosyl donors, PhD Thesis, The University of Toledo, ProQuest Dissertations Publishing, **2008**, 3329486.

36) Debenham, A.D.; Toone, E.J. Regioselective reduction of 4,6-O-benzylidenes using triethylsilane and $BF_3{\cdot}Et_2O$. Tetrahedron:Asymmetry **2000**, *11*, 385-387.

37) Winans, K. A.; King, D. S.; Rao, V. R.; Bertozzi, C. R. A Chemically Synthesized Version of the Insect Antibacterial Glycopeptide, Diptericin, Disrupts Bacterial Membrane Integrity *Biochemistry* **1999**, *38*, 11700−11710.

38) Maria Theresia Cornelia Walvoort, On The Reactivity & Selectivity Of Donor Glycosides In Glycochemistry & Glycobiology, Walvoort, M. T. C., **2012**, Doctoral Thesis, Institute of Chemistry/Bio-Organic Synthesis Department, Faculty of Science, Leiden University p.64

39) A) Augustine R.L., Heterogenous catalysis for the Synthetic Chemist , New York : M. Dekker, **1996**. B) Freifelder M., Practical Catalytic Hydrogenation Techniques and Applications. Wiley-Interscience, New York, N.Y., **1971**. C) Rylander P.N. Catalytic Hydrogenation over Platinum Metals. Academic Press Inc., New York, N.Y. 1967. D) Eley D.D., Advances in Catalysis, Vol. 14, Academic Press Inc. New York, N.Y. **1963** p. 218.

40) Xin Ge, Chenxi Luo, Chao Qian, Zhiping Yua and Xinzhi Chena, RANEY® nickel-catalyzed reductive N-methylation of amines with paraformaldehyde: theoretical and experimental study, *RSC Adv*., **2014**, *4*, 43195-43203.

41) van Boom J.H. & Burgers P.M.J. Use of levulinic acid in protection of oligonucleotides via modified phosphotriester method - Synthesis of decaribonucleotide U-A-U-A-U-A-U-A-U-A. *Tetrahedron Lett.* **1976**, *52*, 4875-4878.

42) Sakagami M. & Hamana H. A selective ring opening reaction of 4,6-*O*-benzylidene acetals in carbohydrates using trialkylsilane derivatives. *Tetrahedron Lett.* **2000**, *41*(29), 5547-5551.

43) Moynihan P.J., Clarke A.J. Assay for peptidoglycan *O*-acetyltransferase: A potential new antibacterial target. *Anal Biochem* **2013**, *439*, 73-79.

44) Riley L.M., et al. Structural and functional characterization of Pseudomonas aeruginosa AlgX. *J Biol Chem.* **2013**, *288*, 22299-22314.

45) Goldschmidt L., Cooper D., Derewenda Z., Eisenberg D. Toward rational protein crystallization: A Web server for the design of crystallizable protein variants *Protein Sci* **2007**, *16*, 1569-1576.

46) Little D.J., Whitney J.C., Robinson H., Yip P., Nitz M., Howell P.L. Combining in situ proteolysis and mass spectrometry to crystallize Escherichia coli PgaB. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **2012**, *68*, 842–845.

47) Kern V.J., Kern J.W., Theriot J.A., Schneewind O., Missiakas D.M. Surface (S)-layer proteins Sap and EA1 govern the binding of the S-layer associated protein BslO at the cell septa of Bacillus anthracis. *J. Bacteriol*. **2012**, *194*, 3833–3840.

48) Moynihan P.J., Clarke A.J. Substrate specificity and kinetic characterization of peptidoglycan O-acetyltransferase B from Neisseria gonorrhoeae. *J Biol Chem* **2014**, *289*, 16748-16760.

CHAPTER 4

CONCLUSIONS

The secondary cell wall polysaccharide (SCWP) of *B. anthracis* serves a variety of functions ranging from providing structural support to mediating cell-cell and host-guest interactions. Through non-covalent interactions, it anchors S-layer proteins (SLPs) and S-layer-associated proteins (BSLs) to the cell surface. Specific functionalities on the terminal non-reducing unit of *B. anthracis* SCWP including a 4,6-*O*-linked ketal pyruvl, a free amine, and O-acetylation are believed to play a role in SLP and BSL protein binding. Proper S-Layer assembly has been shown to be important for cell growth and maintenance functions and has also been shown to play a role in virulence. An efficient synthetic strategy was developed for the purpose of creating a library of complex oligosaccharides derived from the terminal unit of *B. anthracis* SCWP. The library was utilized to identify saccharide motifs that are important for SLP and BSL binding. The use of a 4,6-*O*-pyruvylated Mannosyl thioglycoside donor in combination with Crich β-Mannosylation conditions was found to be essential for effective activation of the donor and good glycosylation yields. It was found necessary to use an ether based protecting group at the O-3 position of the donor in order to obtain some Beta selectivity during disaccharide formation. The choice of amino protecting groups was also a key part of the strategy. A C-2 azido function was used as a non-participating function on the mannoside donor at the C-2 position. The trichlroacetyl function was used at the C-2 amine position of the second building block in order to promote β-selectivity during glycosylation *via* neighboring group participation. The third building block had a t-Butoxycarbonyl protecting group on the C-2 amine and utilized bisprotection of the aglycon amine with Benzyl and Cbz. Following completion of the glycosylation reactions, the azide and TCA functions were reduced and converted to acetamido functions using AIBN and Bu_3SnH radical reduction conditions followed by

treatment with Ac_2O. Following the reduction, the remaining Boc and Cbz/Benzyl amine protecting groups could be manipulated independently of one another. In addition, both Boc and the aromatic protecting groups are resistant to the basic LiOH conditions used to remove the benzyl and acetyl esters.

The overarching questions that were addressed utilizing the oligosaccharide library include: 1) What functional groups or combinations of functional groups are important for SLP and BSL binding? 2) Do all S-layer and S-layer associated proteins have the same ligand requirements for biding? 3) Can O-acetylation be used as a means to modulate the binding of EA1, BslA, and BslO as demonstrated in the genetic studies? To answer these questions, Microarray and ELISA based binding assays were developed that utilized the synthetic target compounds to identify important carbohydrate binding interactions between SCWP and S-layer proteins. It was found that the presence of a 3-*O*-acetyl function on the β-GlcNAc of the trisacchsaride compounds did not directly modulate the binding of EA1, BslO, or BslA proteins as previously suggested in the *patA1*/*patA2* genetic studies. In addition, compounds with 3-*O*-acetylation on the terminal ManNAc did not show any appreciable differences in binding relative to their non-3-*O*-acetylated counterparts indicating that the position is likely not important for SLH-carbohydrate binding interactions. The findings suggest that the O-acetylation is not used for the direct modulation of SLP/BSL-carbohydrate binding interactions. The display of a free amine on the α-$GlcNH_2$ led to a minor decrease in EC_{50} values across the set of proteins. However, the small magnitude of the effect is not likely enough to significantly affect proper binding of SLP and BSL proteins. The 4,6-*O*-pyruvyl ketal was found to be essential for the binding of all SLP and BSL proteins thereby providing definitive evidence in support of previous *csaB* genetic experiments. Unexpctedly, the C-2 acetamido on ManNAc was found to be a vital feature for SLH binding and its substitution with hydroxyl or amine resulted in the complete loss of binding to SLPs and a partial or complete loss of binding to most BSLs. However, several BSL proteins could bind relatively well in its absence demonstrating that SLH binding requirements can vary and showing that in principle, modifications to the C-2 position could be used as

a means to modulate protein binding. Future studies will have to be performed to confirm such modifications exist on *B. anthracis* SCWP in nature. Although a number of BSL proteins have yet to be fully characterized, the data provided *via* this research will be available to provide insight into the carbohydrate binding interactions of these proteins once studies are undertaken.

PatB1 is a putative O-acetyltransferase and is believed to play a role in the 3-*O*-acetylation of the penultimate β-GlcNAc residue on the *Bacillus anthracis* and *B. Cereus* secondary cell wall polysaccharides. PatB1 was found to selectively install a 3-*O*-acetyl onto a synthetic trisaccharide based on the repeating unit of *B. anthracis* SCWP (**1**) with better activity and sepcificty than chitooligosaccharide based substrates. The findings suggested that *B. anthracis* SCWP was a natural substrate for PatB1. The findings proved interesting, but compound **1** lacked many other structural features present on the native substrate. In order to characterize PatB1 specificity and specific activity in the context of its natural environment, a panel of oligosaccharides based on the internal repeating unit was synthesized with galactose substitutions at biologically relevant positions. The synthetic methodology involved the use of an approach whereby a common trisaccharide intermediate was synthesized with levulinoyl (Lev), fluorenylmethyloxycarbonate (Fmoc), and 2-naphthylmethyl (Nap) protecting groups installed at key branching positions. Next it was demonstrated that the orthogonal protecting groups could be regioselectively deprotected allowing for selective installation of galactose at key positions. Compounds **1**-**3** were obtained in good yield using this strategy. For compound **4**, the installation of β-Galactose at the O-4 position of the α-GlcNAc was low yielding. This was likely due to steric crowding at the position. A 2+2 glycosylation strategy was devised to circumvent this problem. A compound based on the non-reducing trisaccharide unit of *B. anthracis* was also synthesized (**5**) in order to determine if the presence of the 4,6-*O*-pyruvyl ketal and/or free amine seen in the natural structure can affect PatB1 enzymatic activity and specificity. The library of compounds was used in PatB1 enzyme assays to determine if the presence of galactose substitutions, a free amine, or 4,6-*O*-pyruvyl ketal can

affect the specific activity and site specificity of O-acetylation. It was found that the presence of galactosylation on the trisaccharide repeating unit did not alter site specificity, but led to a decrease in specific activity relative to the non-galactosylated trisaccharide repeat. MS/MS analysis of the product peaks could only narrow down the site of O-acetylation to the O-3 or O-6 positions on β-GlcNAc. Compound **3** did not react with PatB1 due to the presence of an α-Gal at the O-3 position of β-GlcNAc and served to demonstrate site specificity as no other positions were acetylated. Furthermore, it was found that the trisaccharide bearing the 4,6-*O*-pyruvyl ketal and free amine showed a significant increase in specific activity demonstrating that these features are likely utilized *in vivo* as PatB1 binding epitopes and may help to facilitate 3-*O*-acetylation of the penultimate β-GlcNAc.

APPENDIX A

SUPPLEMENTARY FOR DATA CHAPTER 2

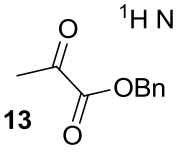
^{1}H and ^{13}C NMR Data: p 294-451

Microarray Data: p 452-454

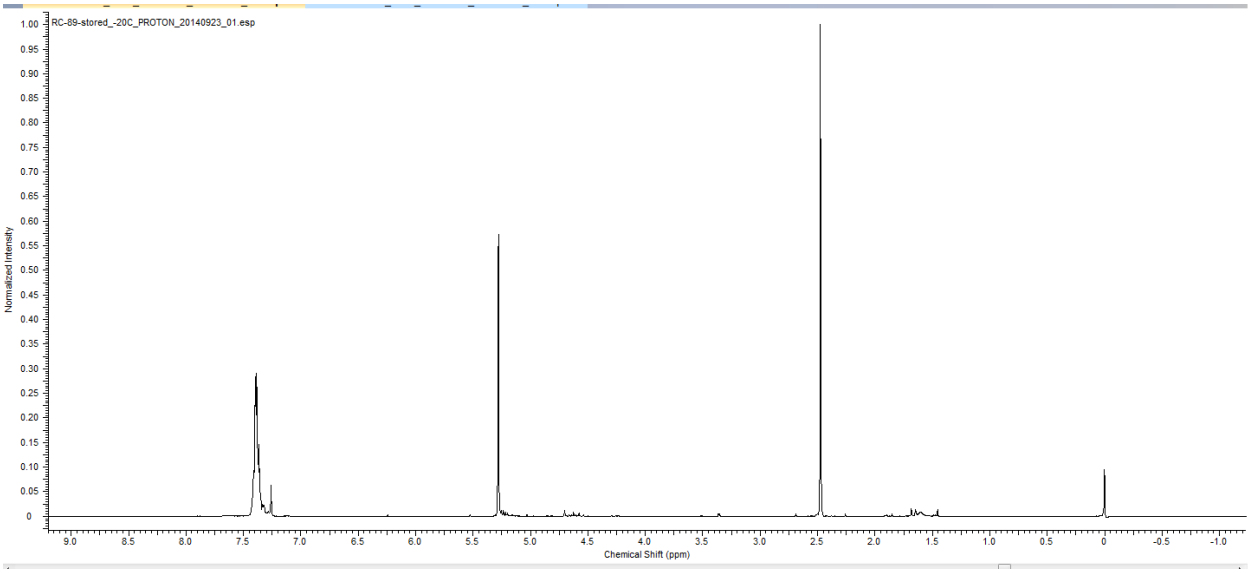
ELISA Data: p 455-460

Table of EC_{50} Values: p 461

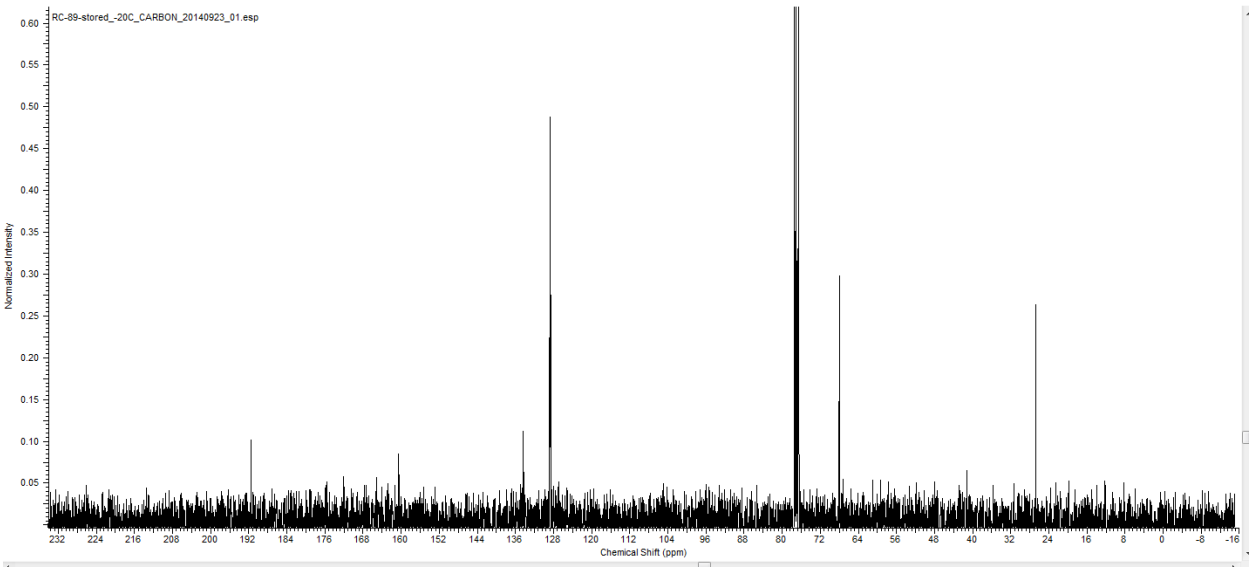
^{1}H NMR (300 MHz, $CDCl_3$)

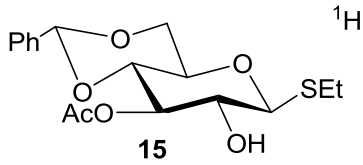


13



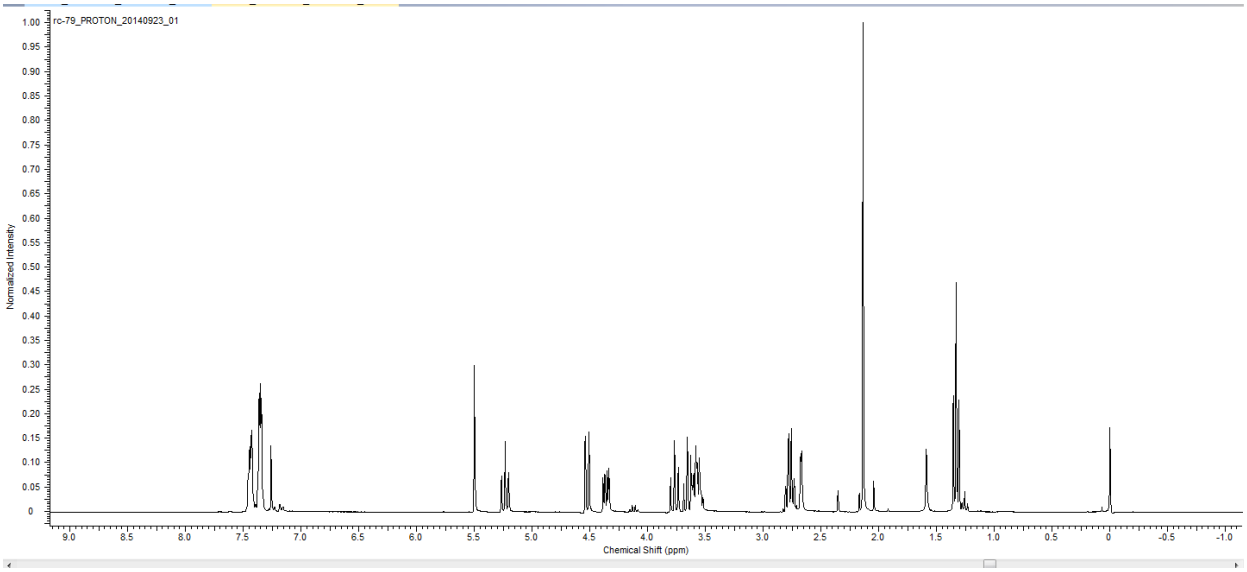
^{13}C NMR (75 MHz, $CDCl_3$)



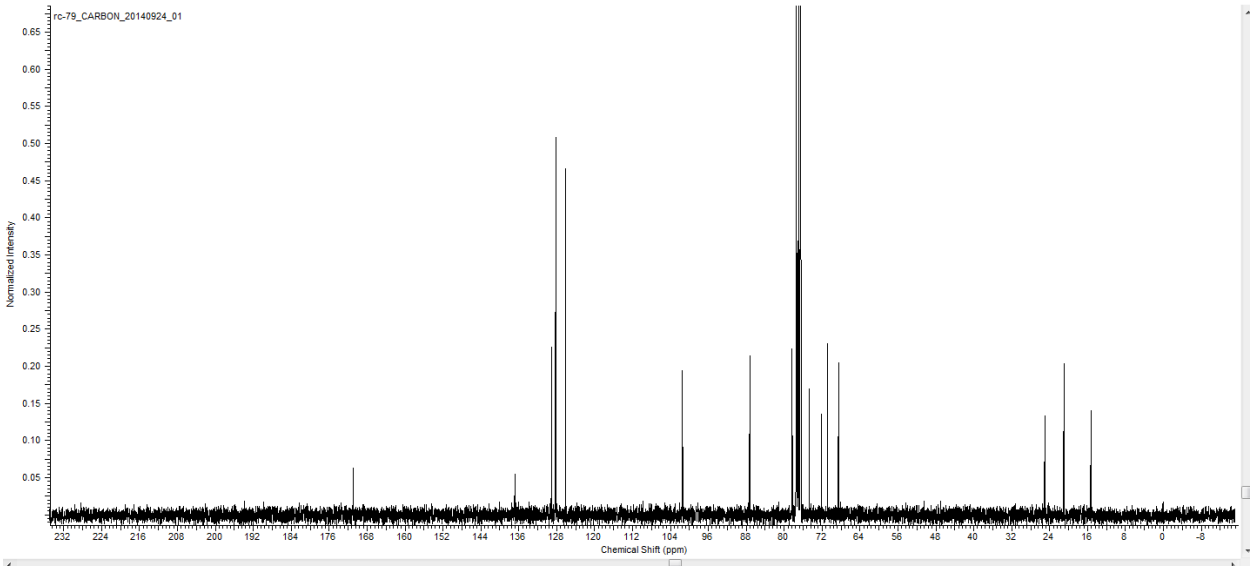


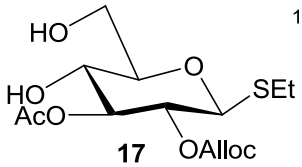
15

^{1}H NMR (300 MHz, $CDCl_3$)



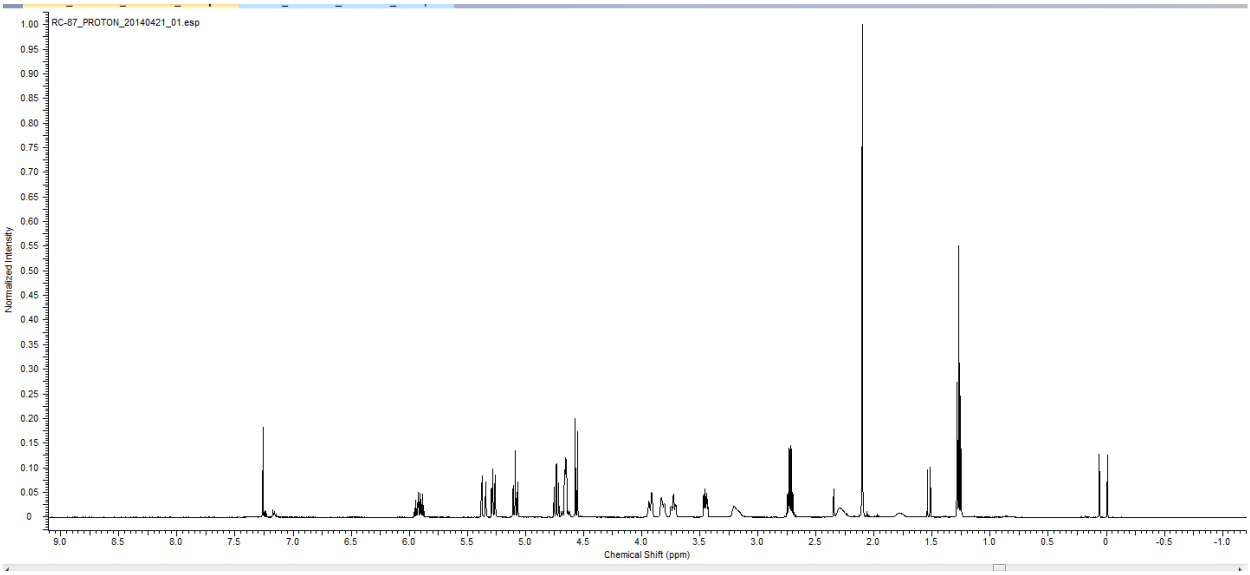
^{13}C NMR (75 MHz, $CDCl_3$)



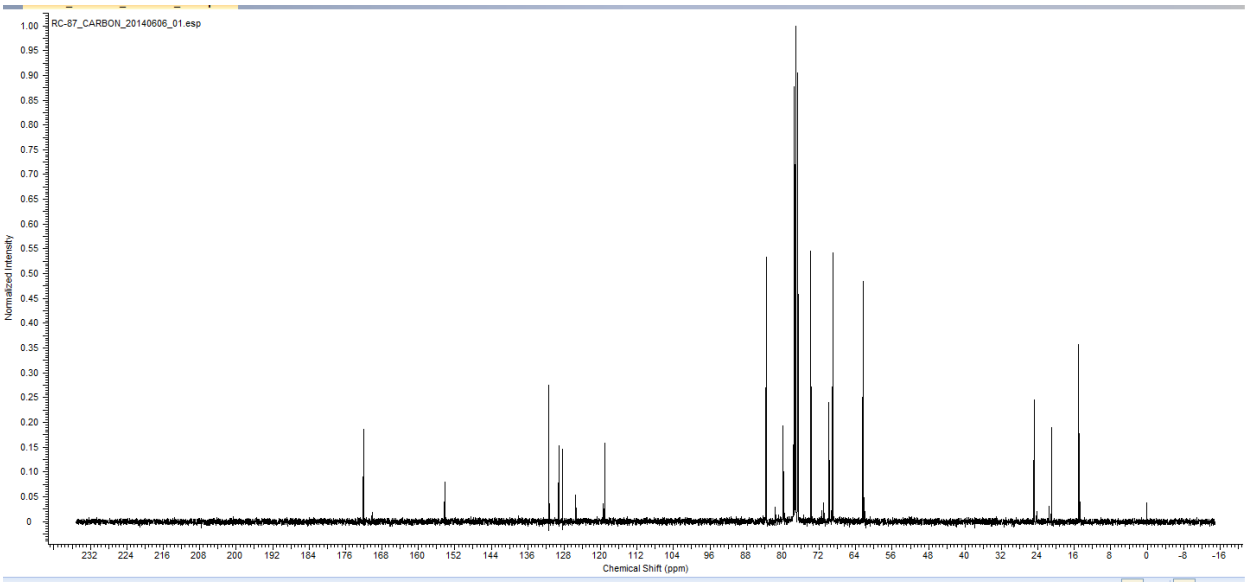


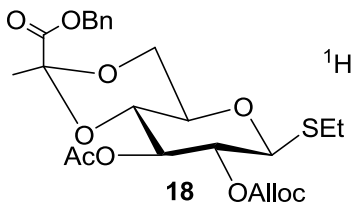
17

^{1}H NMR (300 MHz, $CDCl_3$)

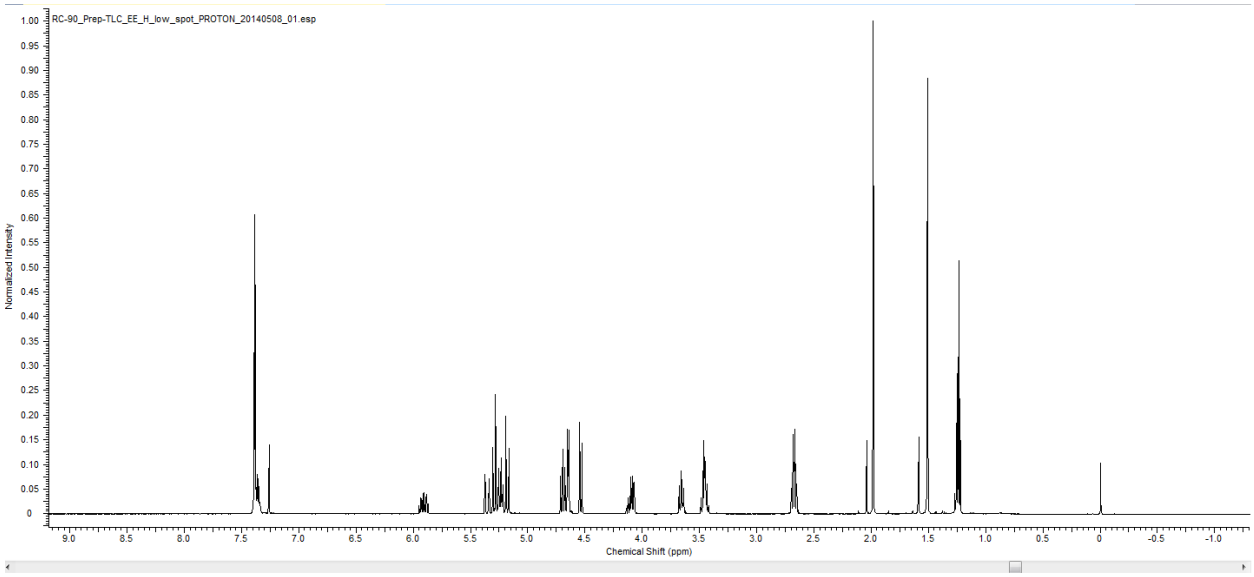


^{13}C NMR (75 MHz, $CDCl_3$)

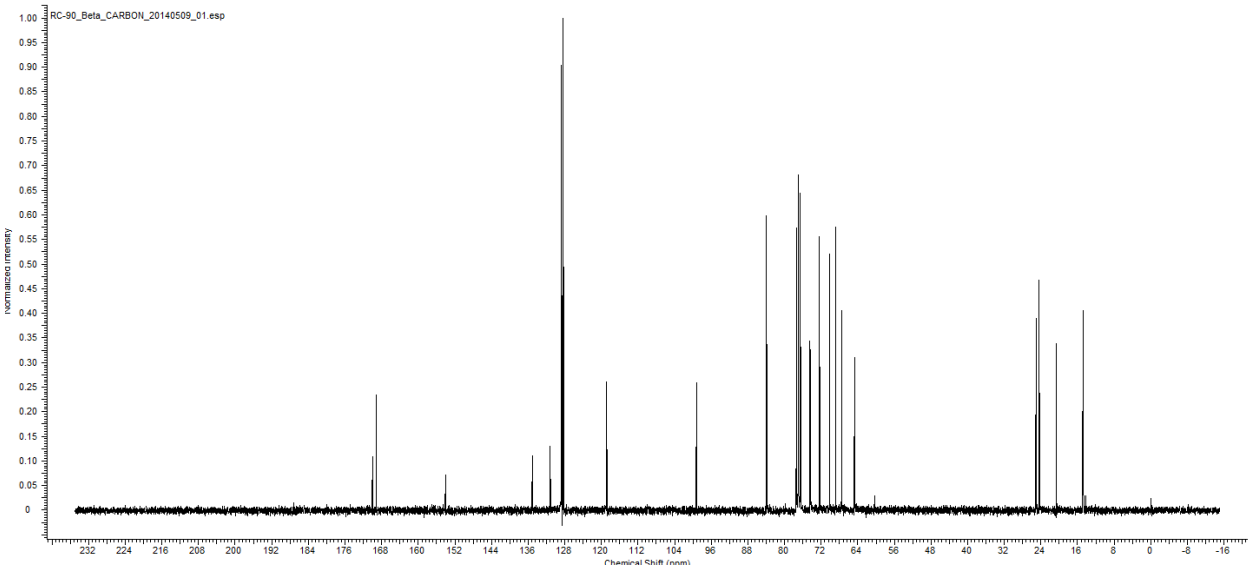




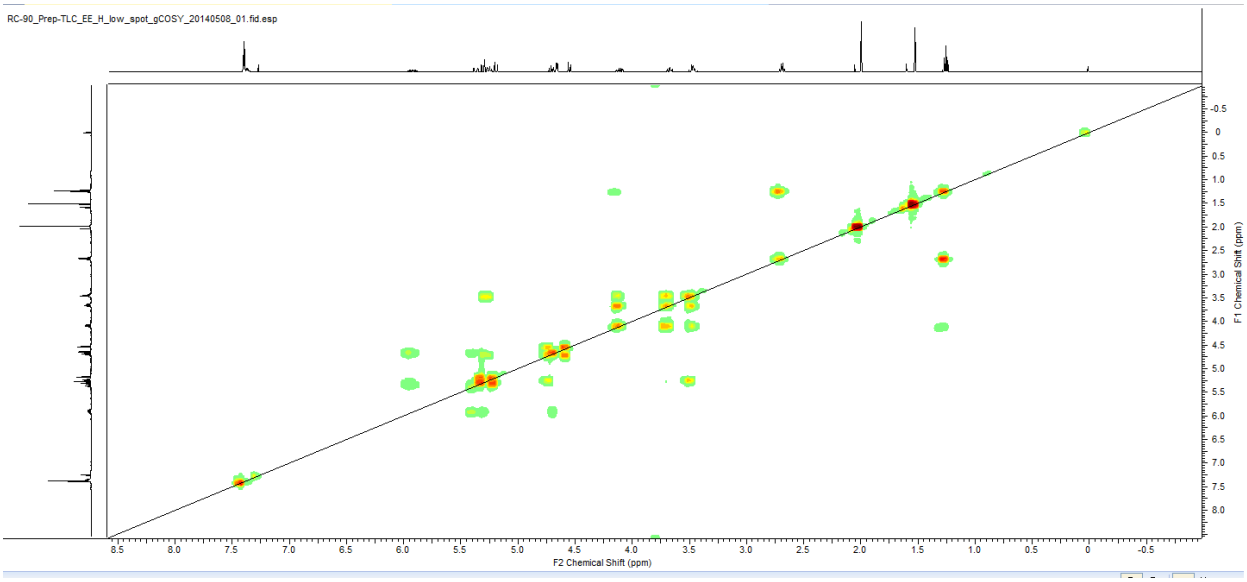
^{1}H NMR (500 MHz, $CDCl_3$)



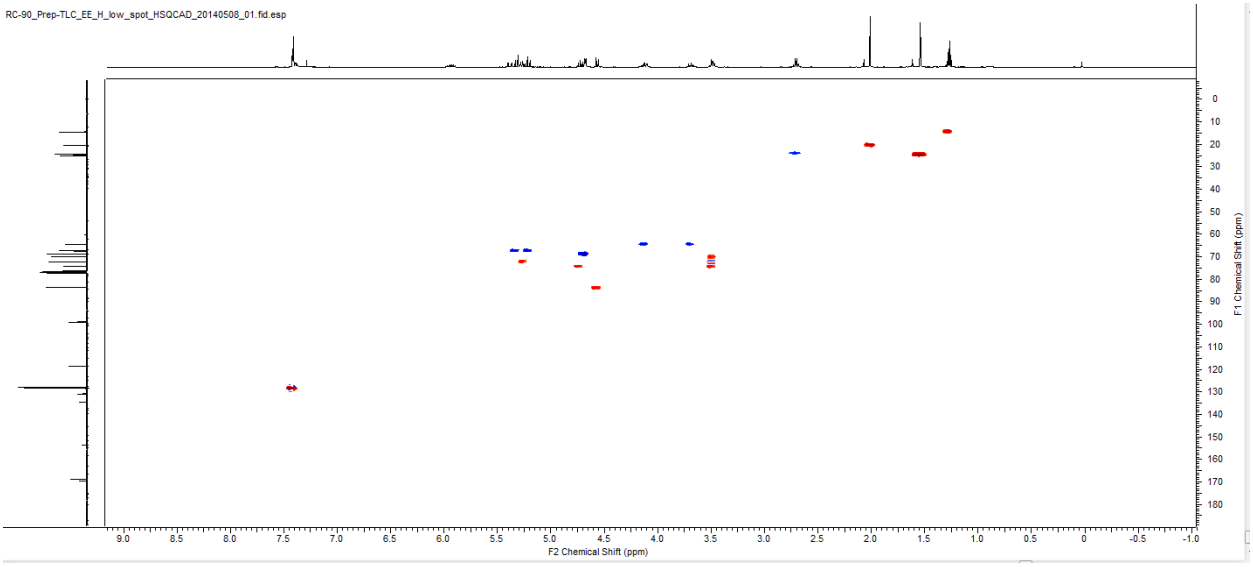
^{13}C NMR (75 MHz, $CDCl_3$)

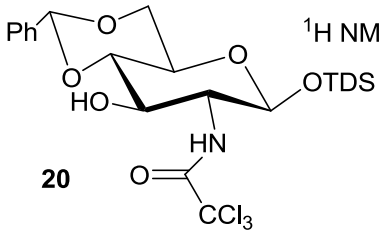


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



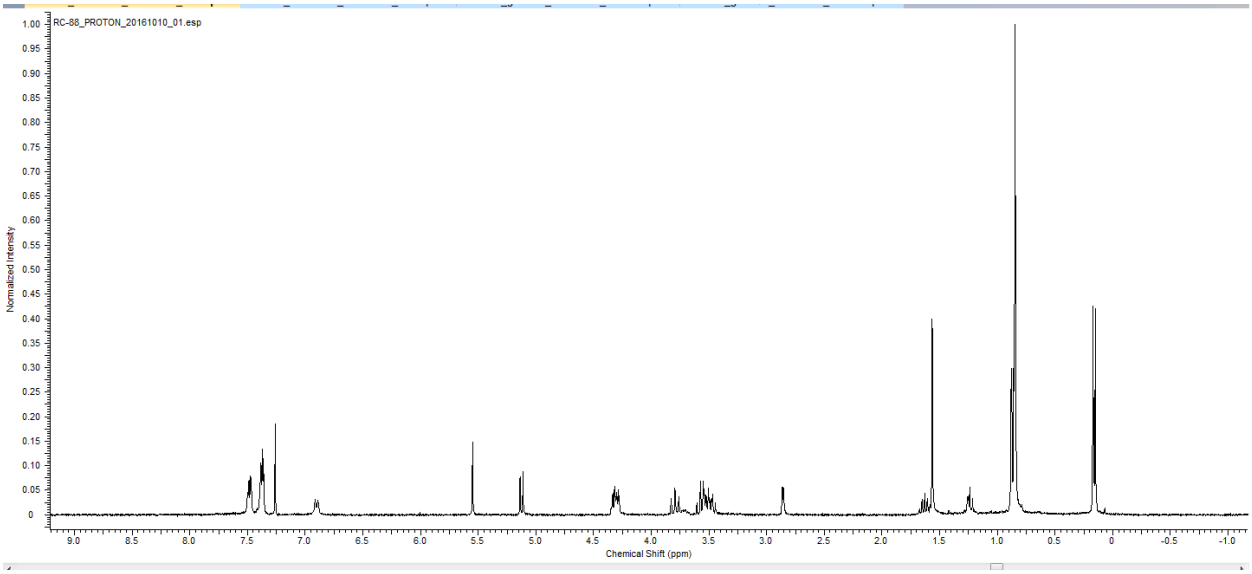
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)



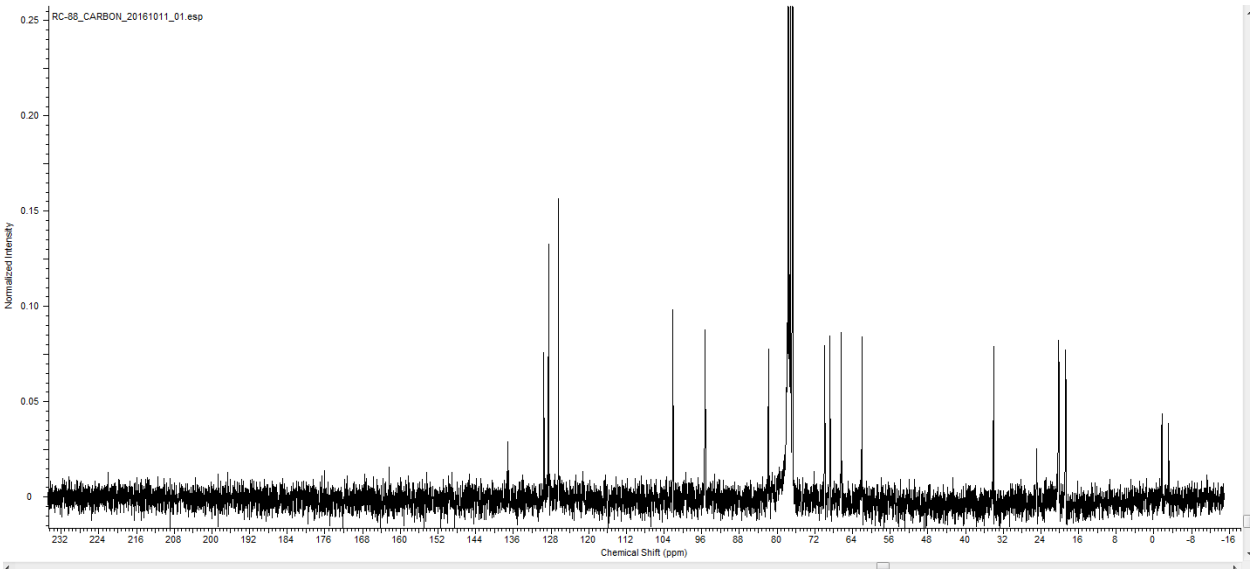


20

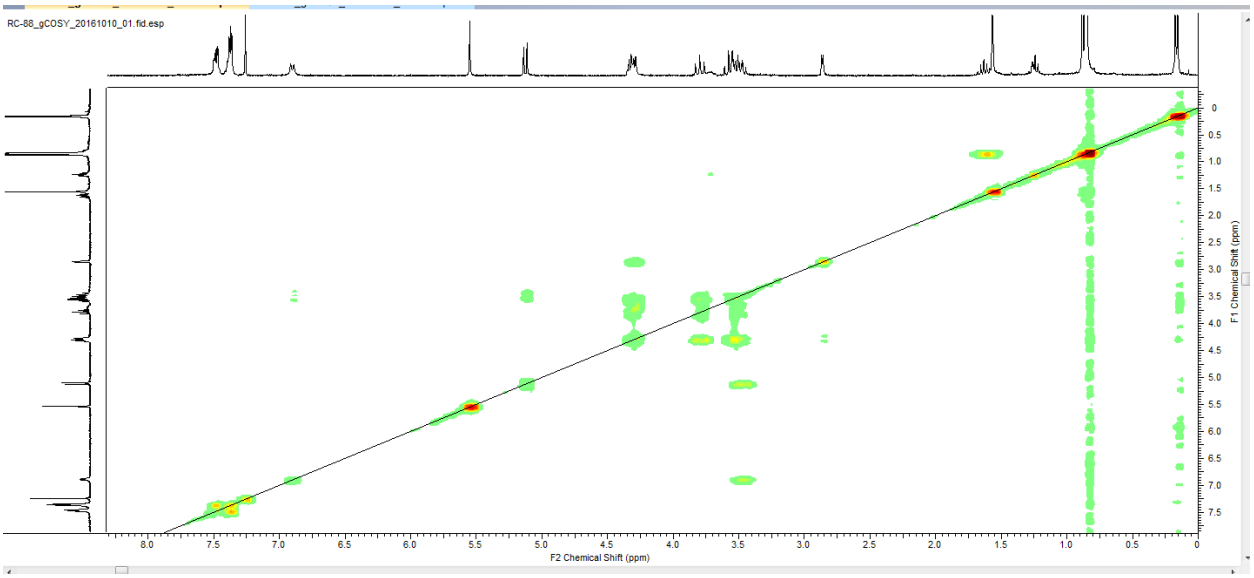
^{1}H NMR (300 MHz, $CDCl_3$)



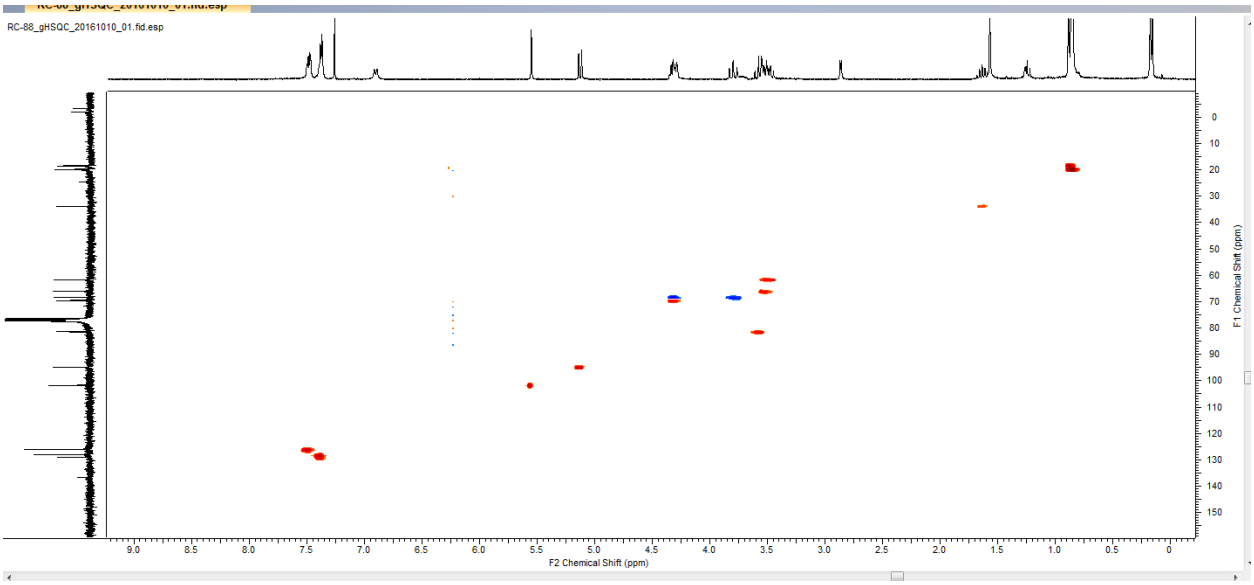
^{13}C NMR (75 MHz, $CDCl_3$)

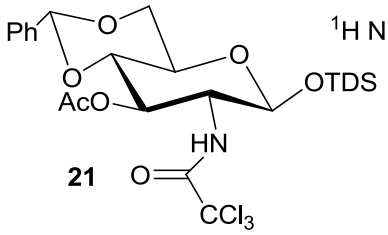


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



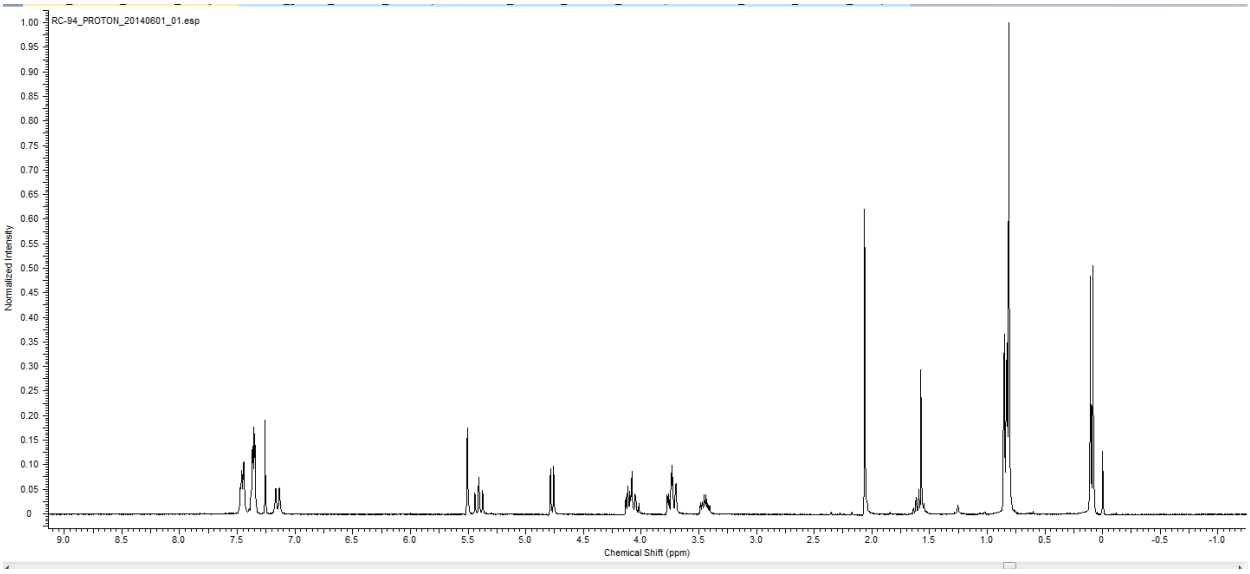
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



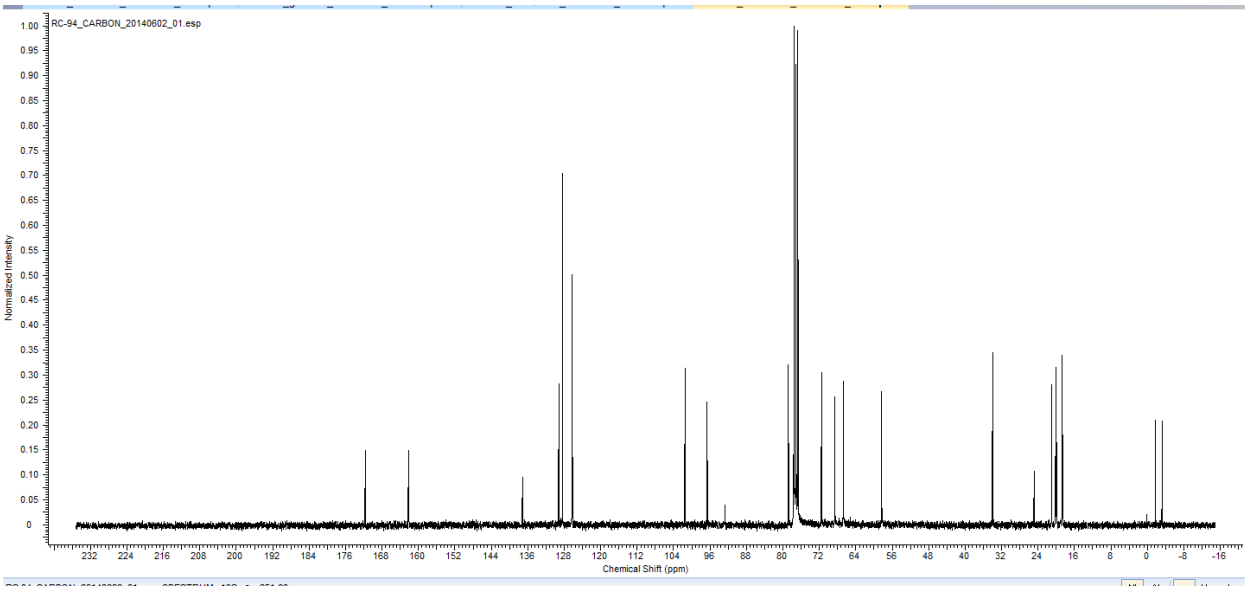


21

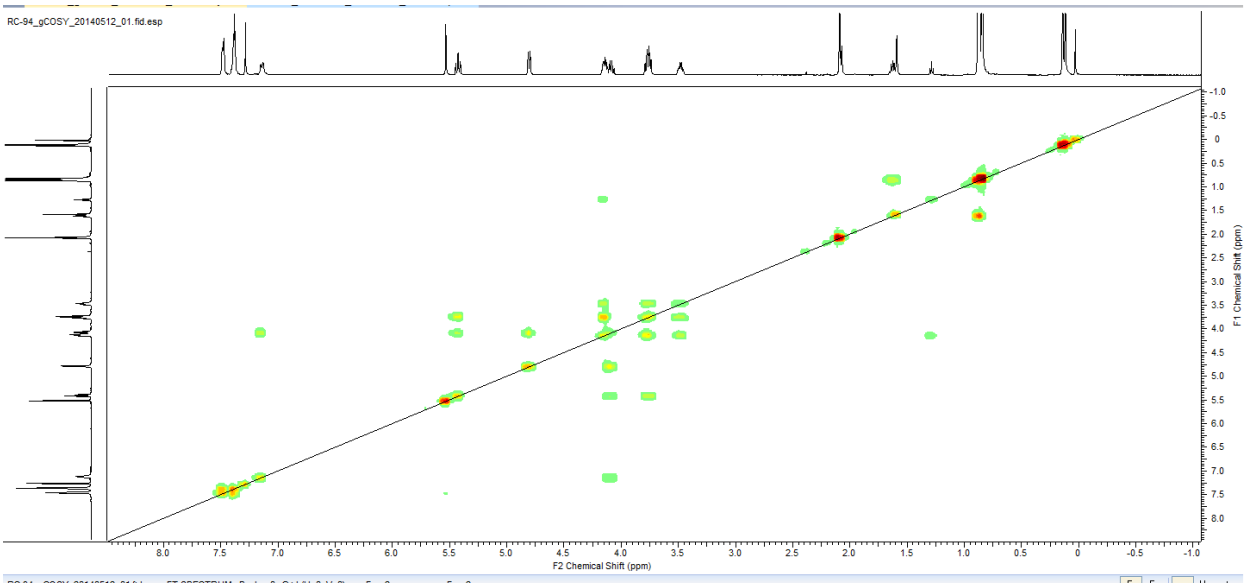
^{1}H NMR (300 MHz, $CDCl_3$)



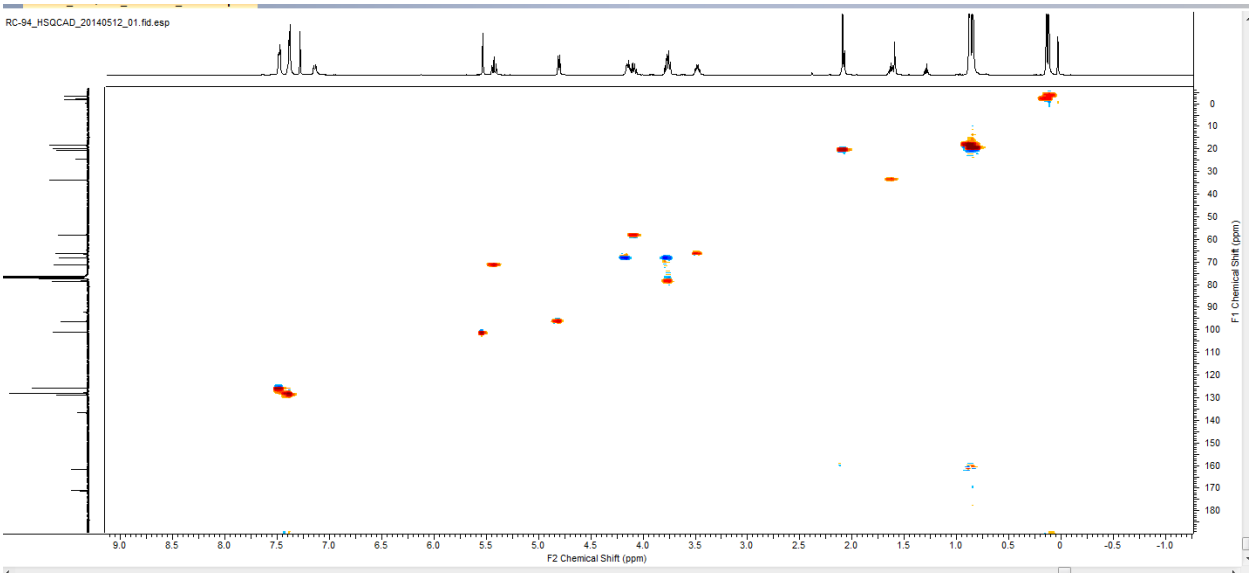
^{13}C NMR (75 MHz, $CDCl_3$)

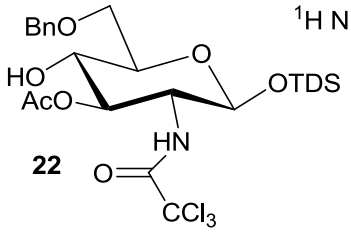


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



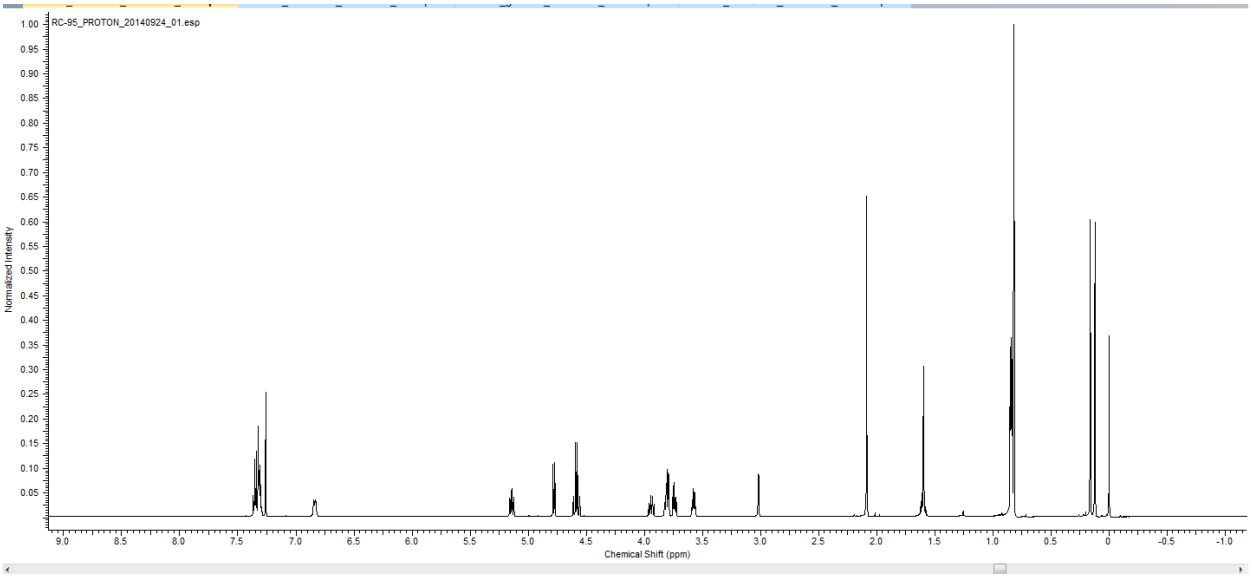
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



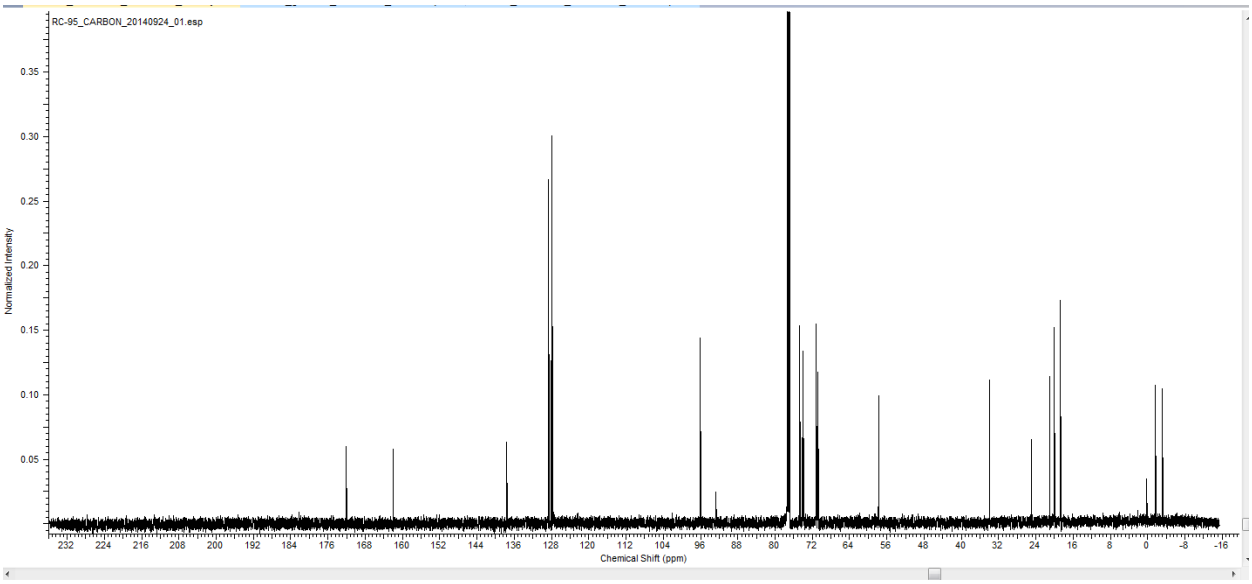


22

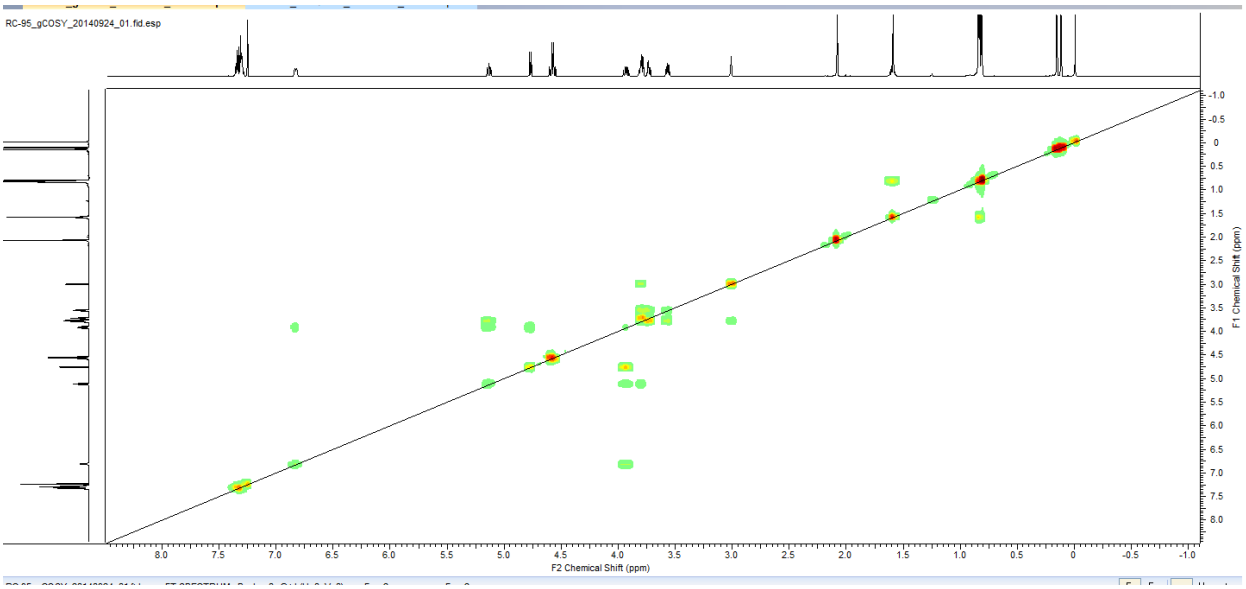
^{1}H NMR (300 MHz, $CDCl_3$)



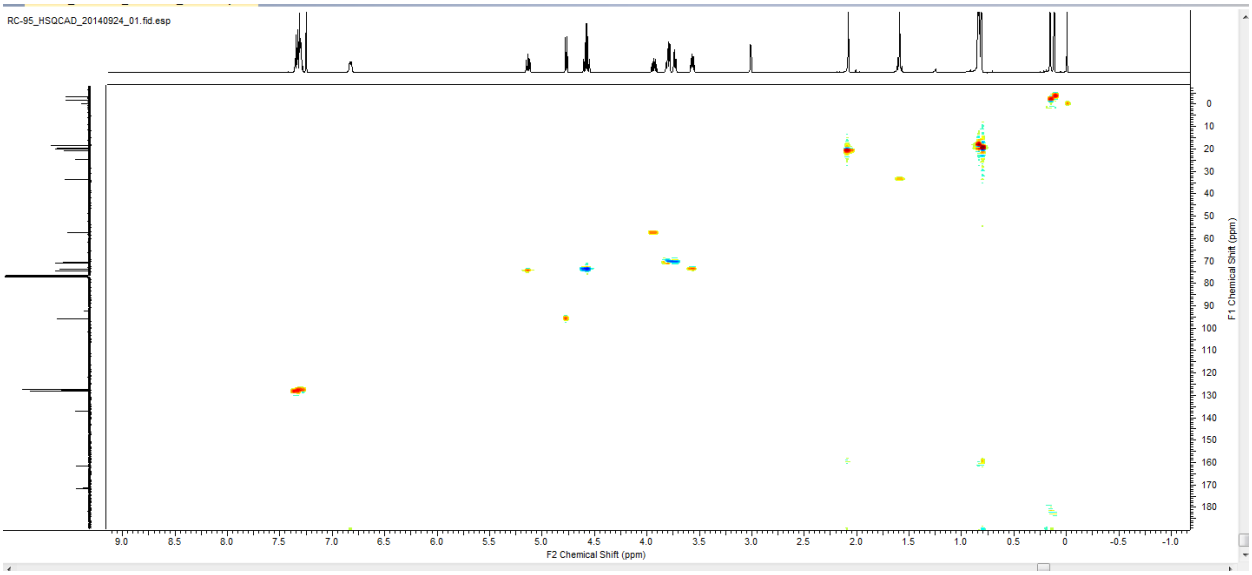
^{13}C NMR (75 MHz, $CDCl_3$)

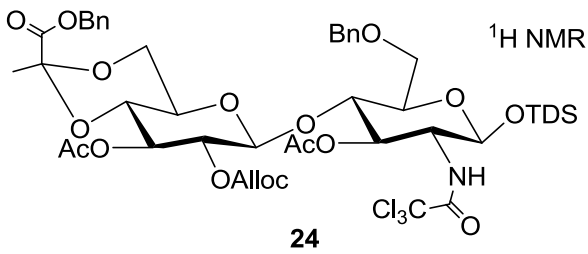


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



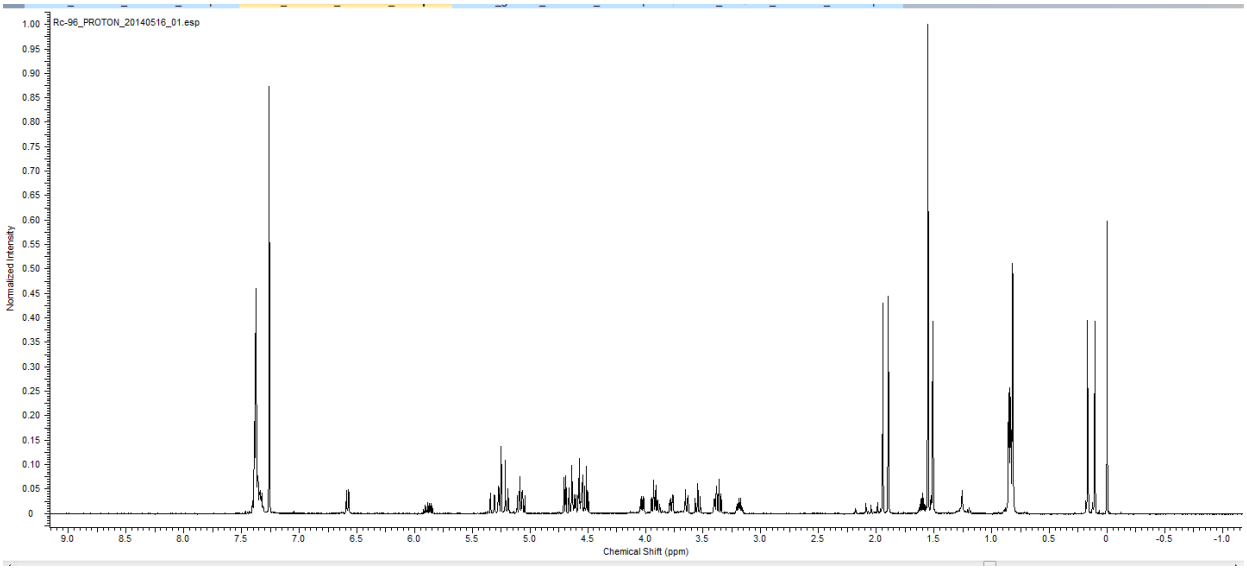
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



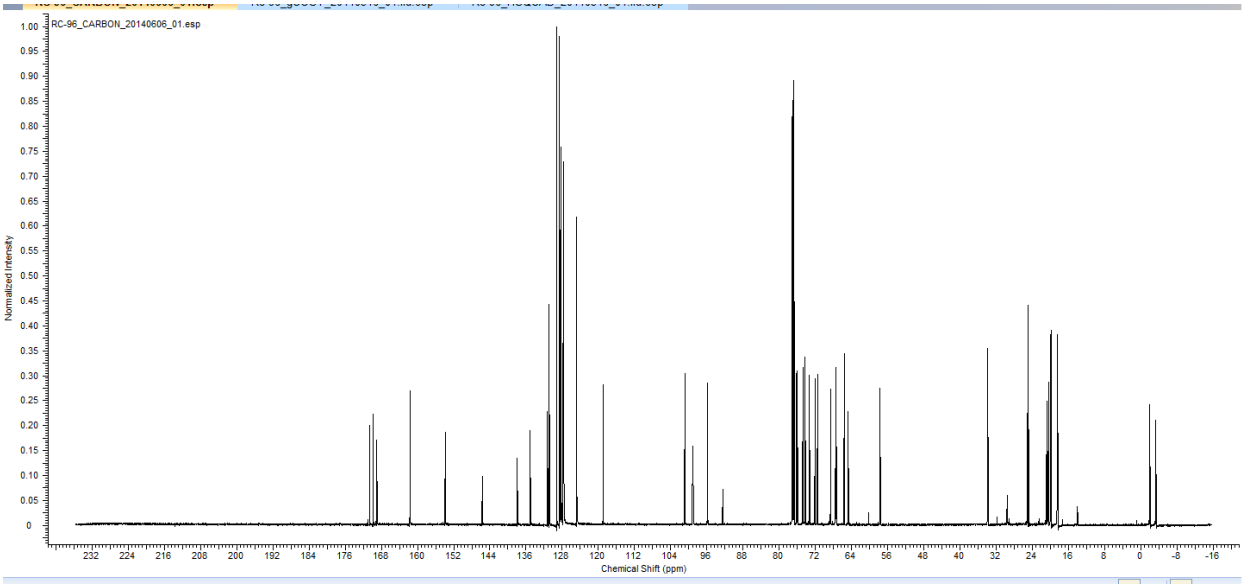


24

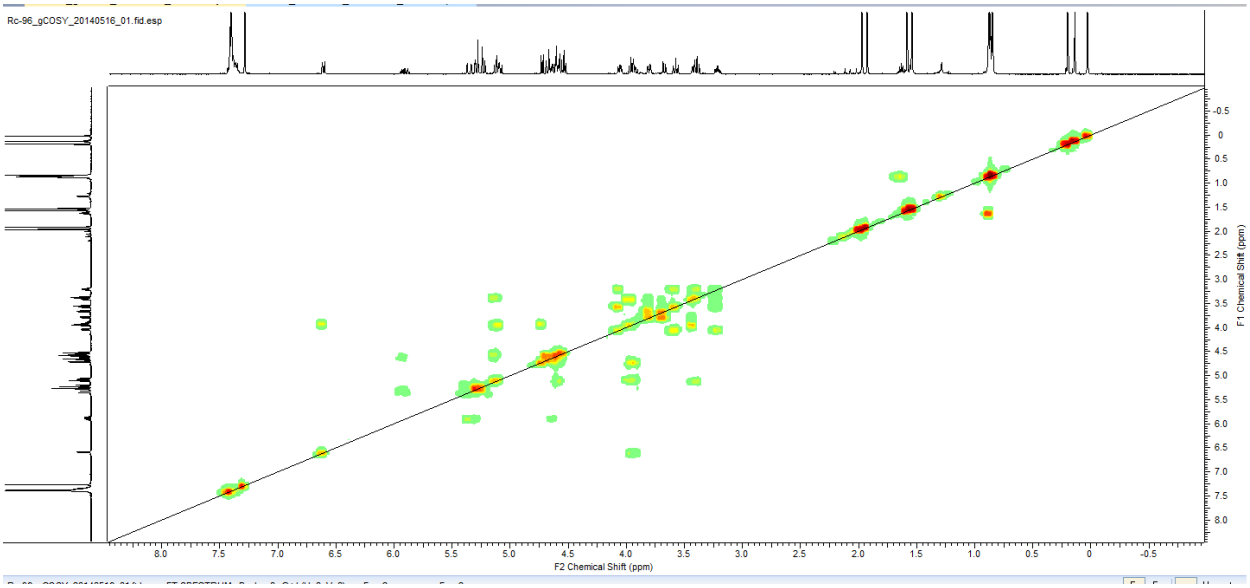
^{1}H NMR (600 MHz, $CDCl_3$)



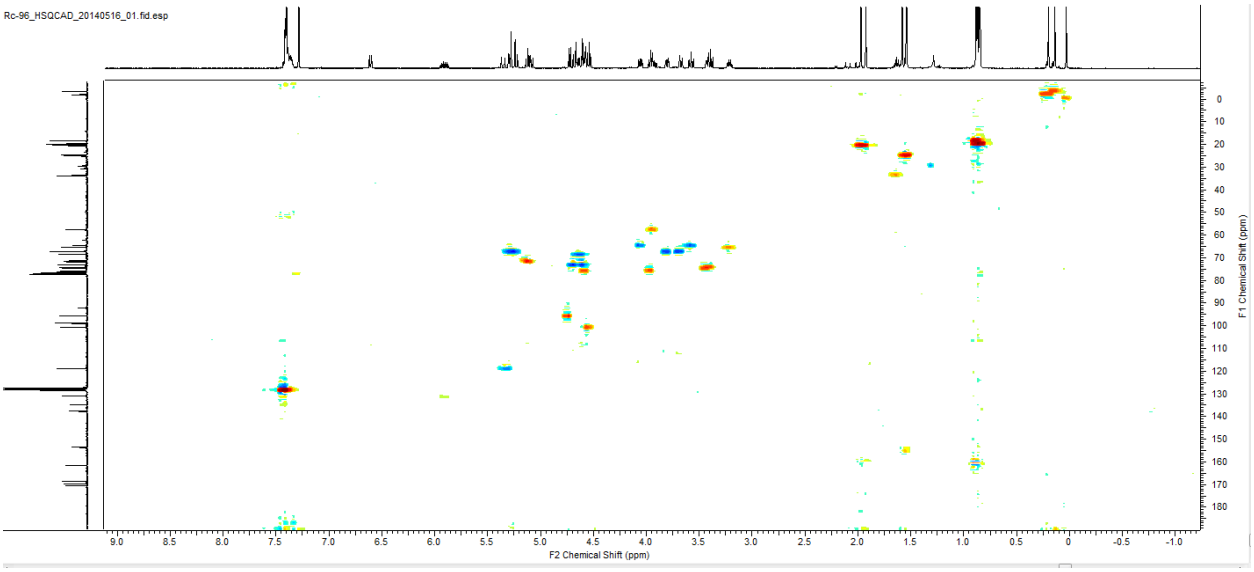
^{13}C NMR (150 MHz, $CDCl_3$)

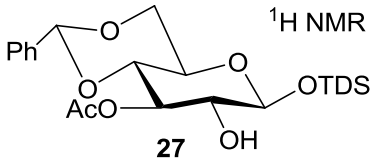


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



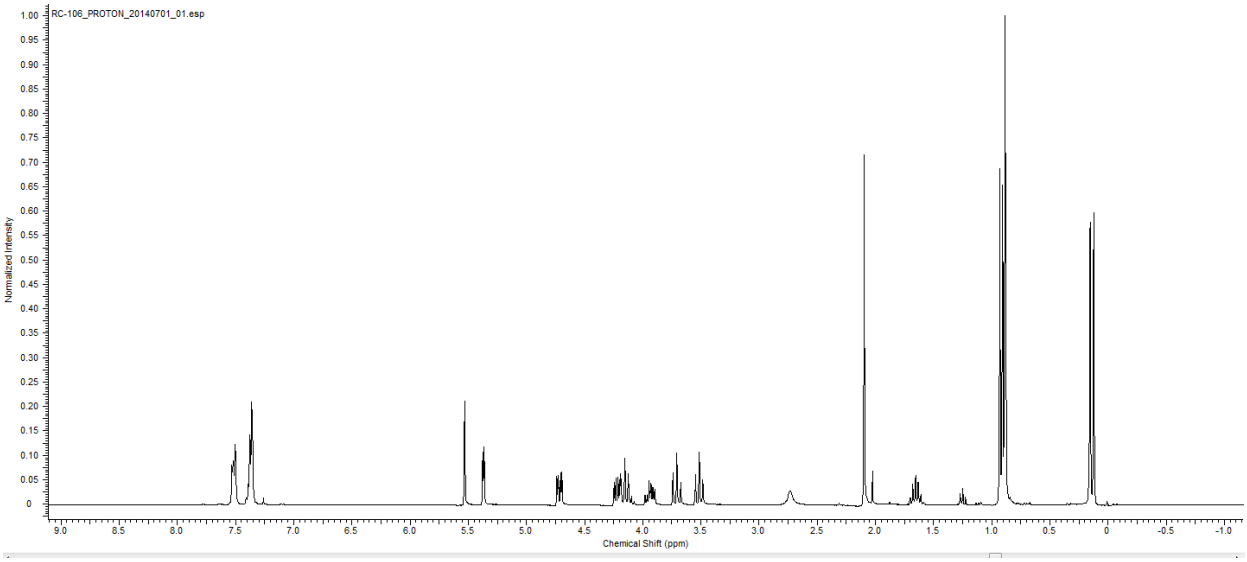
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



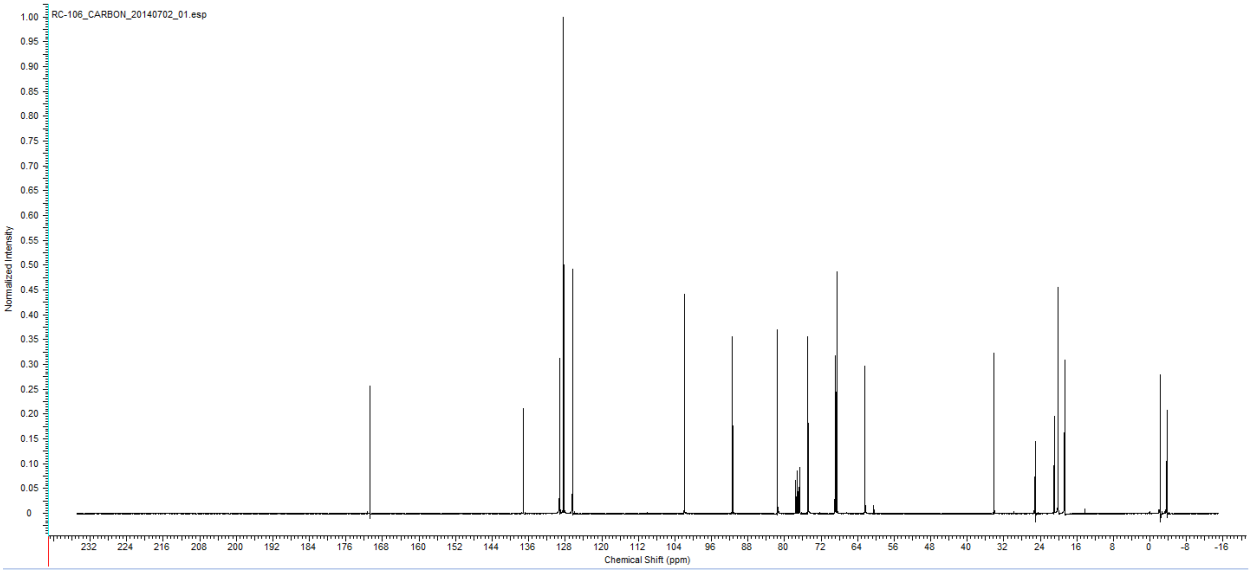


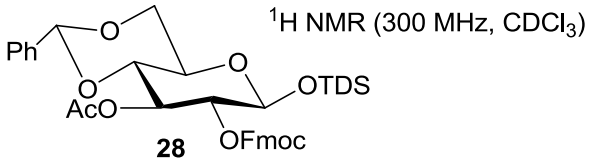
27

^{1}H NMR (300 MHz, $CDCl_3$)



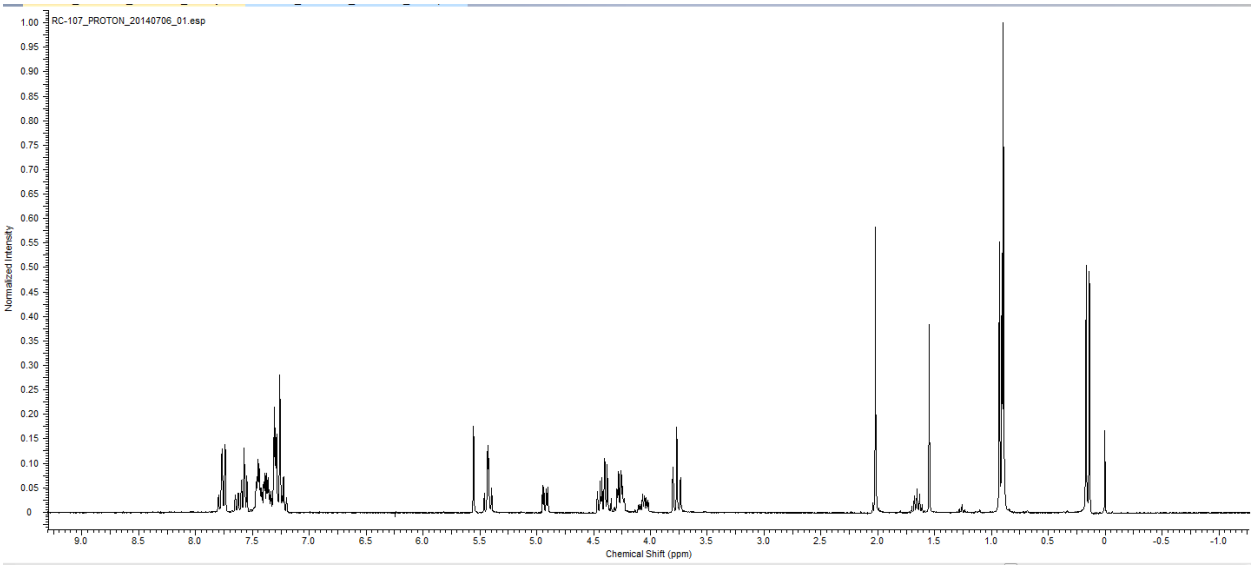
^{13}C NMR (150 MHz, $CDCl_3$)



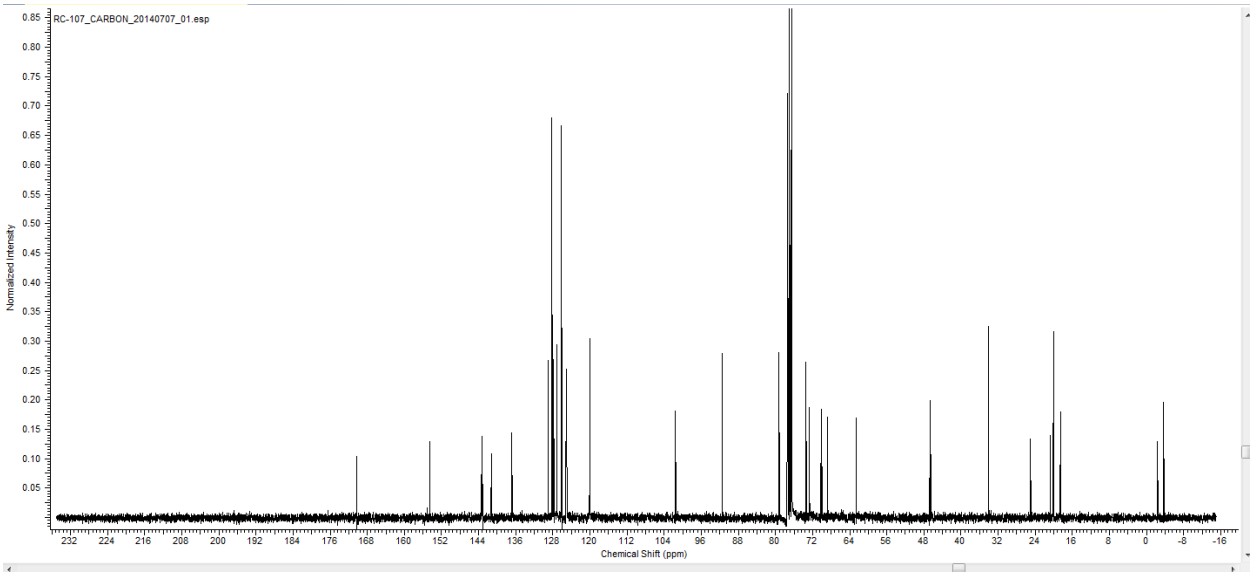


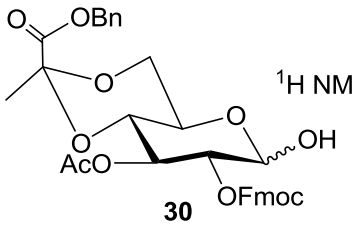
28

^{1}H NMR (300 MHz, $CDCl_3$)



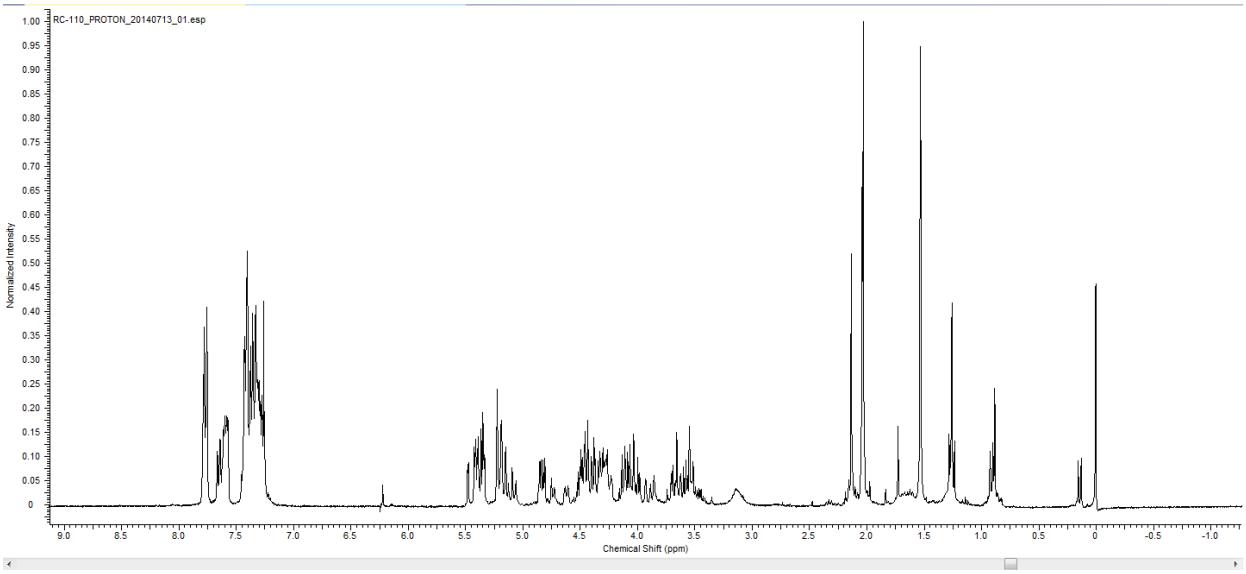
^{13}C NMR (75 MHz, $CDCl_3$)



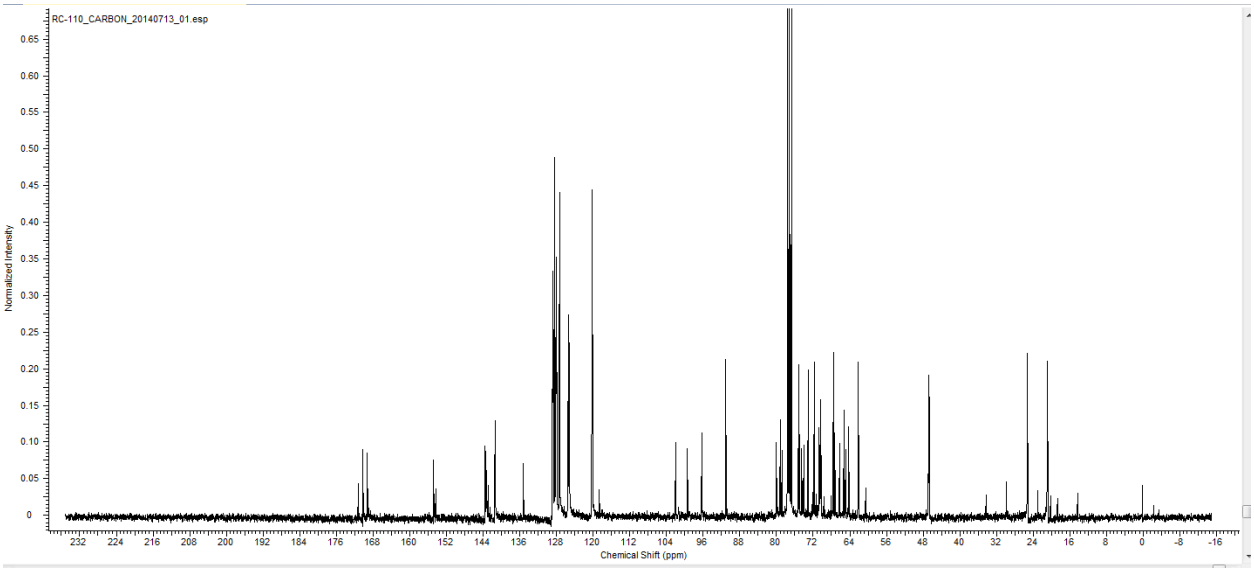


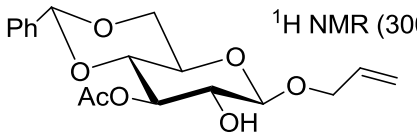
30

^{1}H NMR (300 MHz, $CDCl_3$)



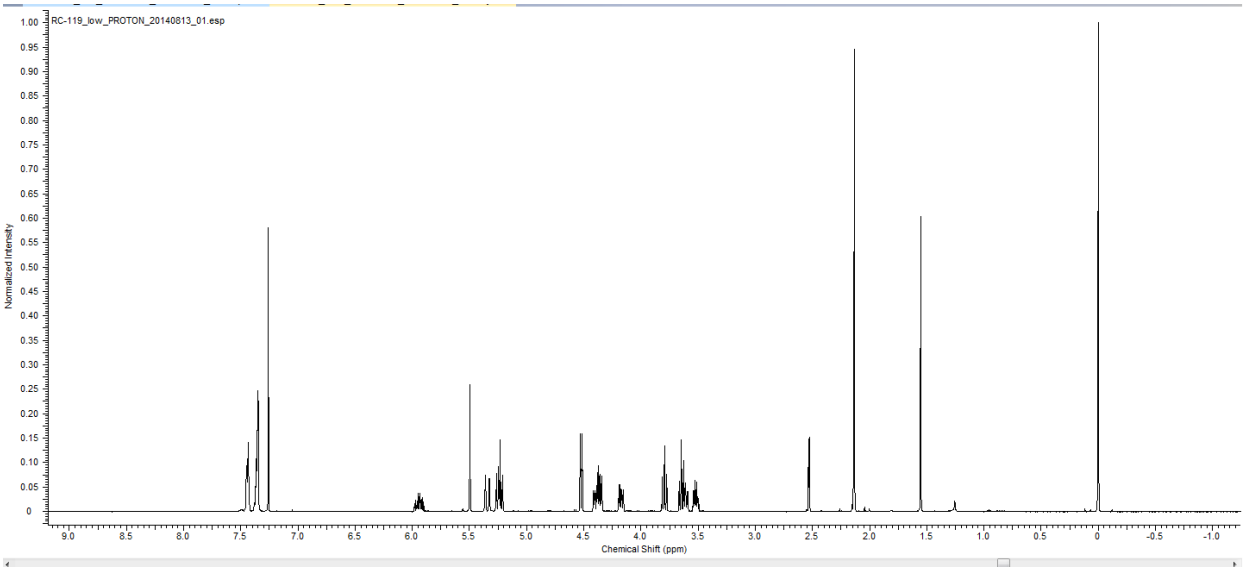
^{13}C NMR (75 MHz, $CDCl_3$)



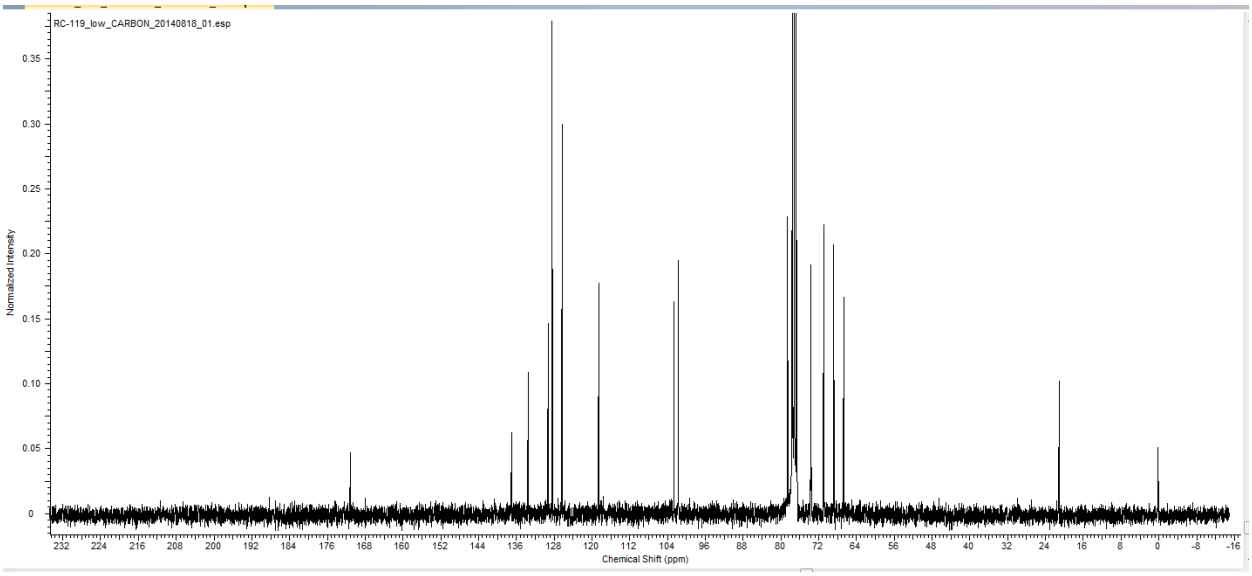


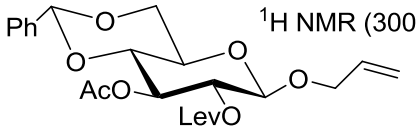
35

^{1}H NMR (300 MHz, $CDCl_3$)



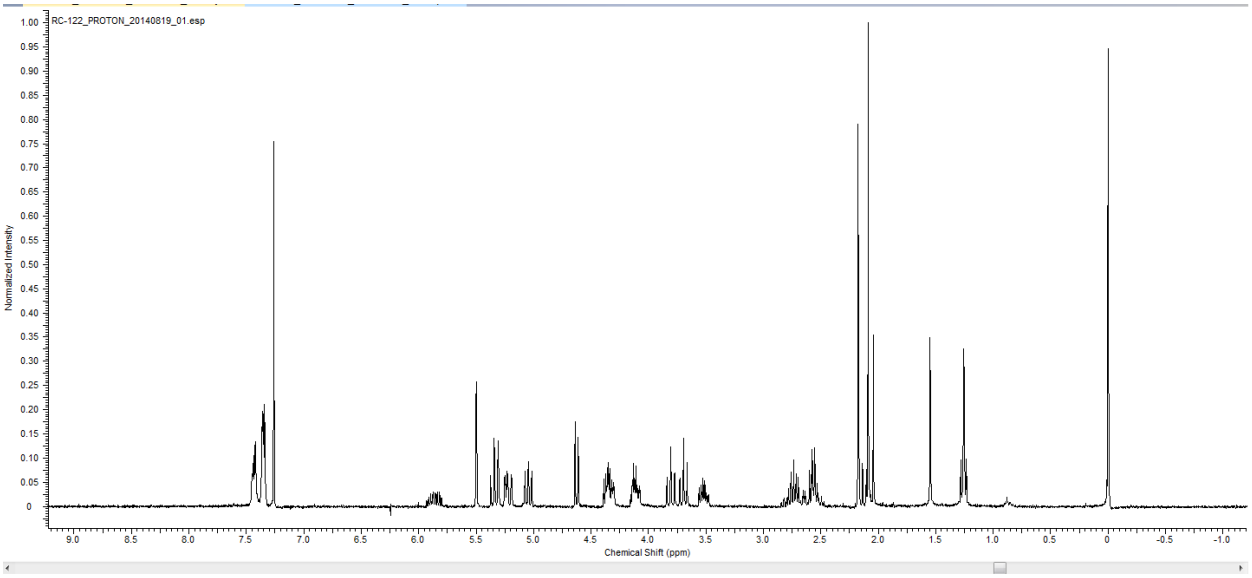
^{13}C NMR (75 MHz, $CDCl_3$)



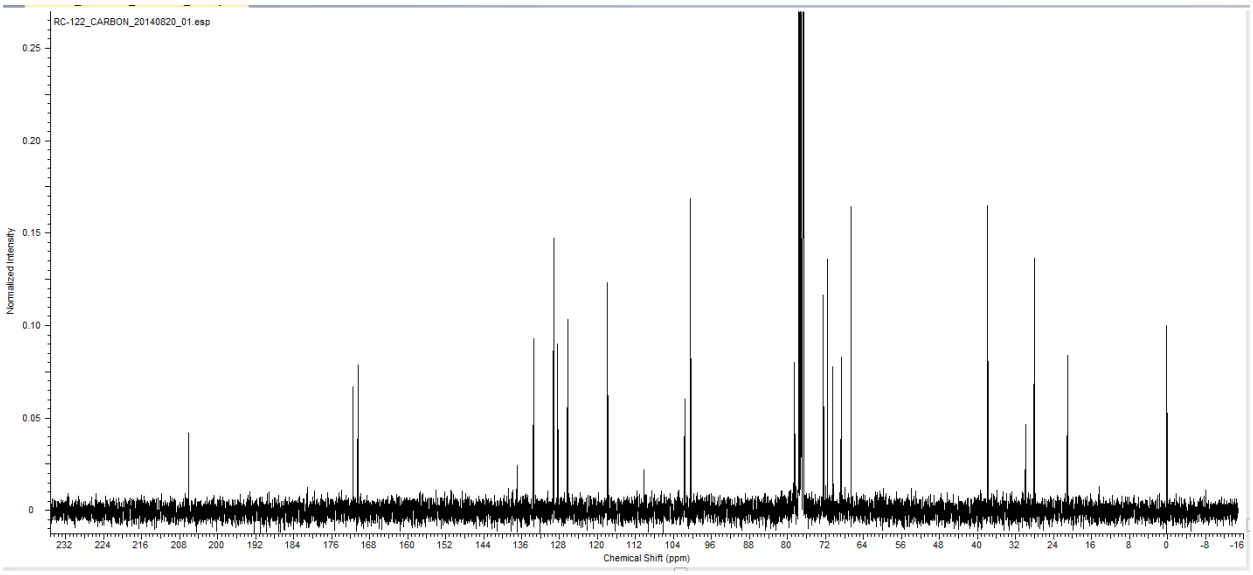


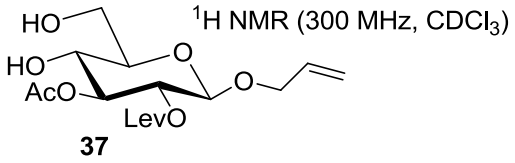
36

^{1}H NMR (300 MHz, $CDCl_3$)



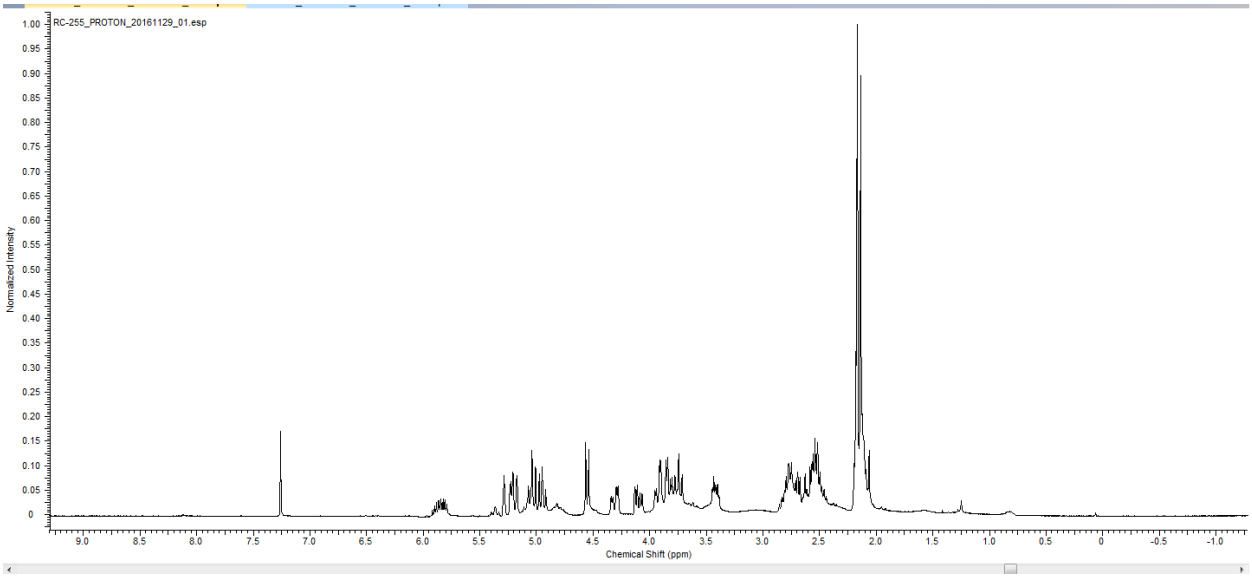
^{13}C NMR (75 MHz, $CDCl_3$)



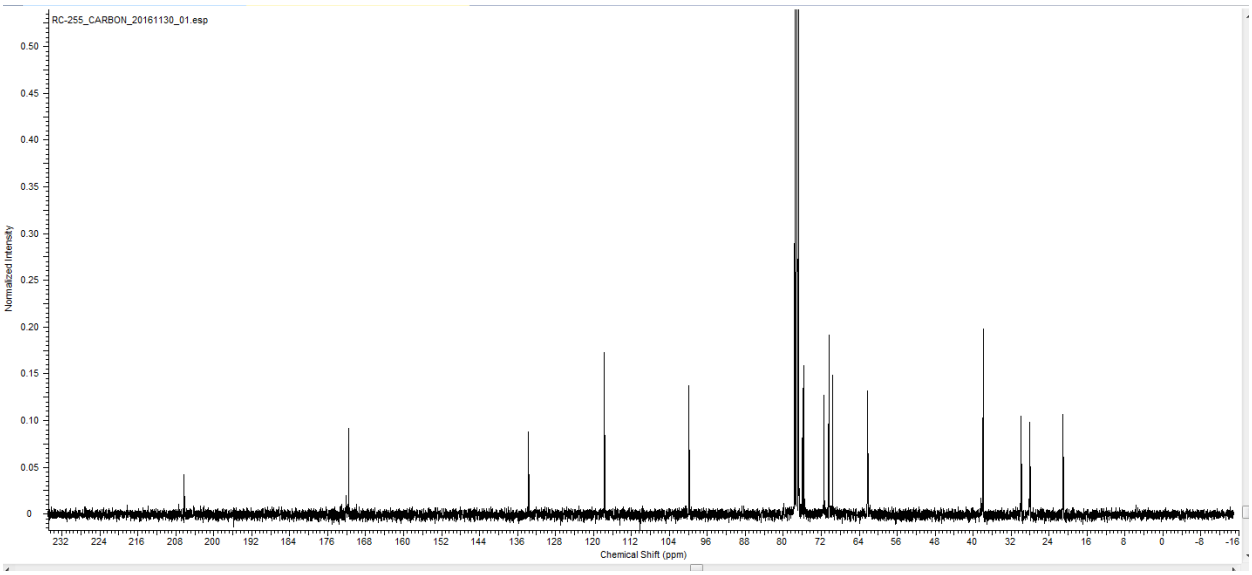


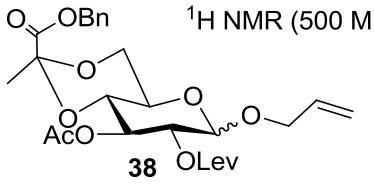
37

^{1}H NMR (300 MHz, $CDCl_3$)



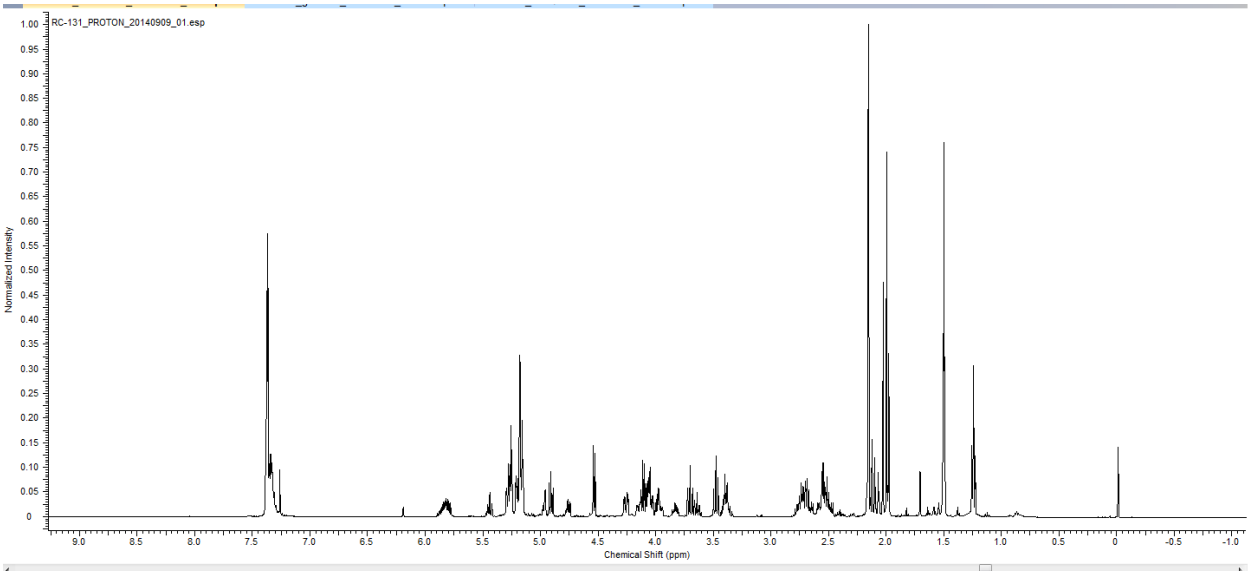
^{13}C NMR (75 MHz, $CDCl_3$)



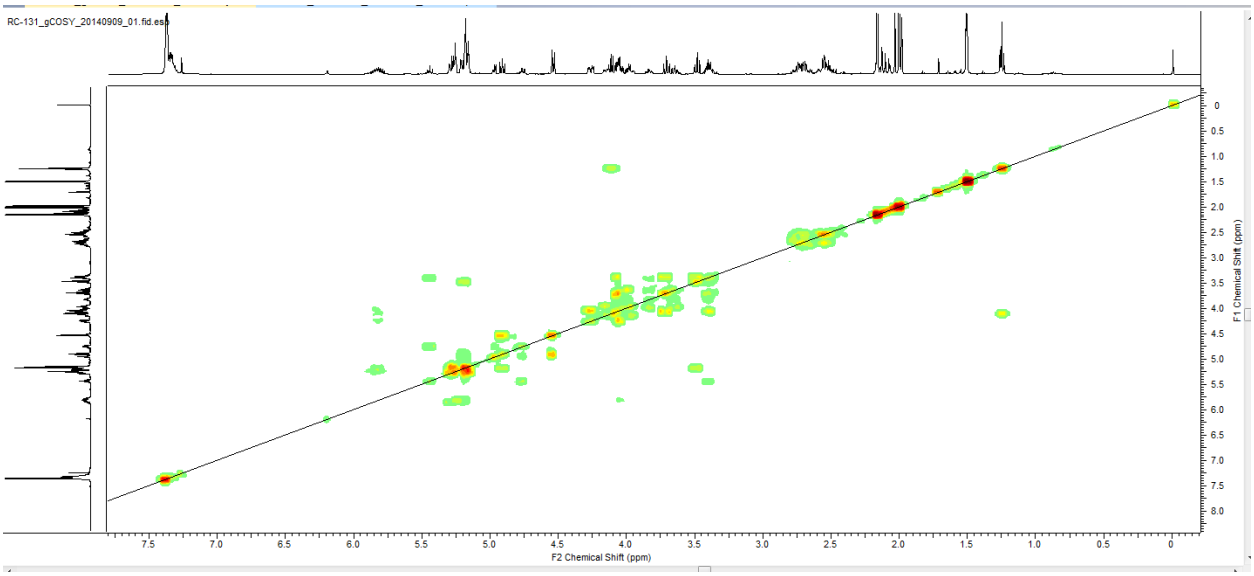


38

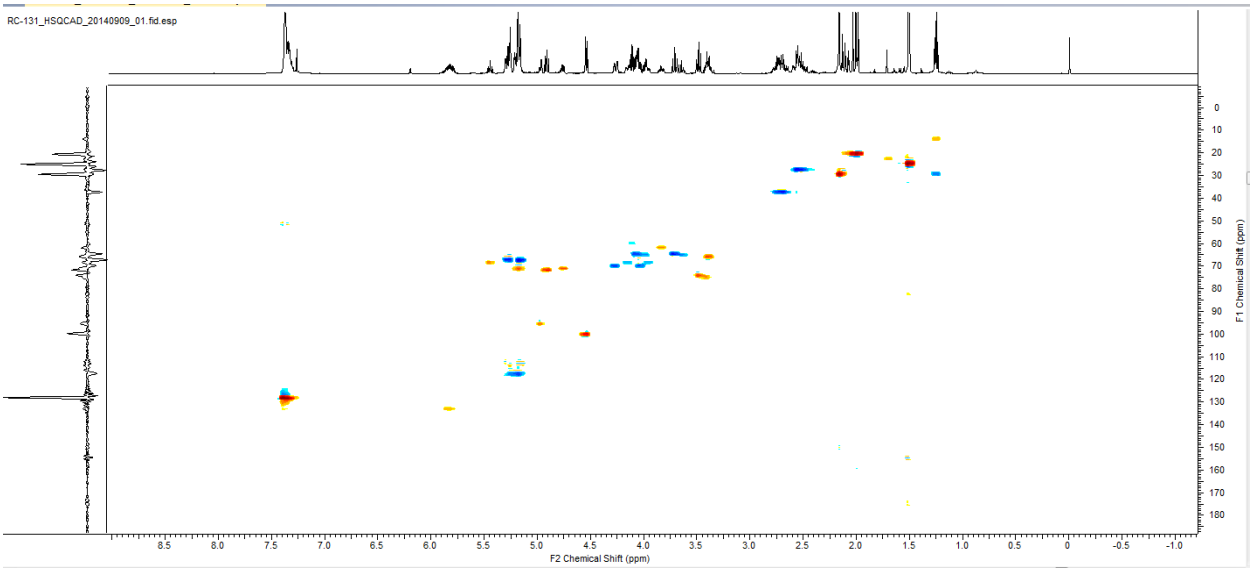
^{1}H NMR (500 MHz, $CDCl_3$)

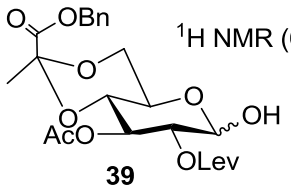


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



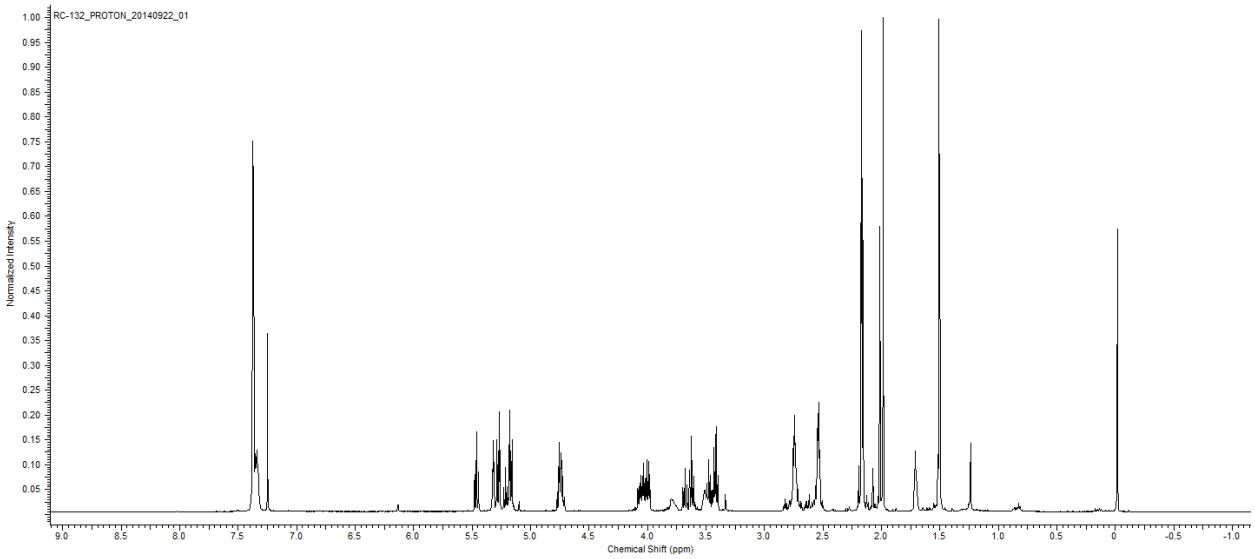
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)



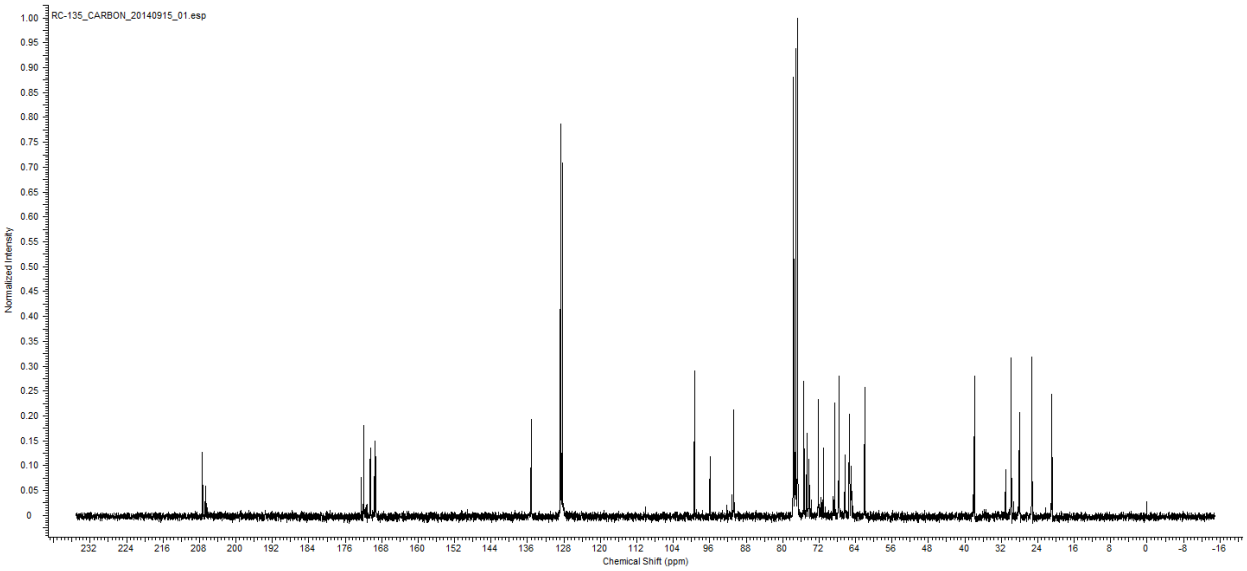


39

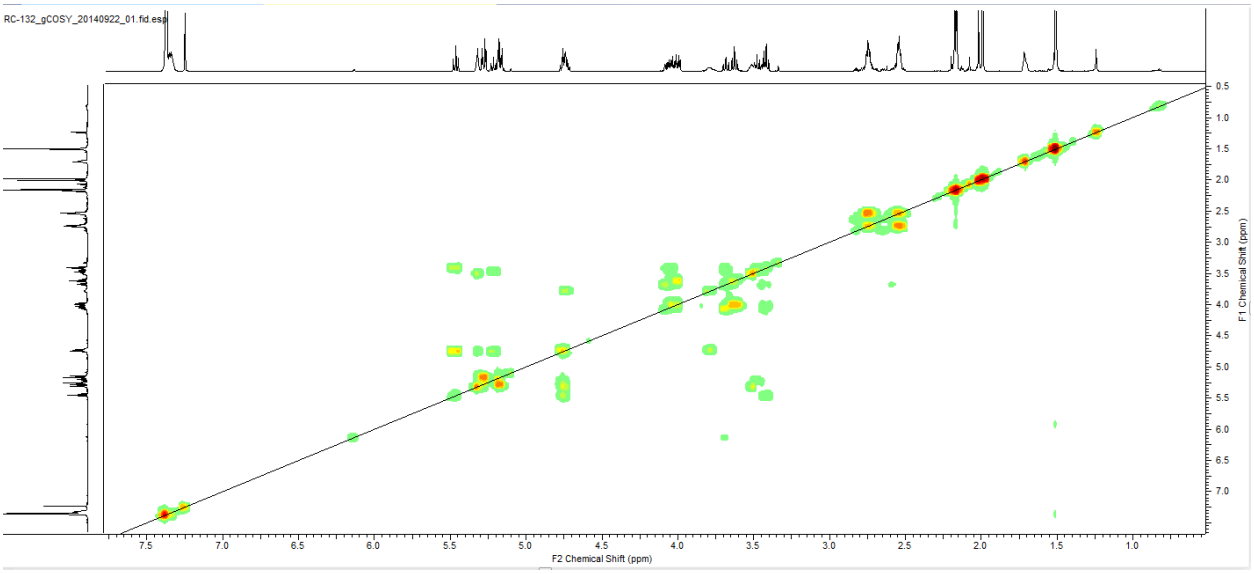
^{1}H NMR (600 MHz, $CDCl_3$)



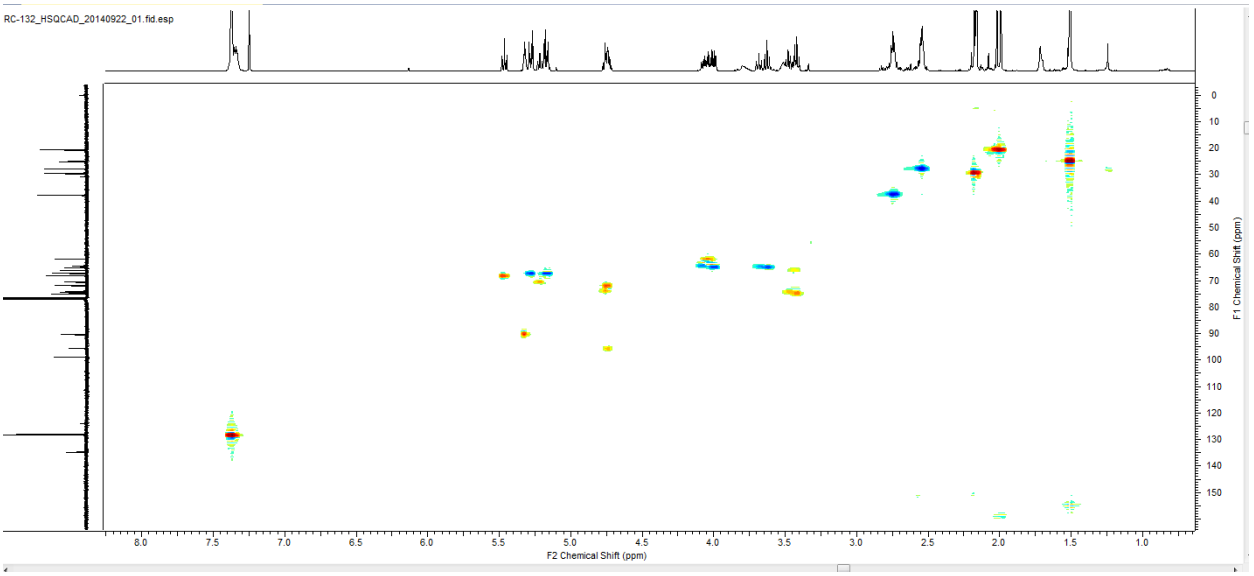
^{13}C NMR (150 MHz, $CDCl_3$)



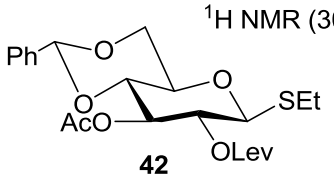
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



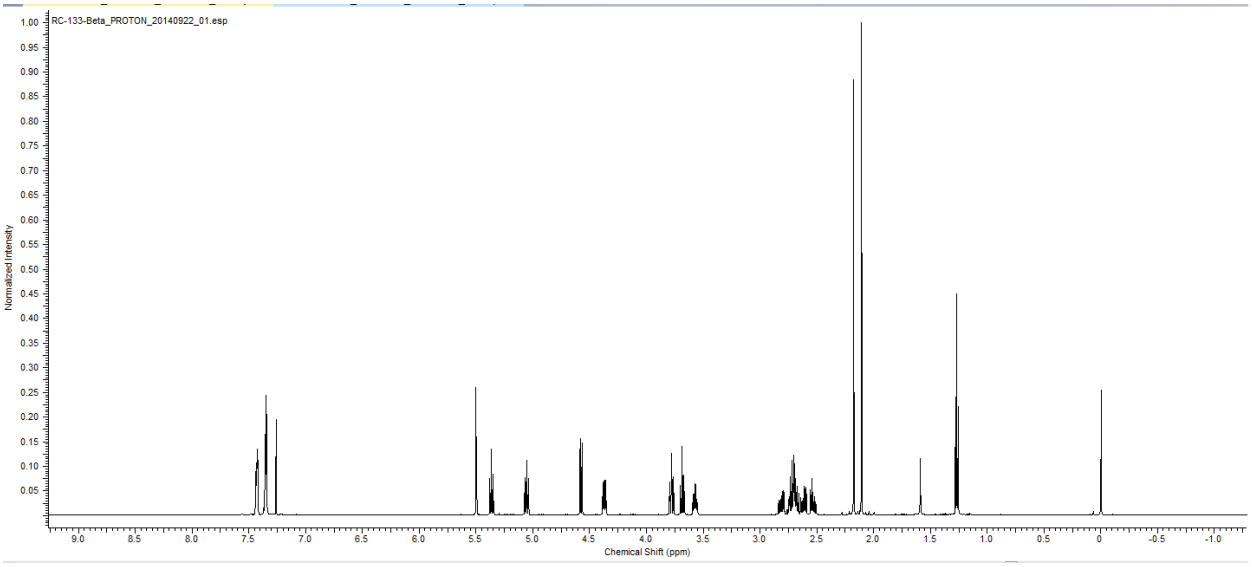
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



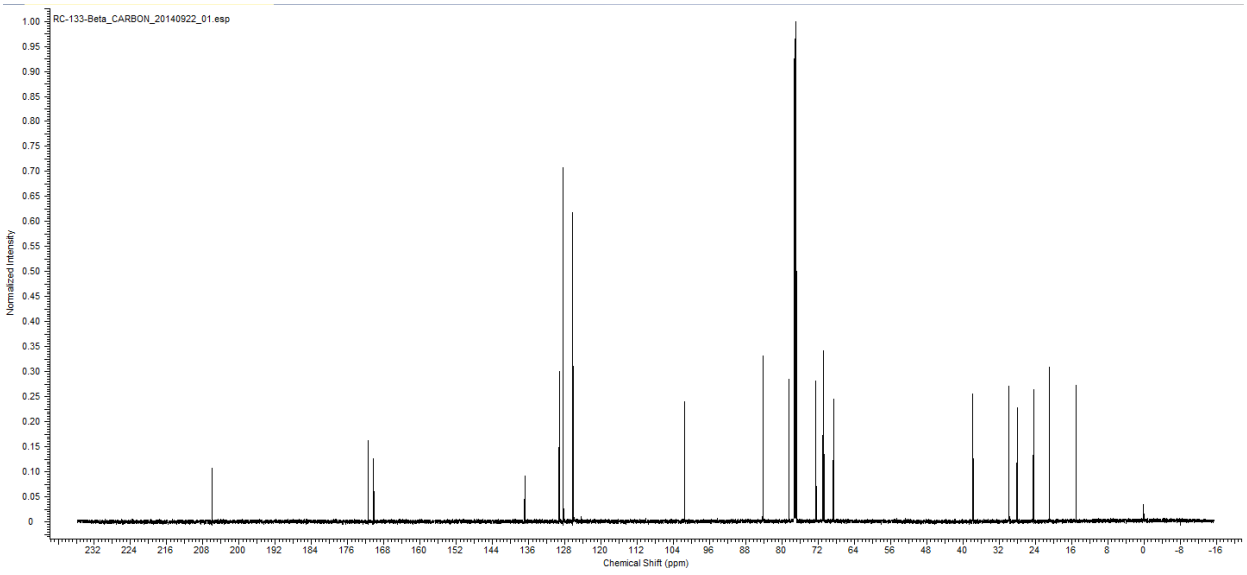
^{1}H NMR (300 MHz, $CDCl_3$)

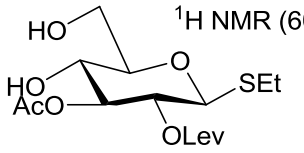


42



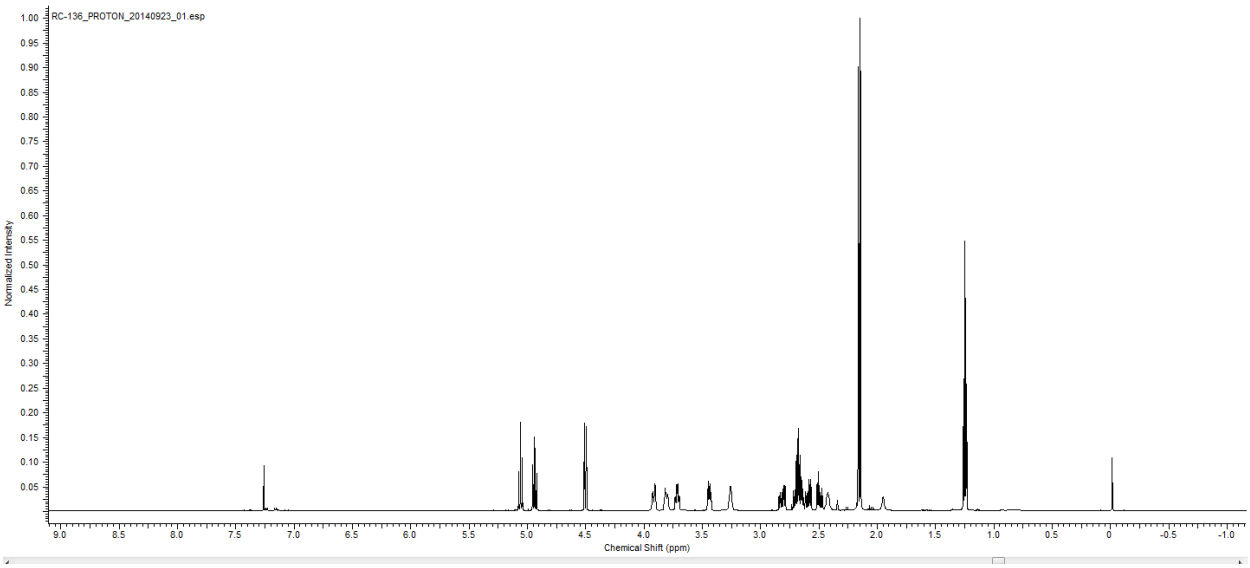
^{13}C NMR (75 MHz, $CDCl_3$)



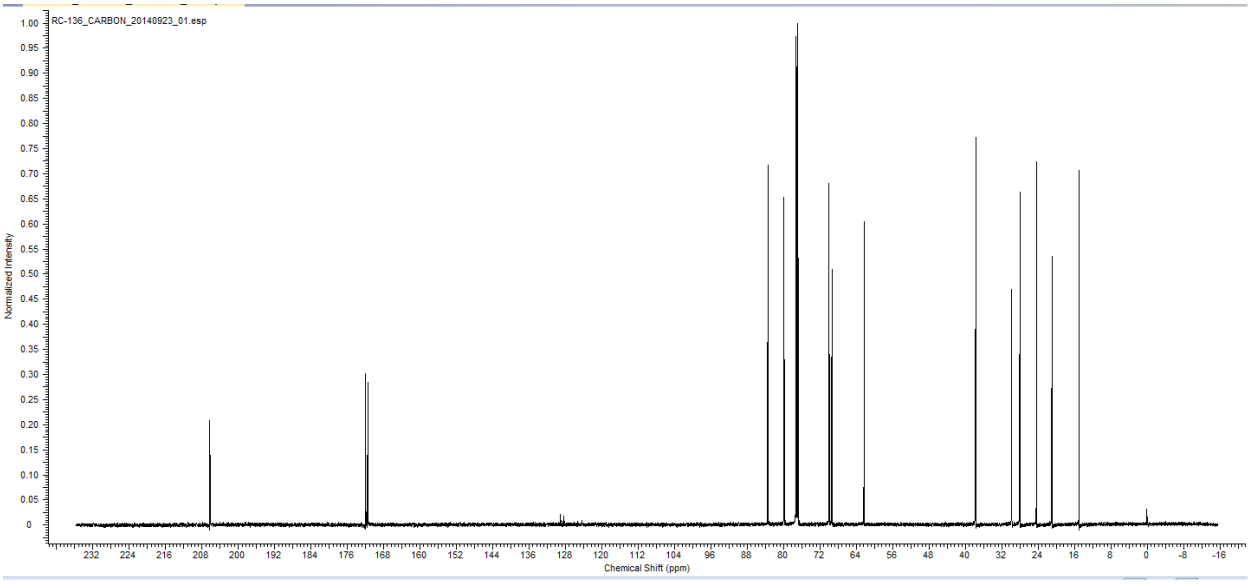


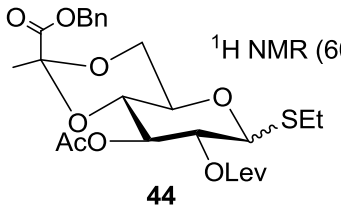
43

^{1}H NMR (600 MHz, $CDCl_3$)



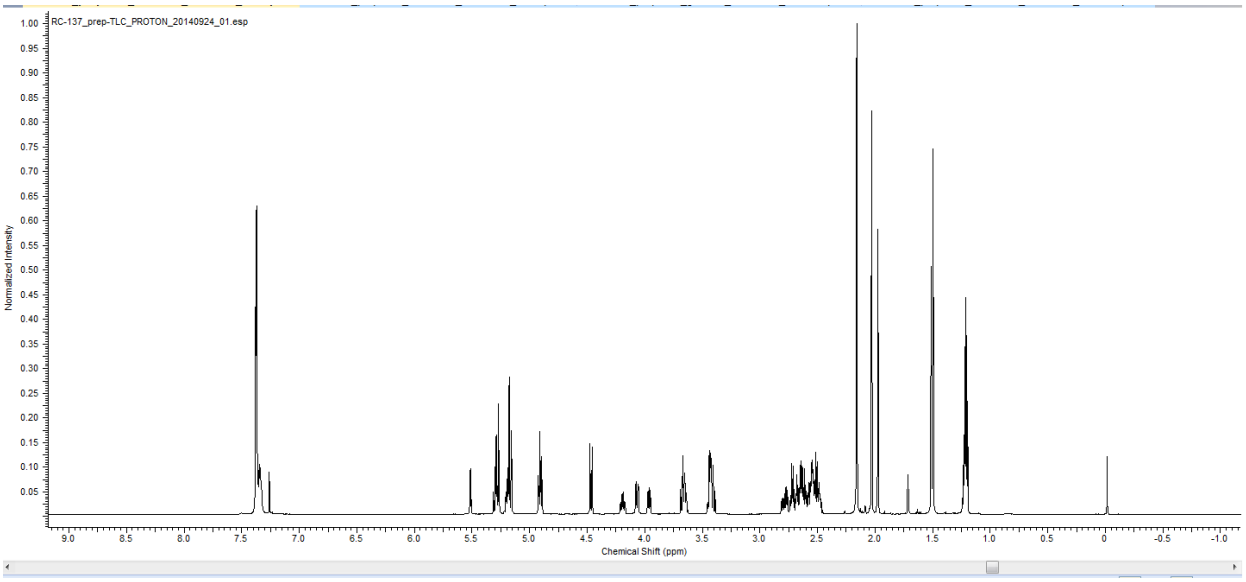
^{13}C NMR (150 MHz, $CDCl_3$)



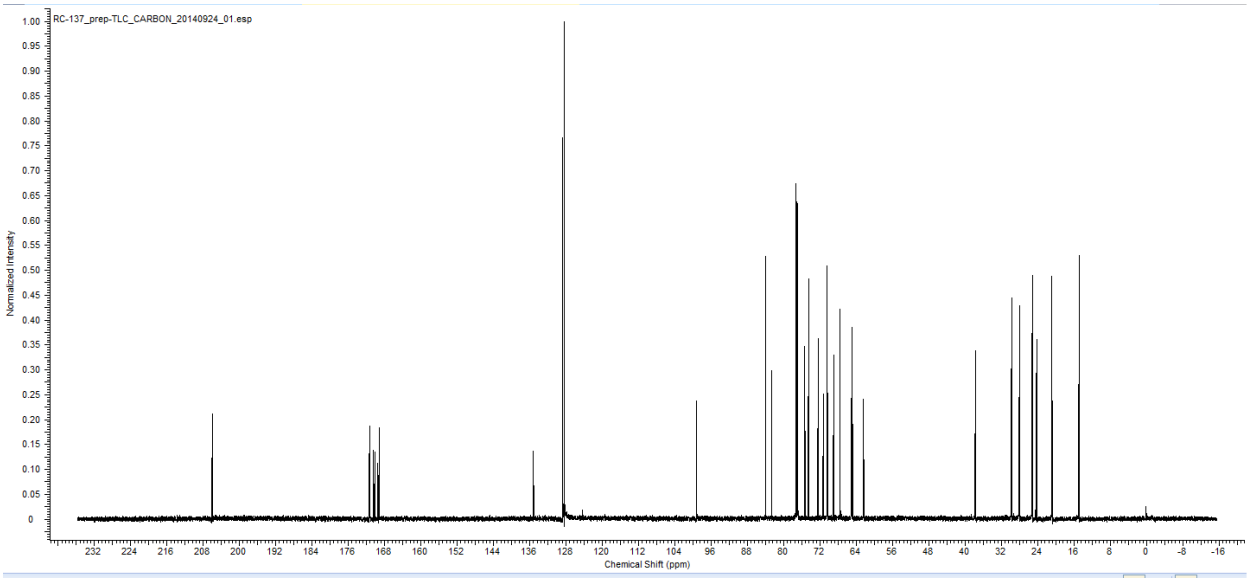


44

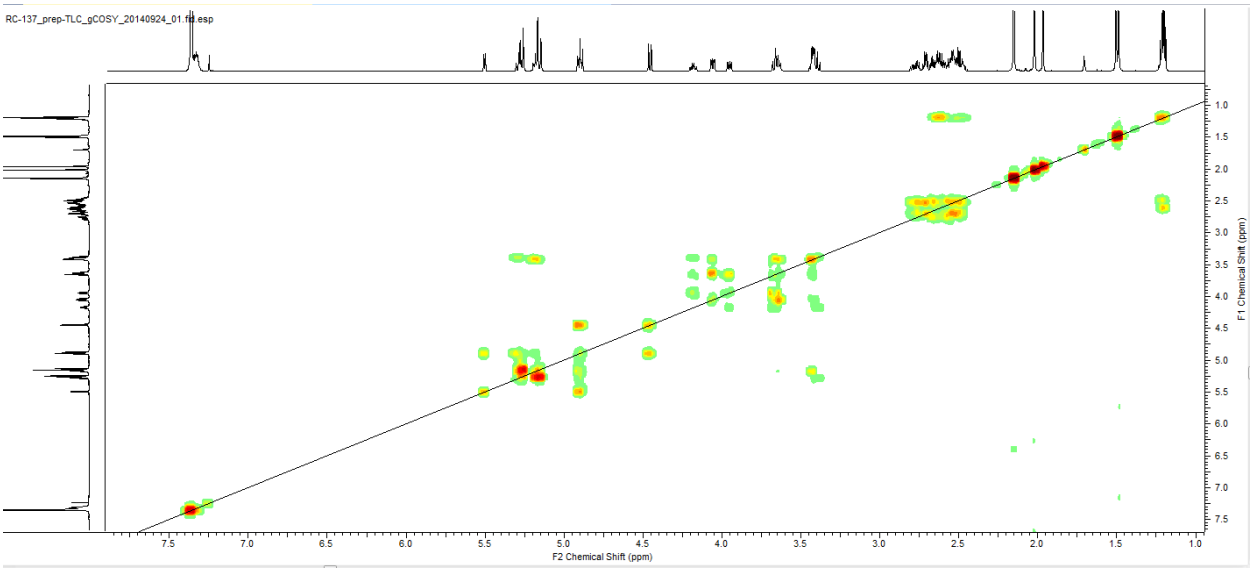
^{1}H NMR (600 MHz, $CDCl_3$)



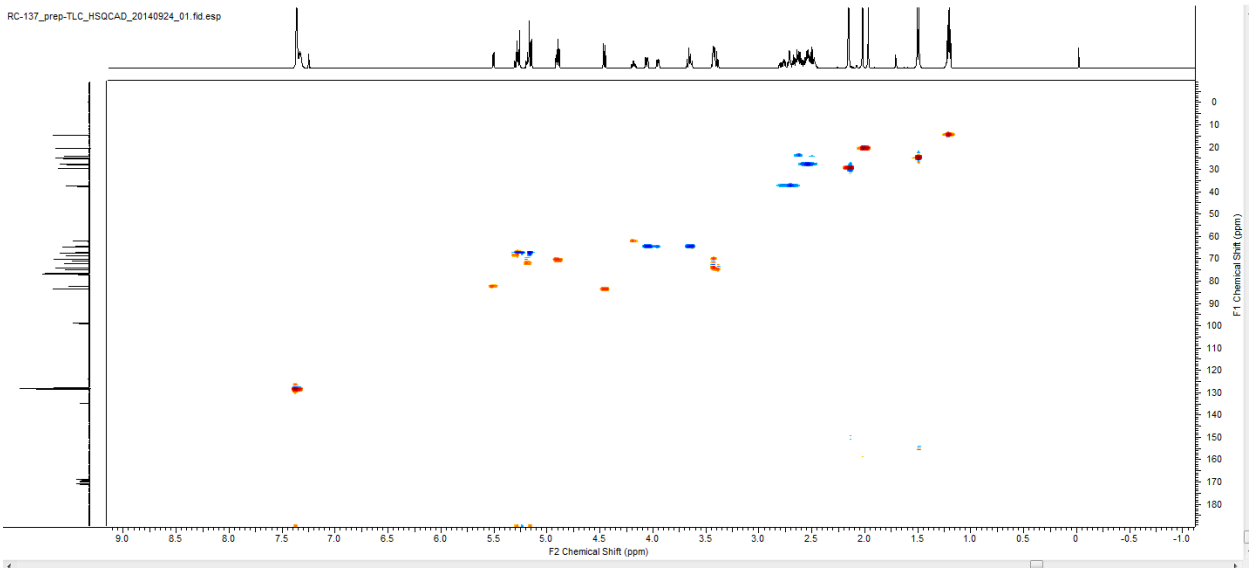
^{13}C NMR (150 MHz, $CDCl_3$)



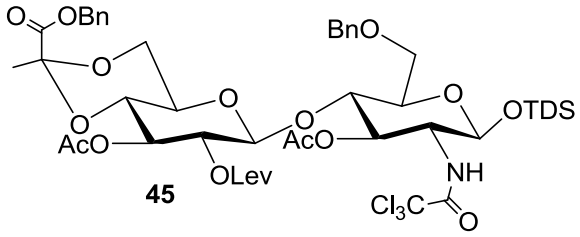
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



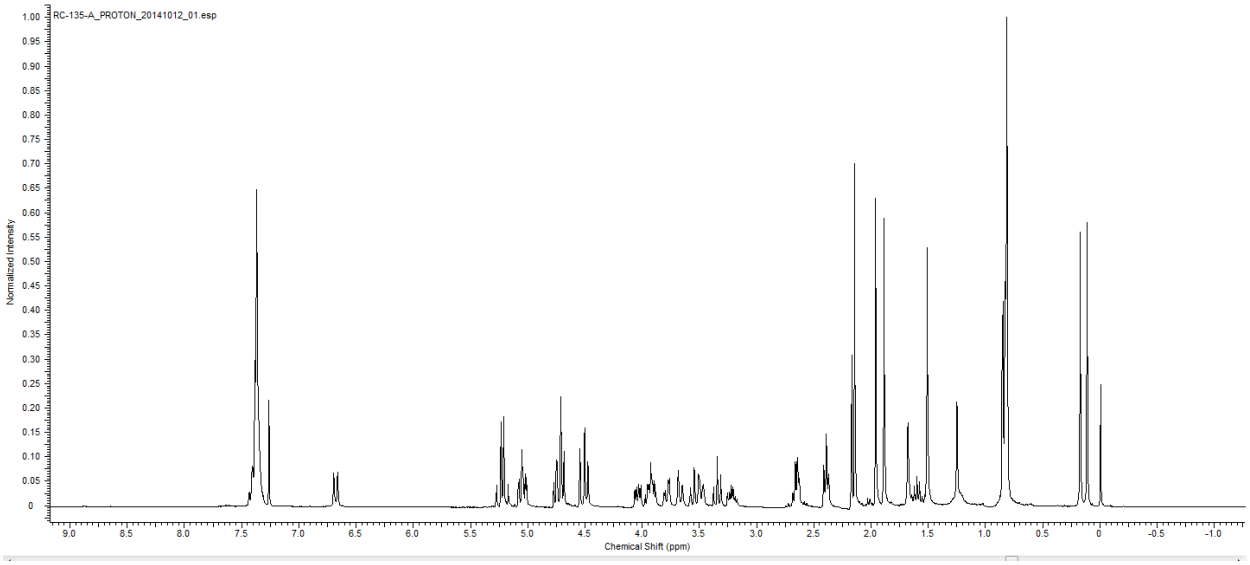
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



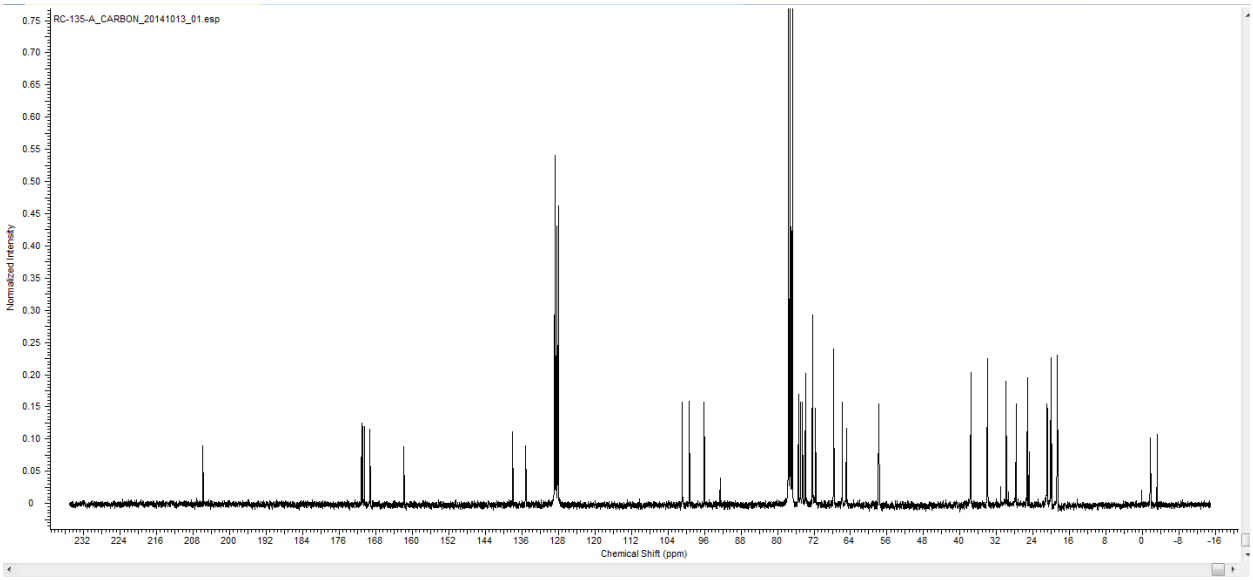
^{1}H NMR (600 MHz, $CDCl_3$)



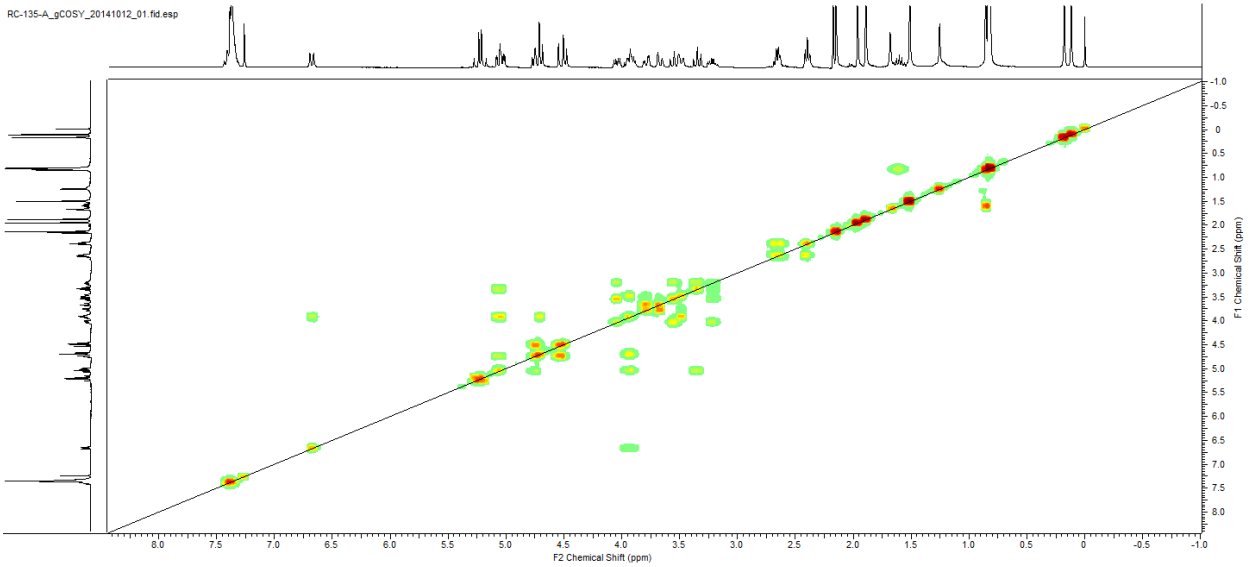
45



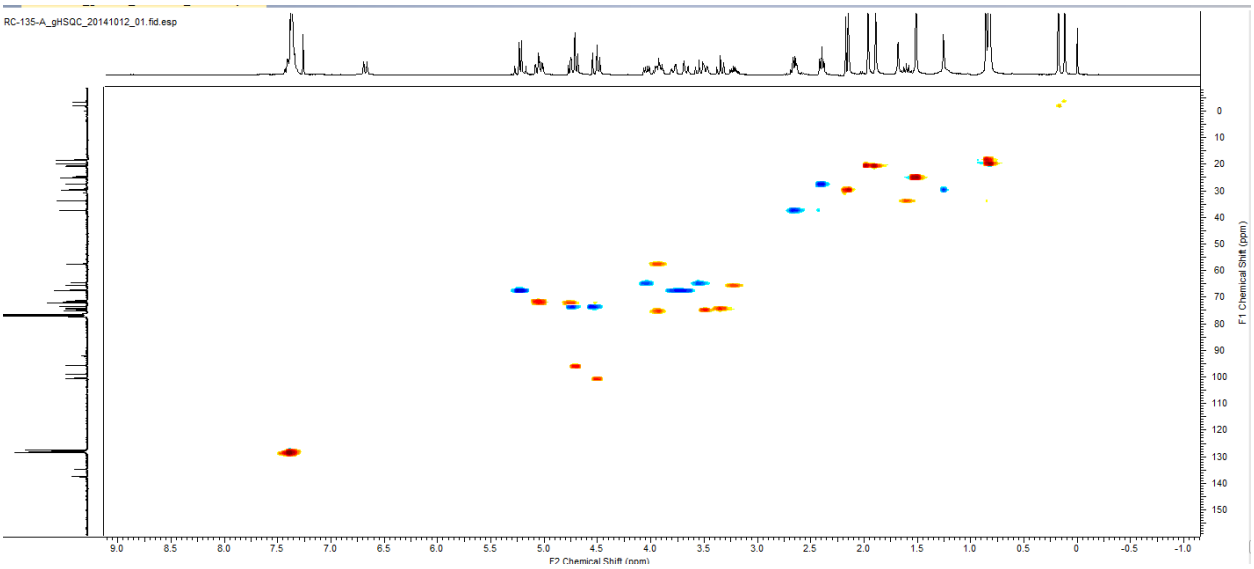
^{13}C NMR (150 MHz, $CDCl_3$)



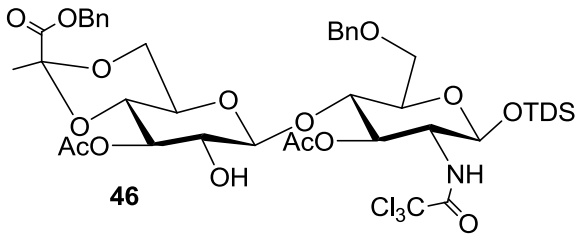
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



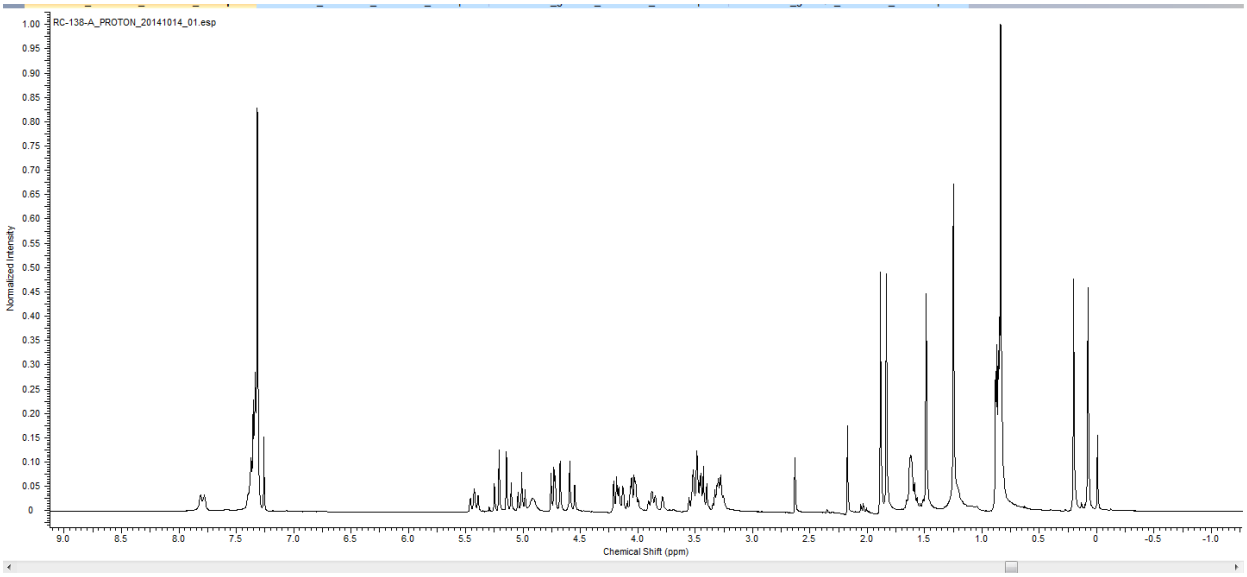
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



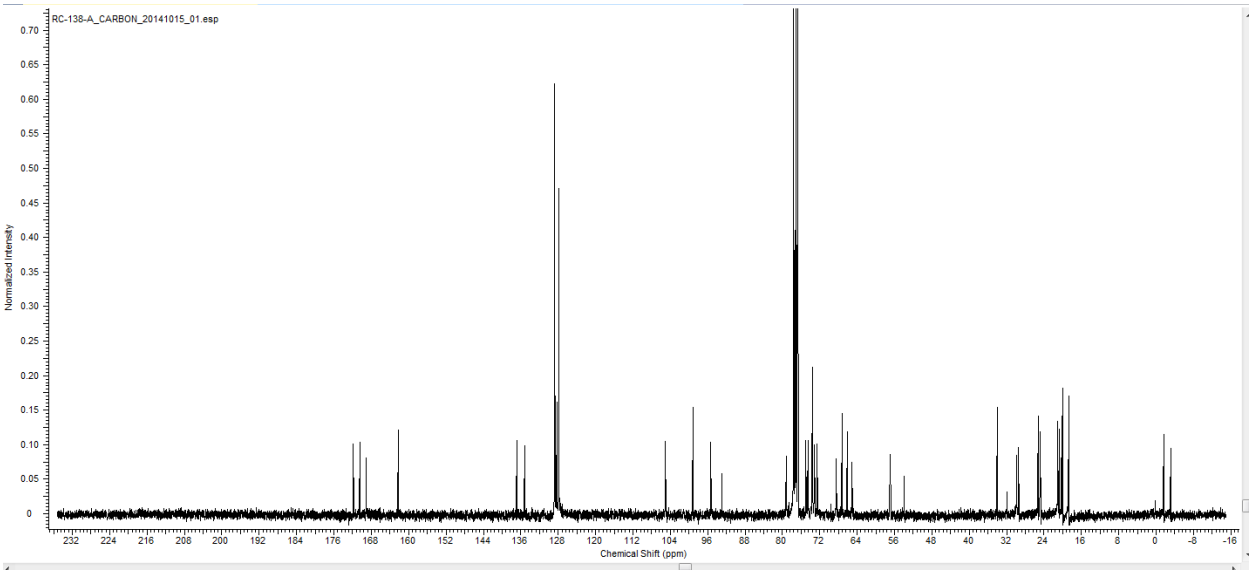
^{1}H NMR (600 MHz, $CDCl_3$)



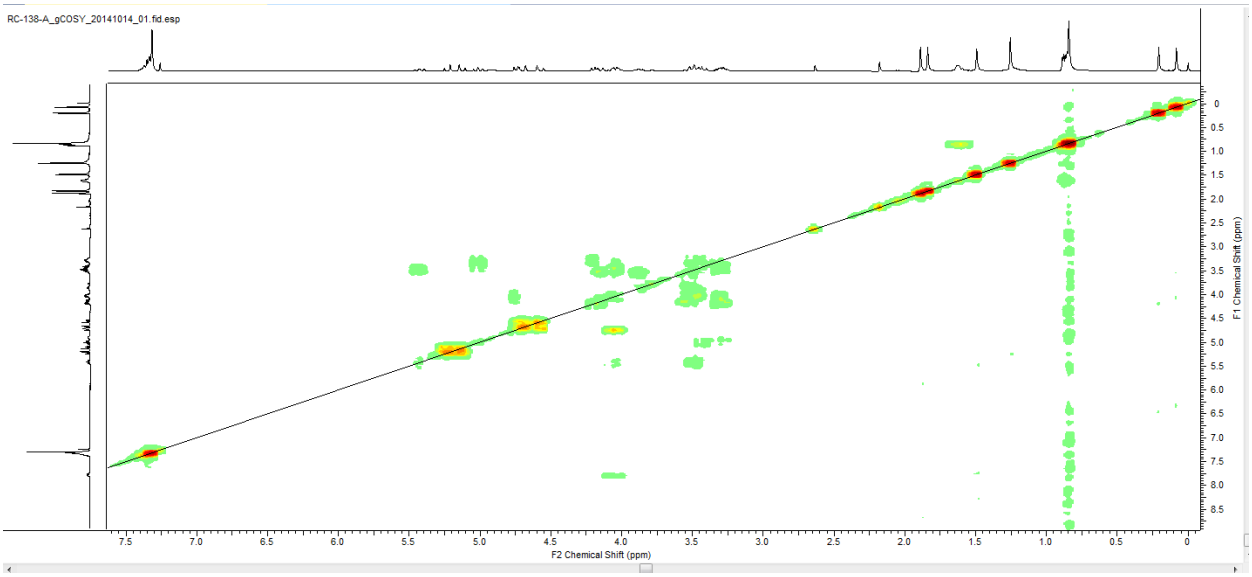
46



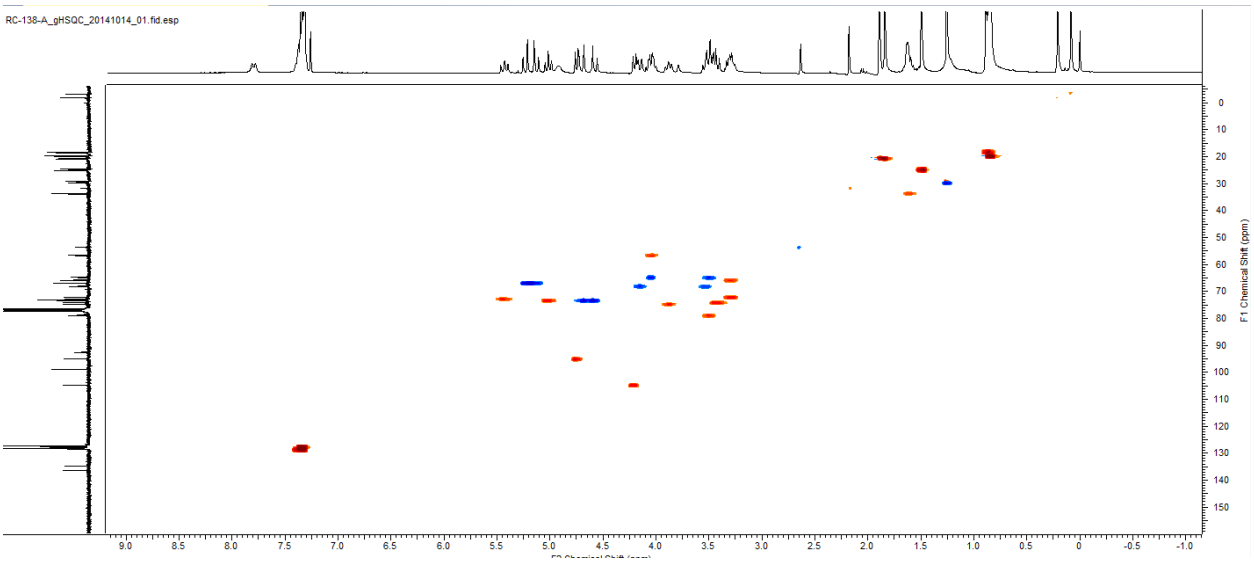
^{13}C NMR (150 MHz, $CDCl_3$)



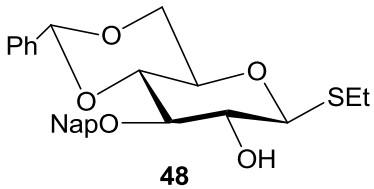
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



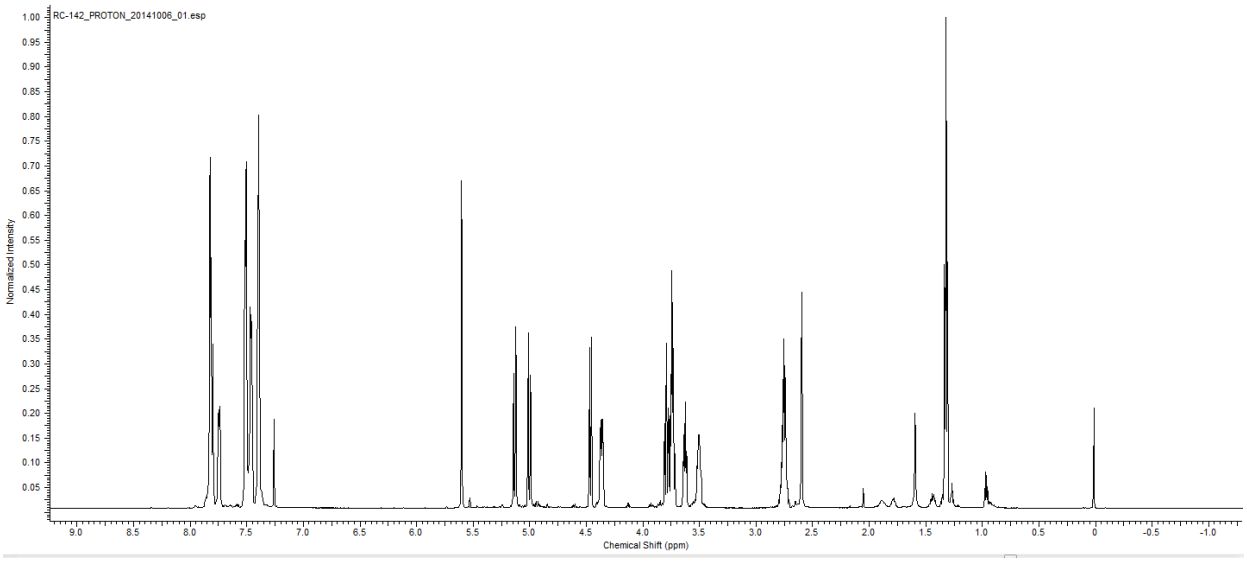
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



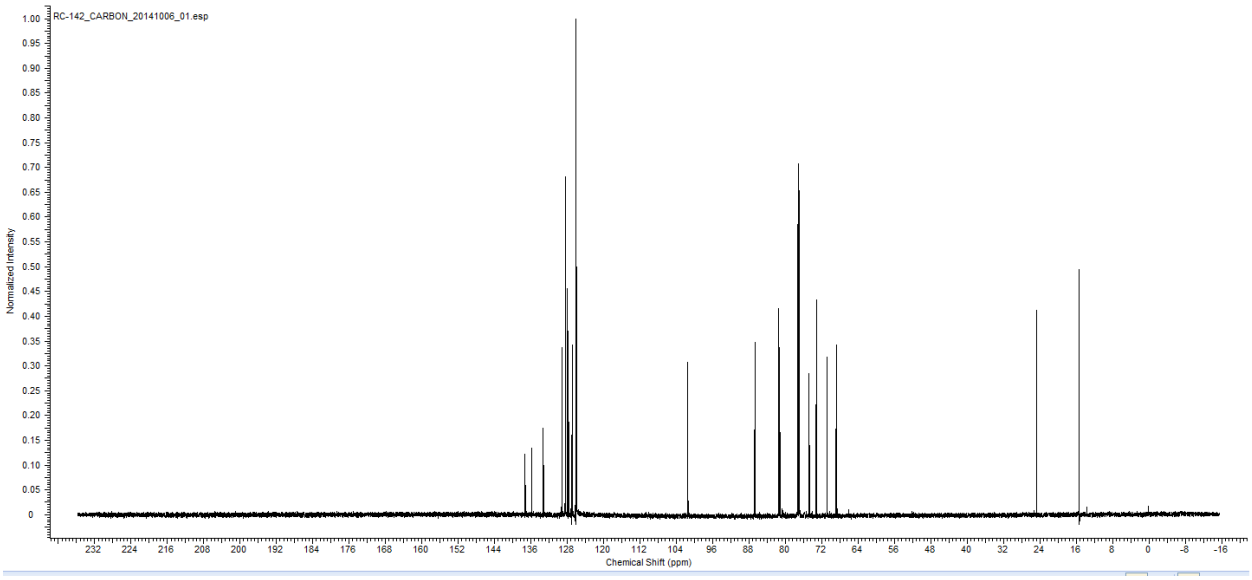
^{1}H NMR (600 MHz, $CDCl_3$)



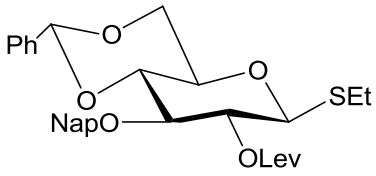
48



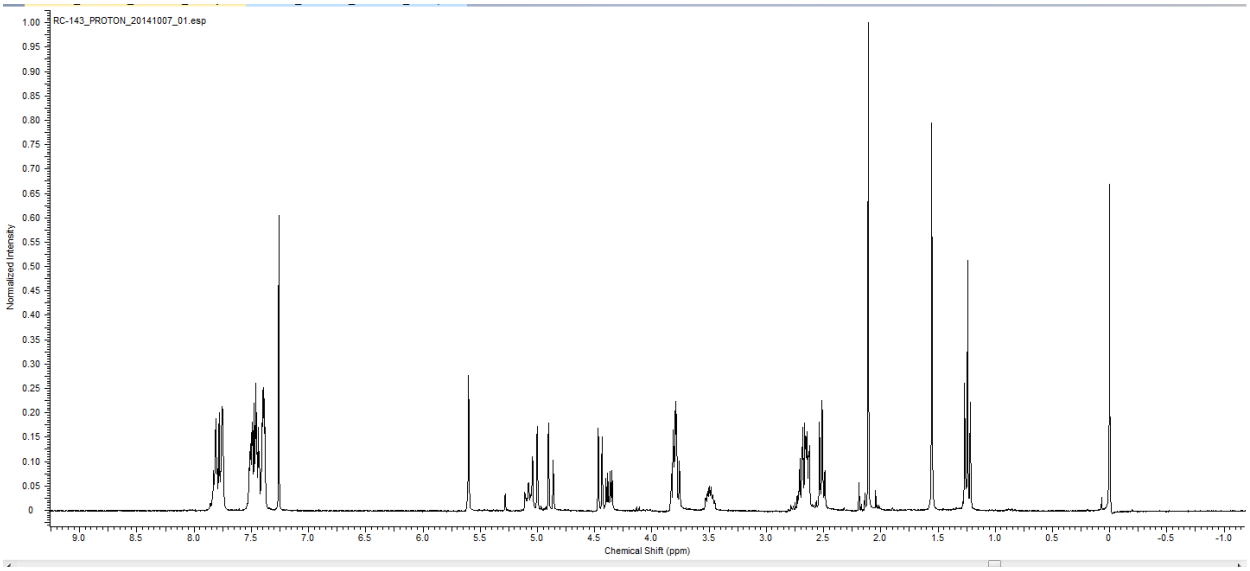
^{13}C NMR (150 MHz, $CDCl_3$)



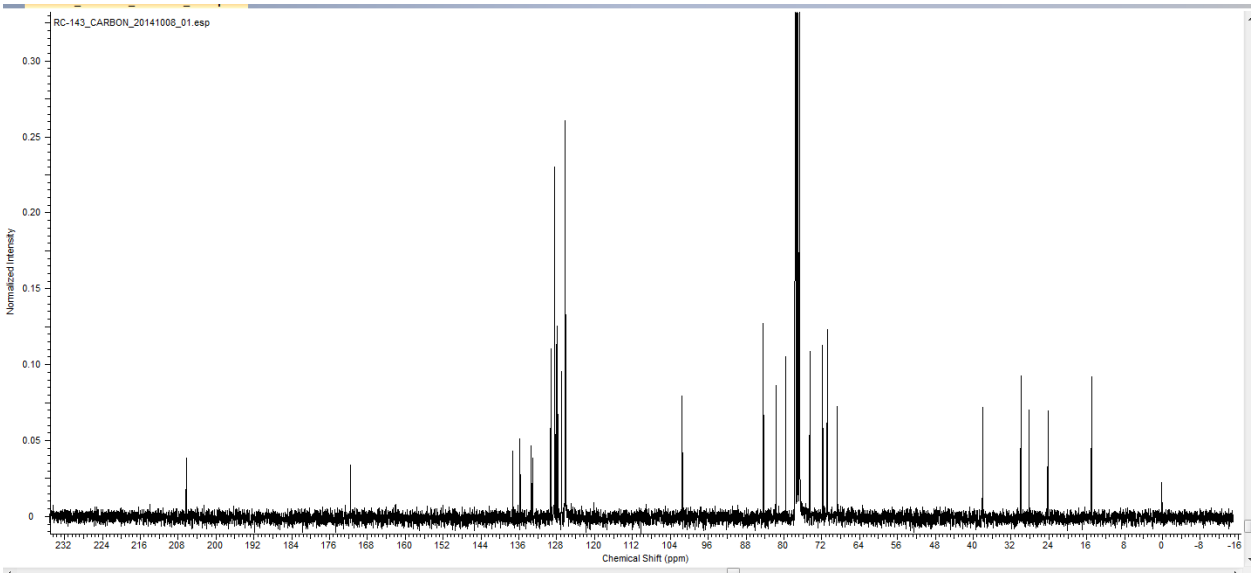
^{1}H NMR (300 MHz, $CDCl_3$)



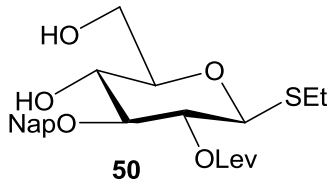
49



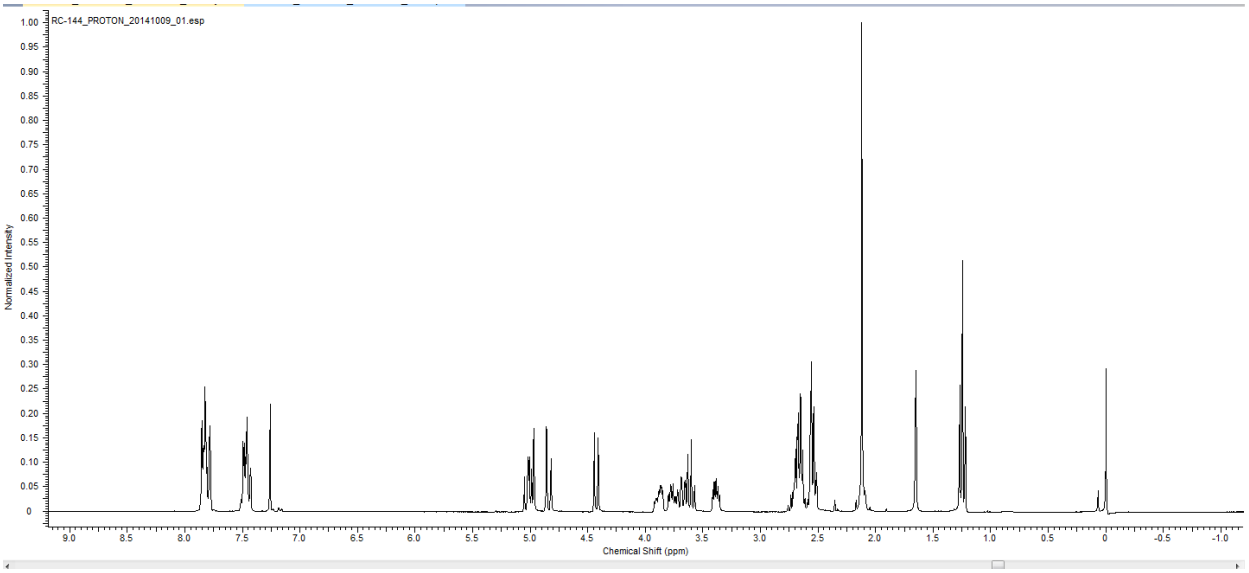
^{13}C NMR (75 MHz, $CDCl_3$)



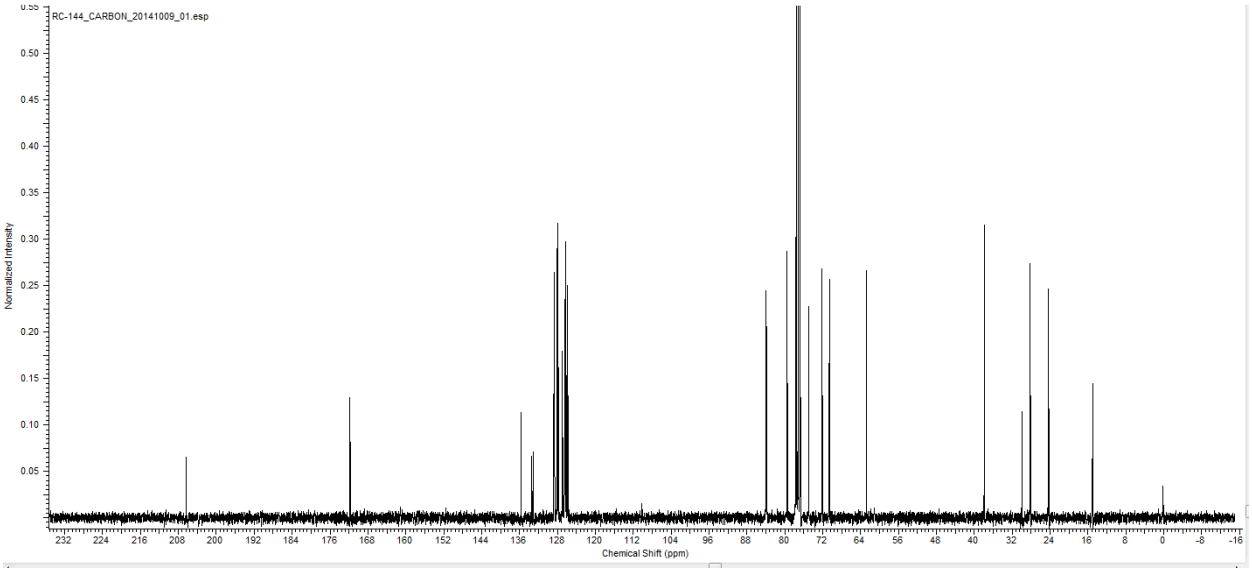
^{1}H NMR (300 MHz, $CDCl_3$)



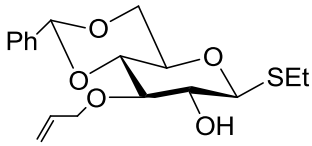
50



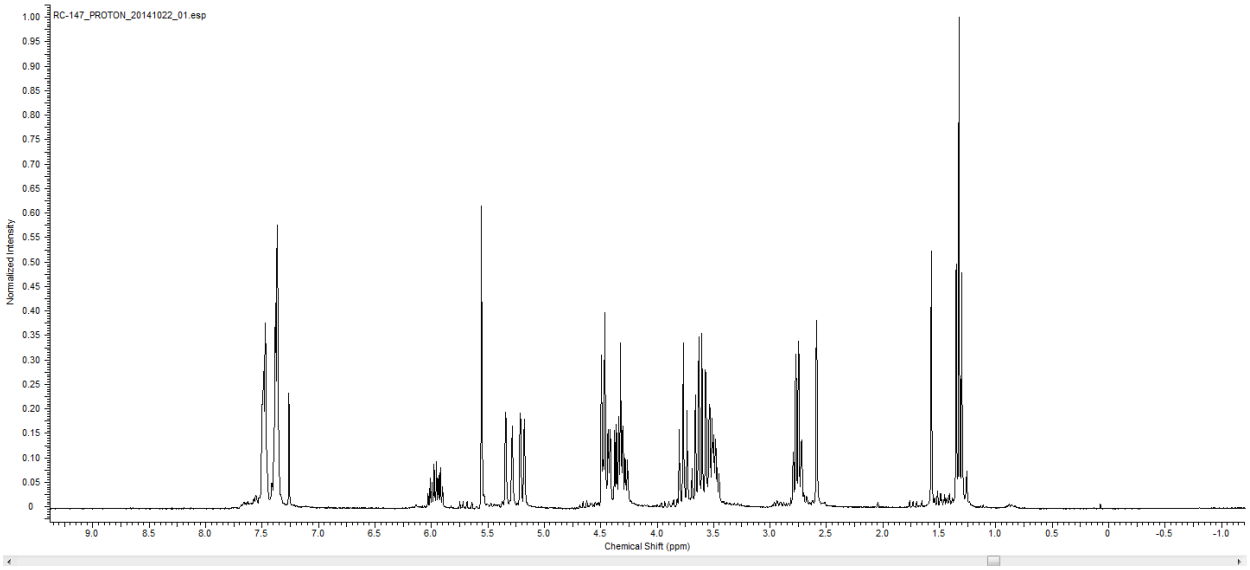
^{13}C NMR (75 MHz, $CDCl_3$)



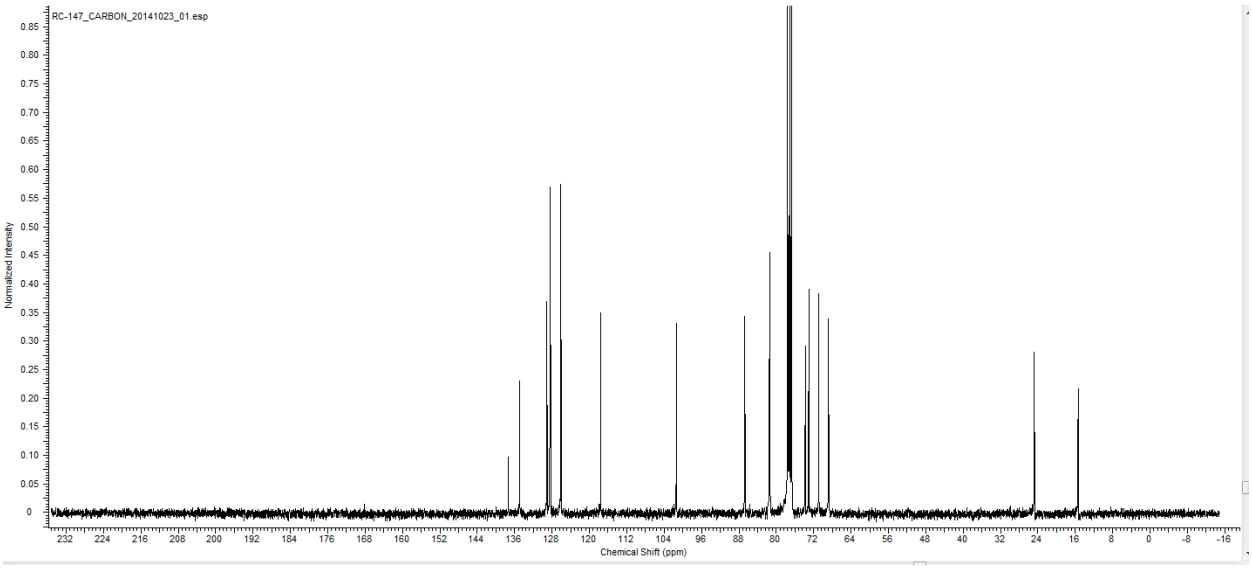
^{1}H NMR (300 MHz, $CDCl_3$)



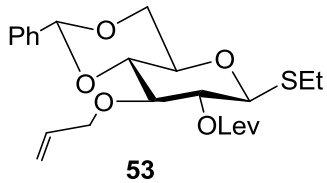
52



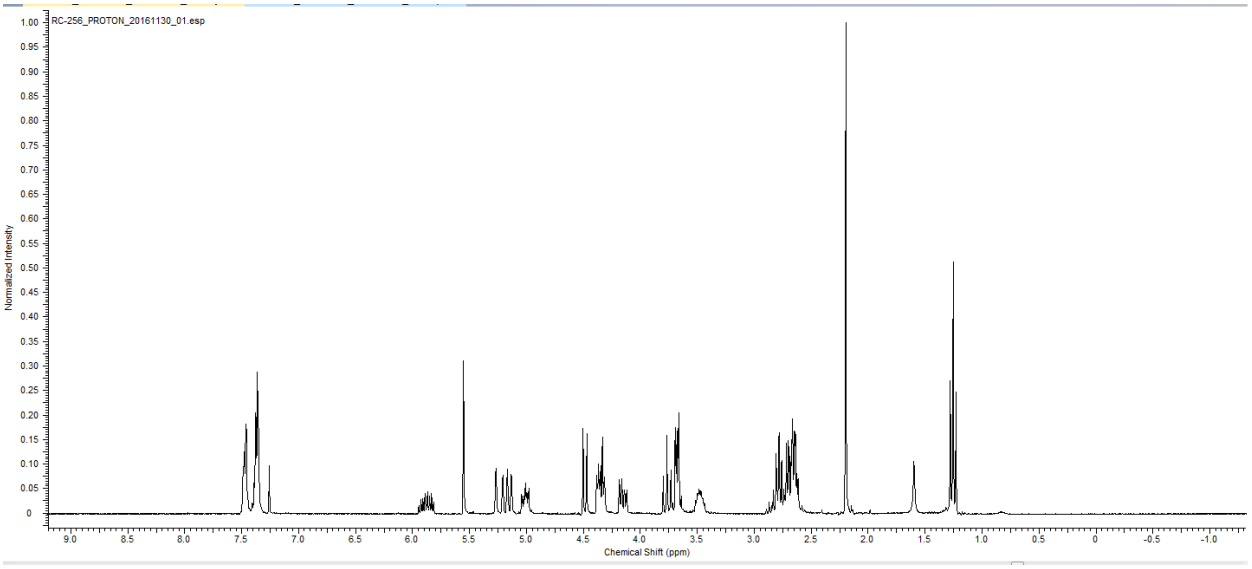
^{13}C NMR (75 MHz, $CDCl_3$)



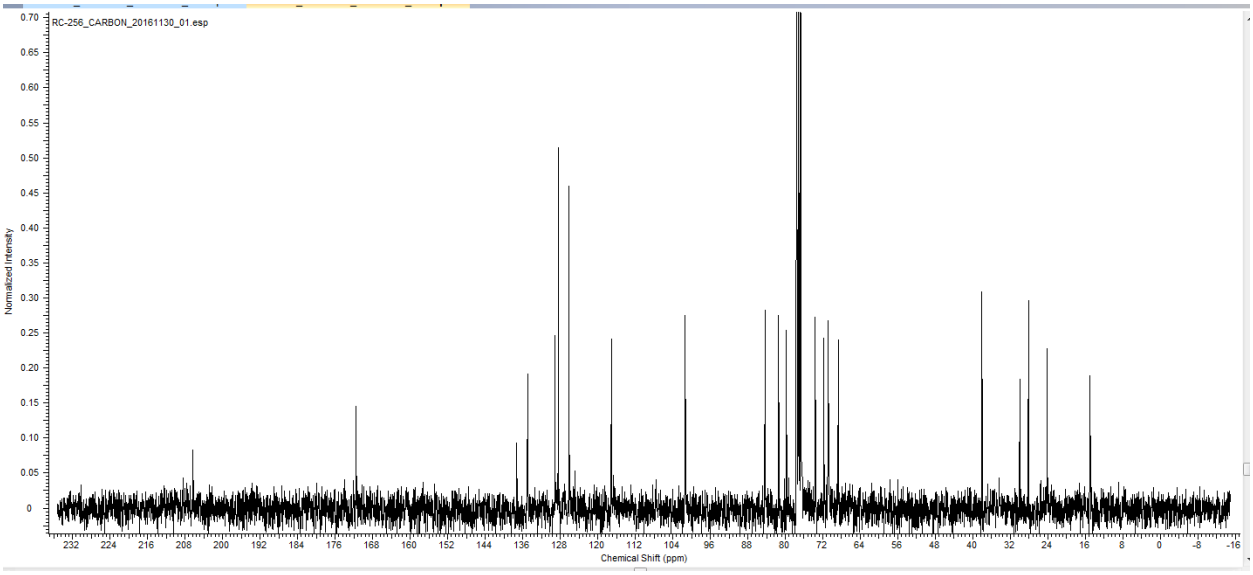
^{1}H NMR (300 MHz, $CDCl_3$)



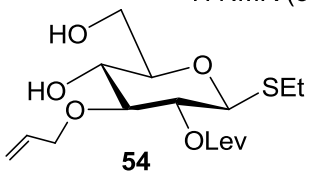
53



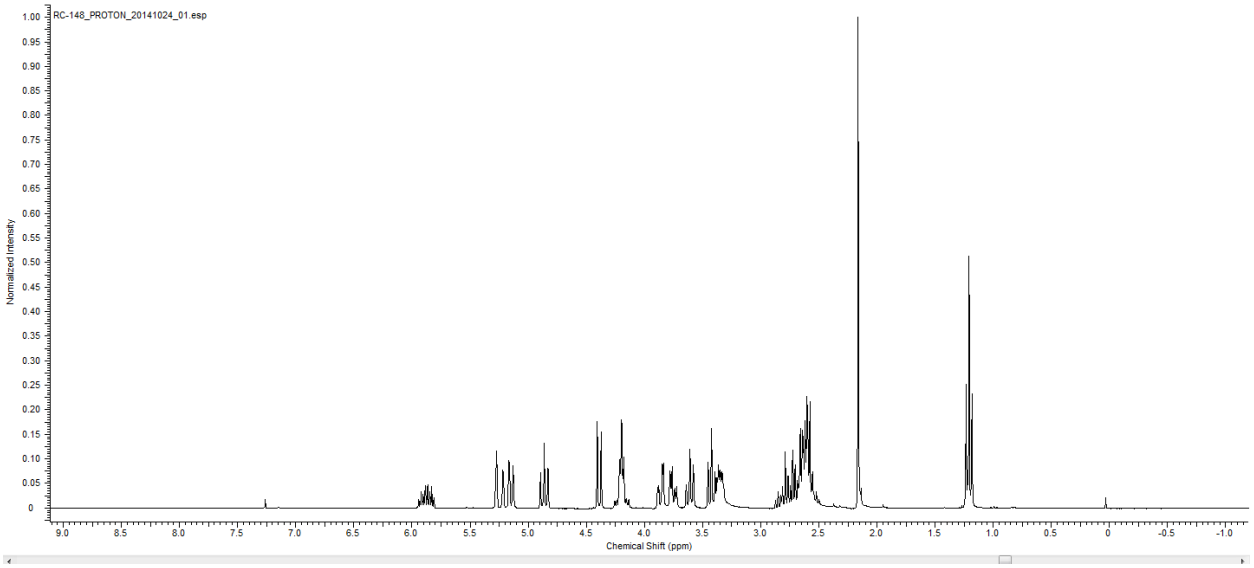
^{13}C NMR (75 MHz, $CDCl_3$)



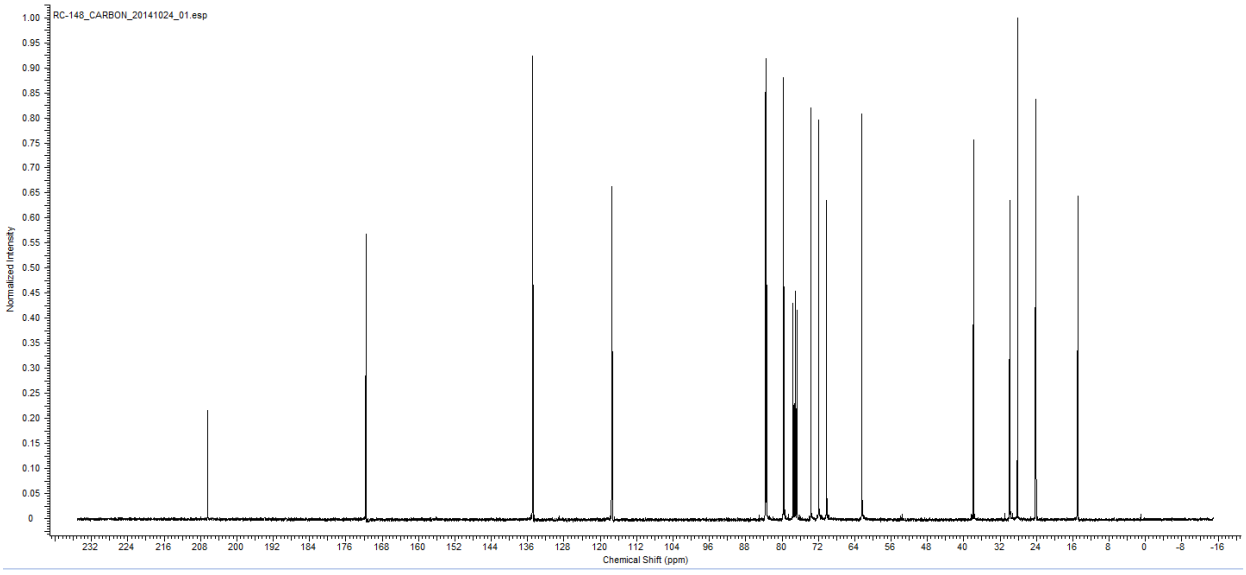
^{1}H NMR (300 MHz, $CDCl_3$)

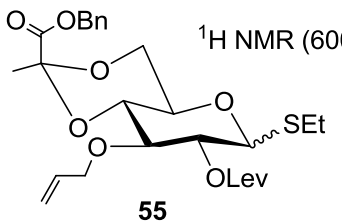


54



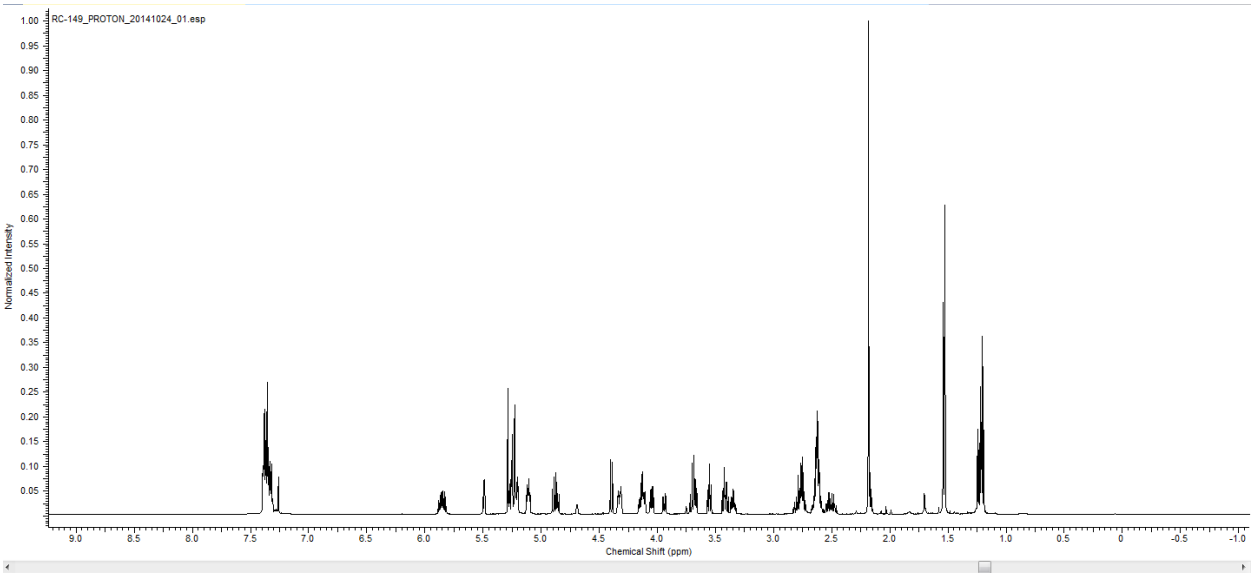
^{13}C NMR (75 MHz, $CDCl_3$)



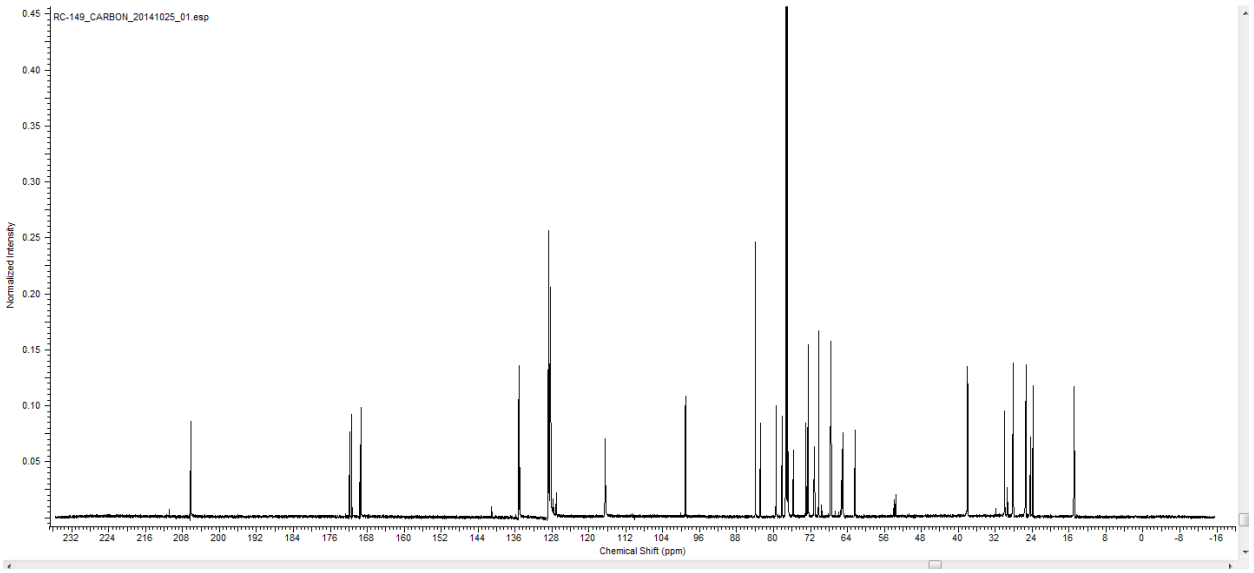


55

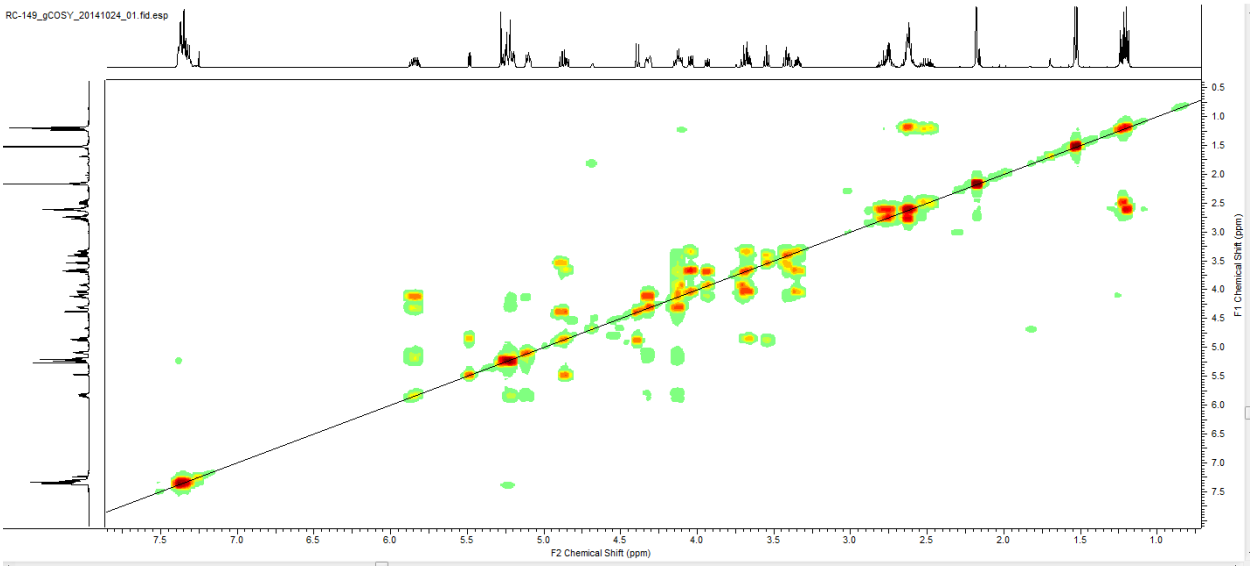
^{1}H NMR (600 MHz, $CDCl_3$)



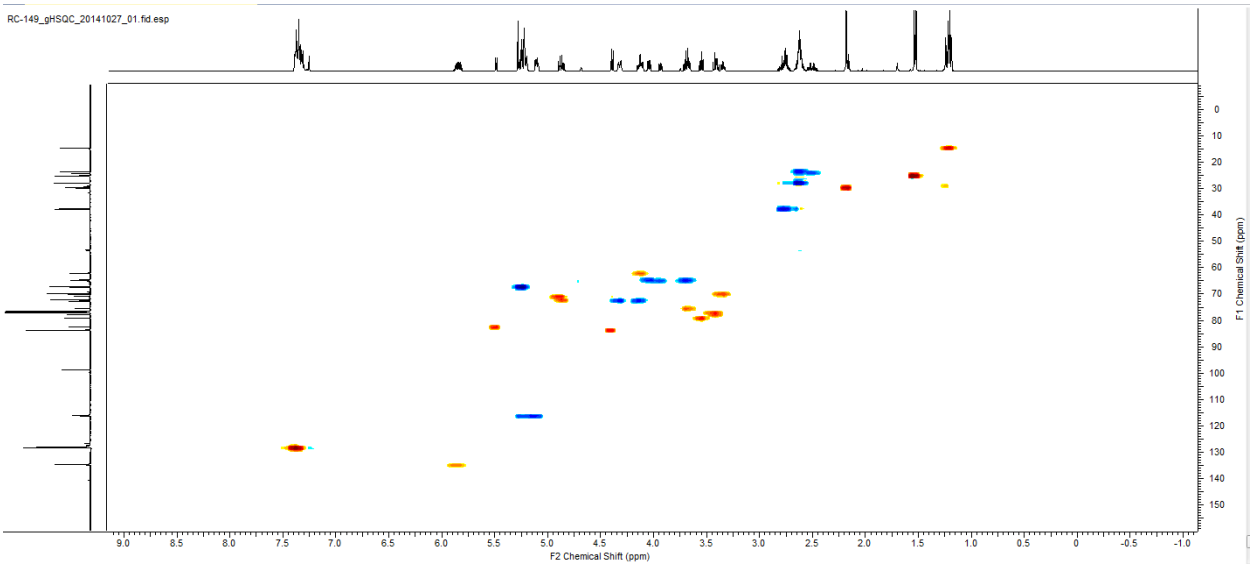
^{13}C NMR (150 MHz, $CDCl_3$)



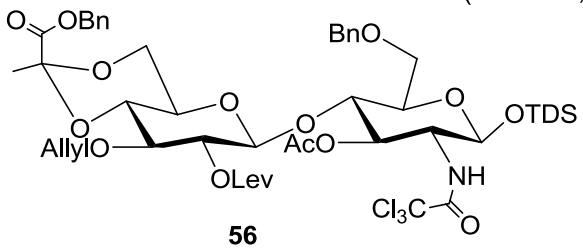
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



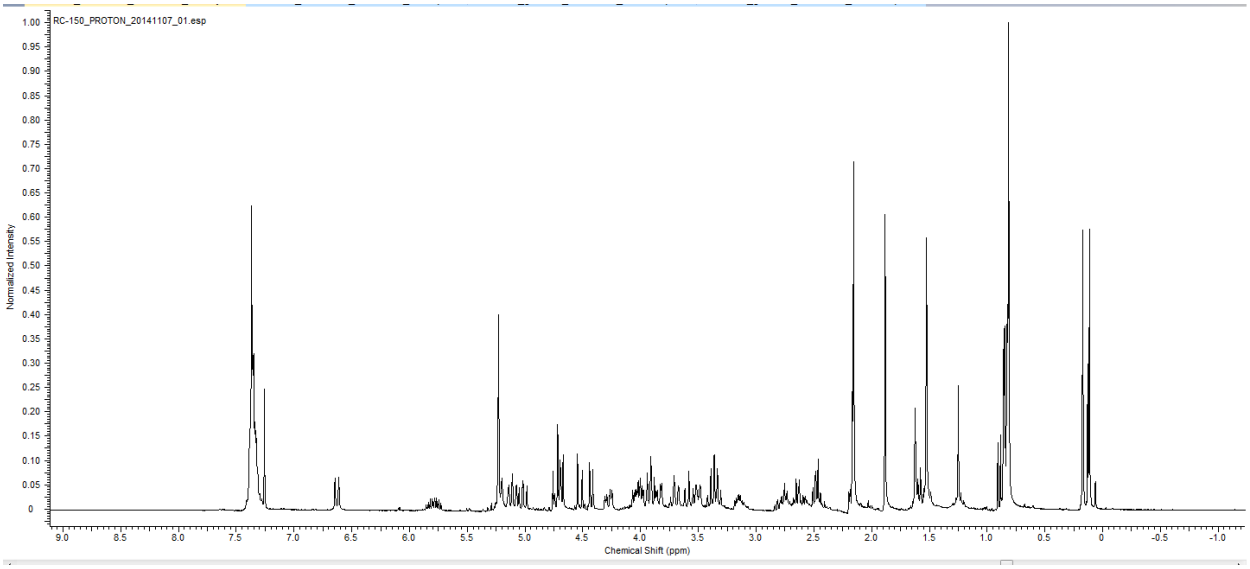
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



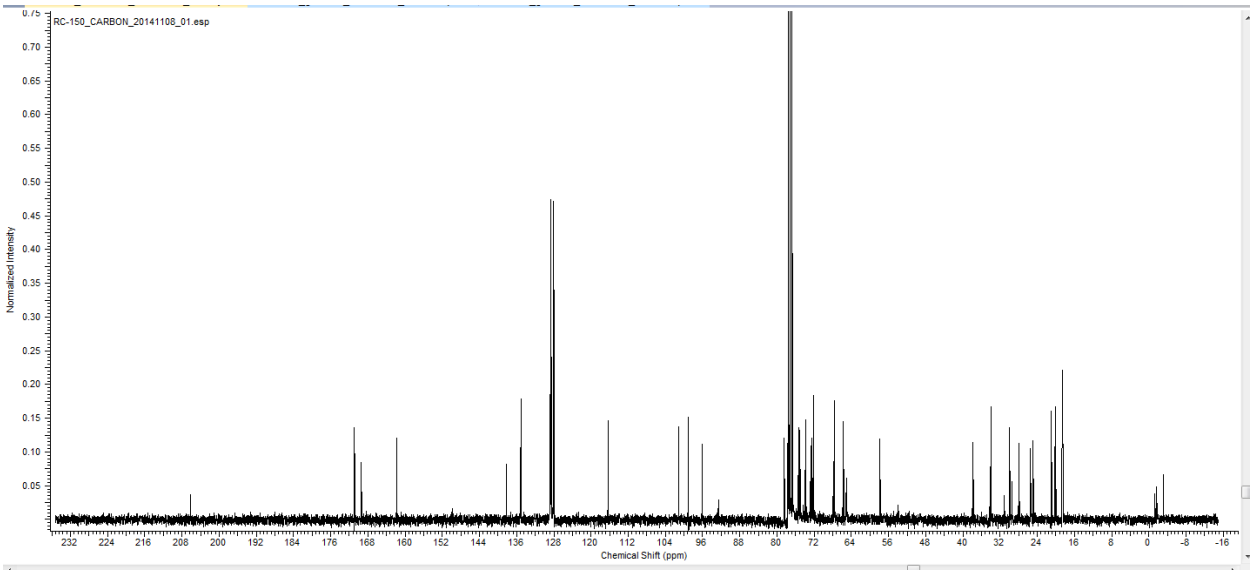
^{1}H NMR (600 MHz, $CDCl_3$)



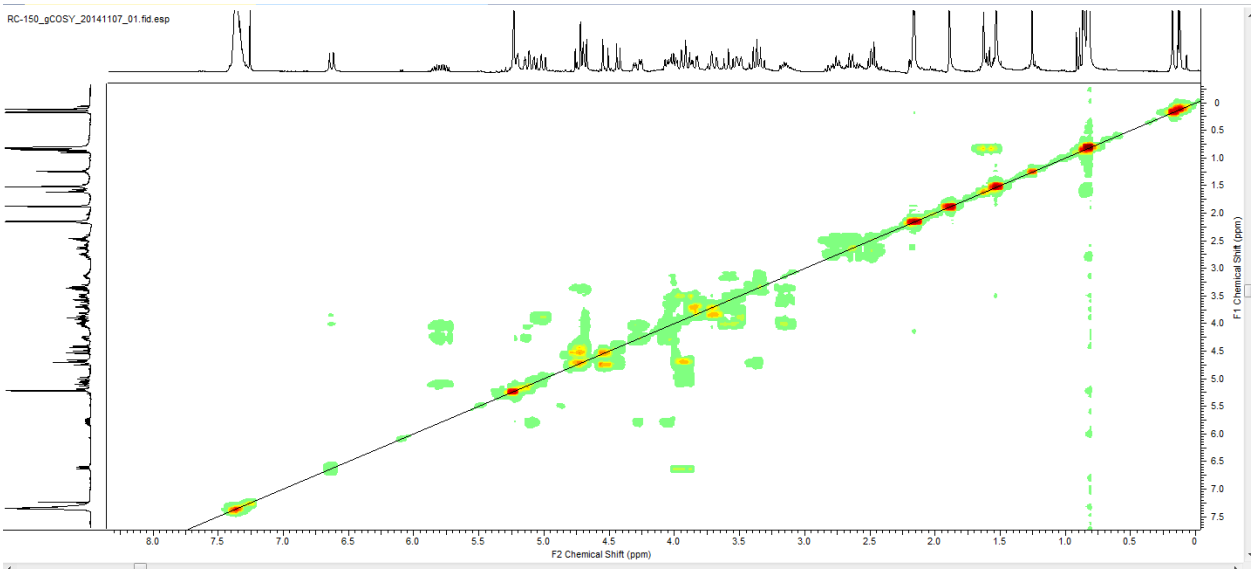
56



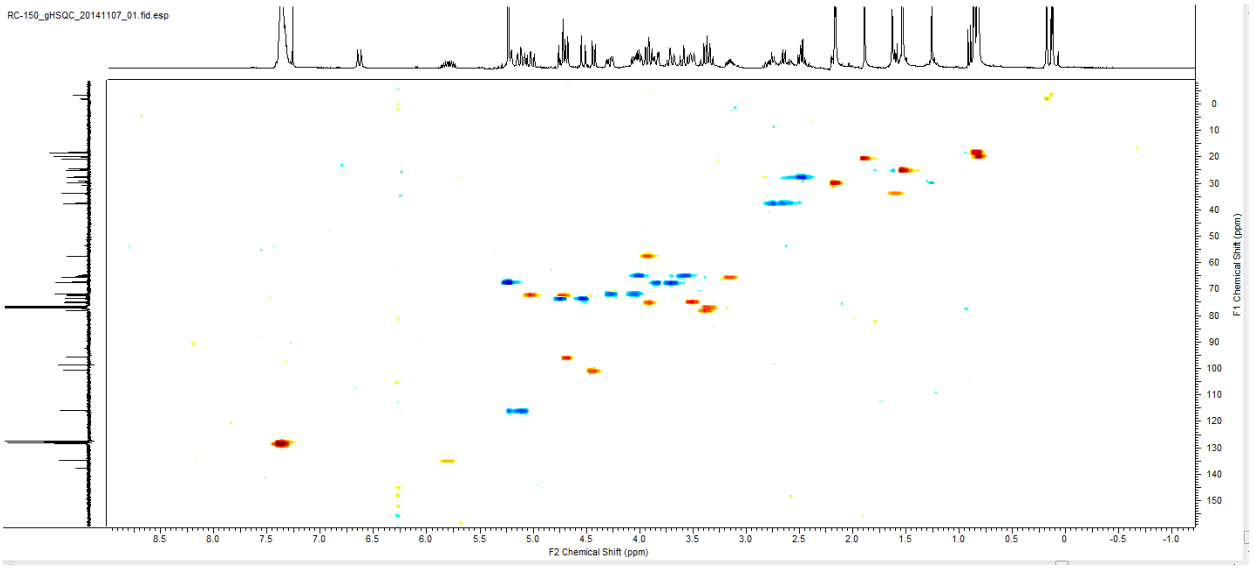
^{13}C NMR (150 MHz, $CDCl_3$)



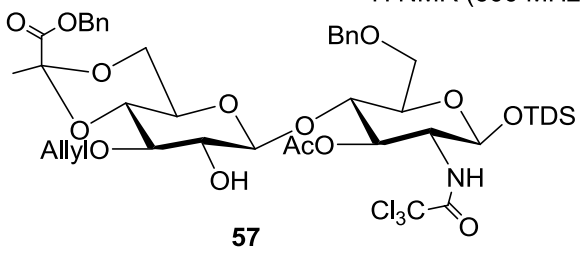
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



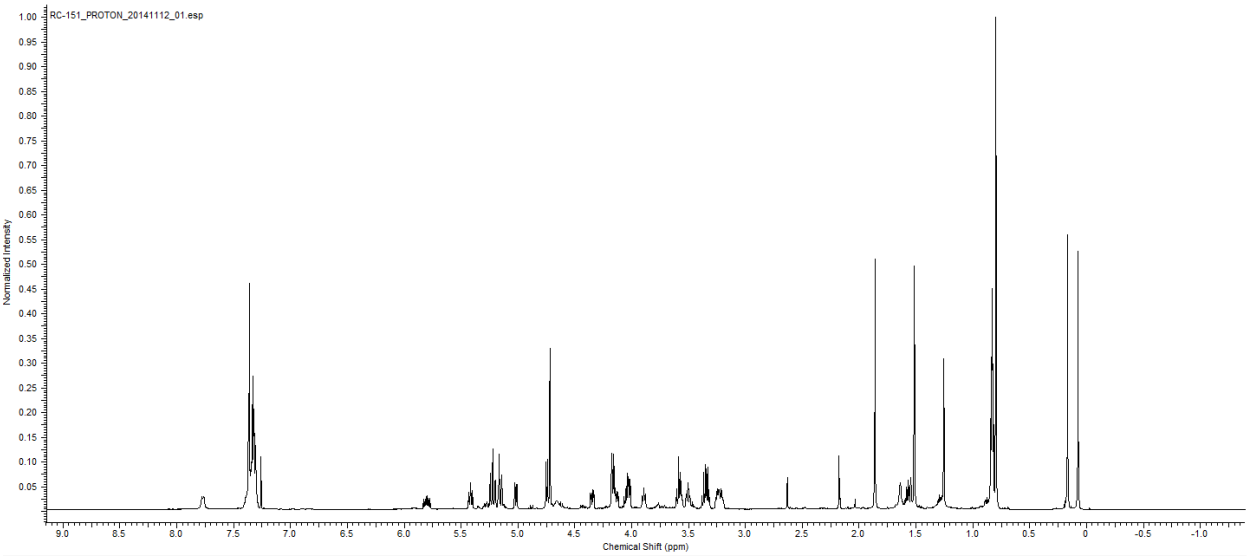
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



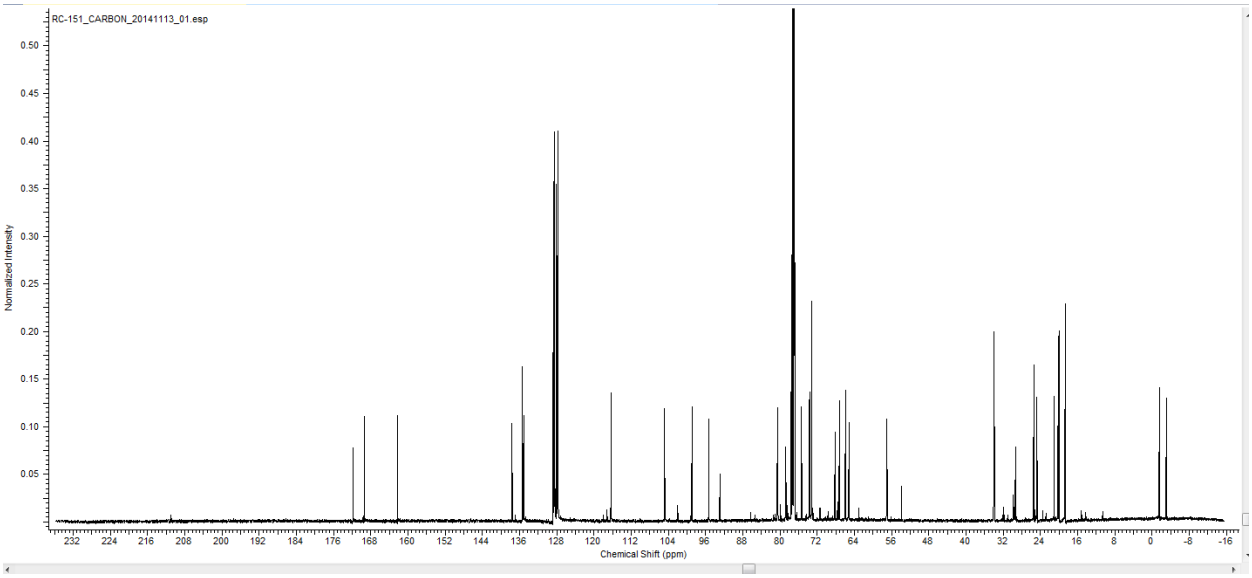
^{1}H NMR (600 MHz, $CDCl_3$)



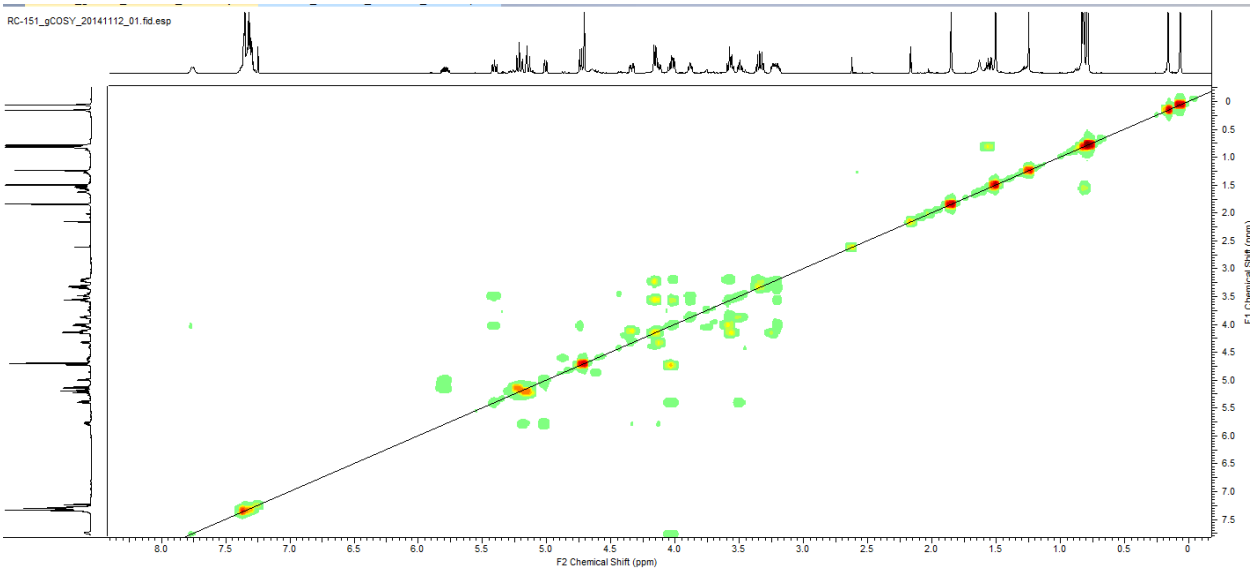
57



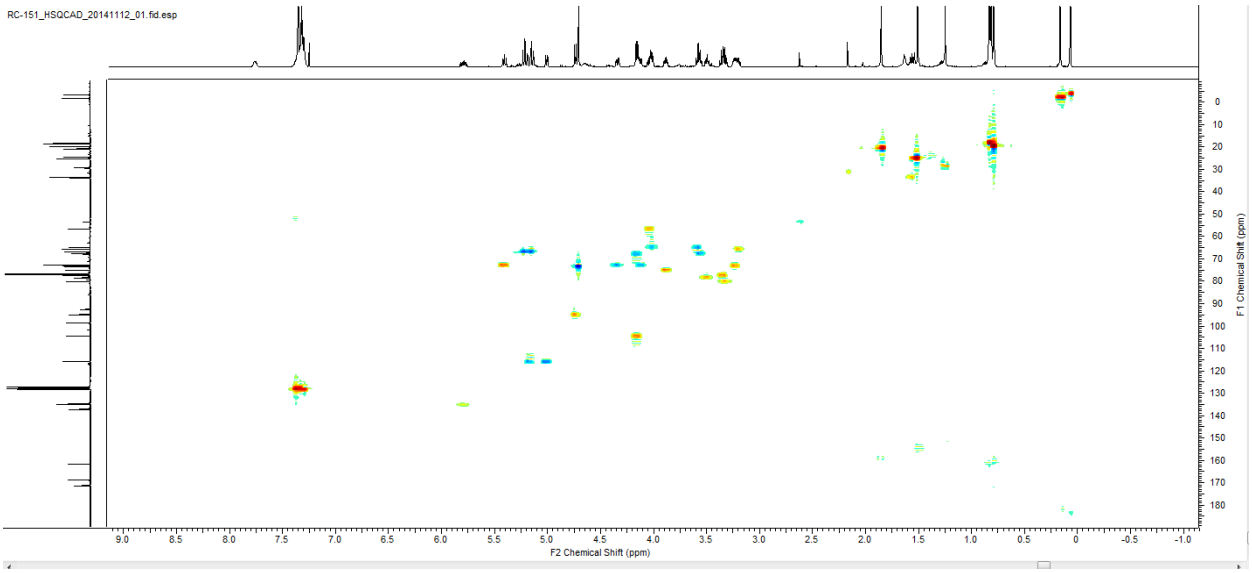
^{13}C NMR (150 MHz, $CDCl_3$)



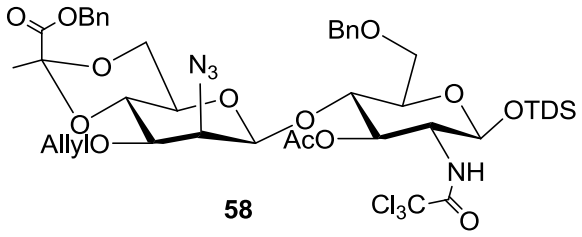
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



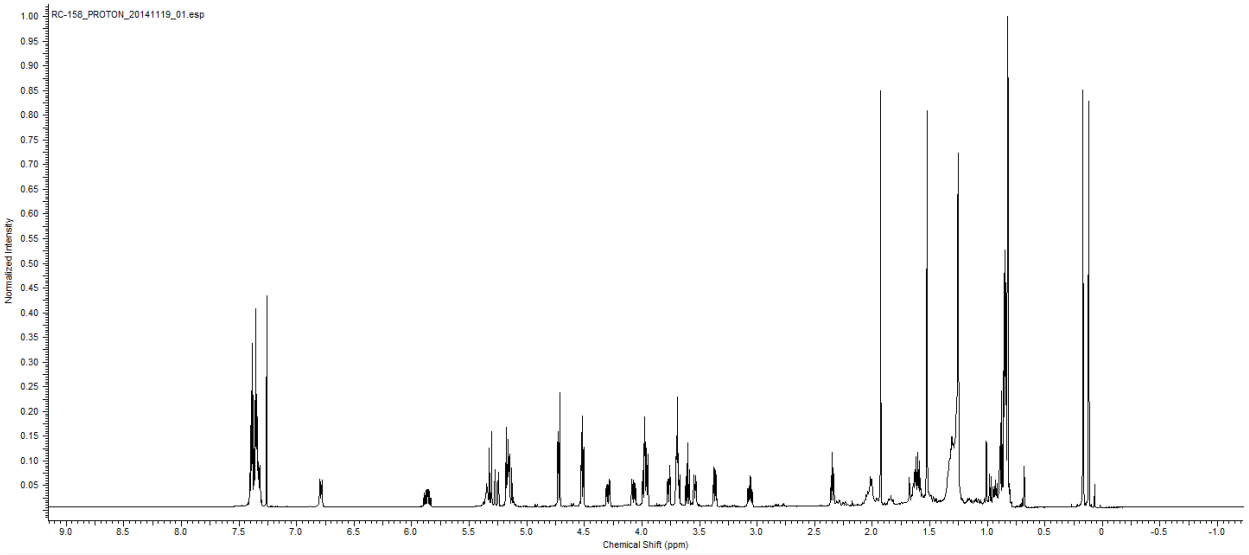
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



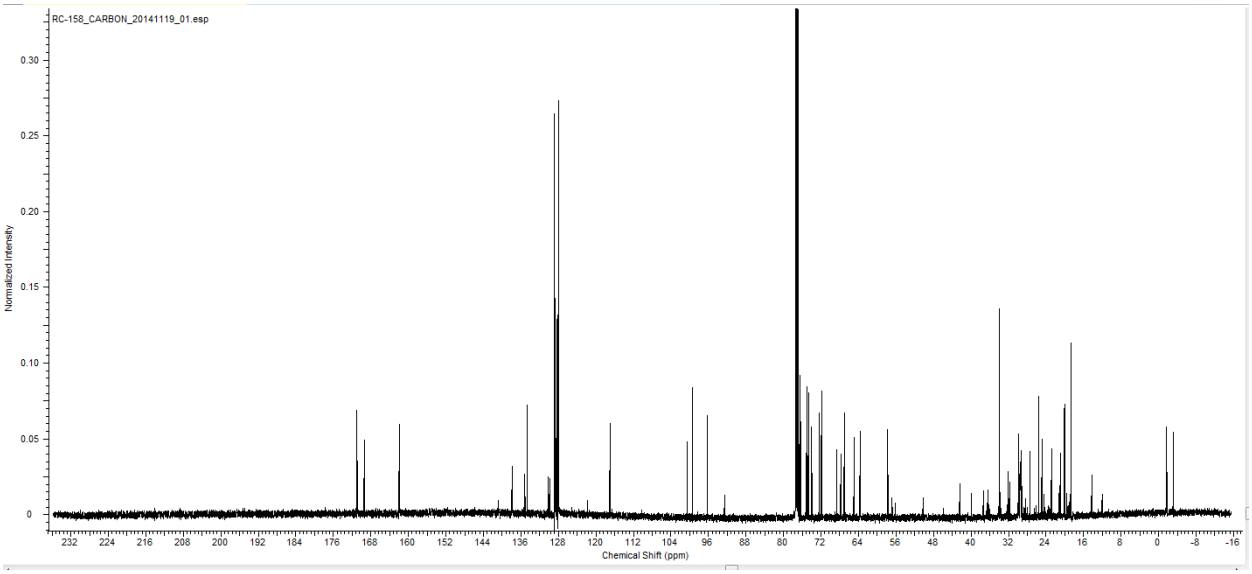
^{1}H NMR (600 MHz, $CDCl_3$)



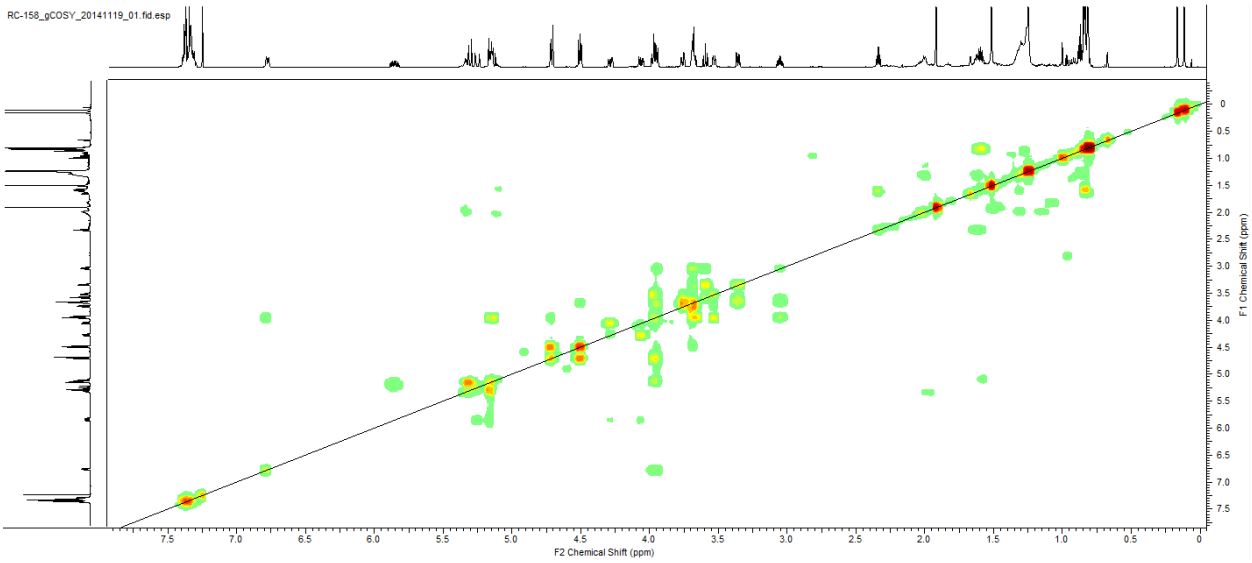
58



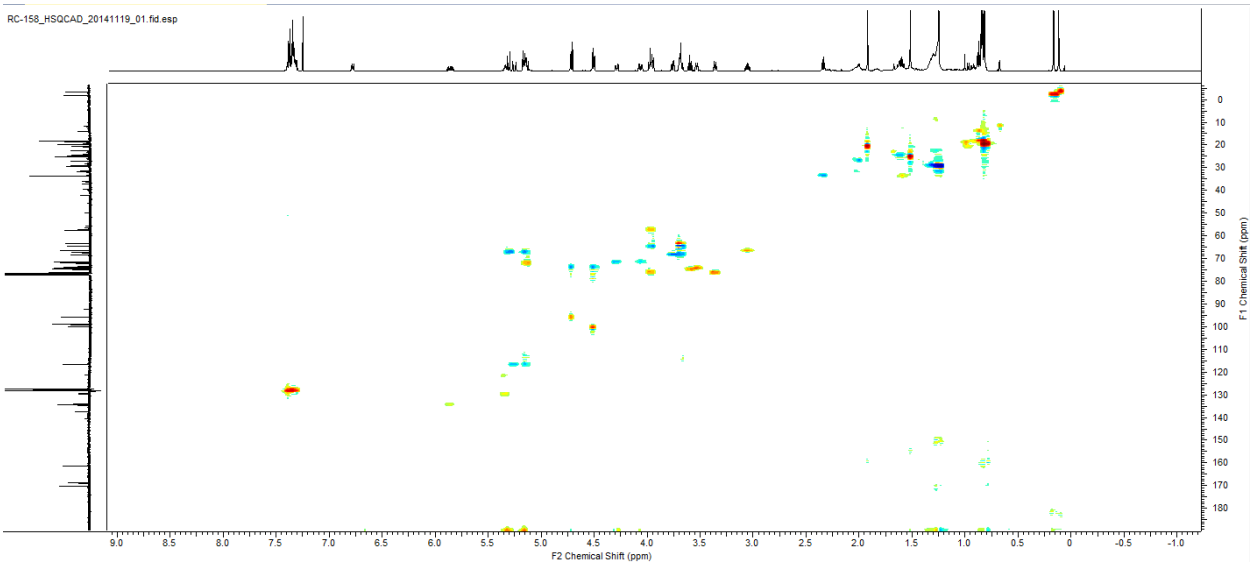
^{13}C NMR (150 MHz, $CDCl_3$)



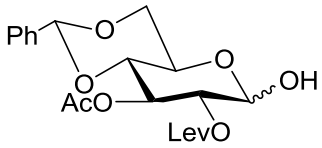
^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



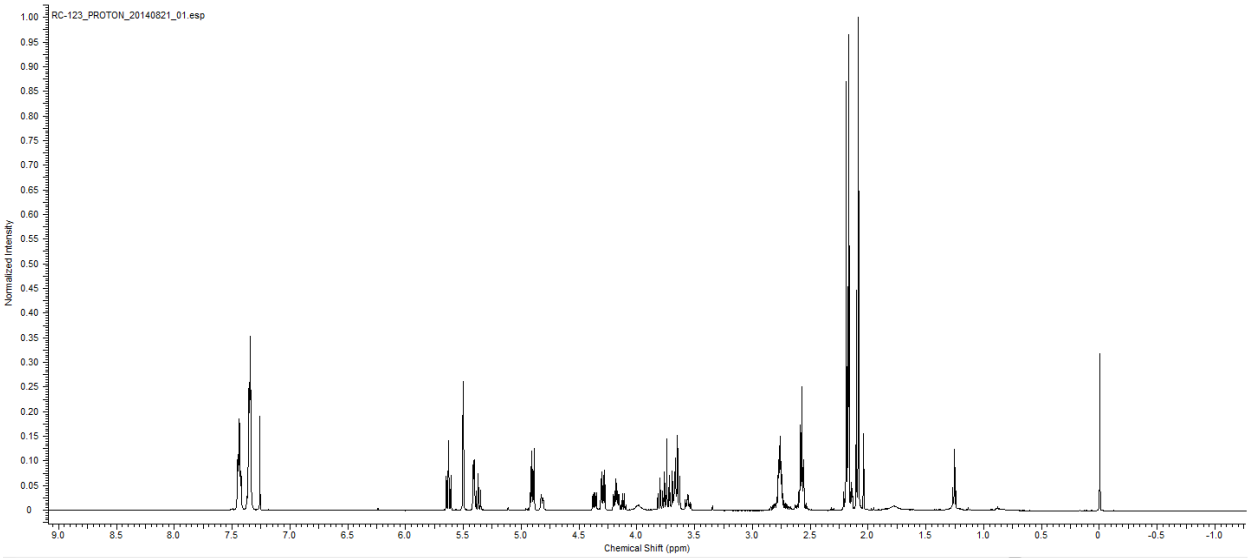
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



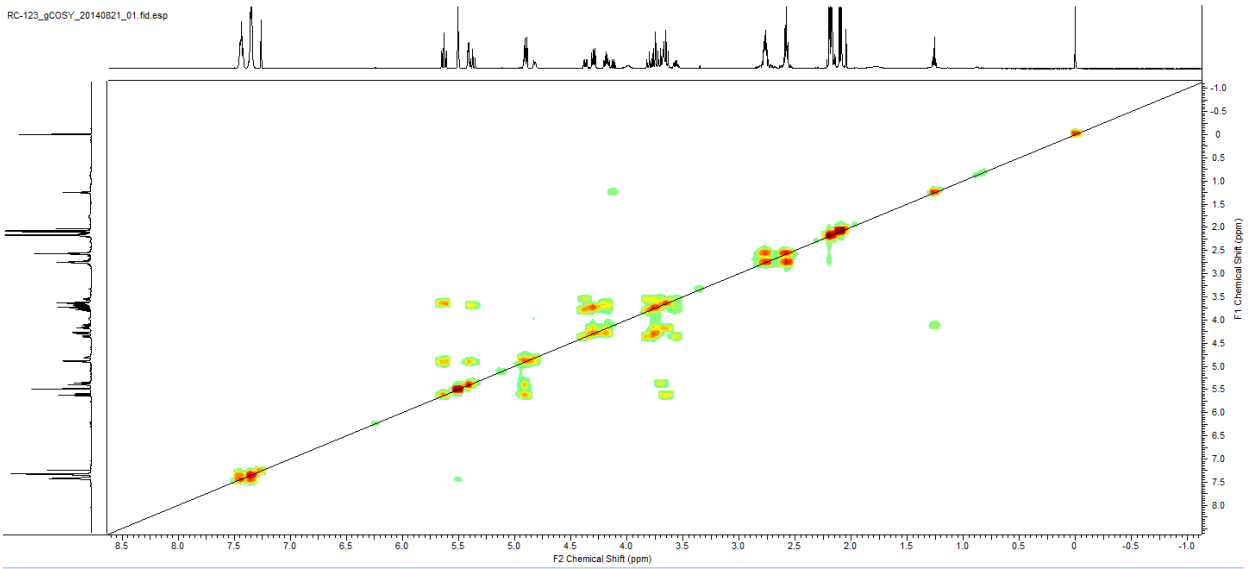
^{1}H NMR (300 MHz, $CDCl_3$)



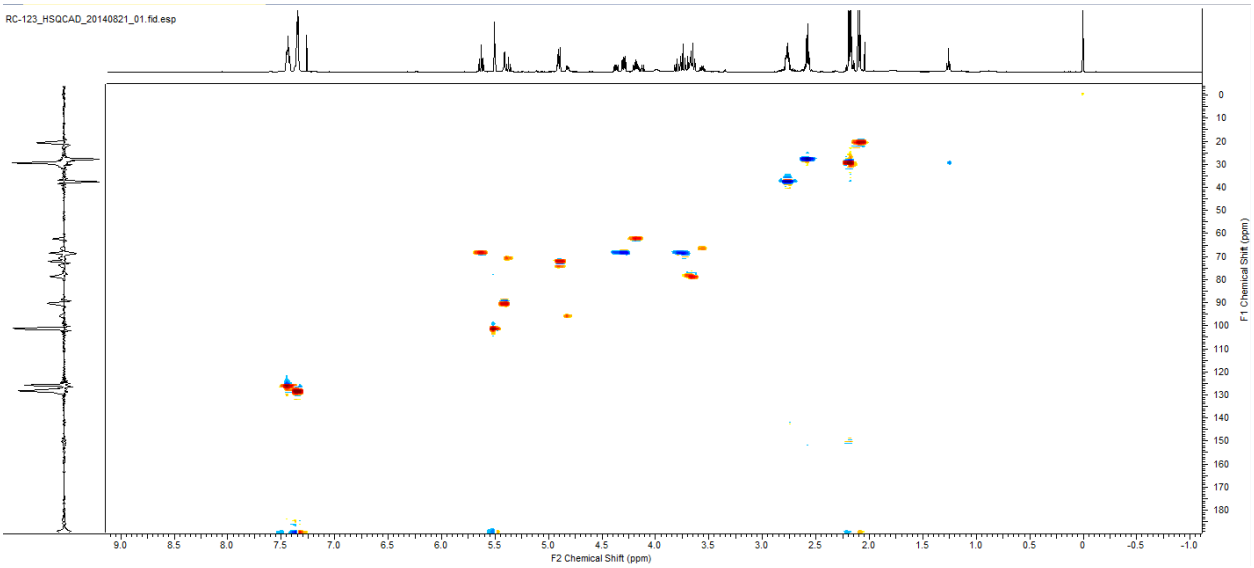
59



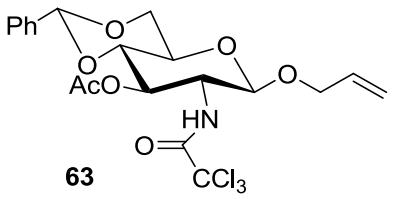
^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



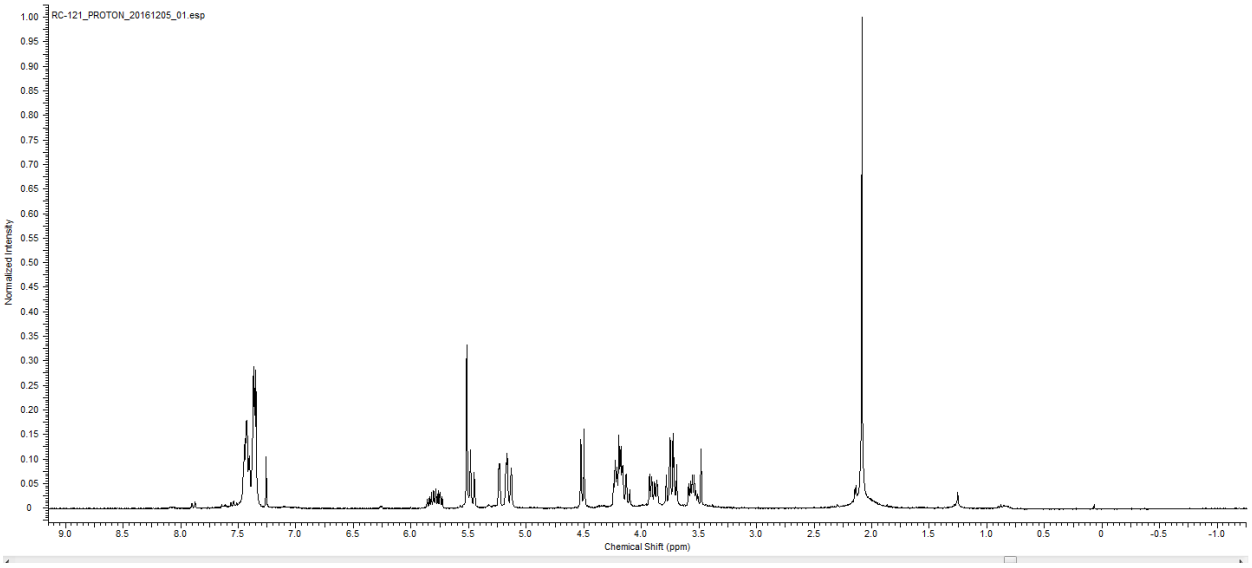
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



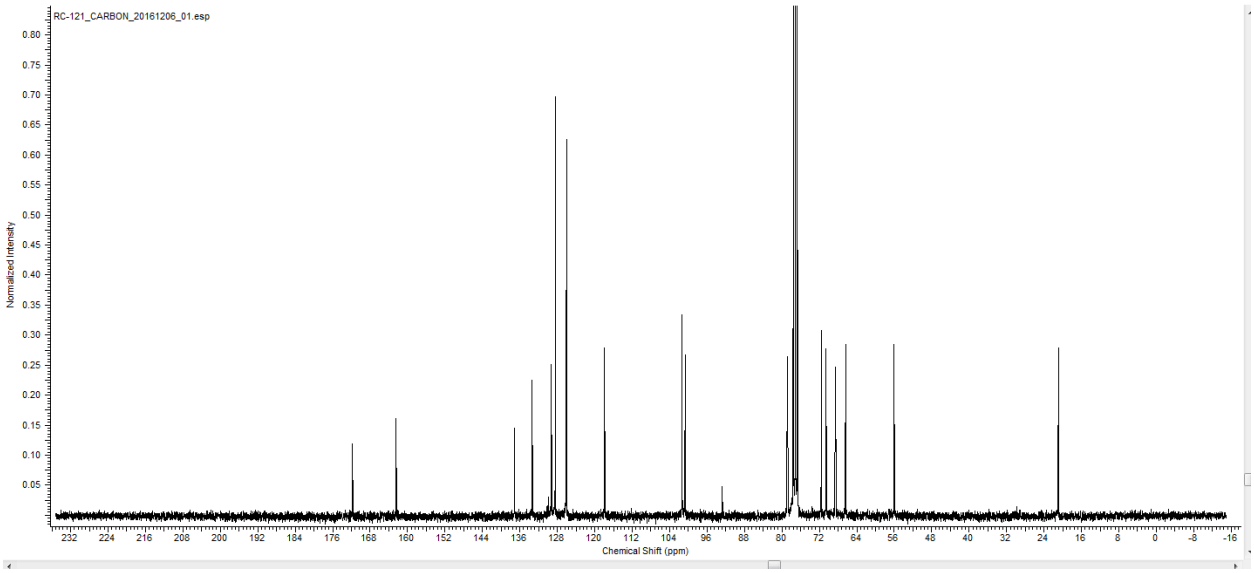
^{1}H NMR (300 MHz, $CDCl_3$)



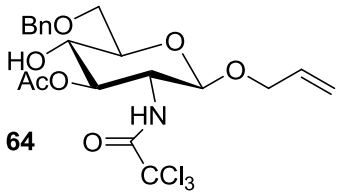
63



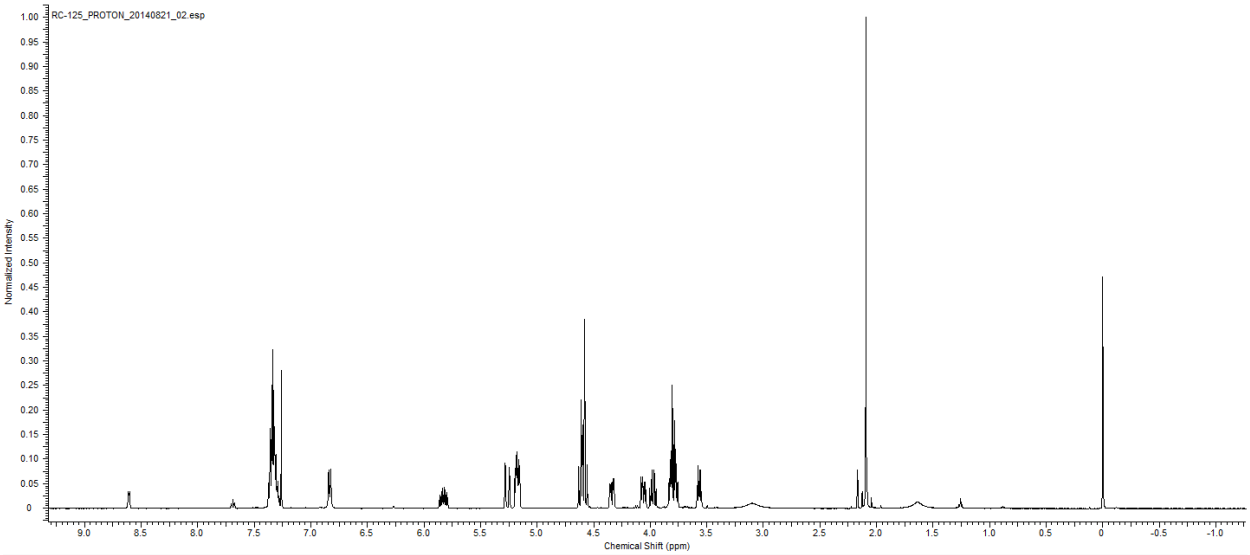
^{13}C NMR (75 MHz, $CDCl_3$)



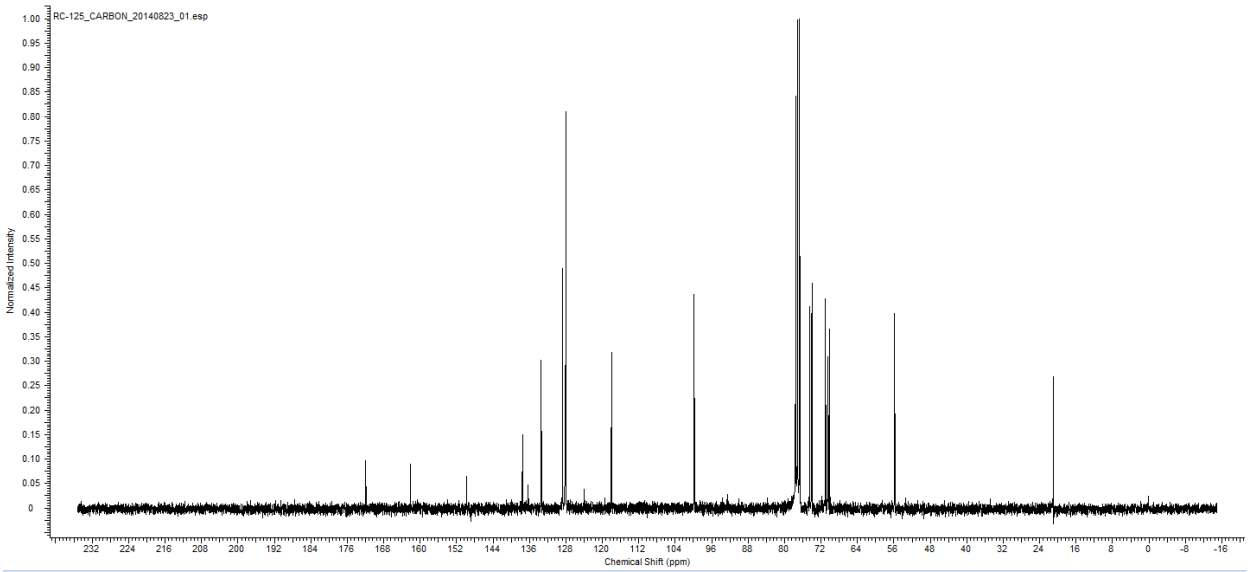
^{1}H NMR (500 MHz, $CDCl_3$)



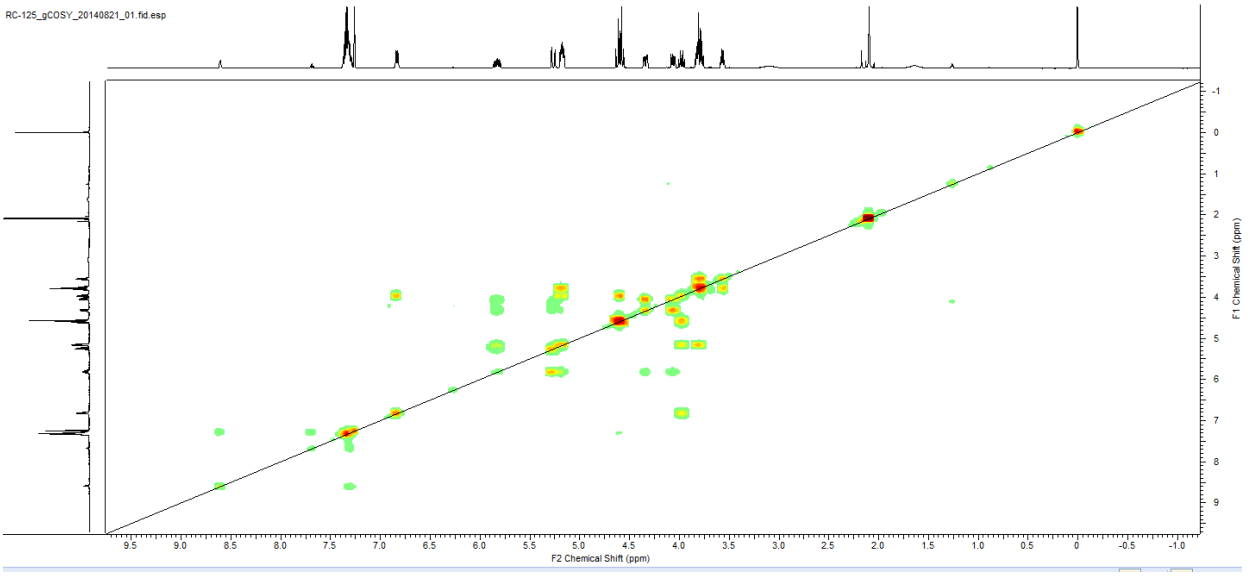
64



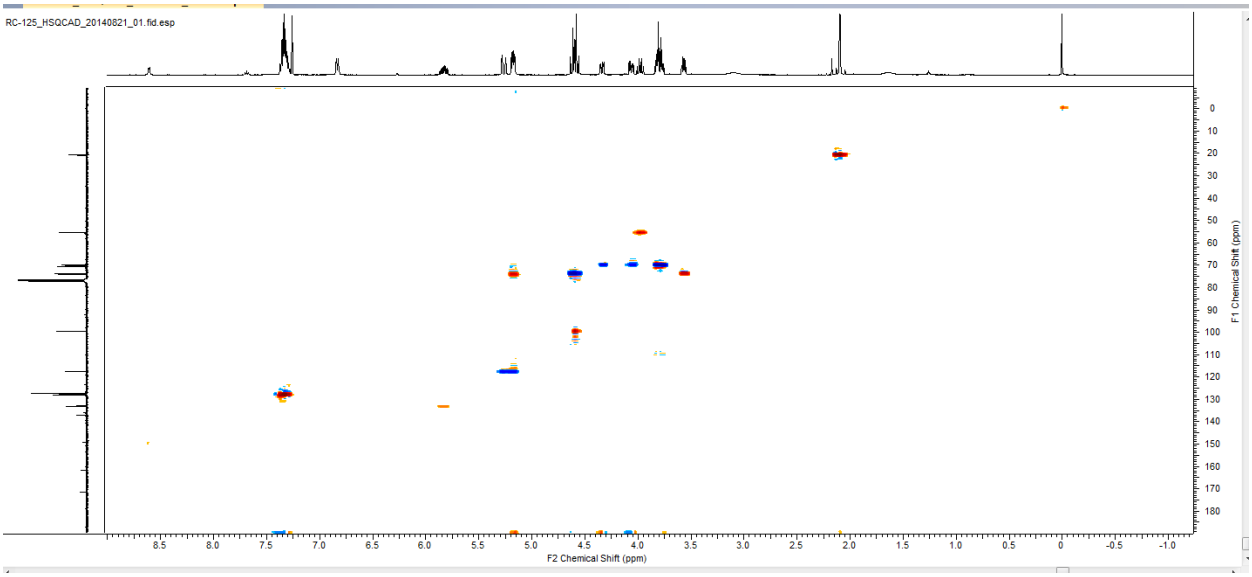
^{13}C NMR (75 MHz, $CDCl_3$)

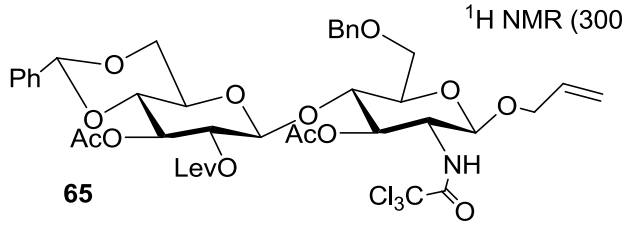


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



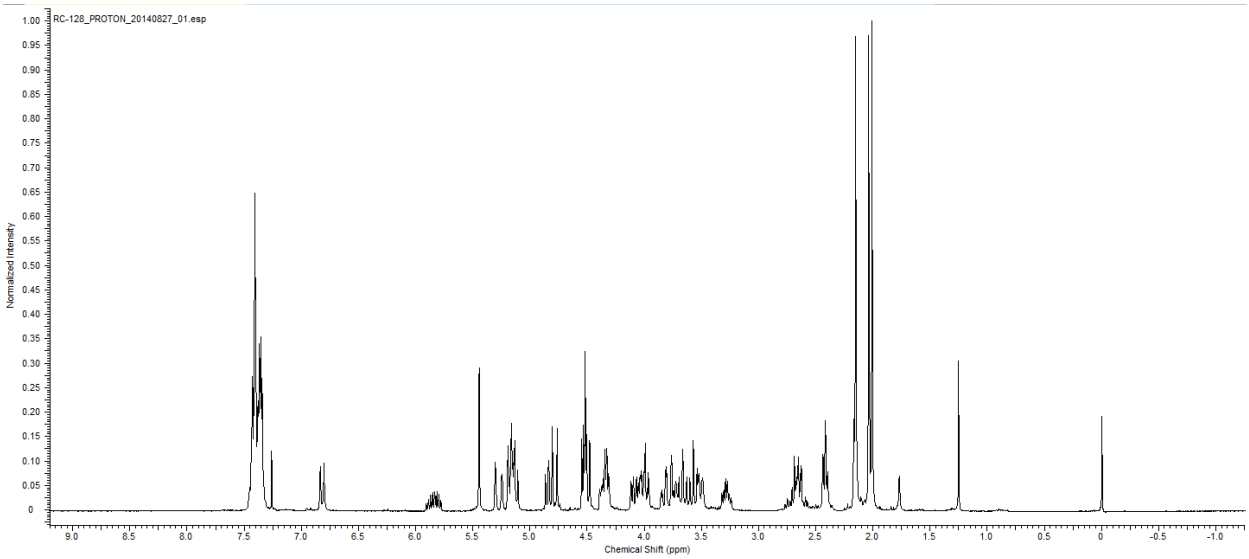
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)



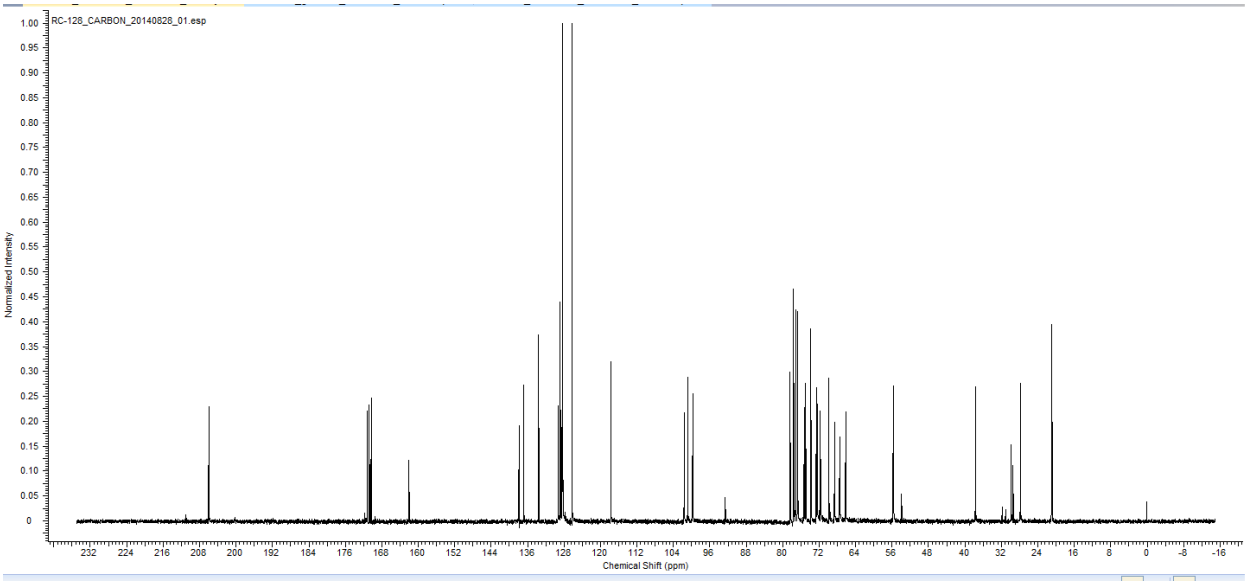


65

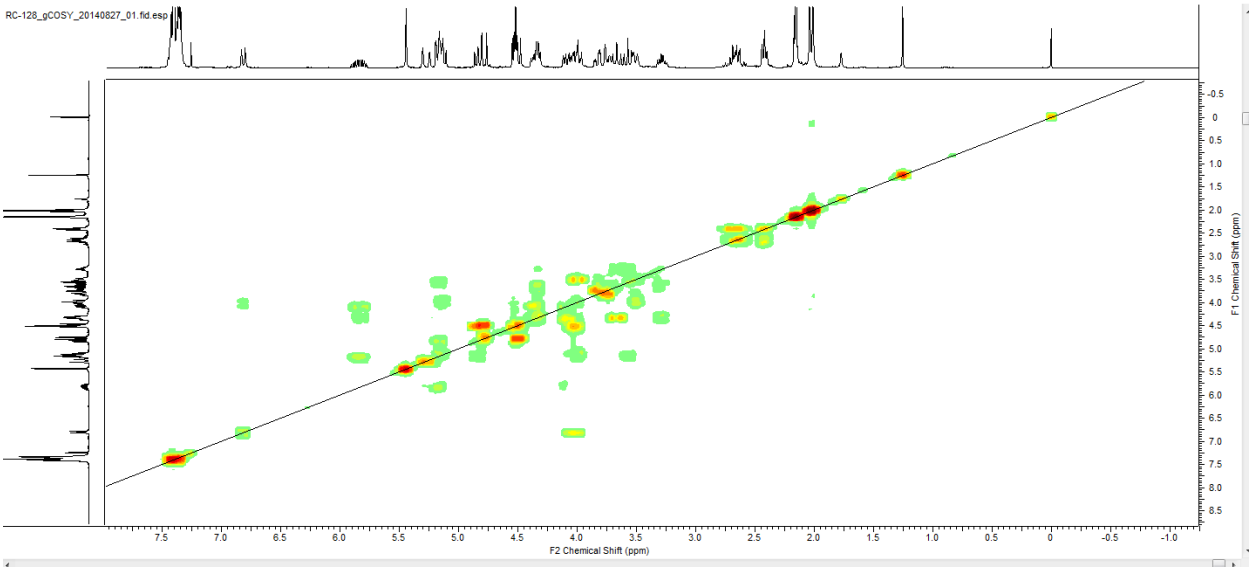
^{1}H NMR (300 MHz, $CDCl_3$)



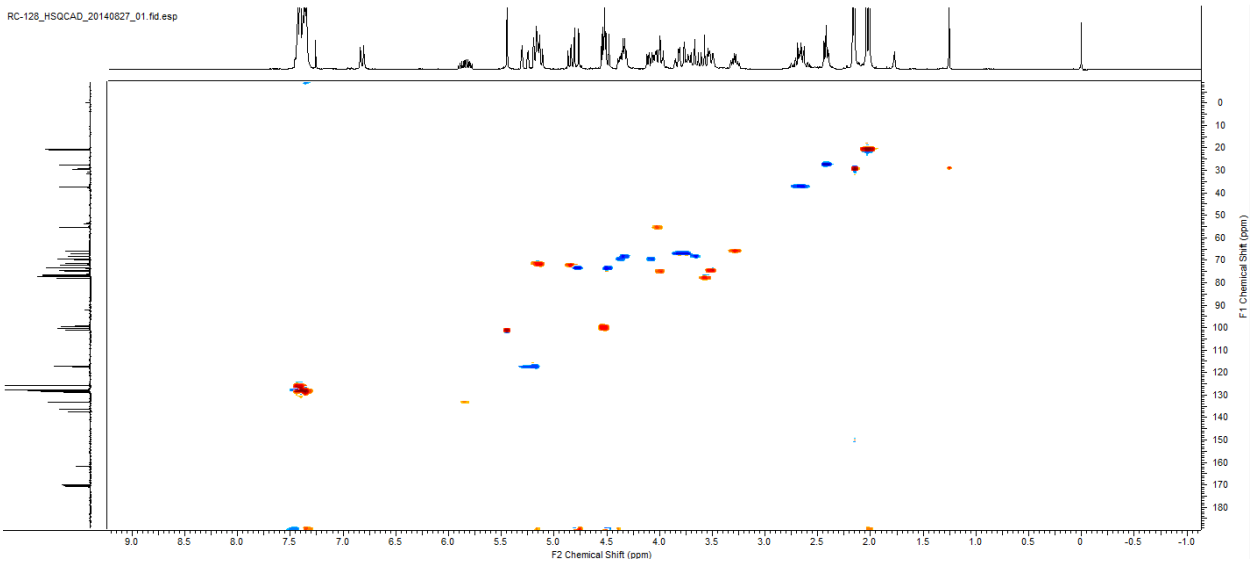
^{13}C NMR (75 MHz, $CDCl_3$)

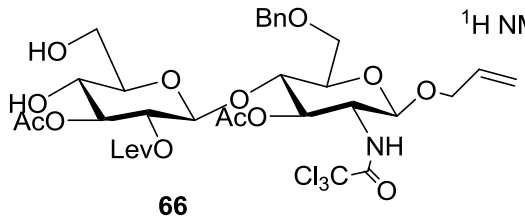


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



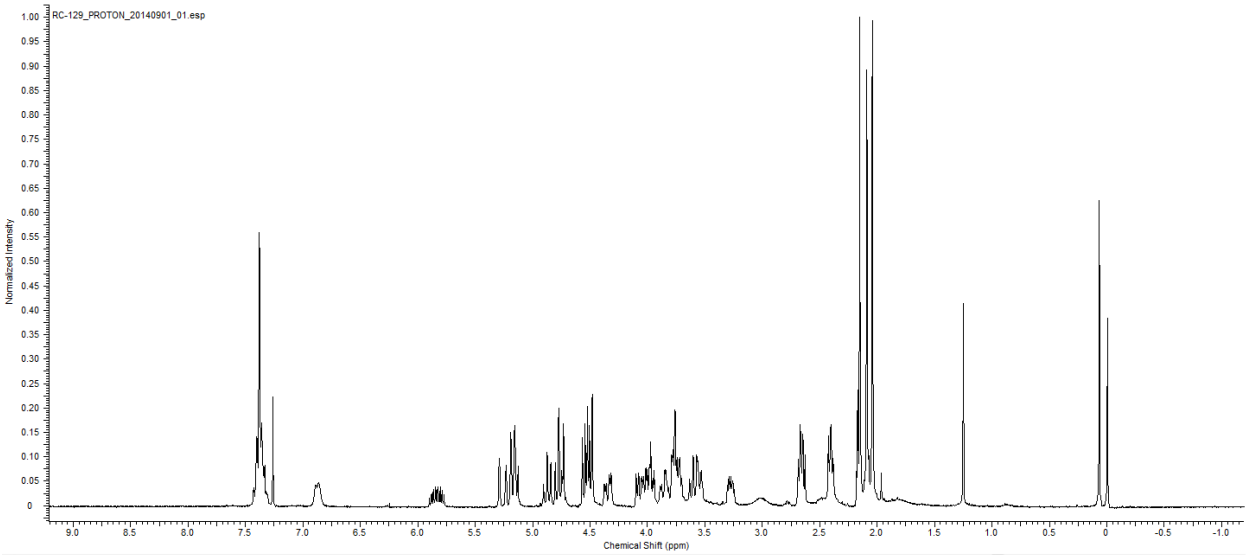
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



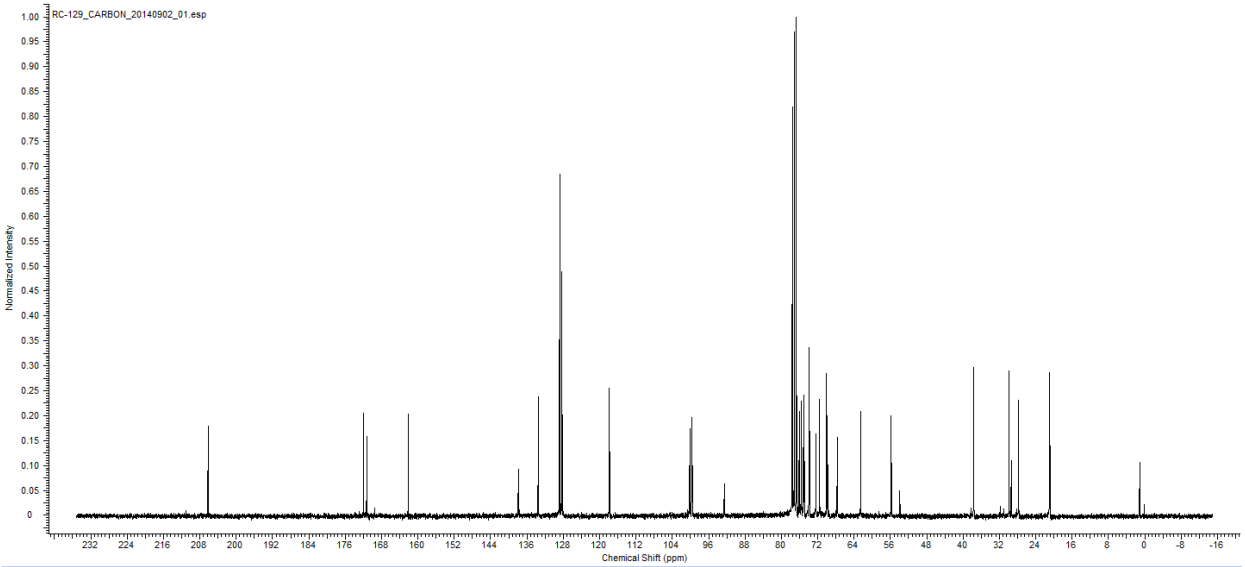


66

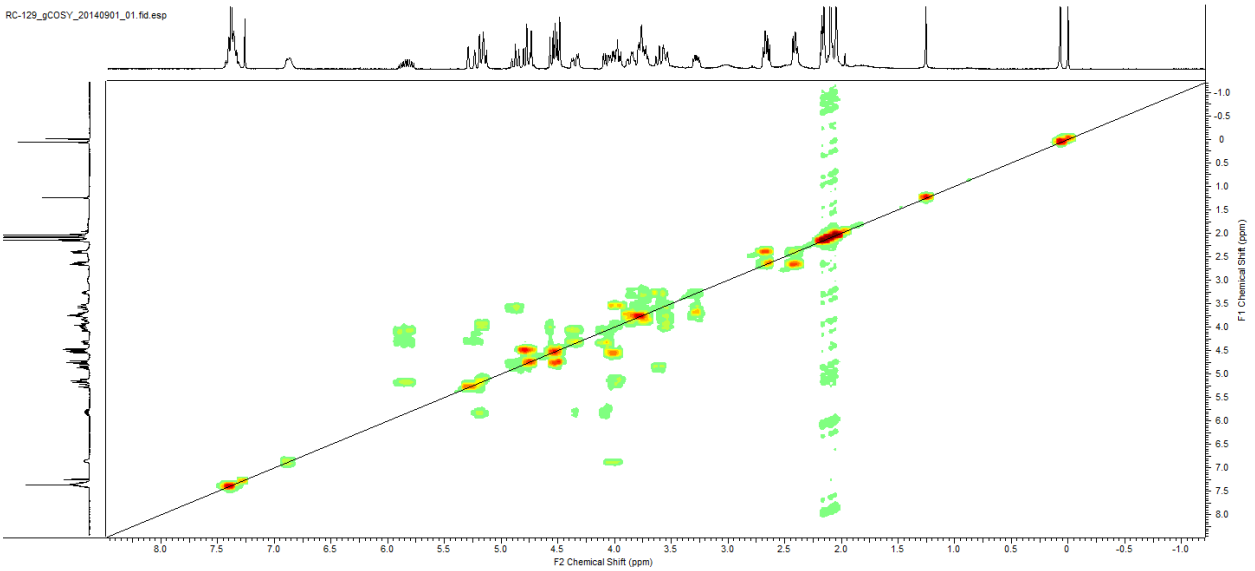
^{1}H NMR (300 MHz, $CDCl_3$)



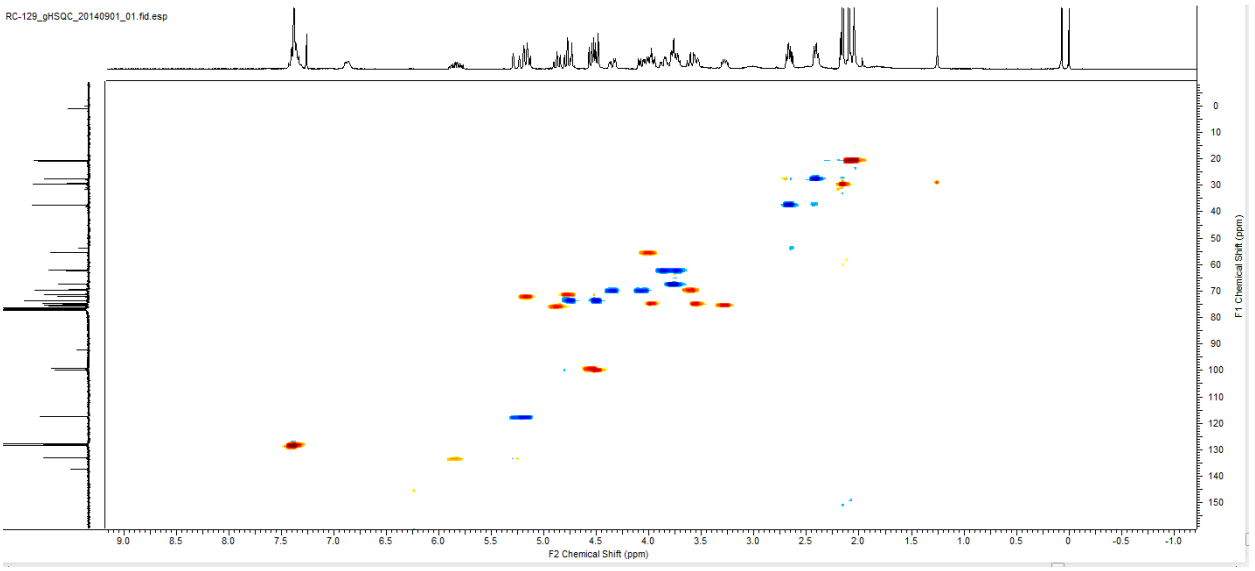
^{13}C NMR (75 MHz, $CDCl_3$)

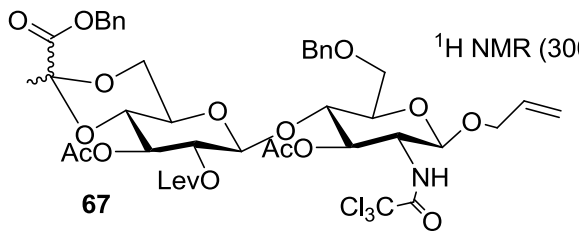


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



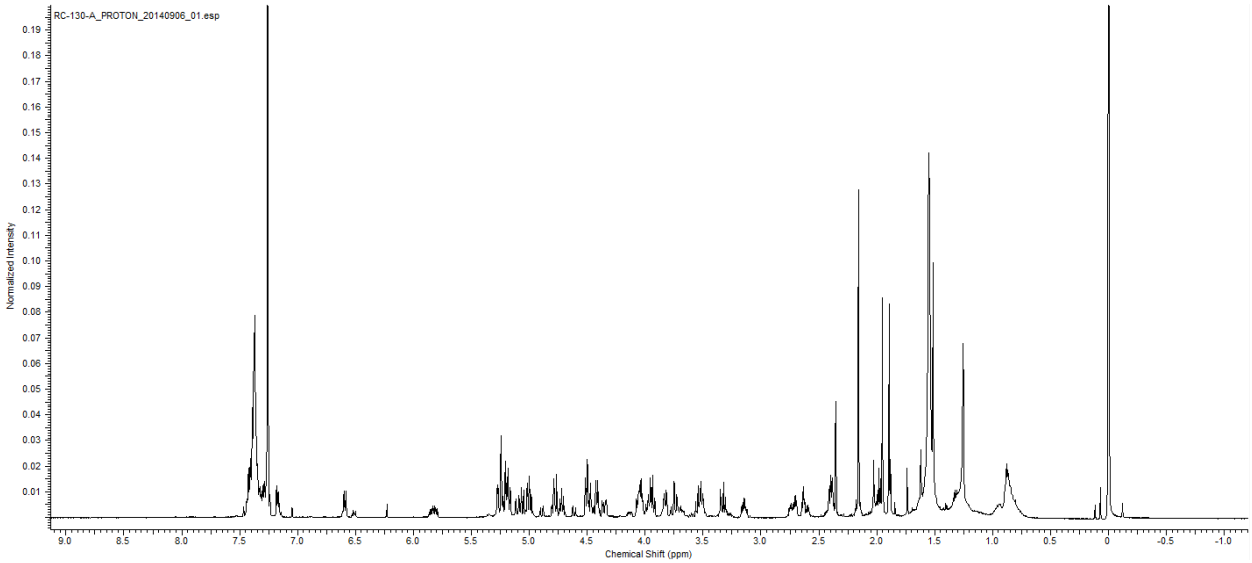
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



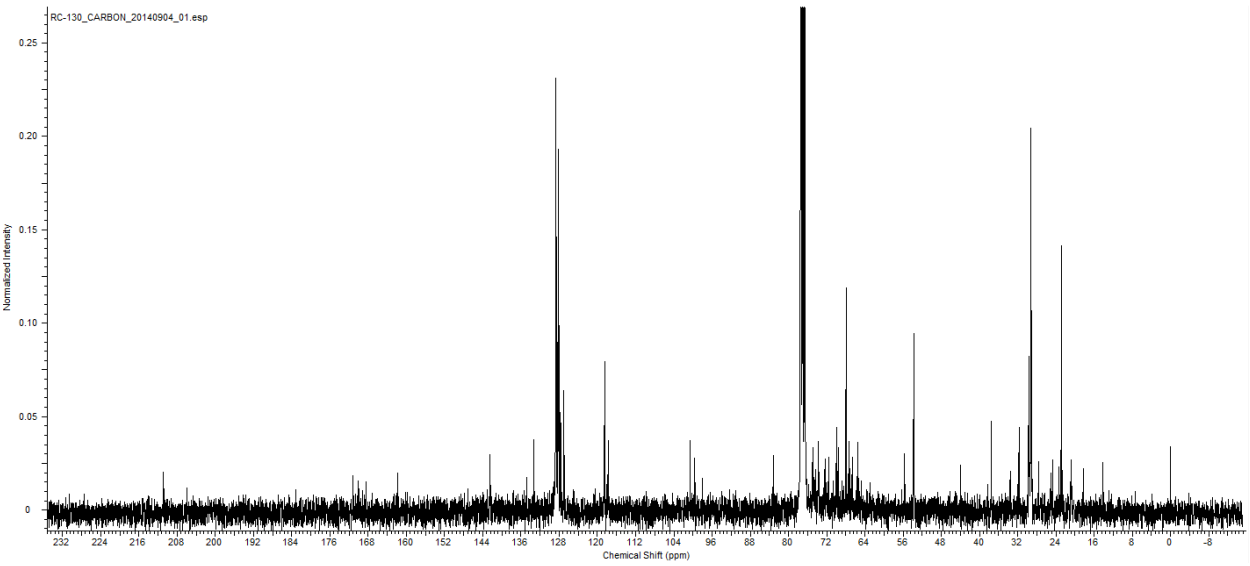


67

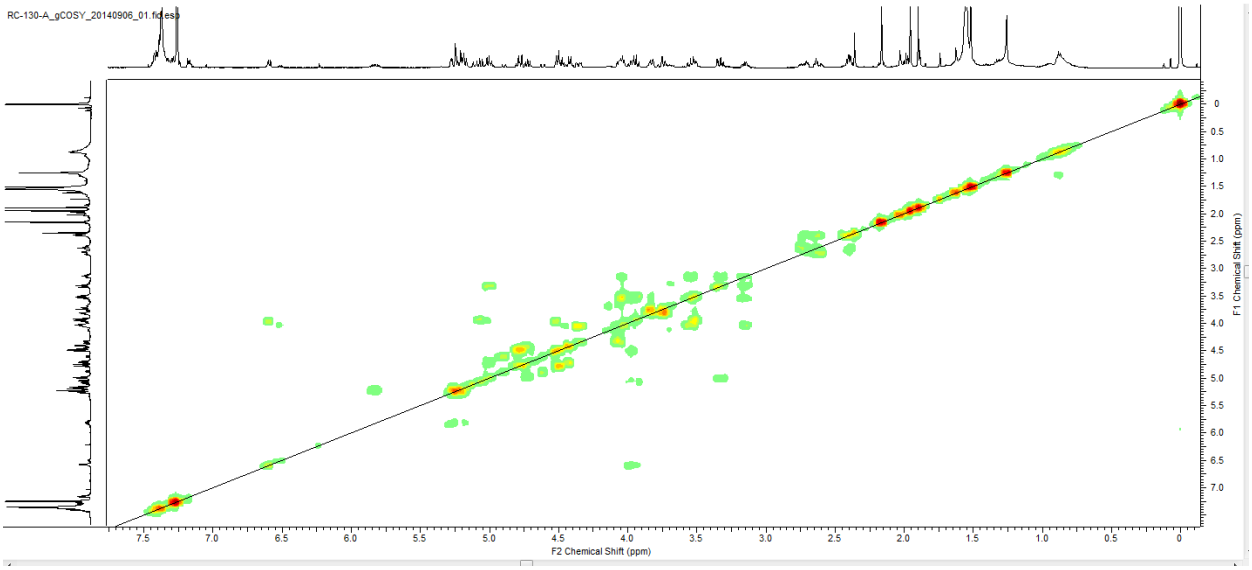
^{1}H NMR (300 MHz, $CDCl_3$)



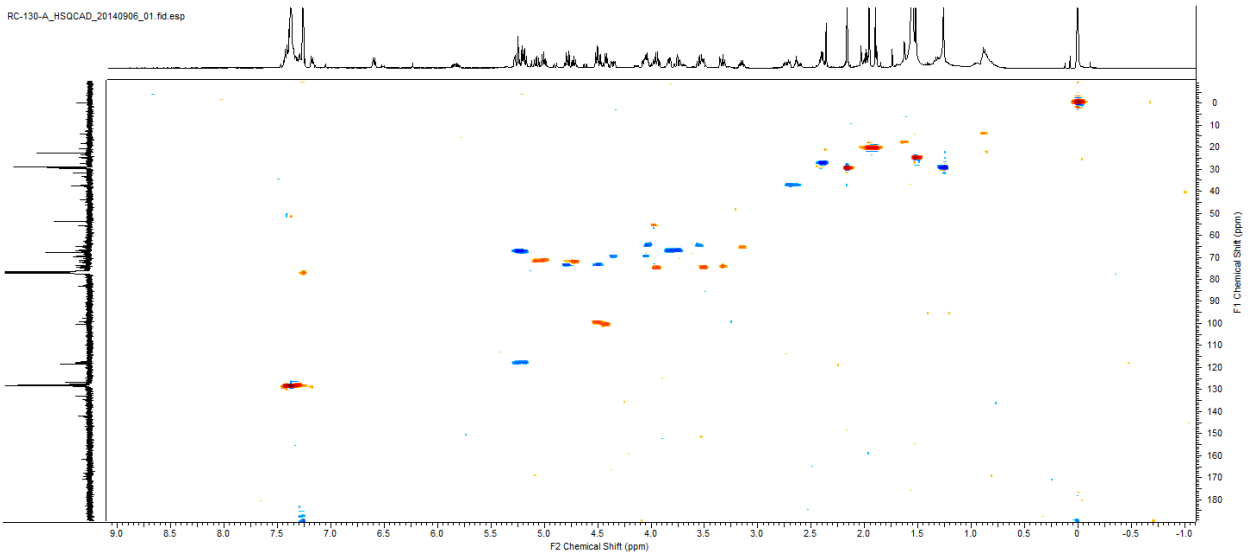
^{13}C NMR (75 MHz, $CDCl_3$)

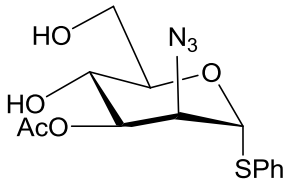


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



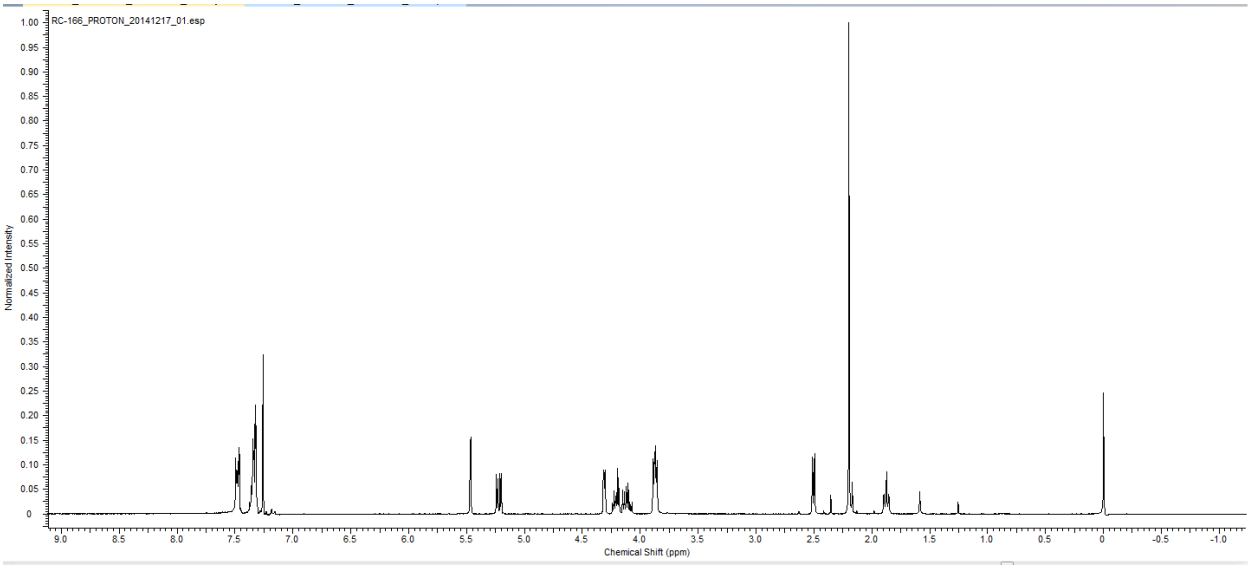
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



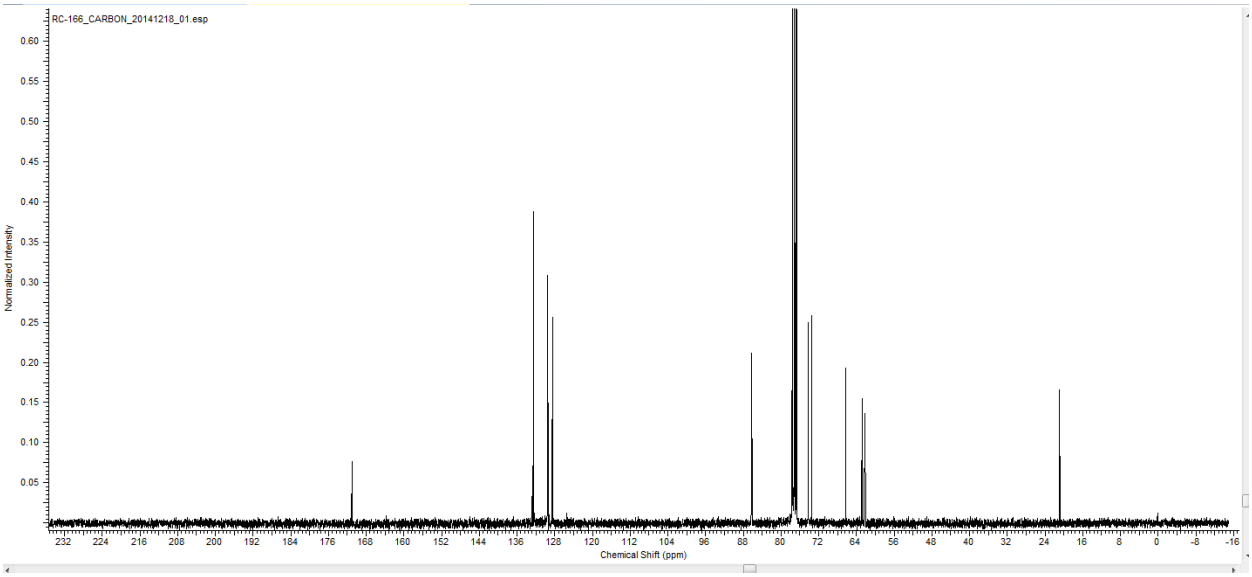


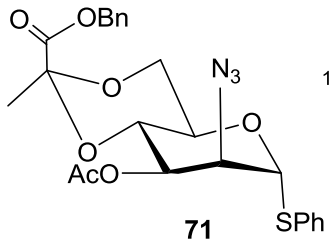
70

^{1}H NMR (300 MHz, $CDCl_3$)



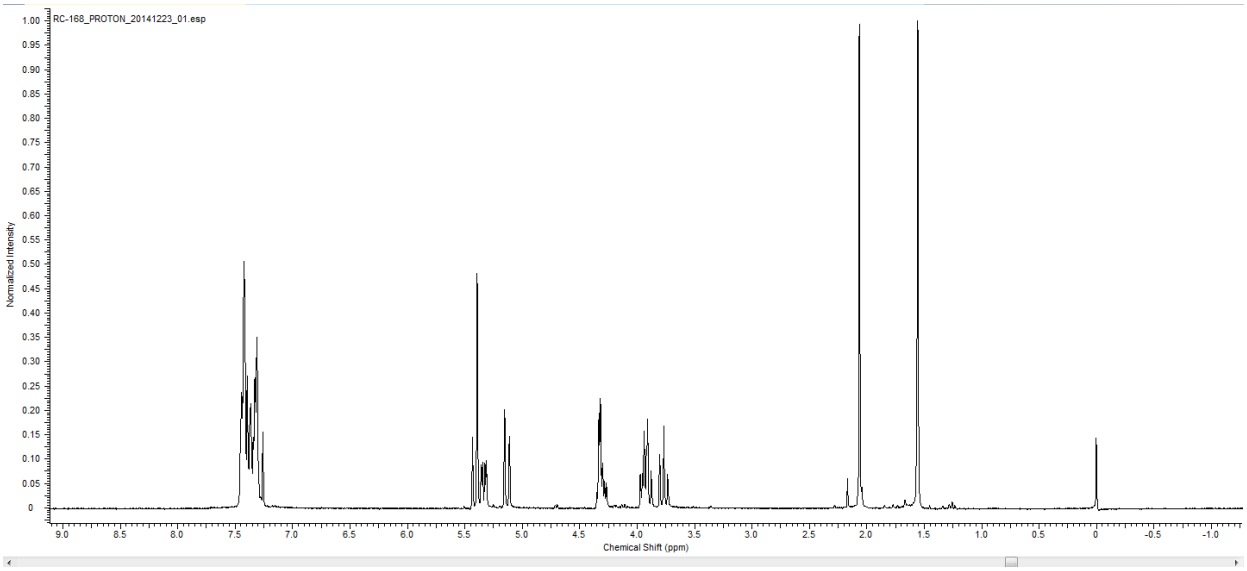
^{13}C NMR (75 MHz, $CDCl_3$)



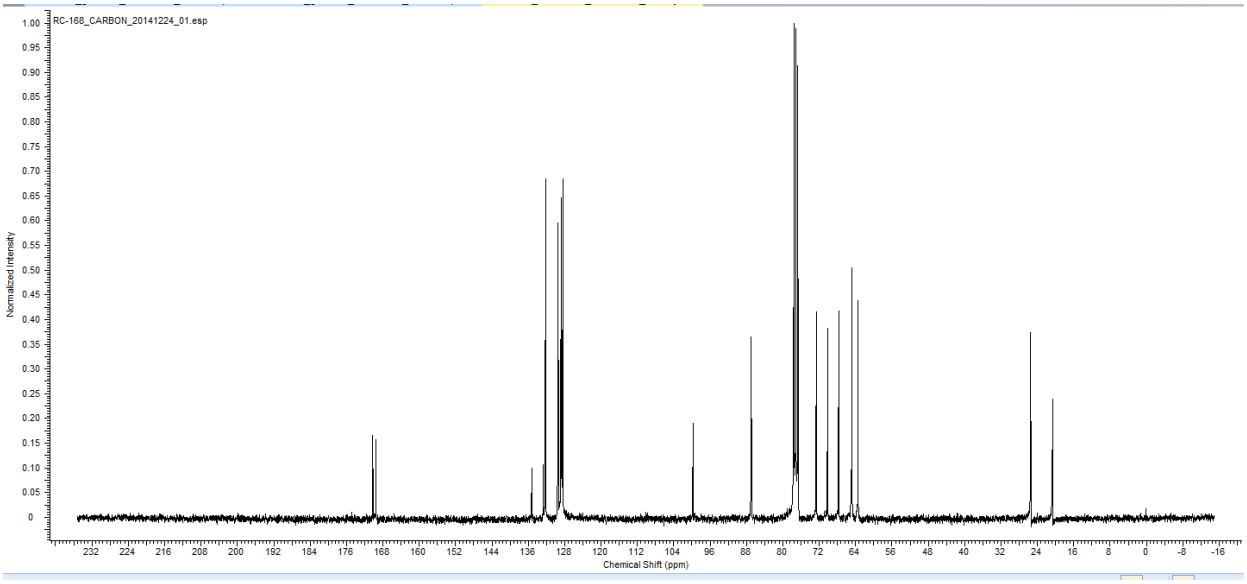


71

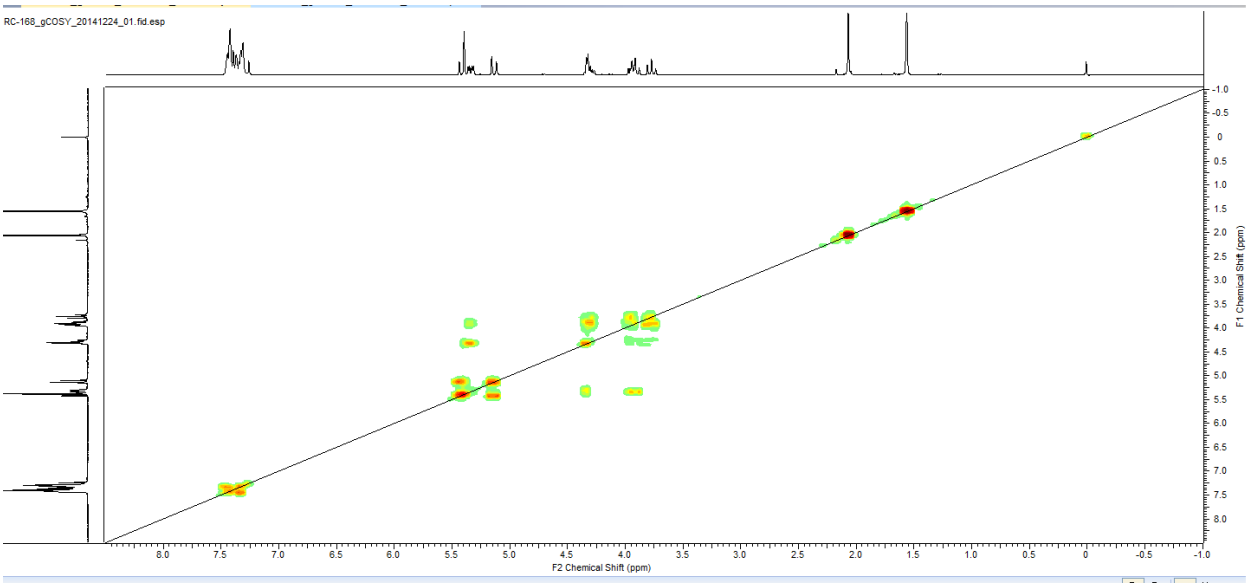
^{1}H NMR (300 MHz, $CDCl_3$)



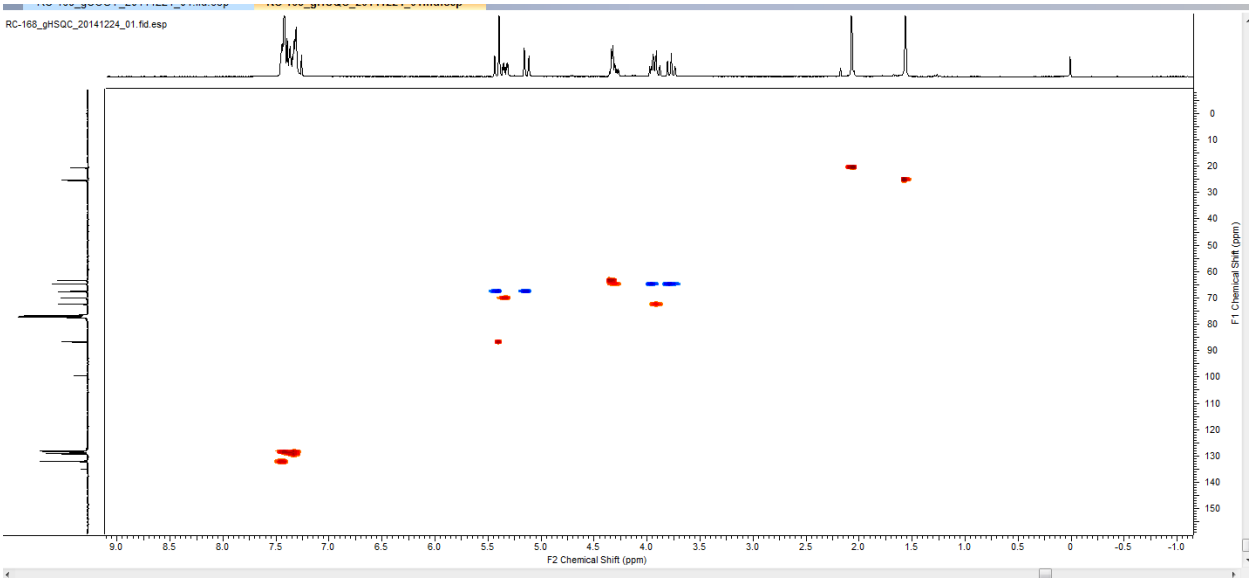
^{13}C NMR (75 MHz, $CDCl_3$)

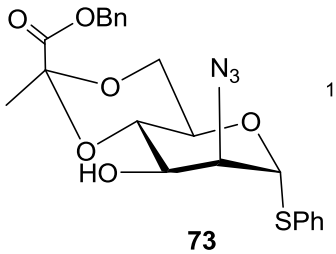


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



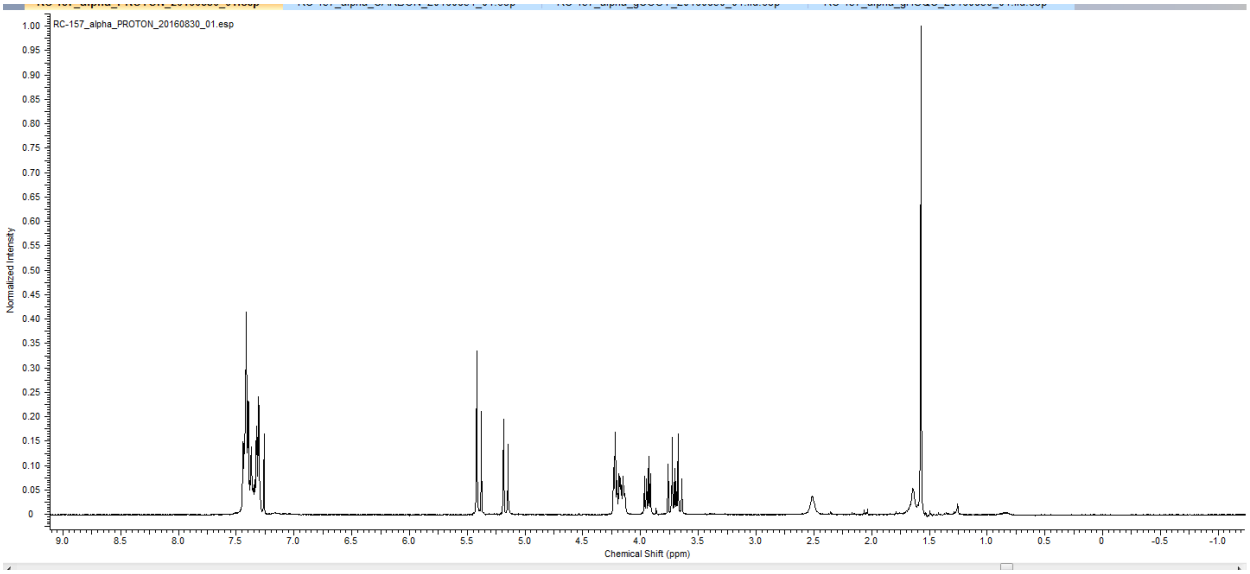
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



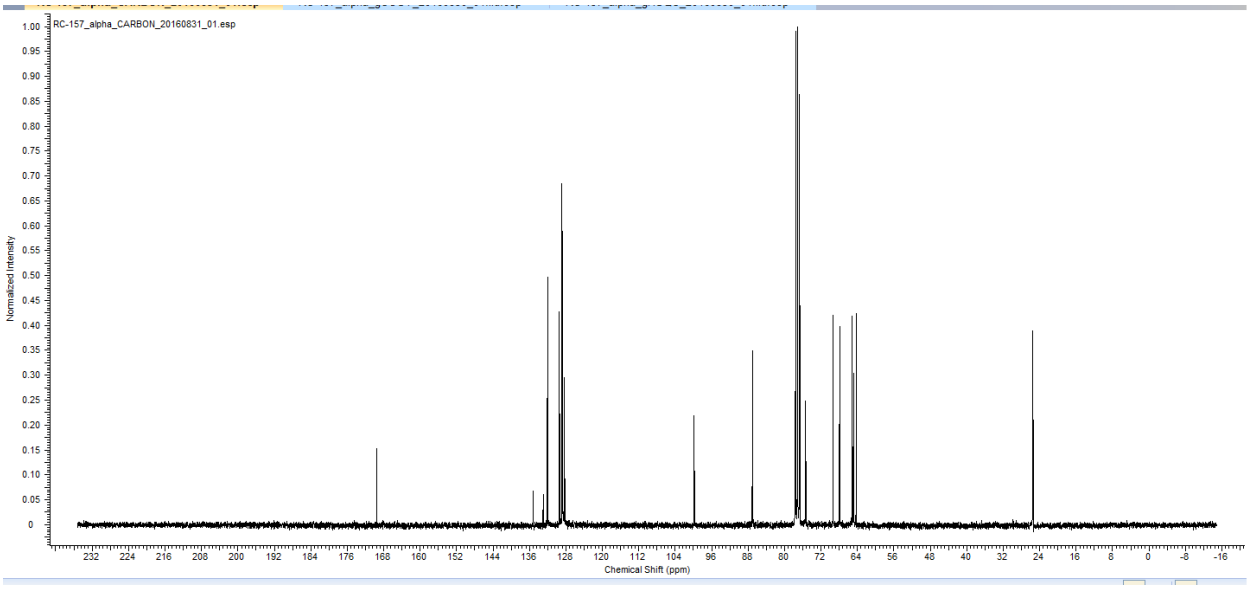


73

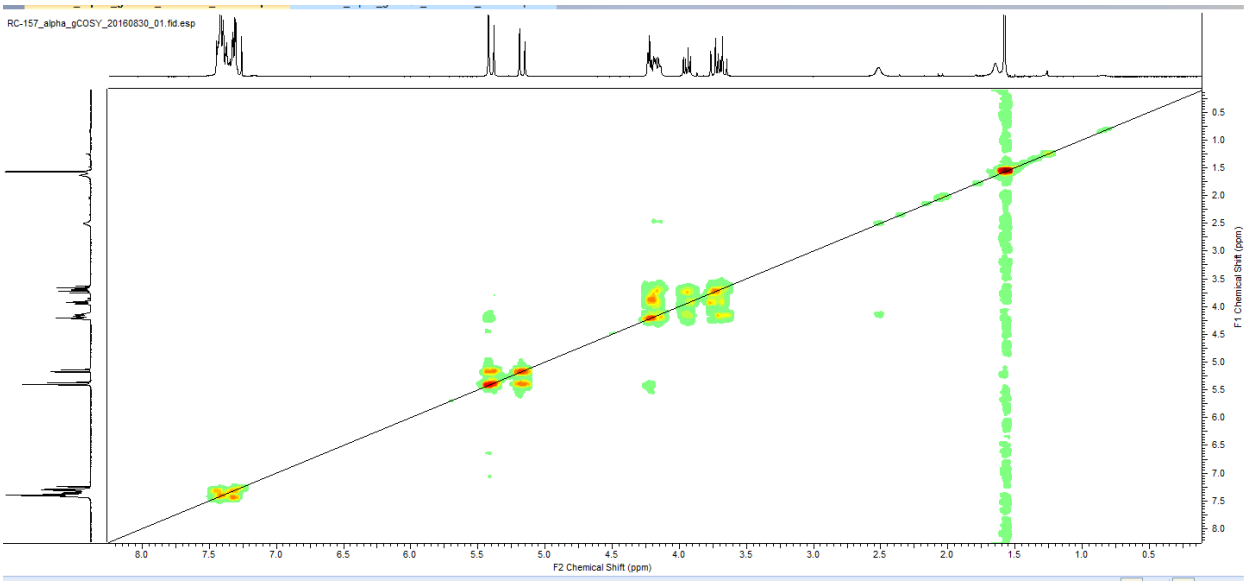
^{1}H NMR (300 MHz, $CDCl_3$)



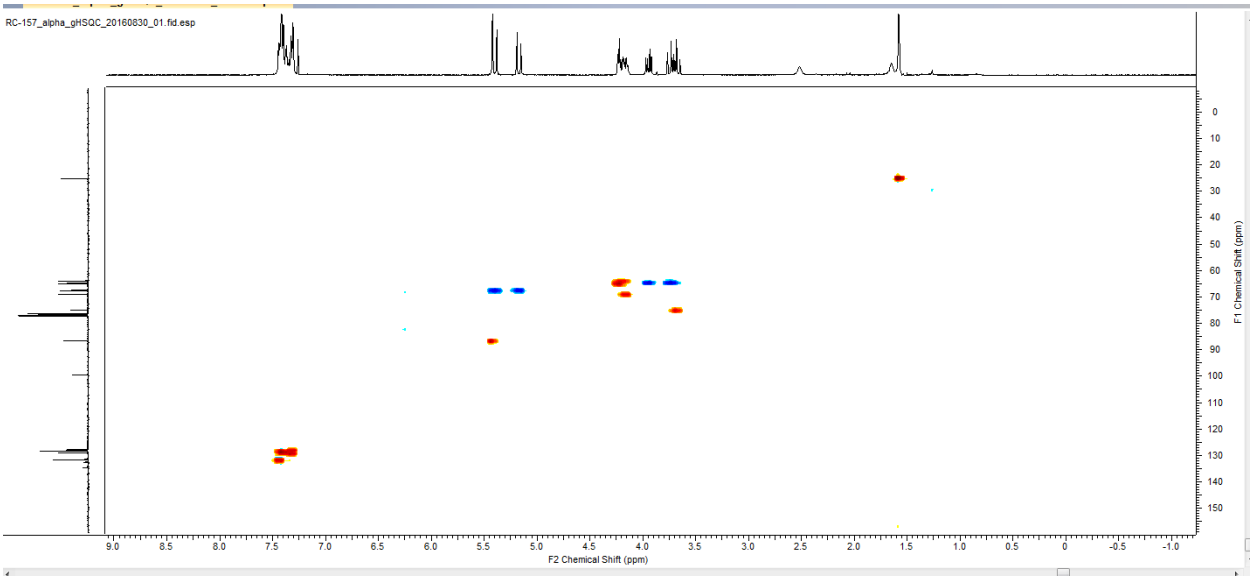
^{13}C NMR (75 MHz, $CDCl_3$)

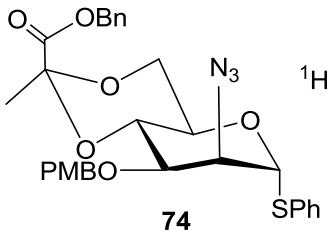


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



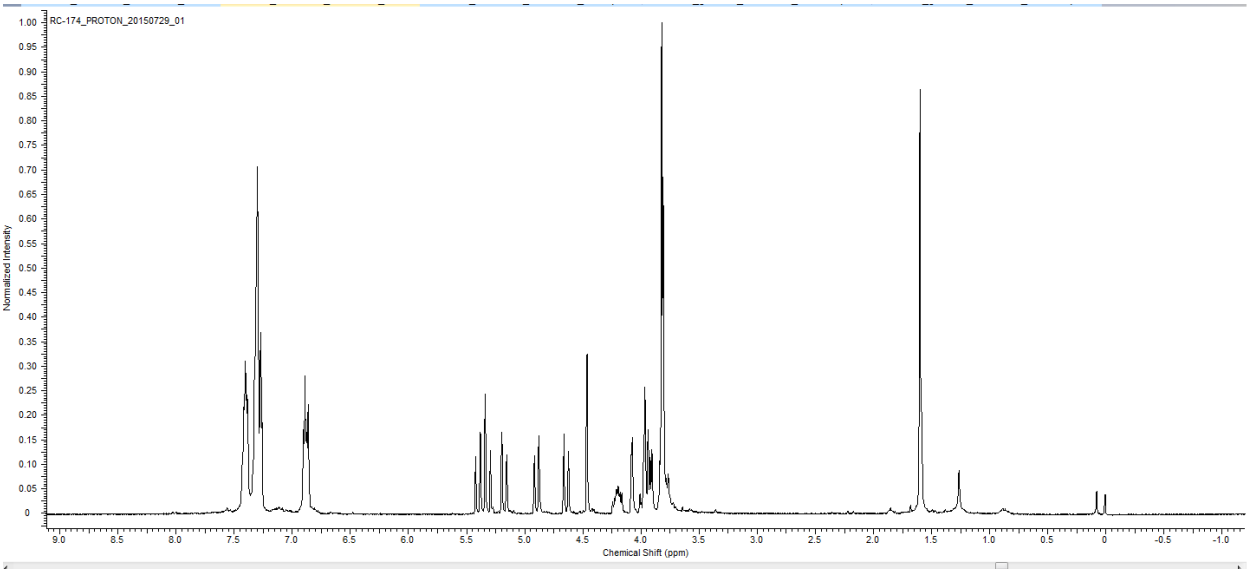
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



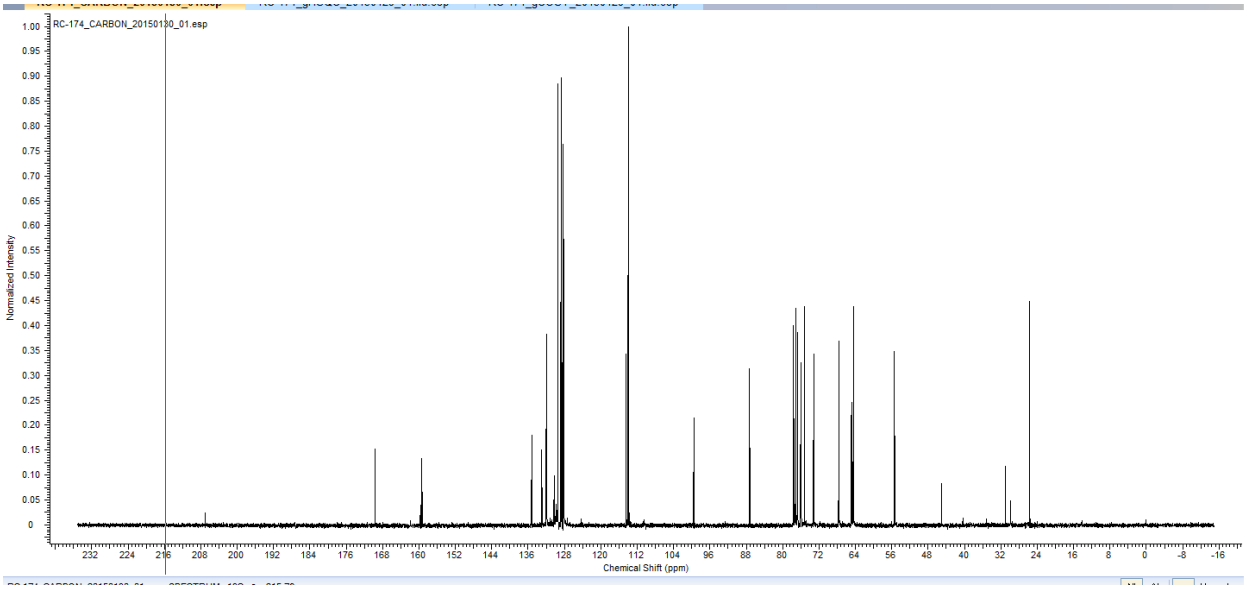


74

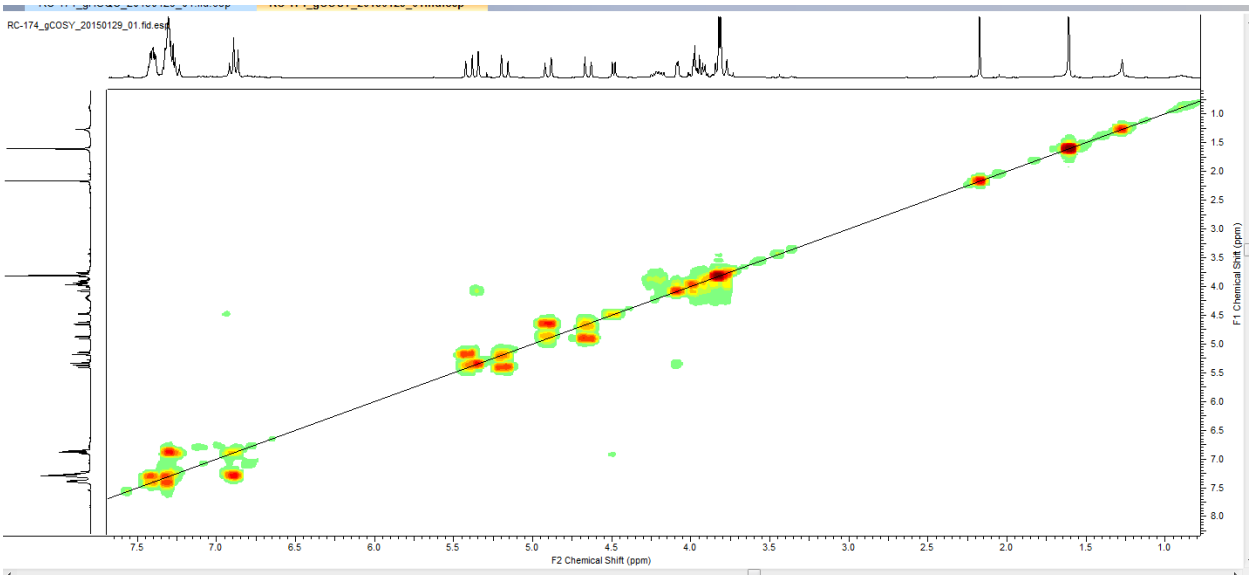
^{1}H NMR (300 MHz, $CDCl_3$)



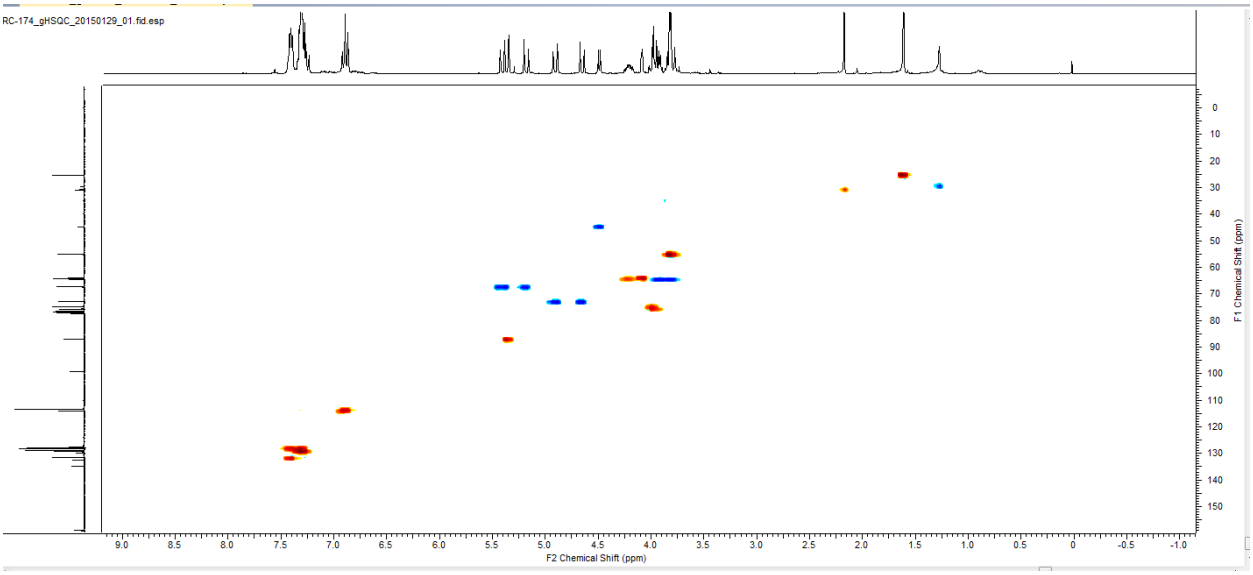
^{13}C NMR (75 MHz, $CDCl_3$)

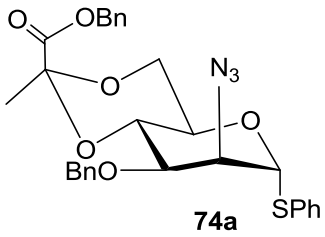


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



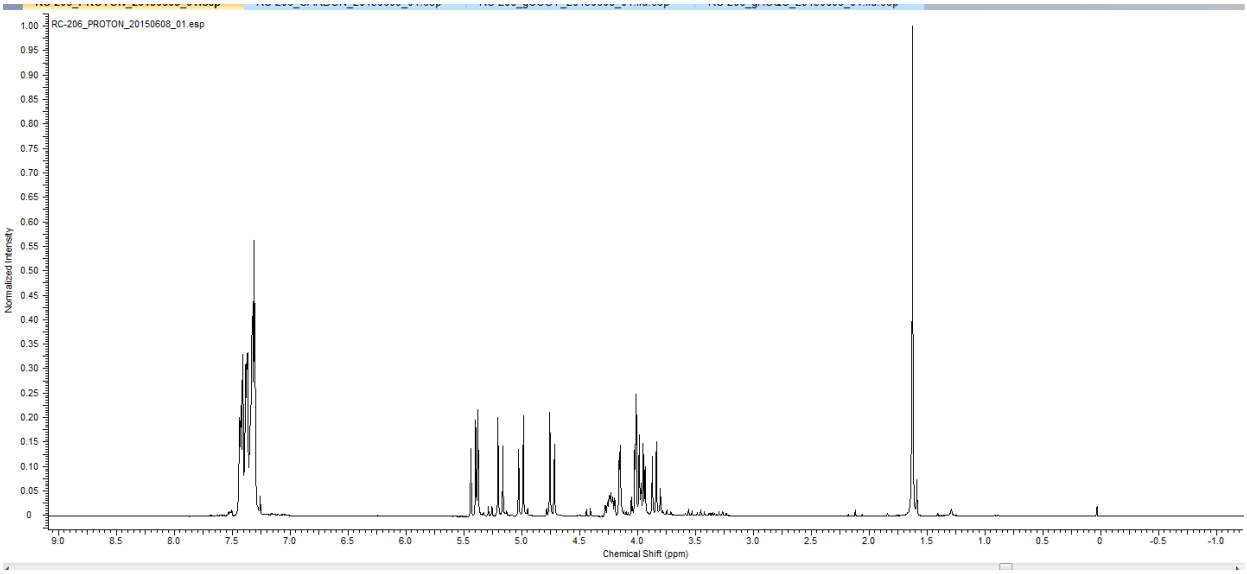
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



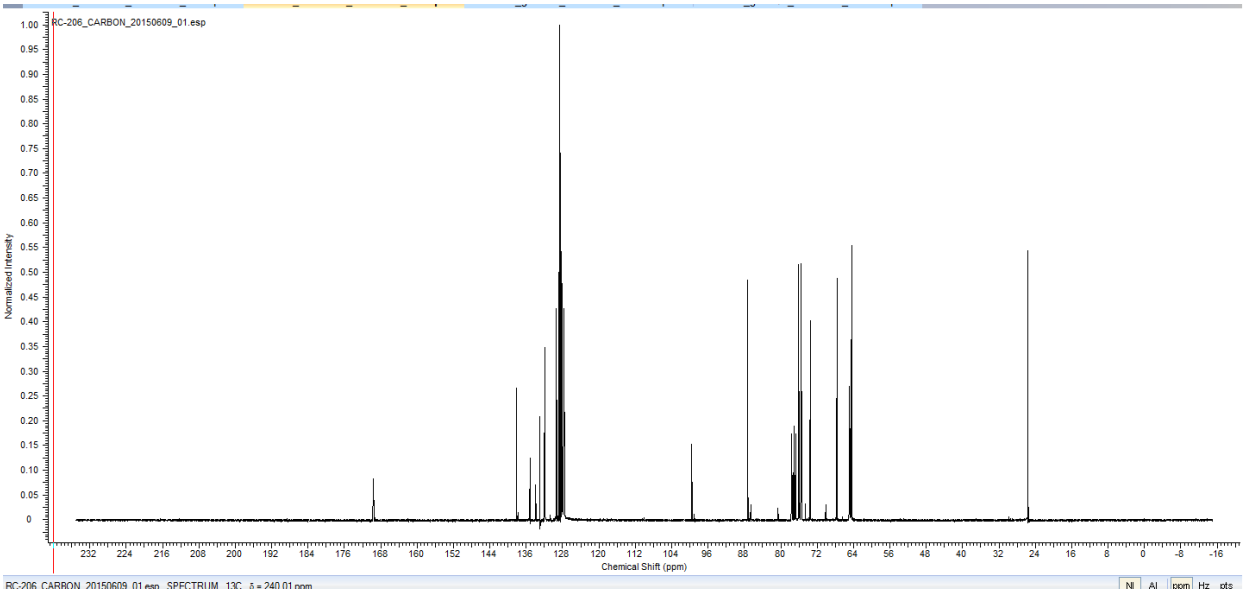


74a

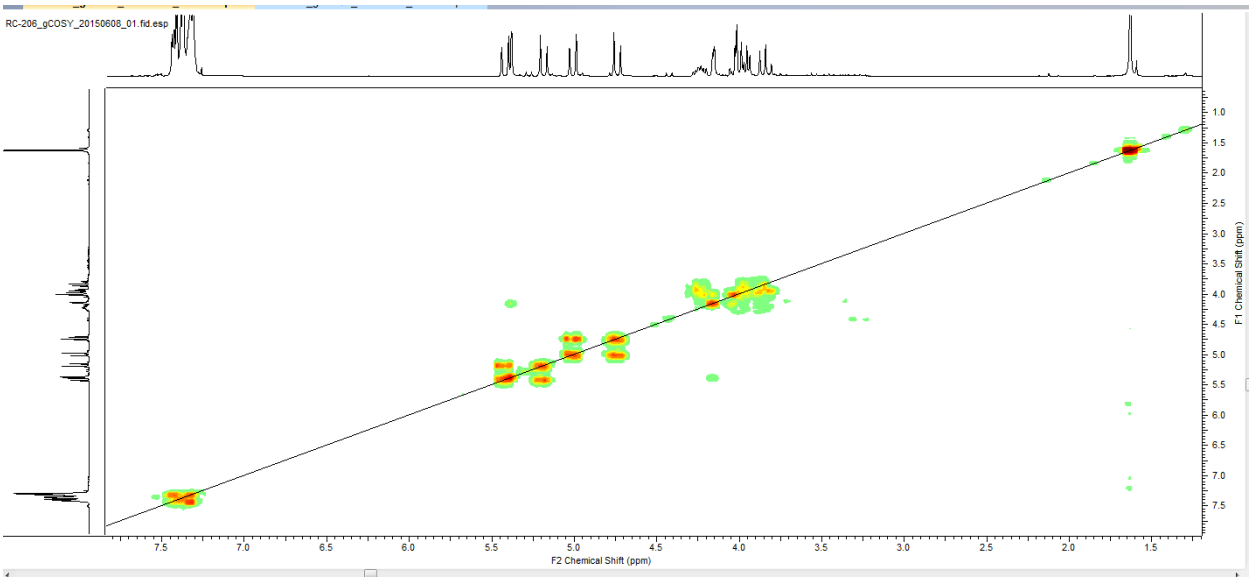
^{1}H NMR (300 MHz, $CDCl_3$)



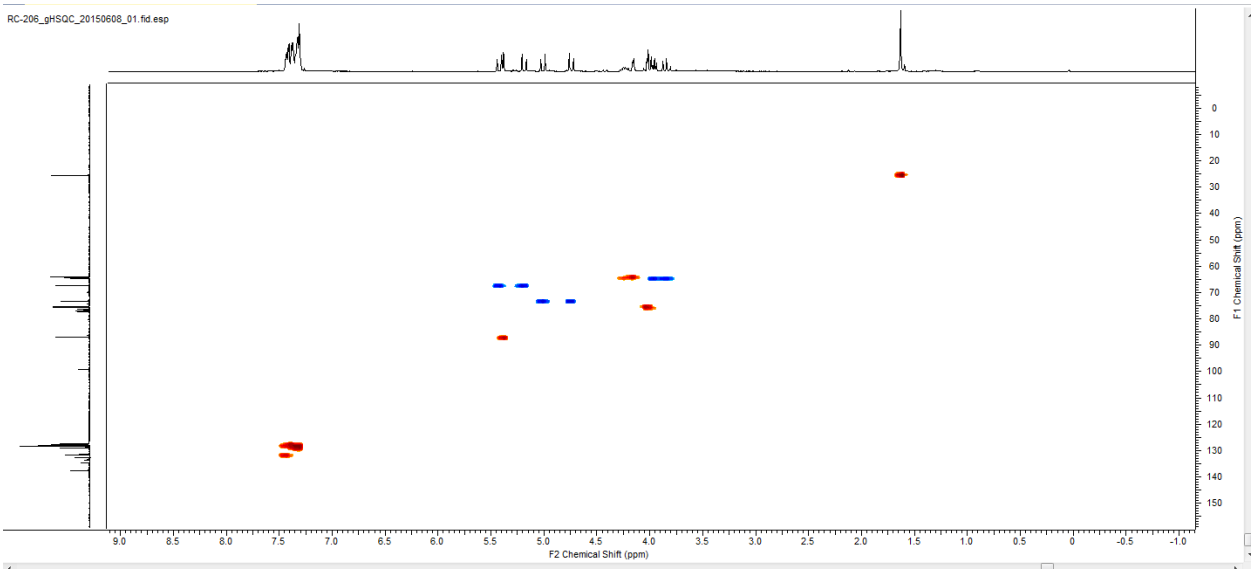
^{13}C NMR (75 MHz, $CDCl_3$)

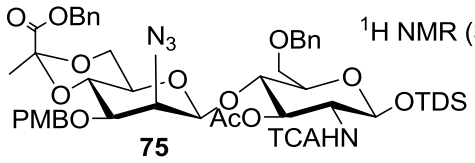


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



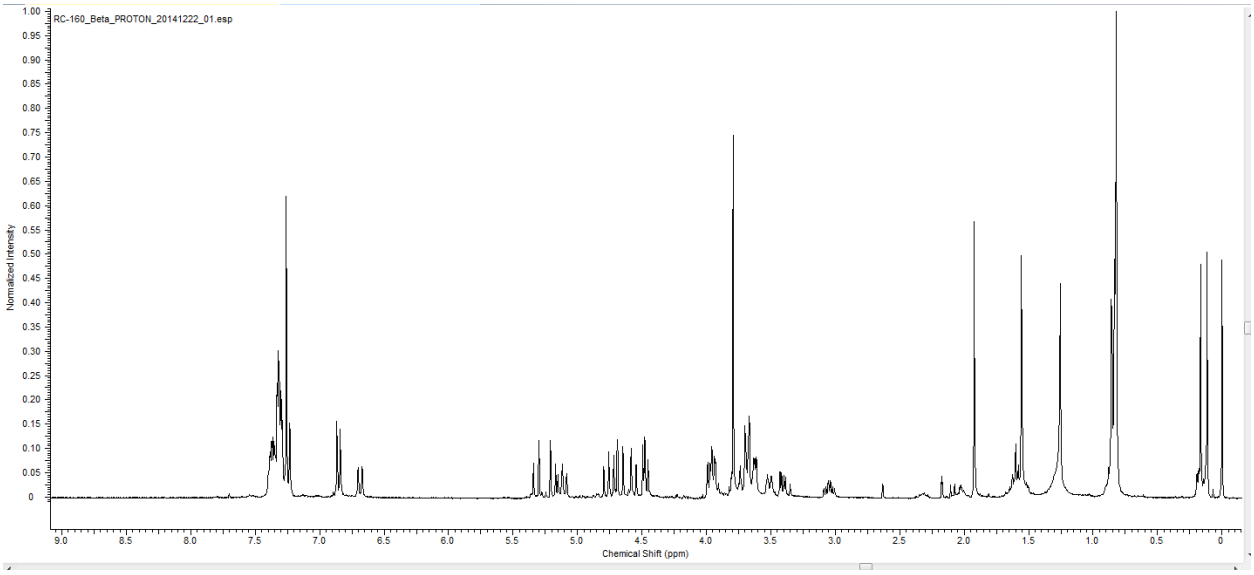
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



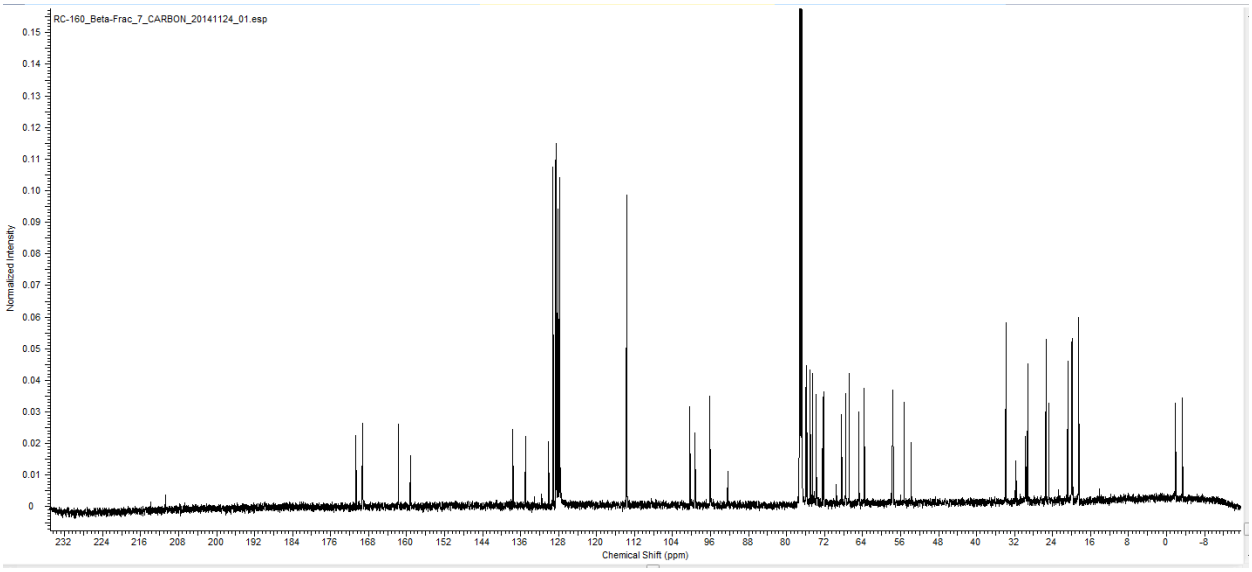


75

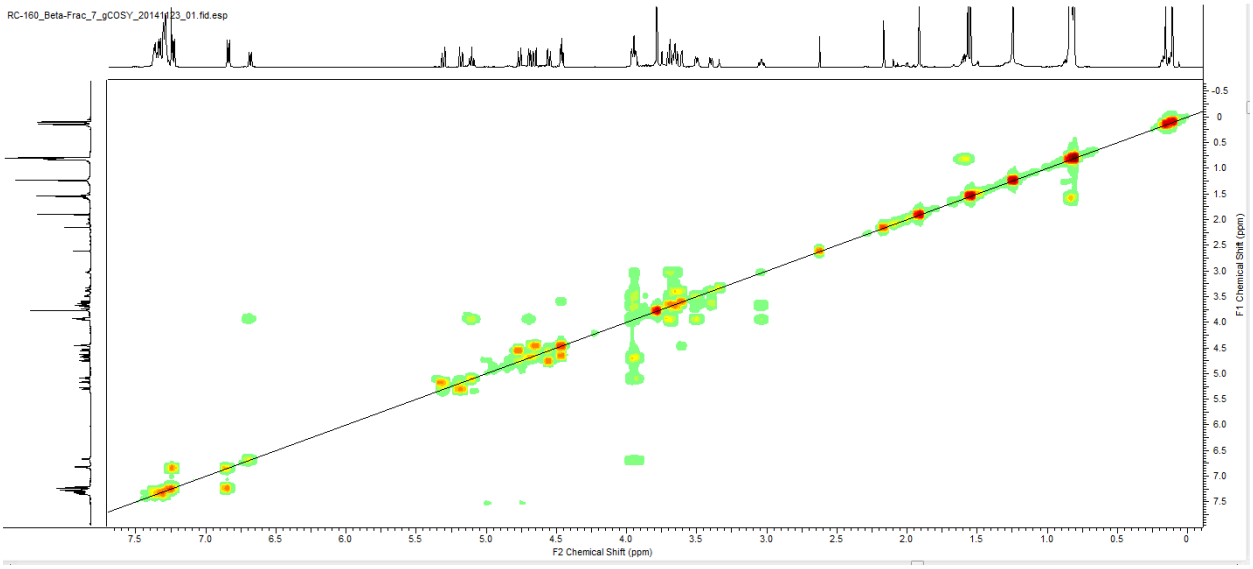
^{1}H NMR (300 MHz, $CDCl_3$)



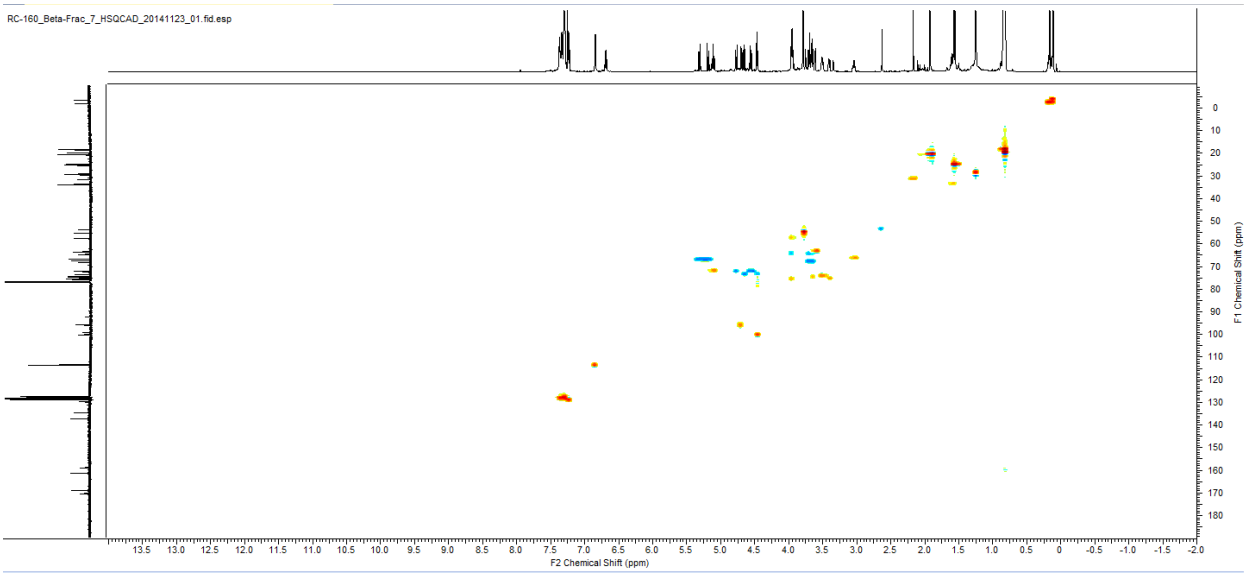
^{13}C NMR (150 MHz, $CDCl_3$)

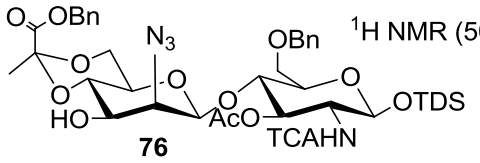


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



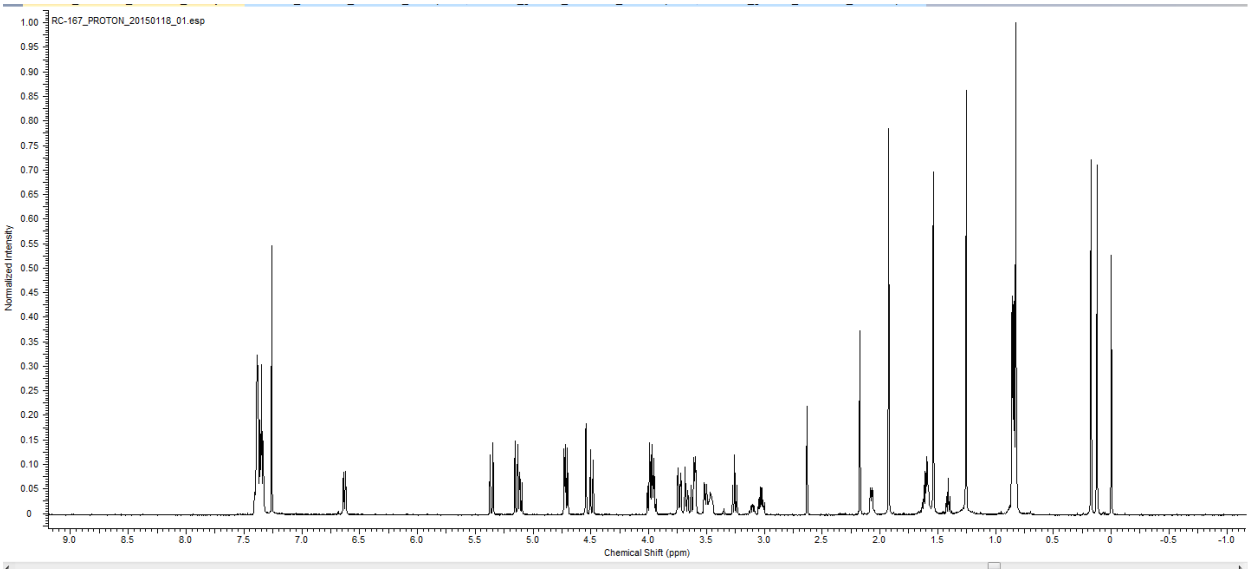
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



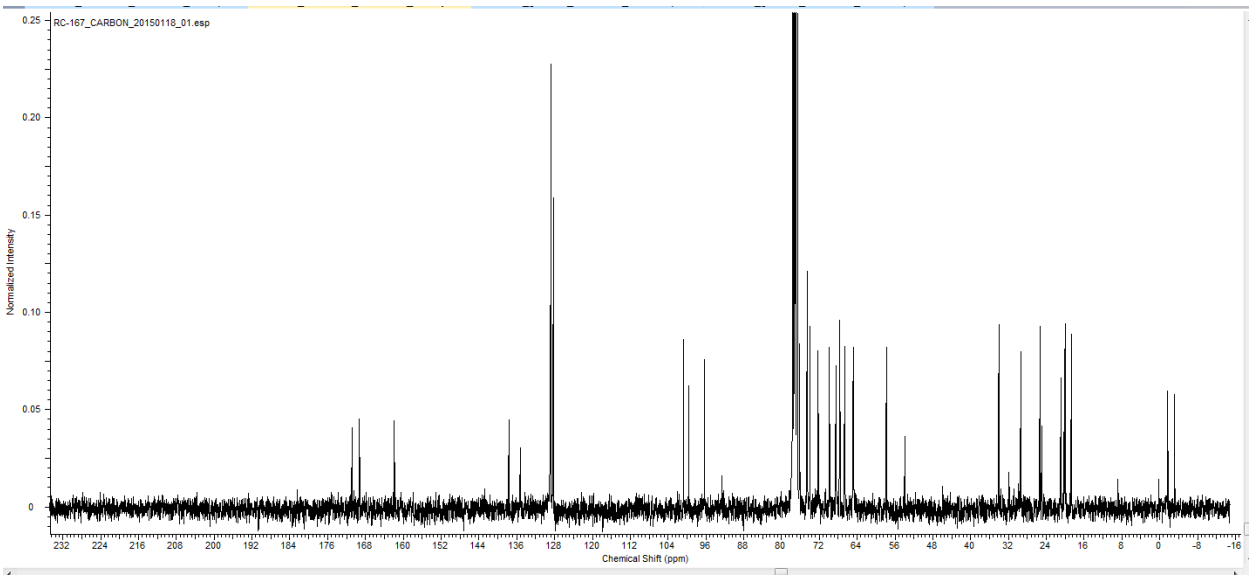


76

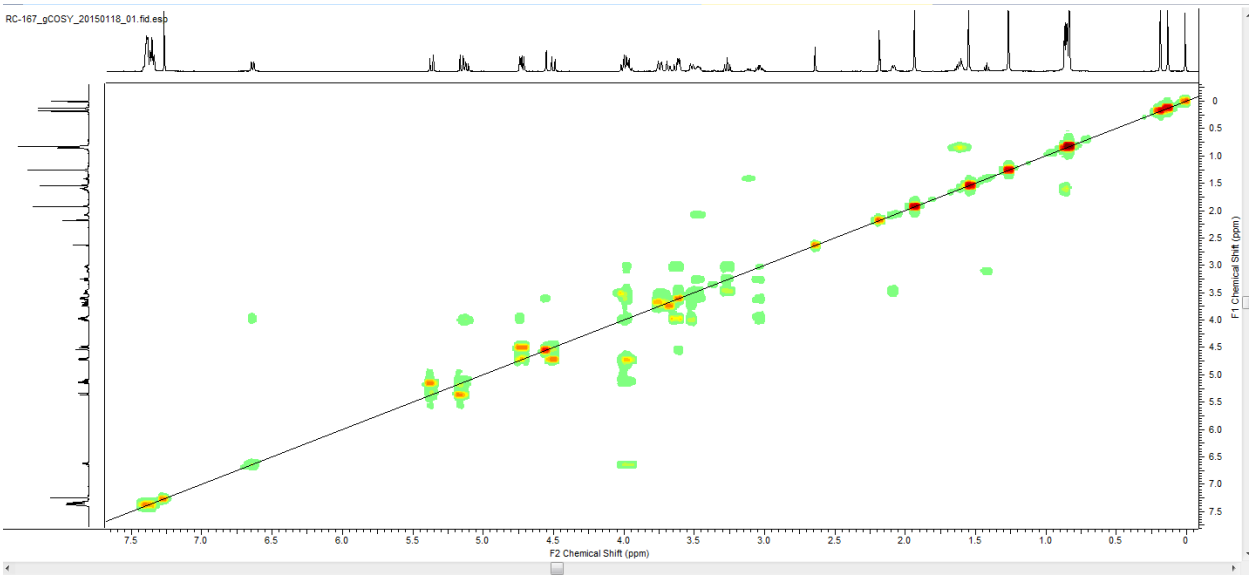
^{1}H NMR (500 MHz, $CDCl_3$)



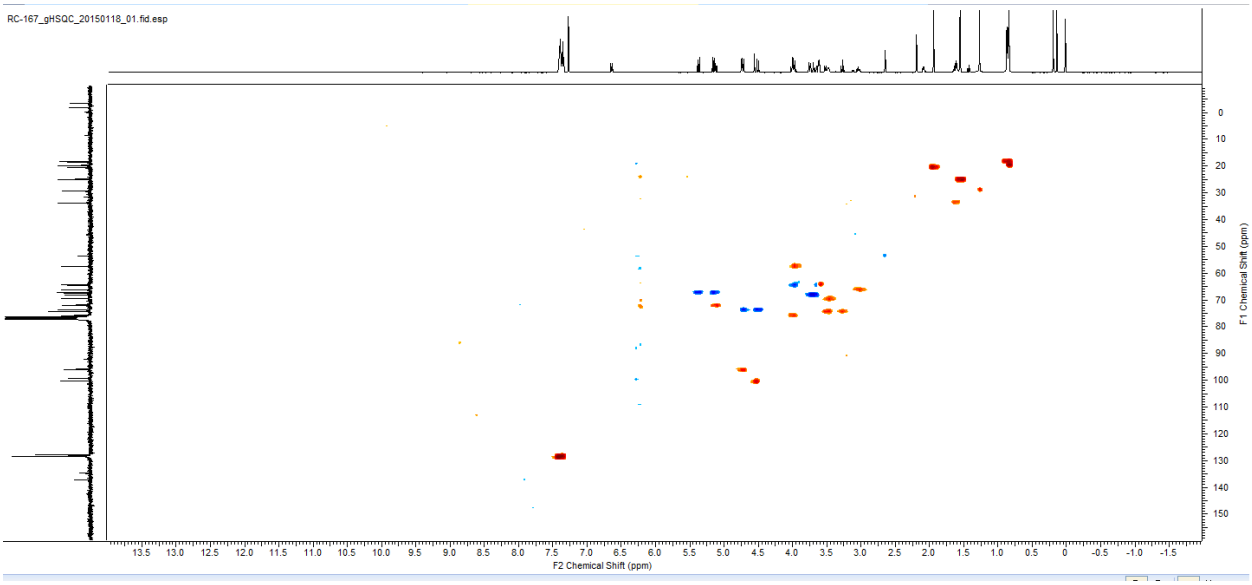
^{13}C NMR (75 MHz, $CDCl_3$)

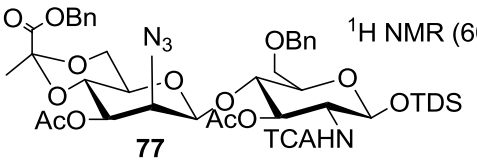


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



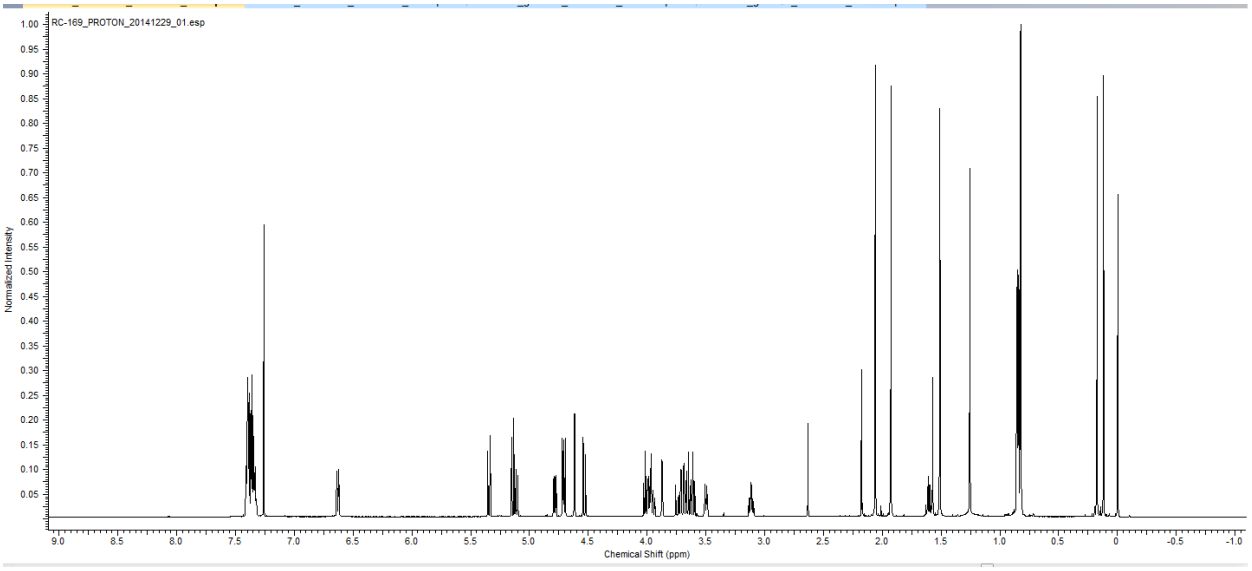
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



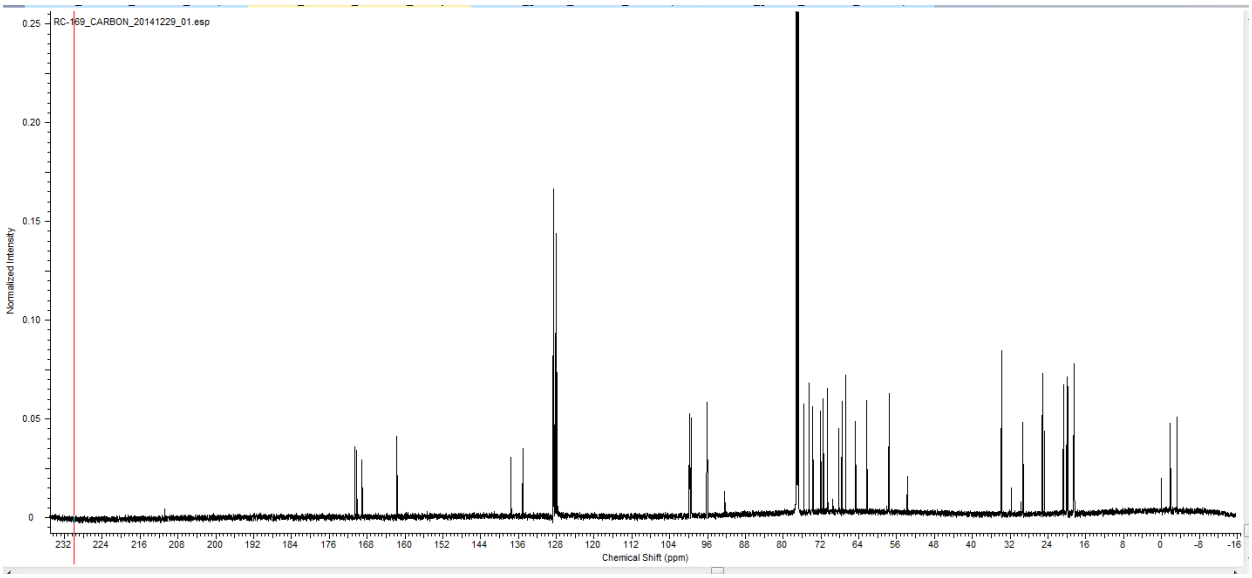


77

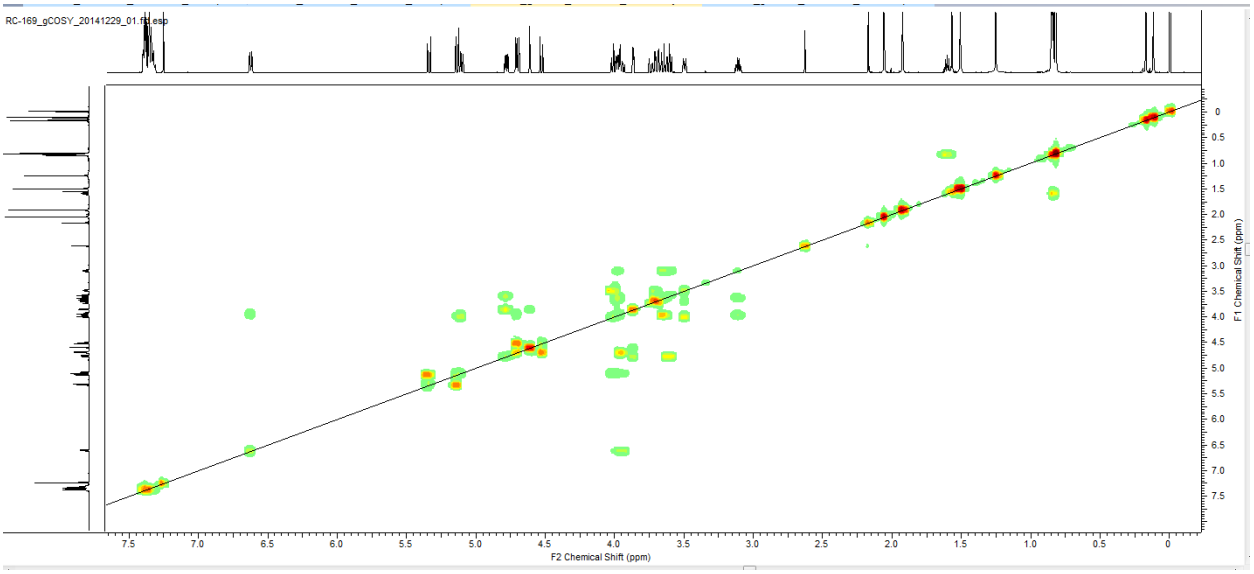
^{1}H NMR (600 MHz, $CDCl_3$)



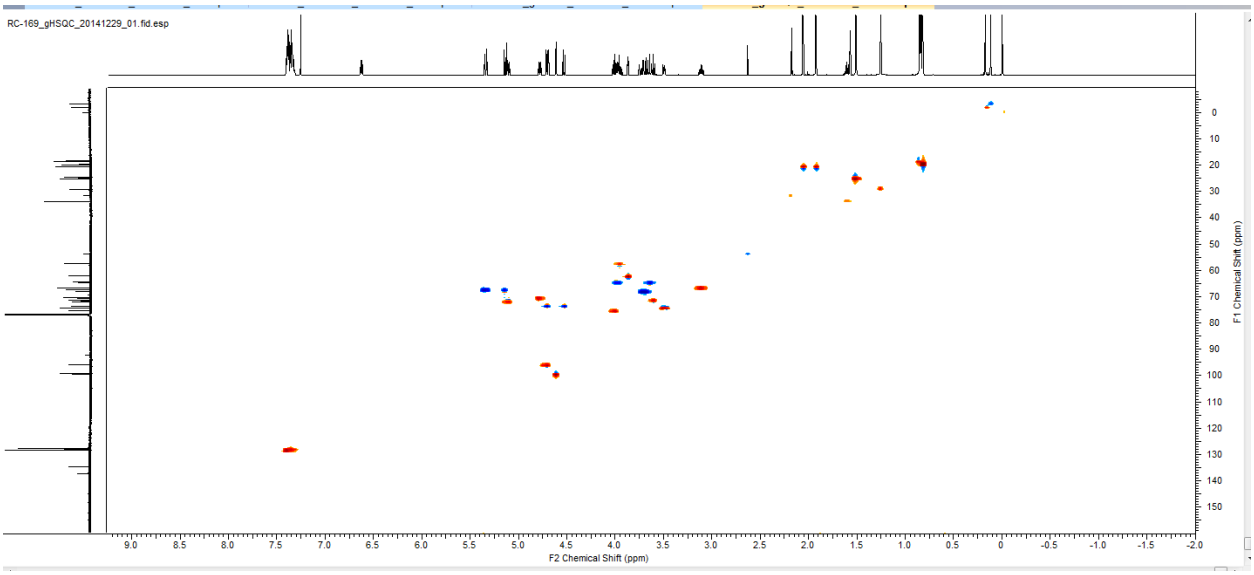
^{13}C NMR (150 MHz, $CDCl_3$)

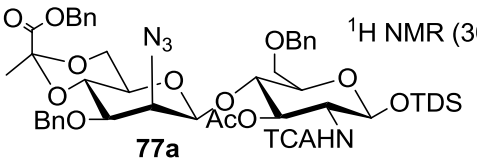


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



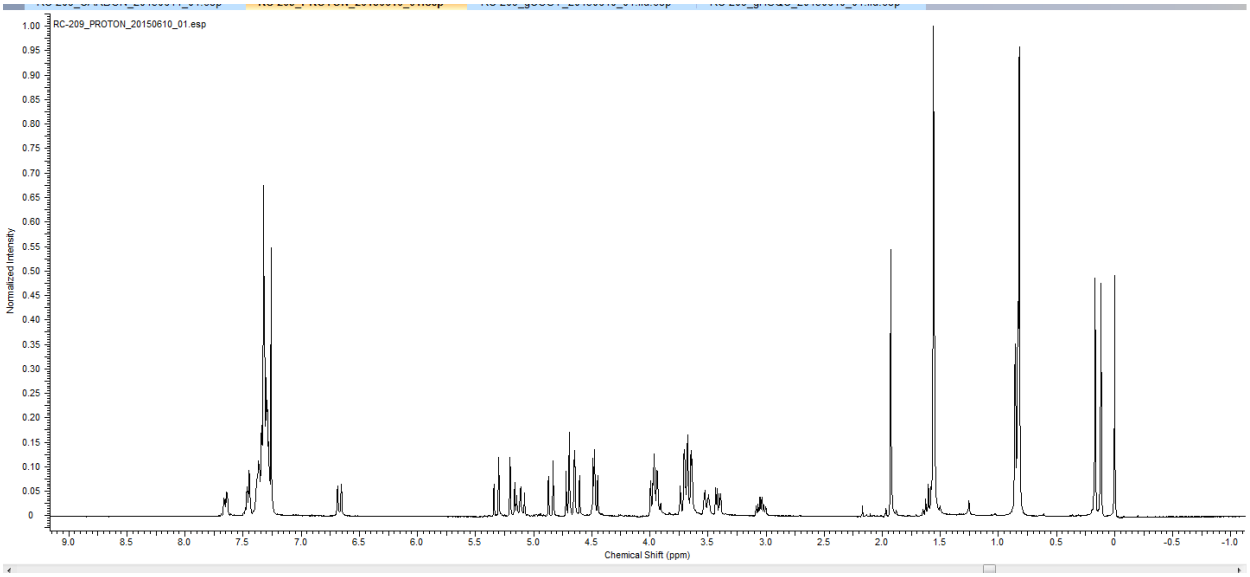
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



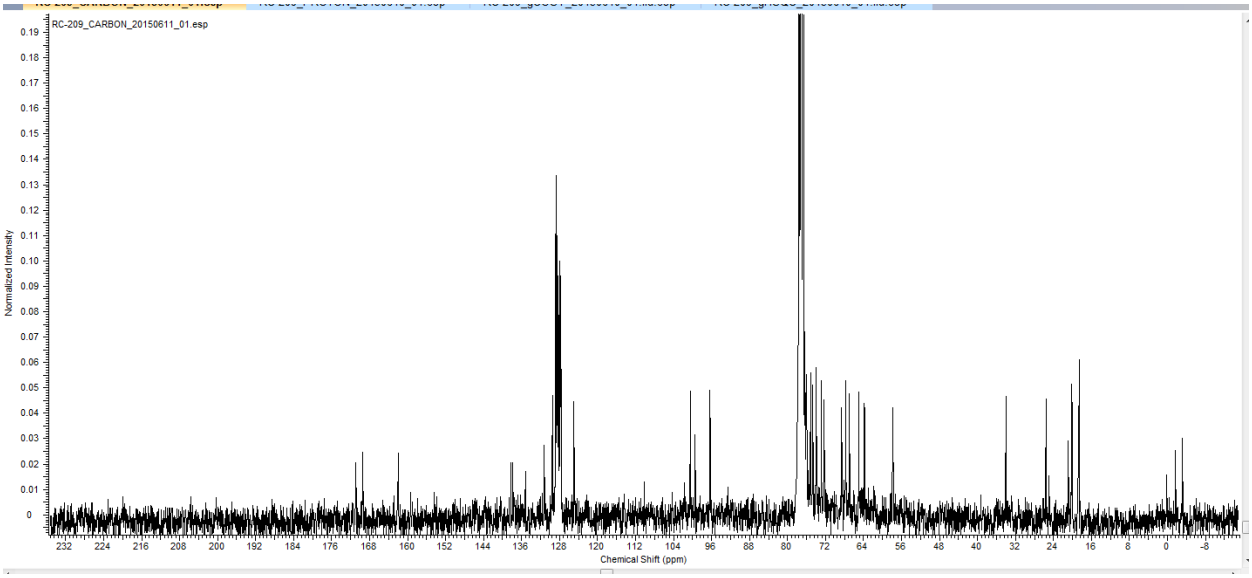


77a

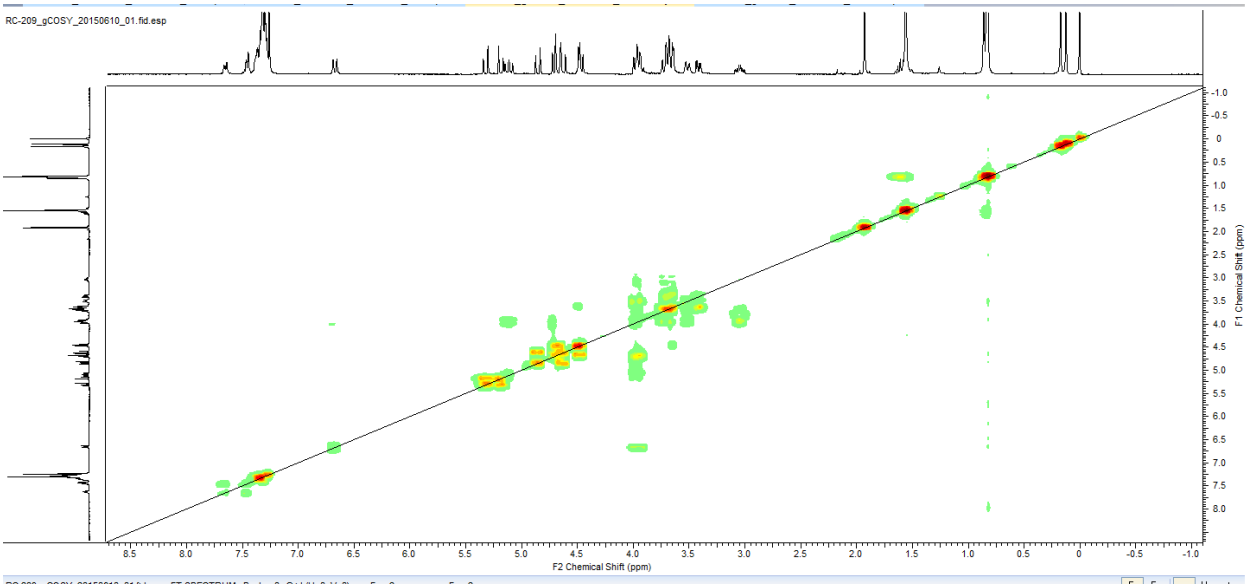
^{1}H NMR (300 MHz, $CDCl_3$)



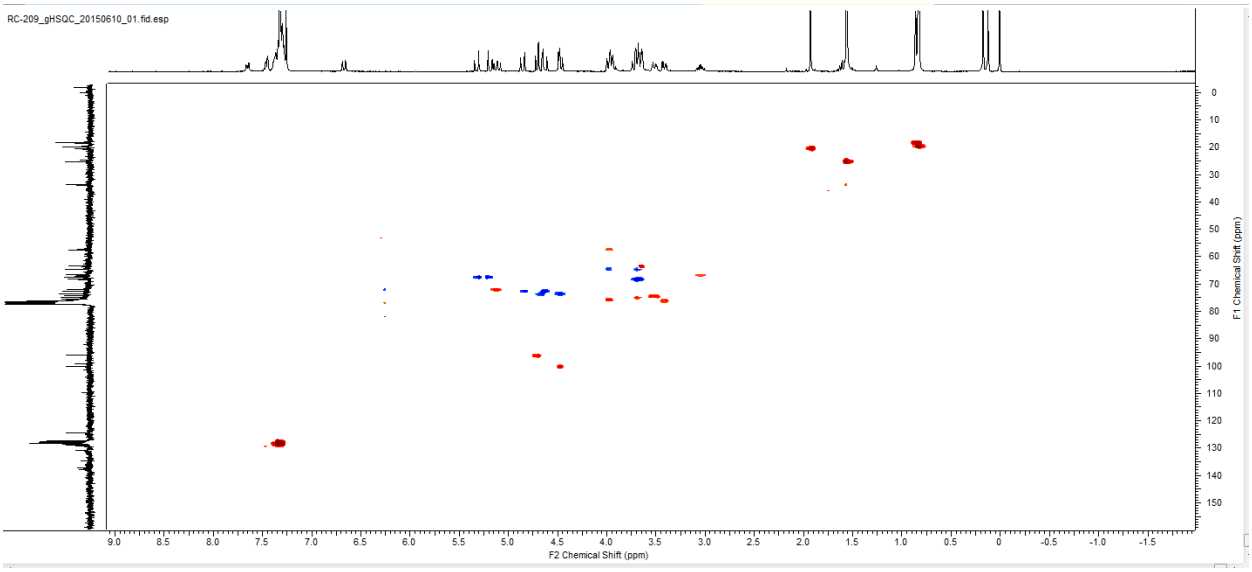
^{13}C NMR (75 MHz, $CDCl_3$)

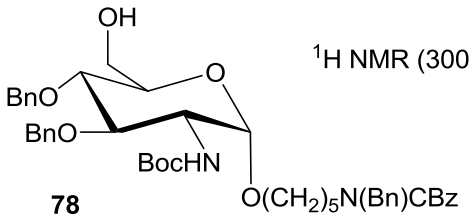


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



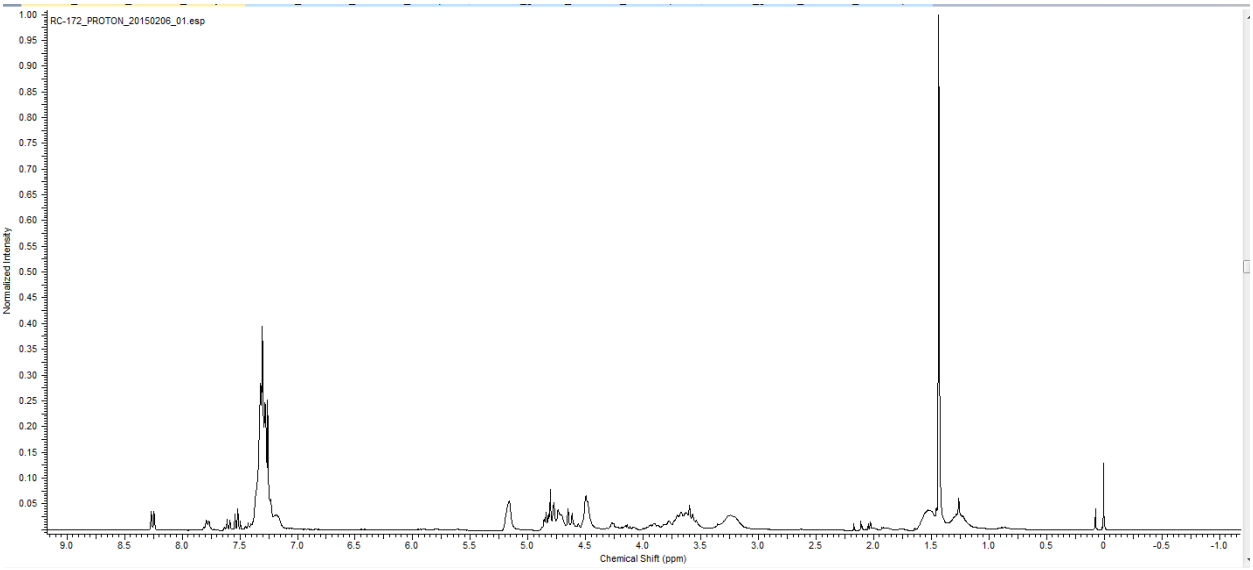
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



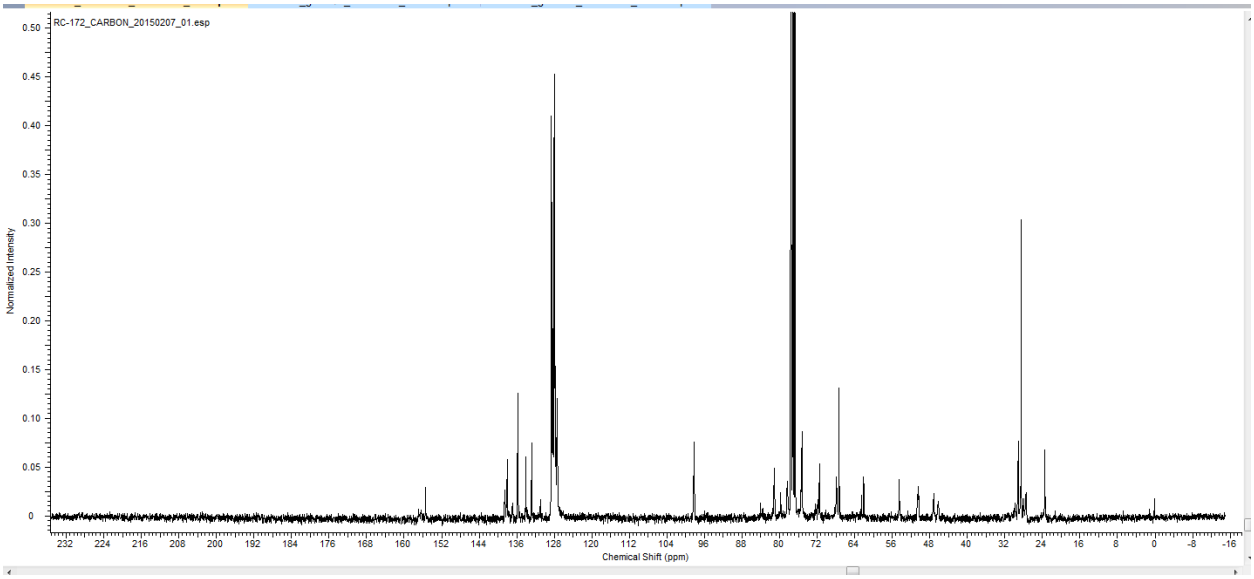


78

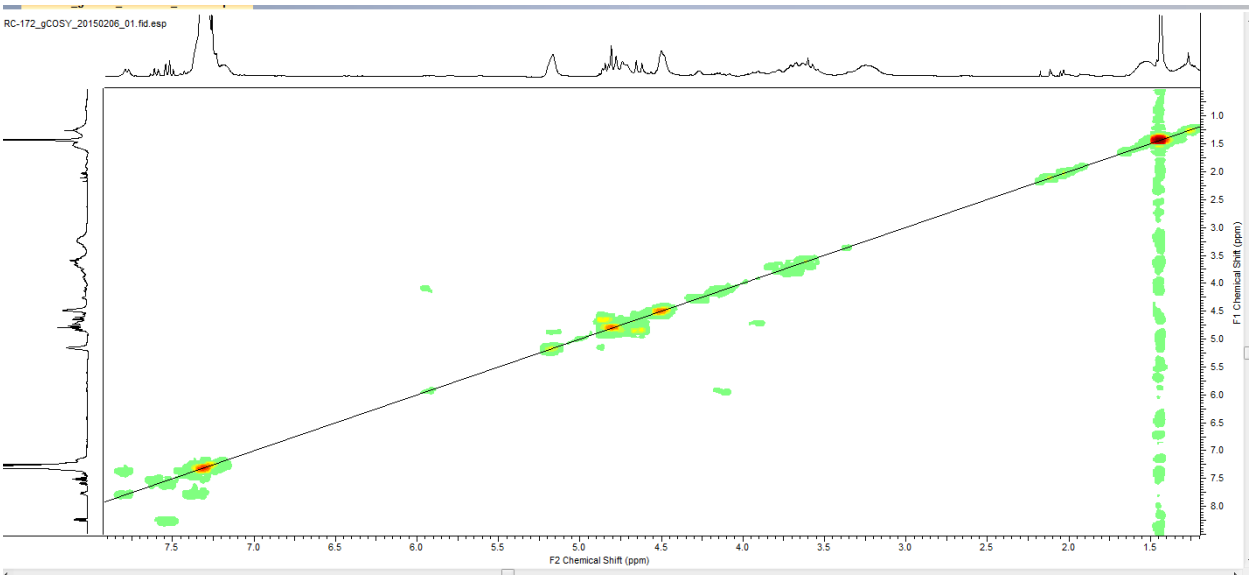
^{1}H NMR (300 MHz, $CDCl_3$)



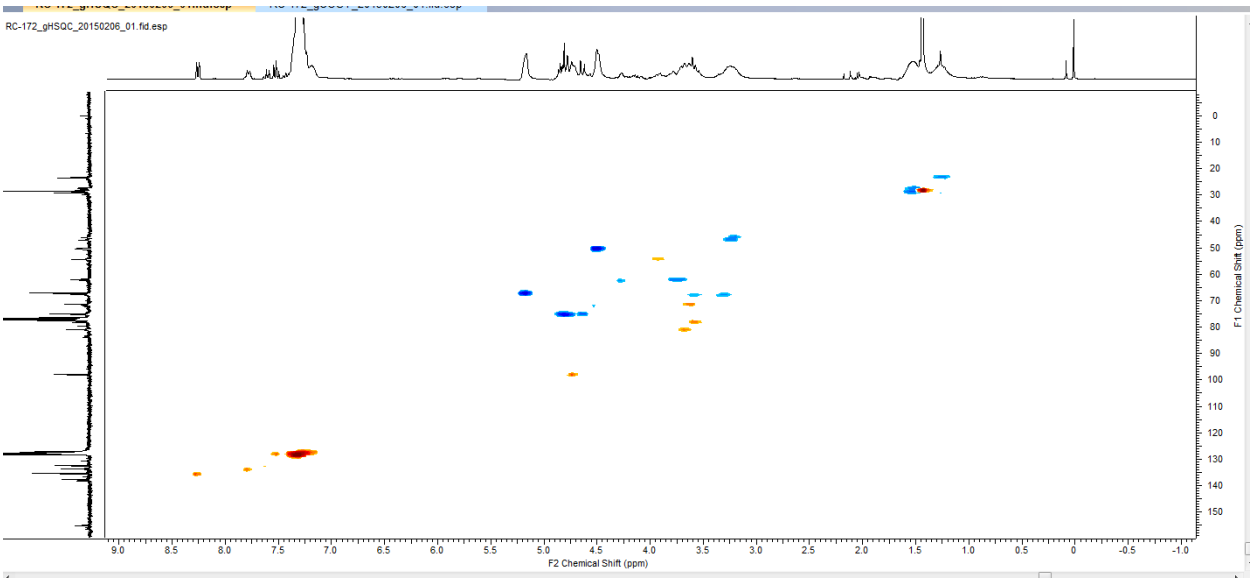
^{13}C NMR (75 MHz, $CDCl_3$)

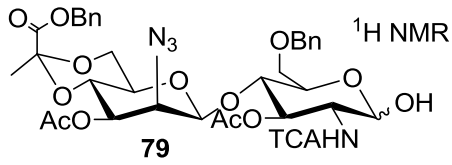


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



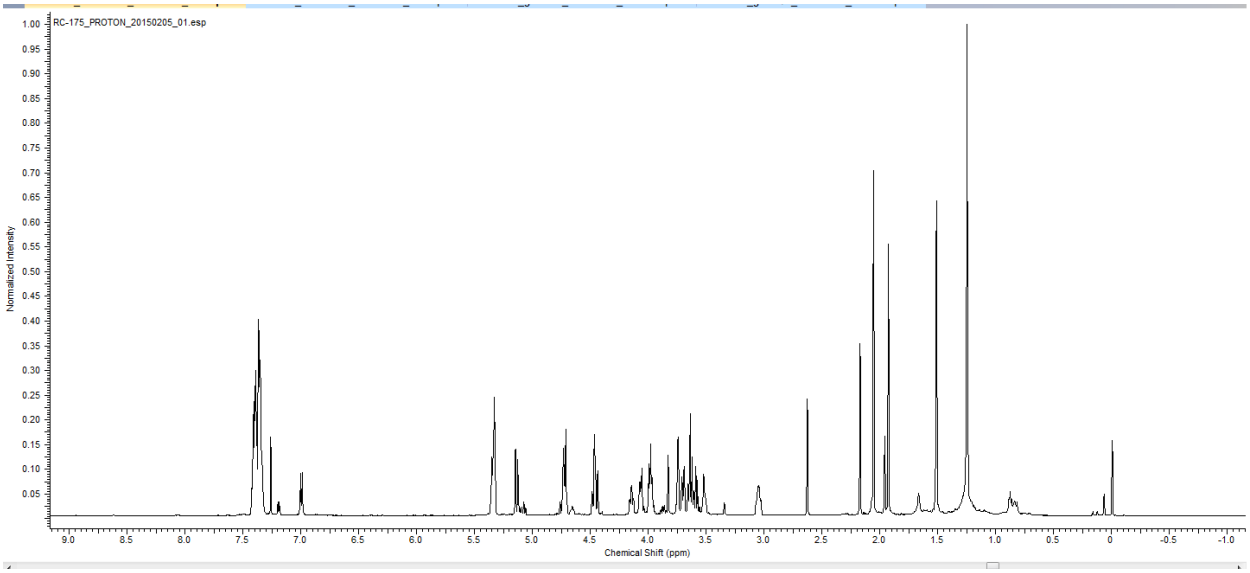
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



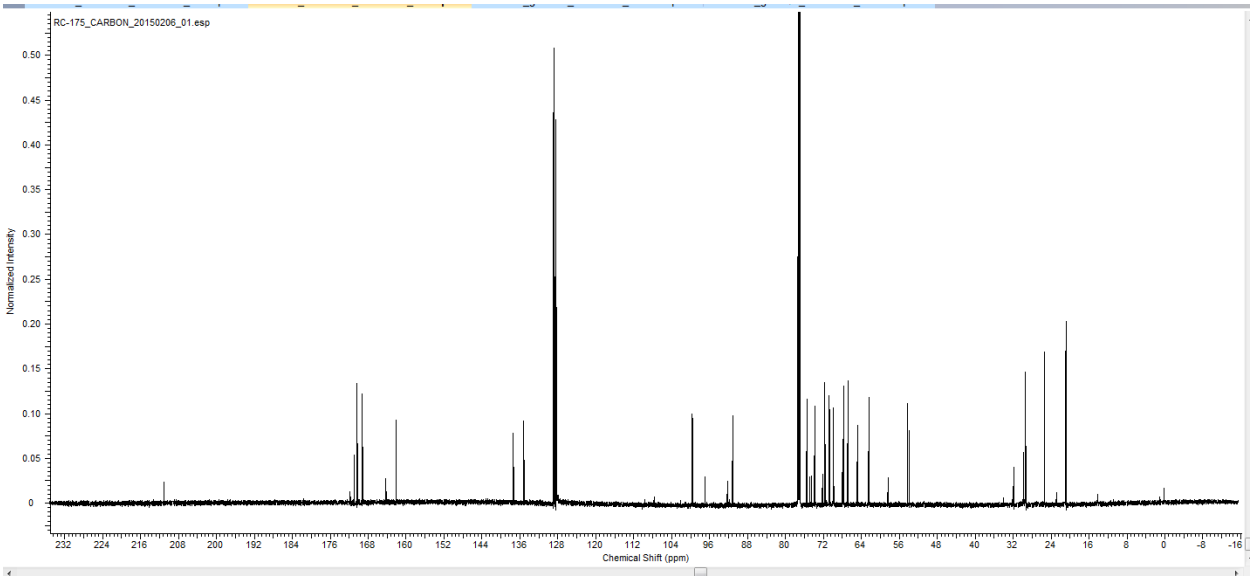


79

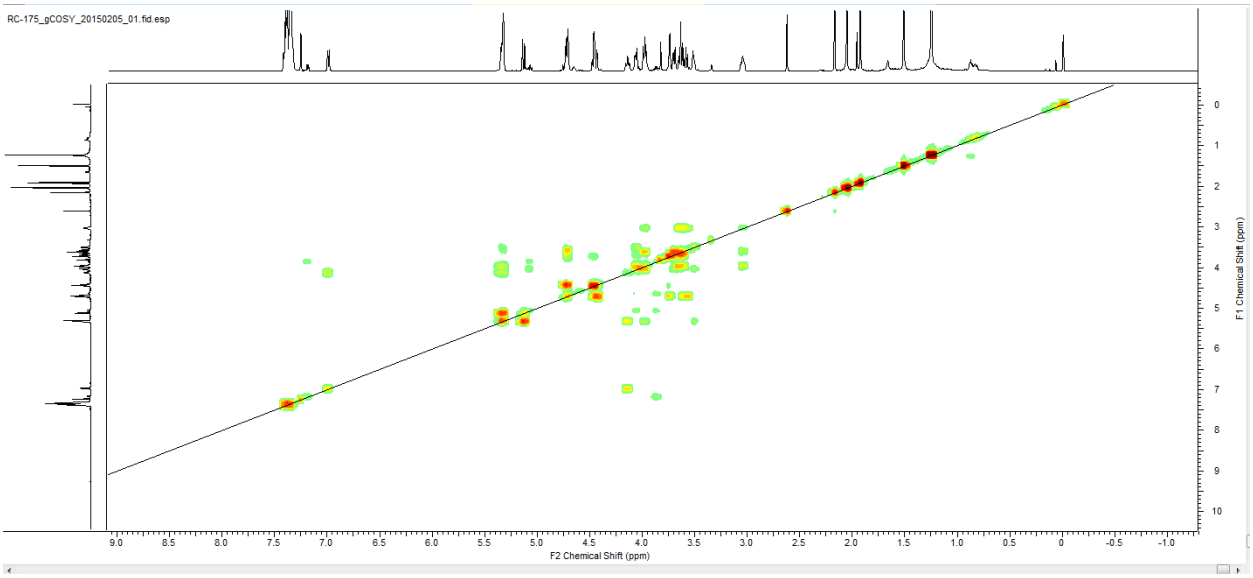
^{1}H NMR (600 MHz, $CDCl_3$)



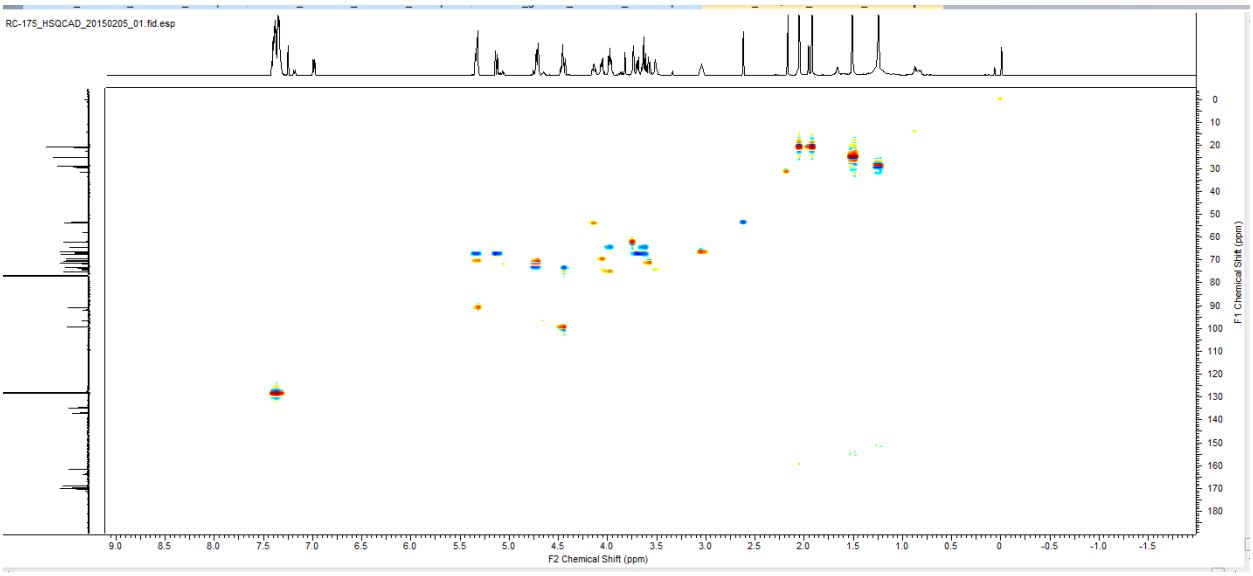
^{13}C NMR (150 MHz, $CDCl_3$)

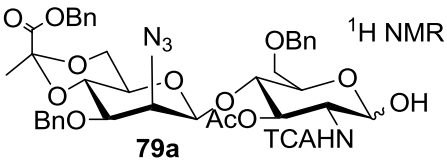


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



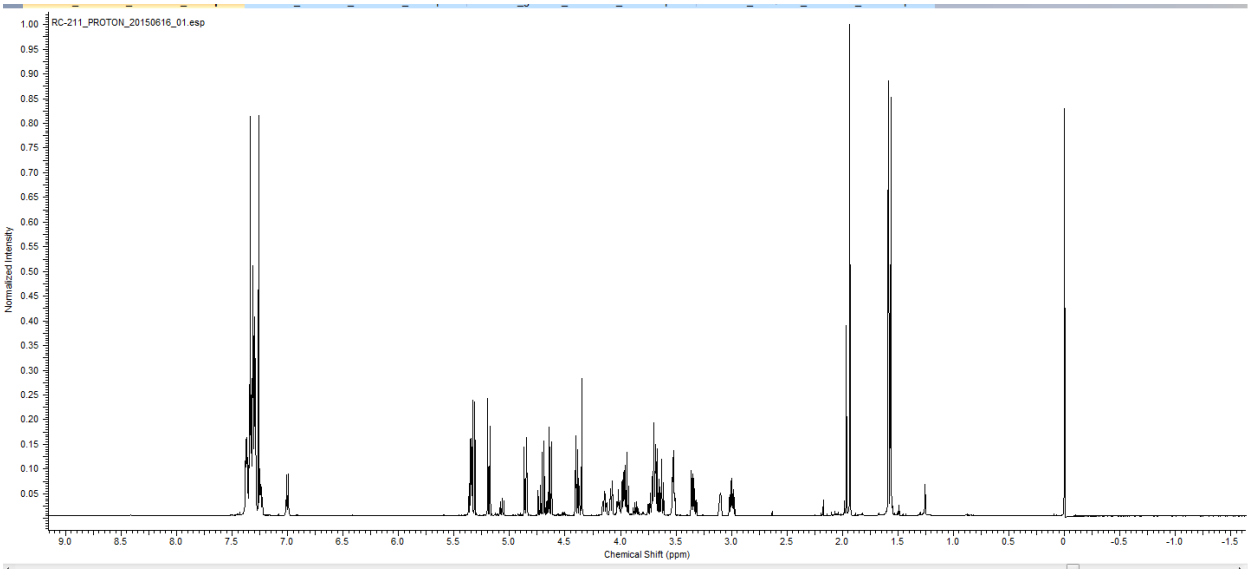
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



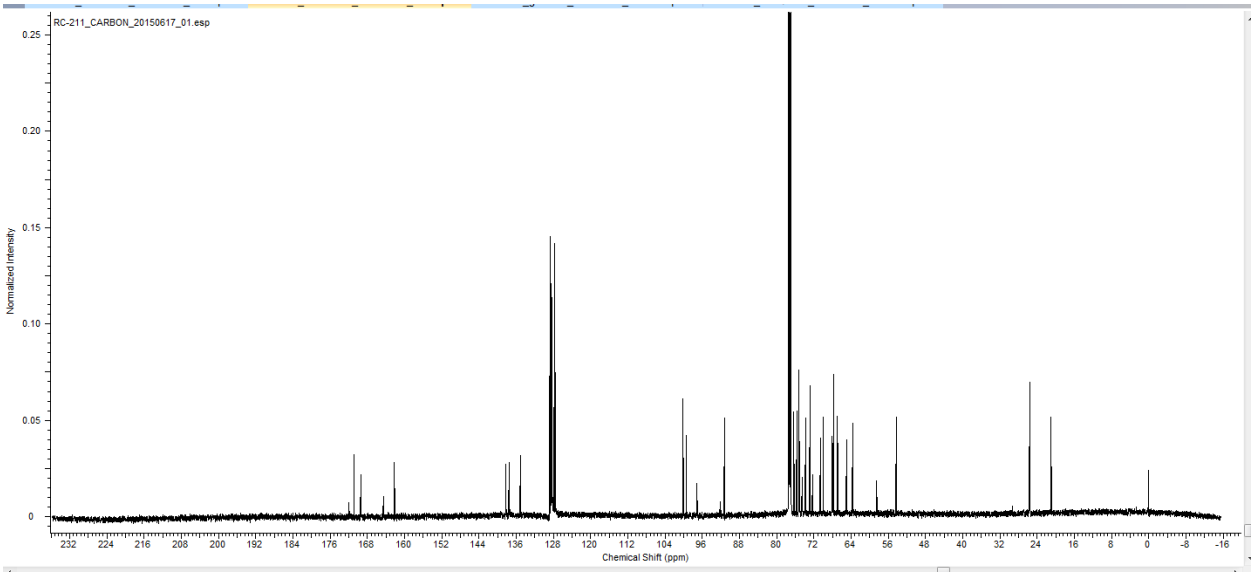


79a

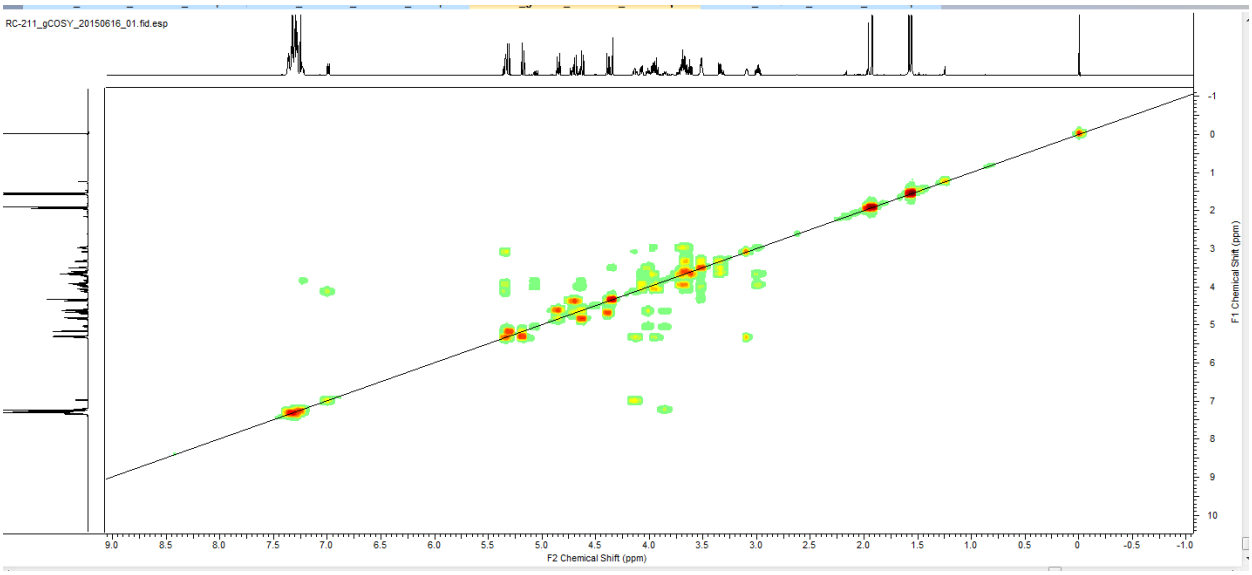
^{1}H NMR (600 MHz, $CDCl_3$)



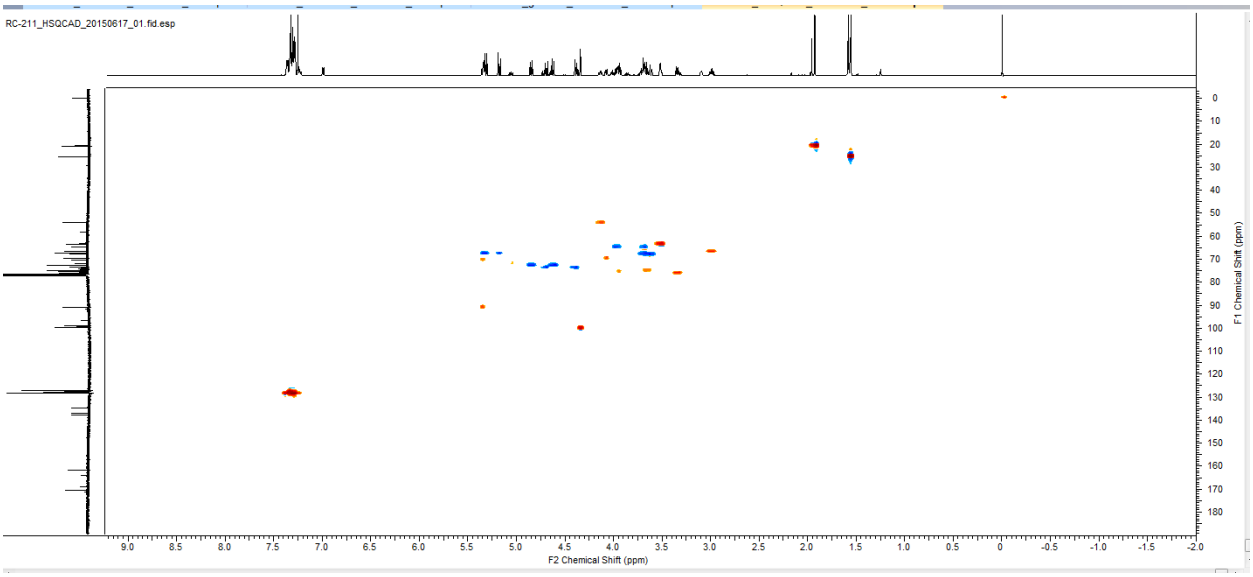
^{13}C NMR (150 MHz, $CDCl_3$)

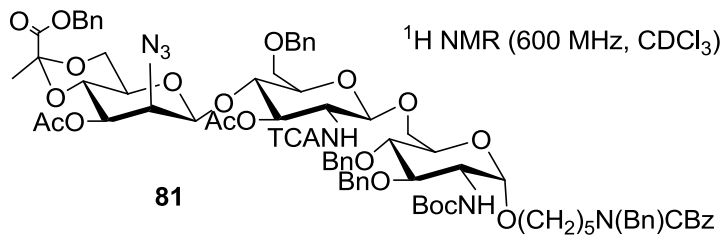


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



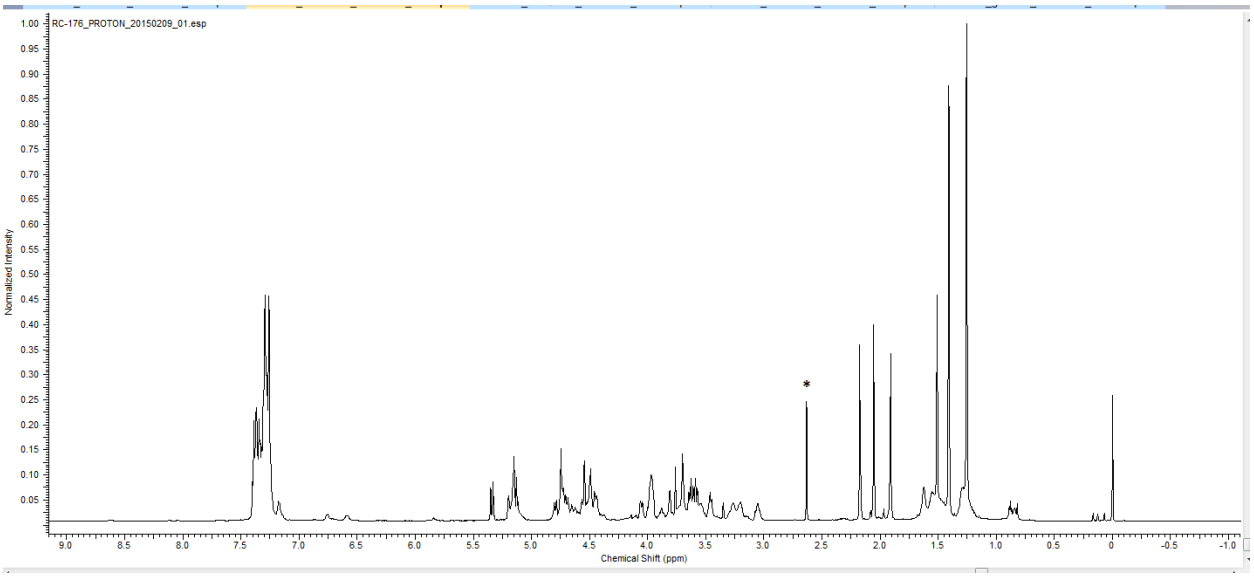
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



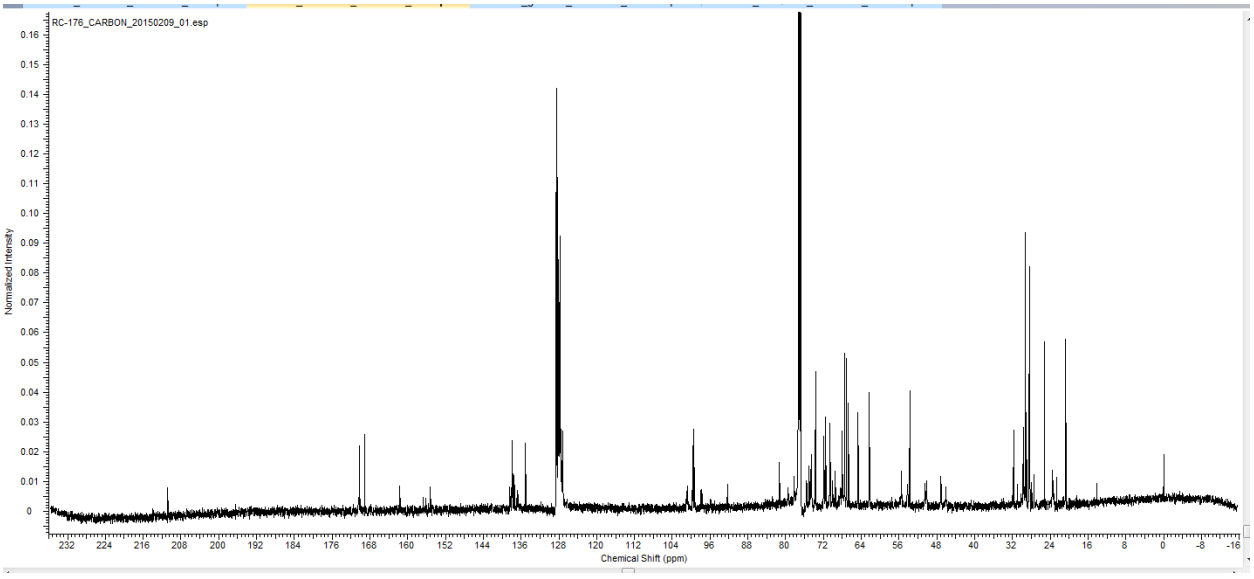


81

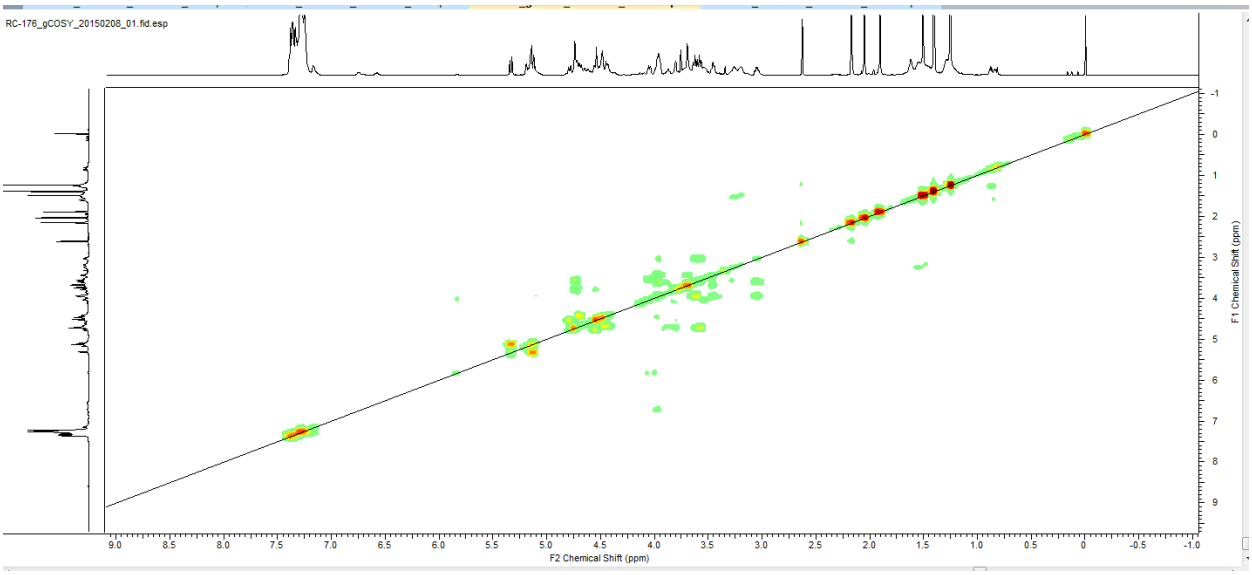
^{1}H NMR (600 MHz, $CDCl_3$)



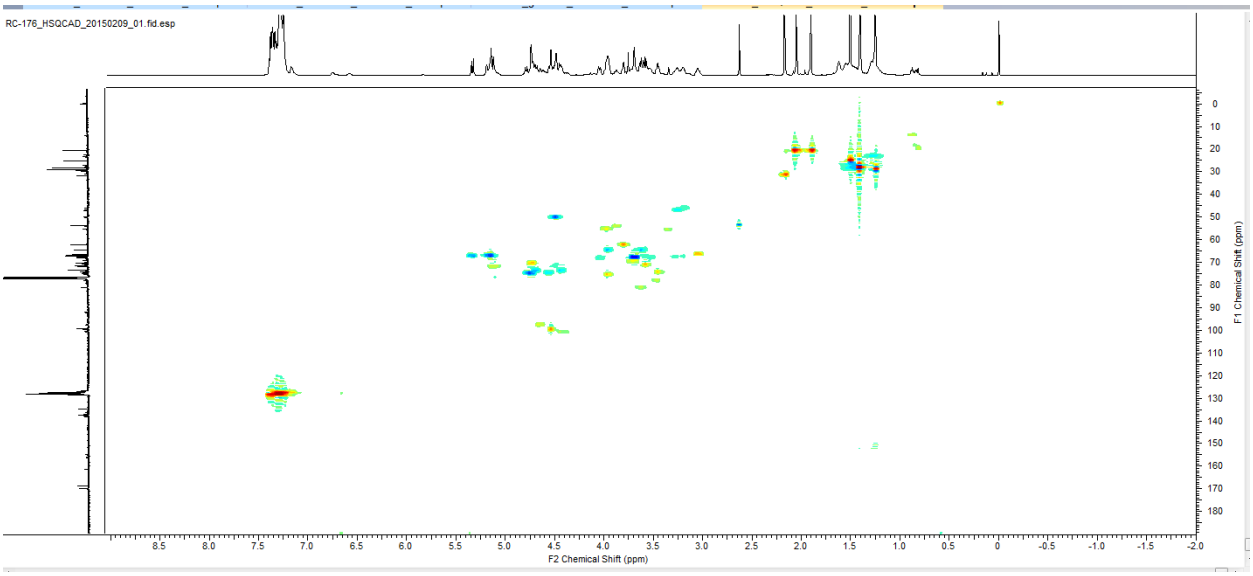
^{13}C NMR (150 MHz, $CDCl_3$)

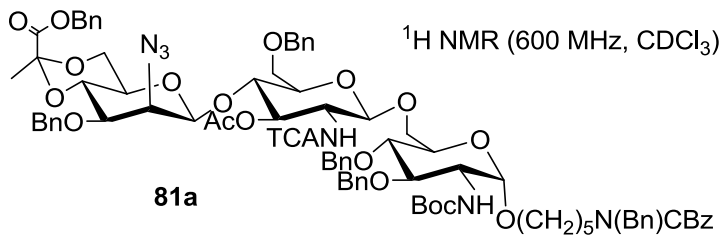


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



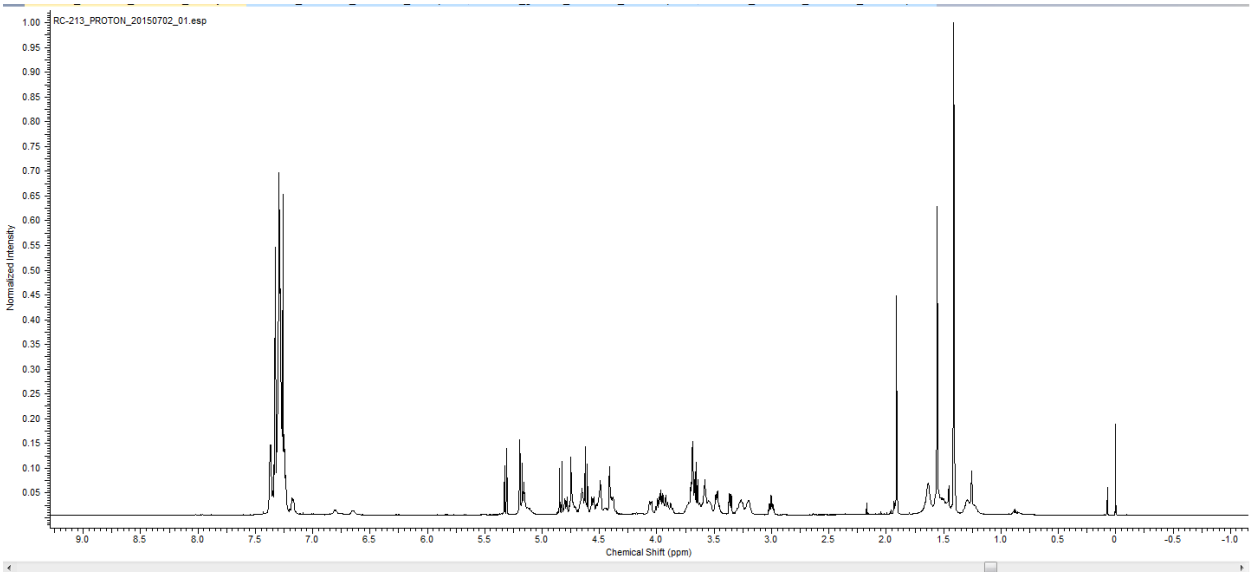
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



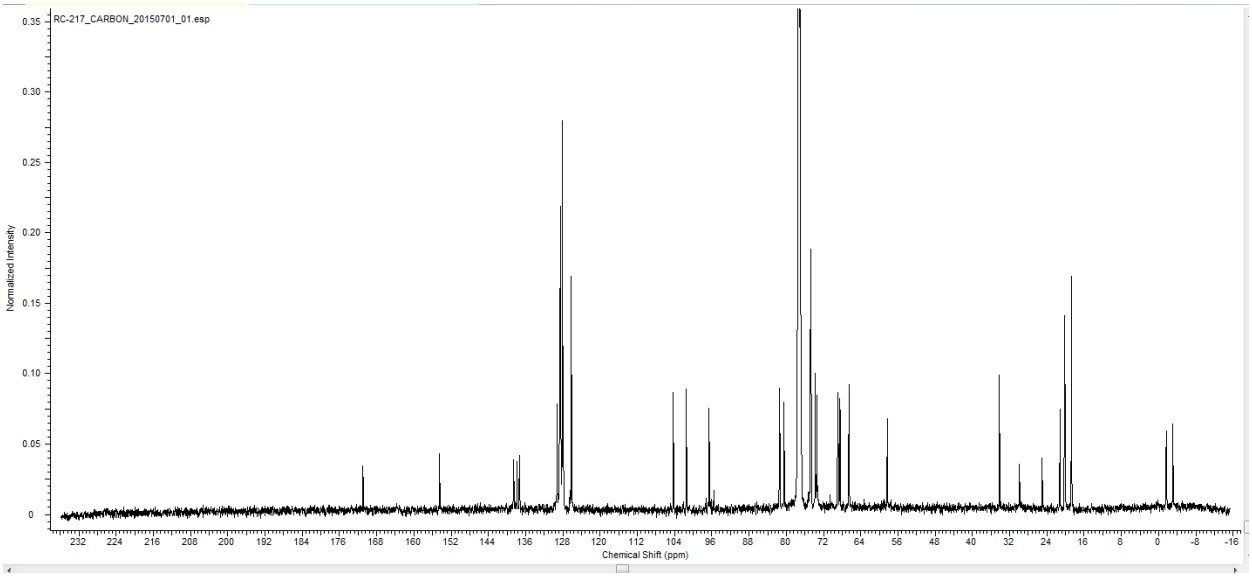


81a

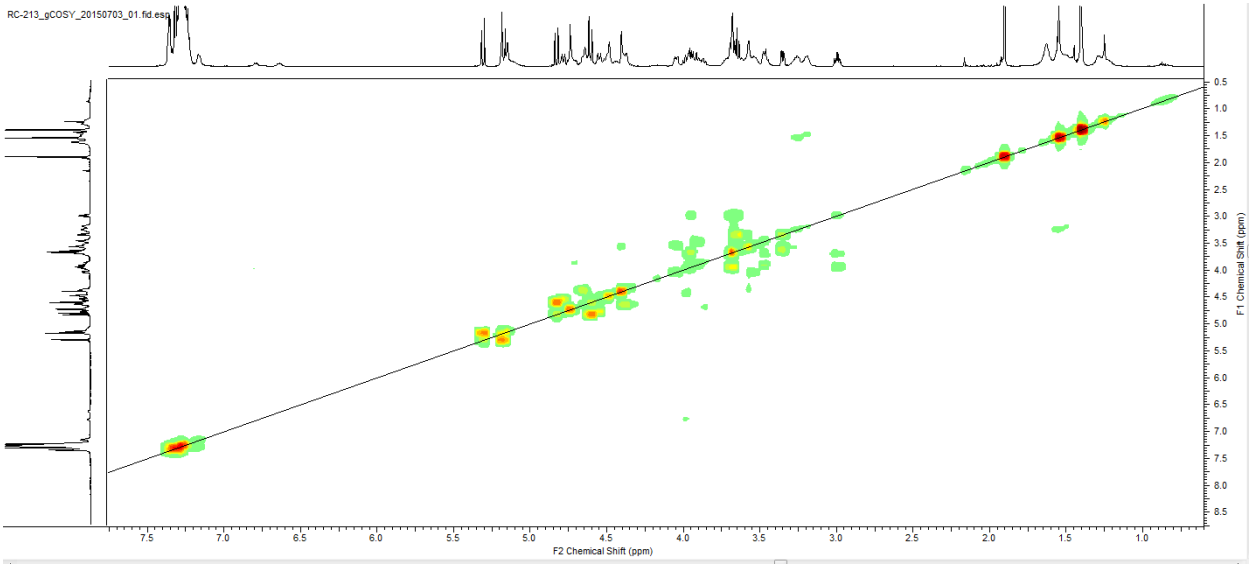
^{1}H NMR (600 MHz, $CDCl_3$)



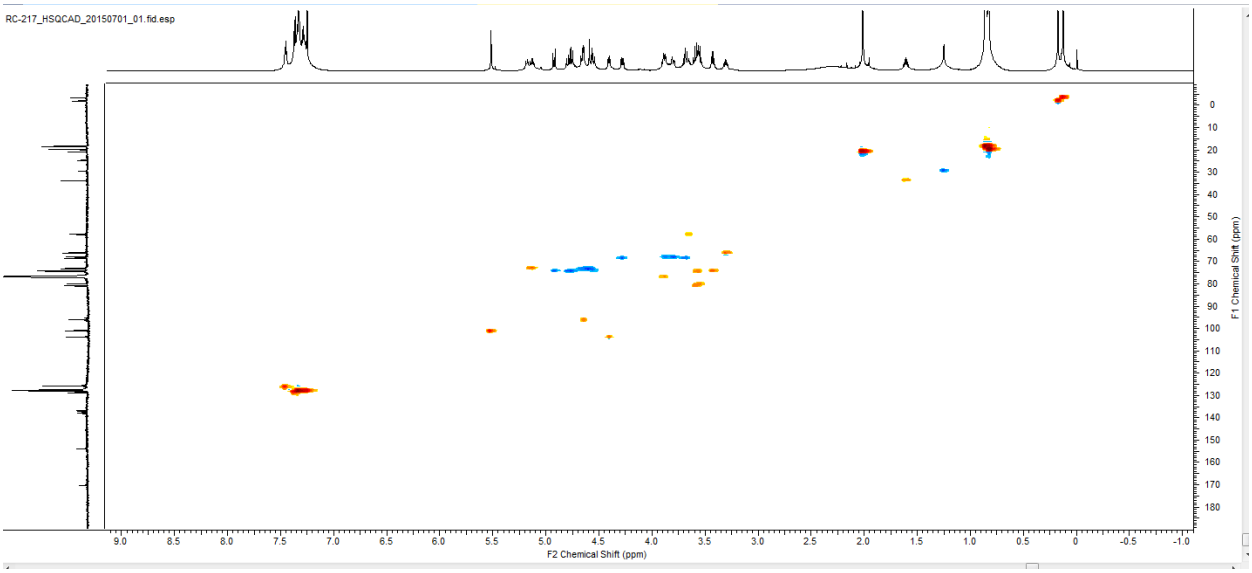
^{13}C NMR (150 MHz, $CDCl_3$)

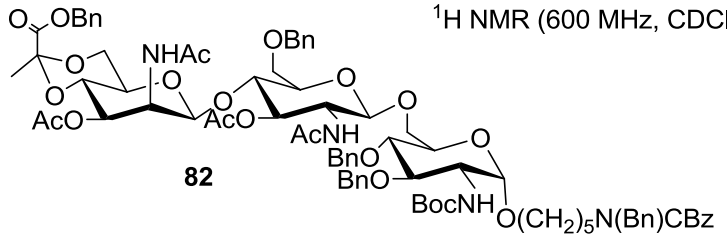


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



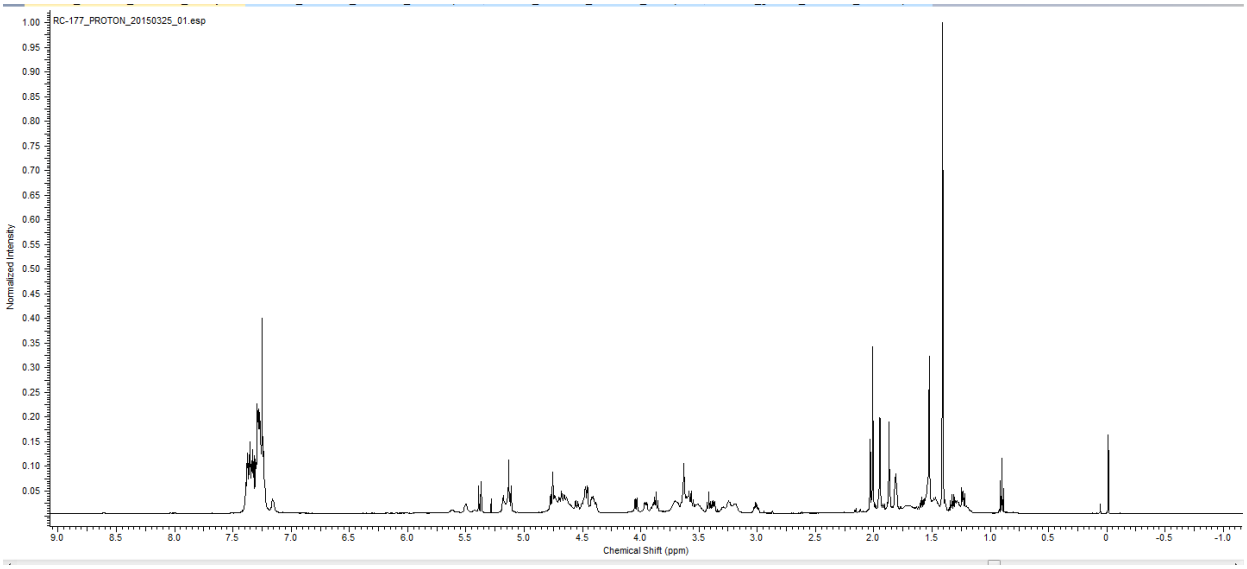
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



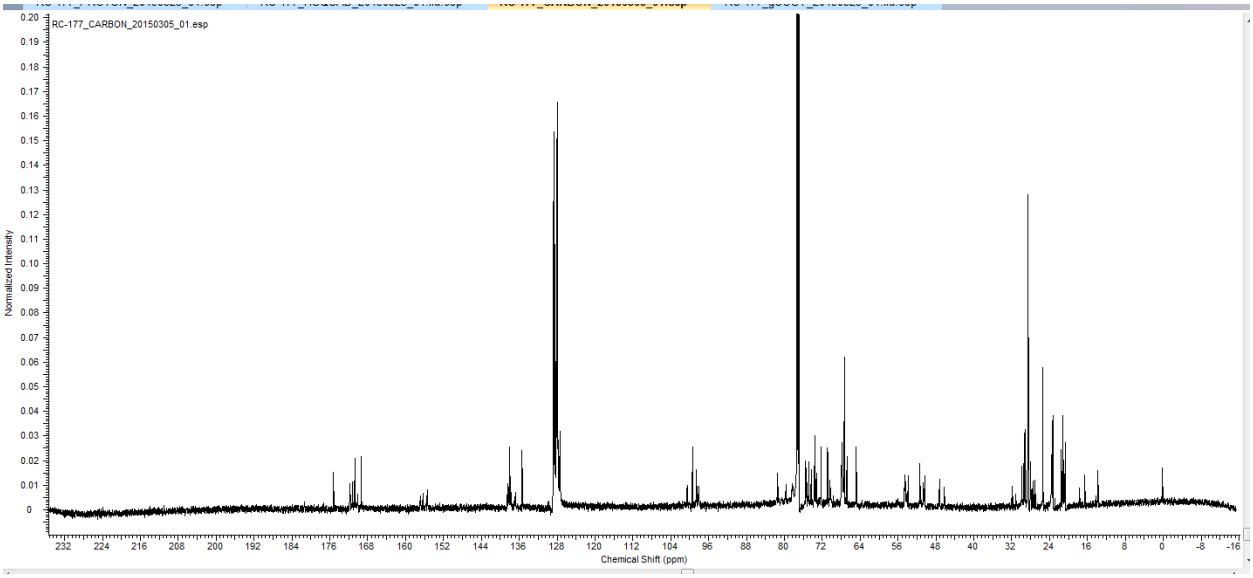


82

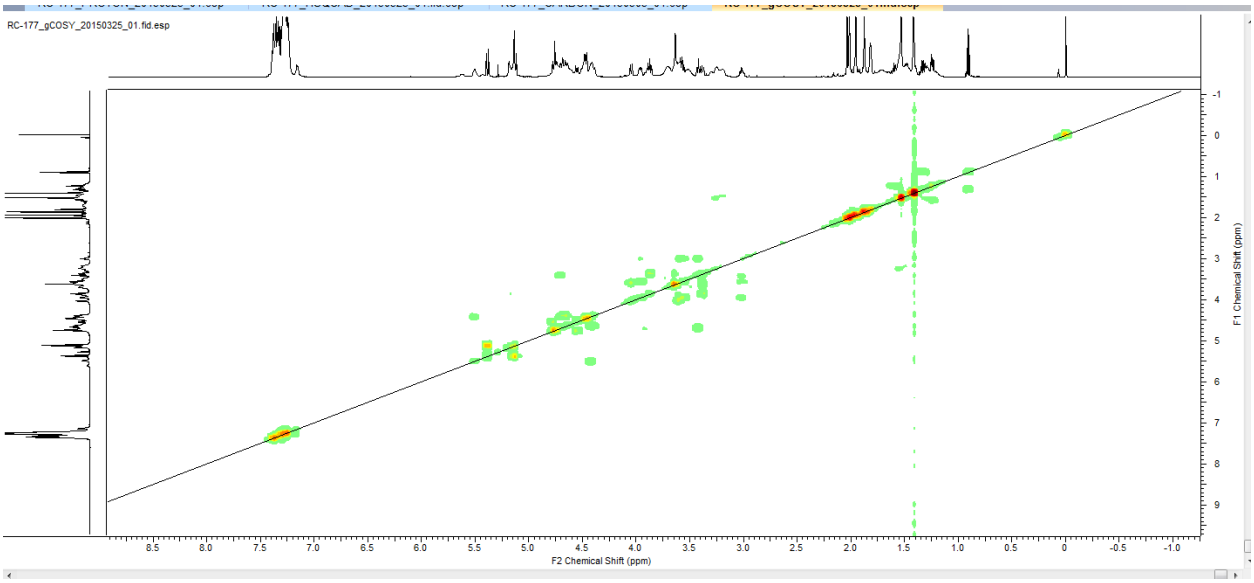
^{1}H NMR (600 MHz, $CDCl_3$)



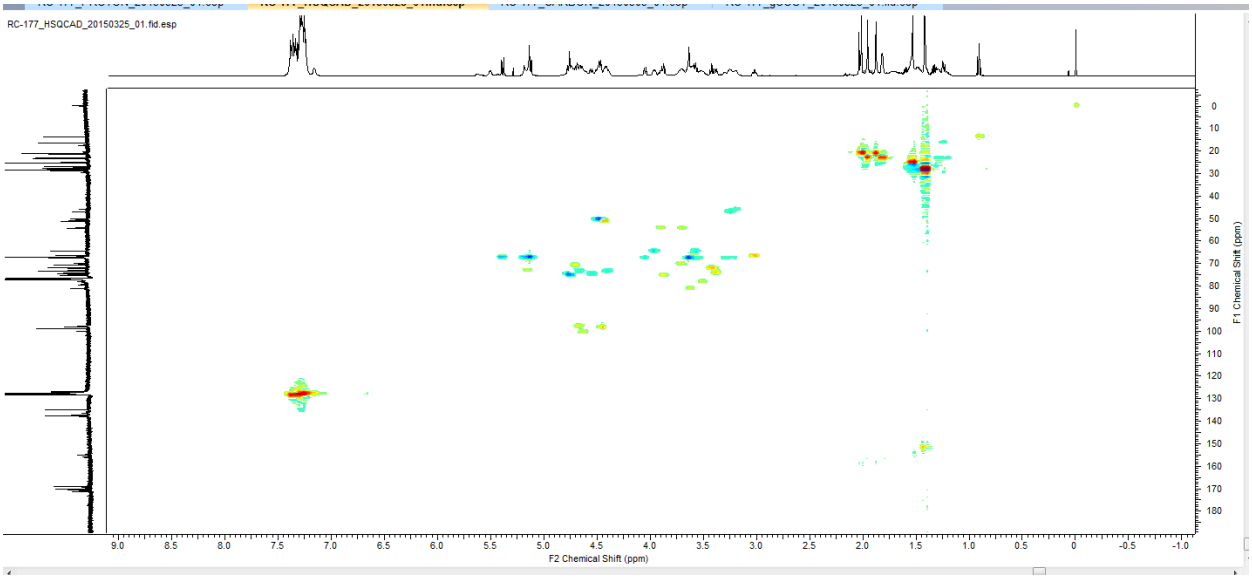
^{13}C NMR (150 MHz, $CDCl_3$)

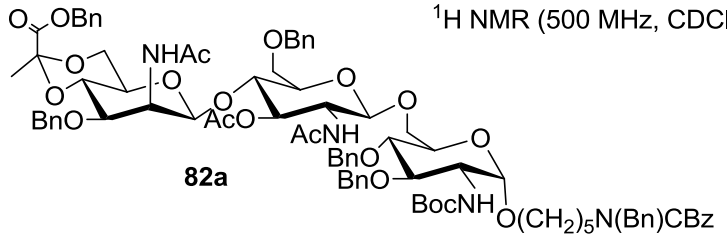


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



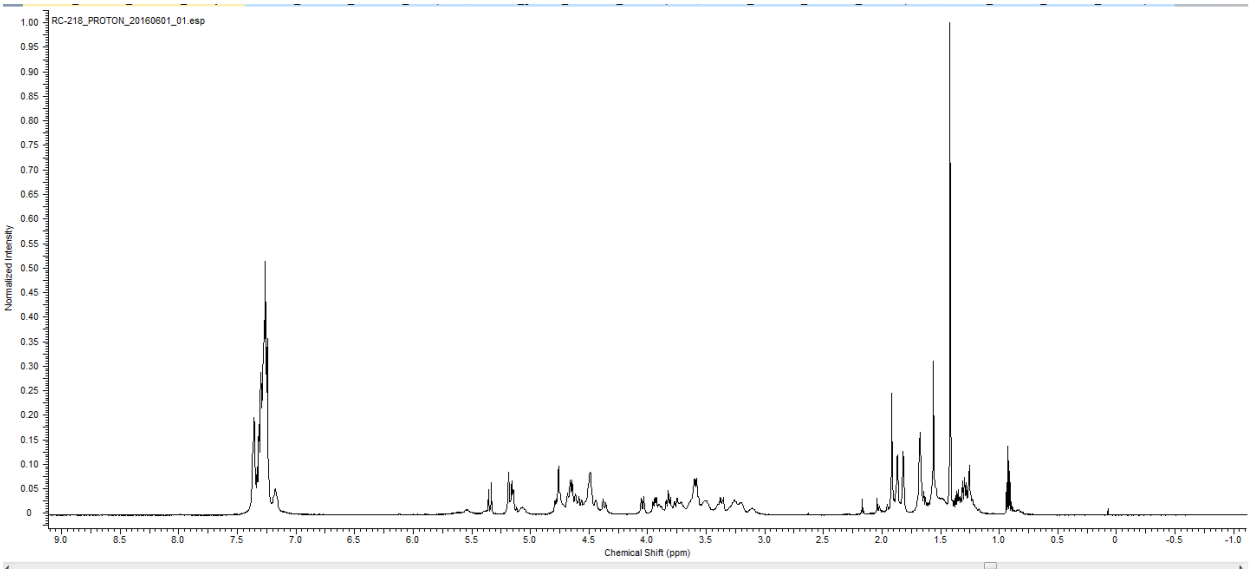
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



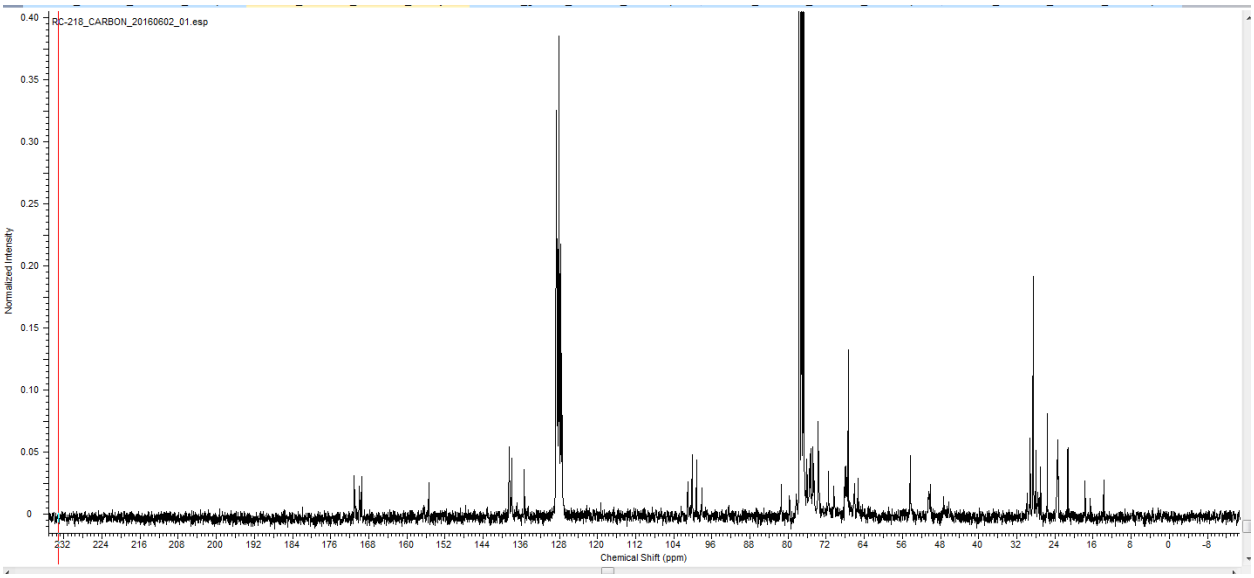


82a

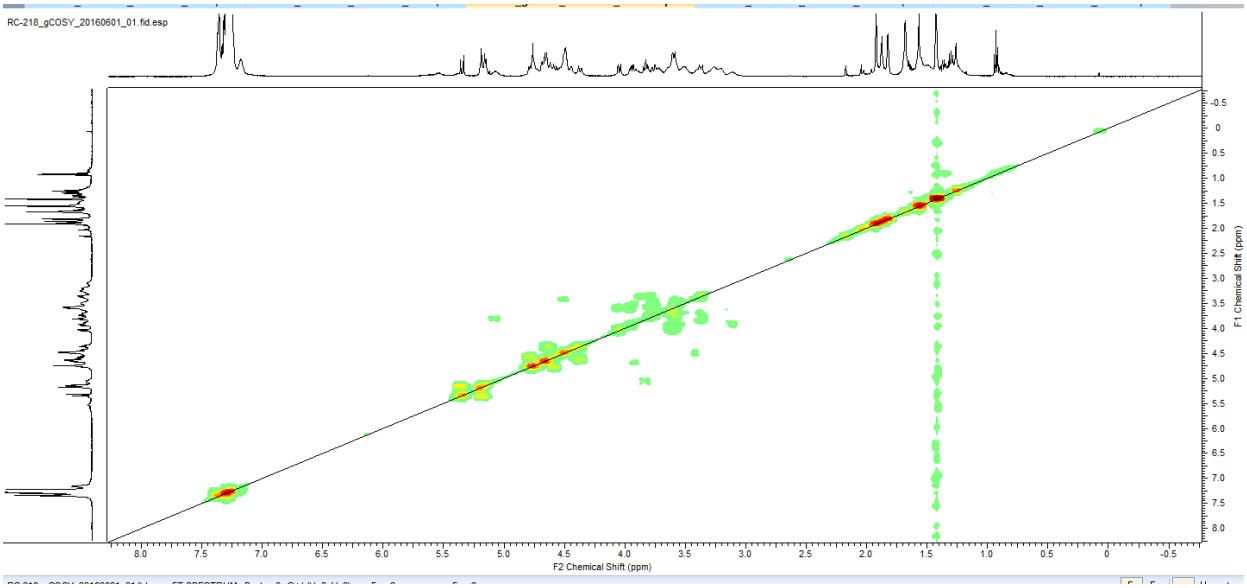
^{1}H NMR (500 MHz, $CDCl_3$)



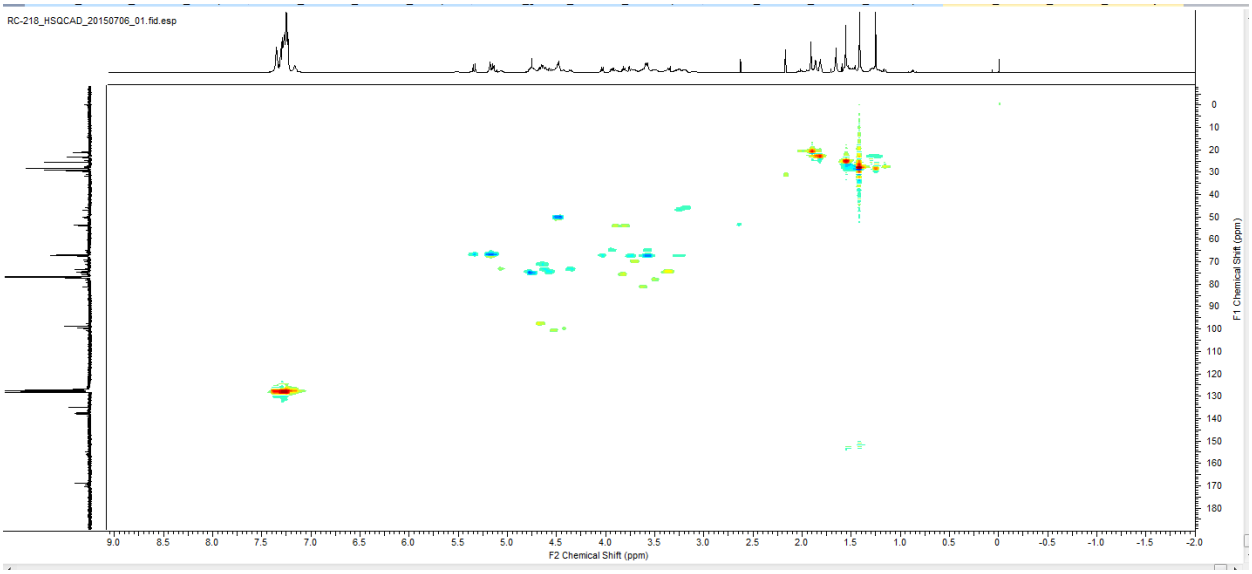
^{13}C NMR (75 MHz, $CDCl_3$)

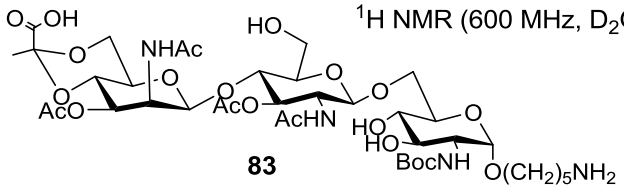


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



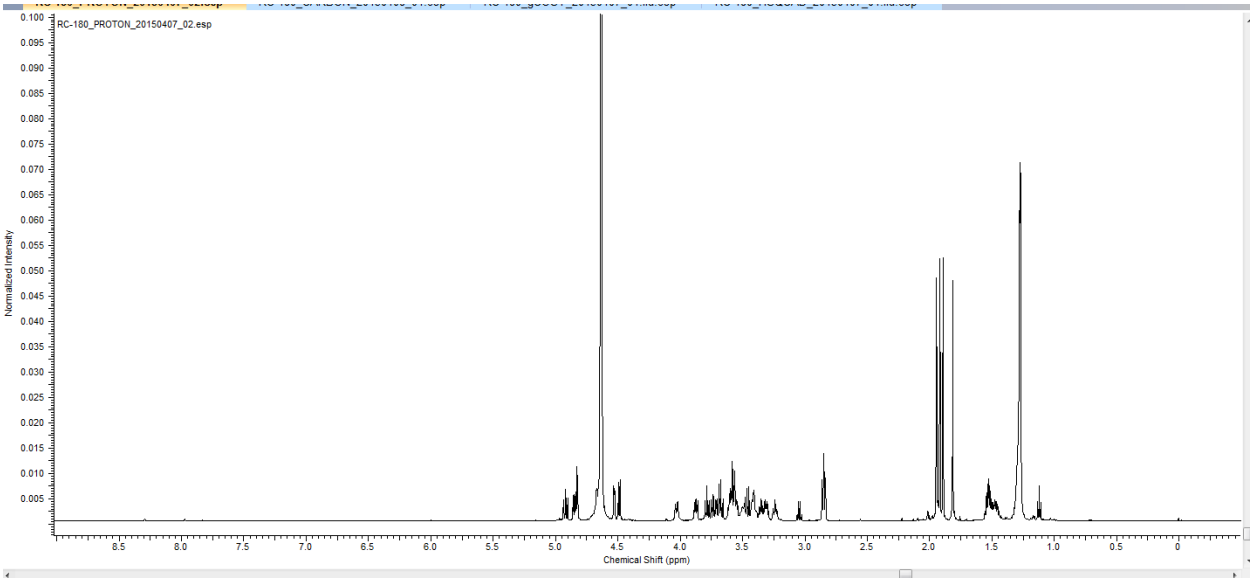
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



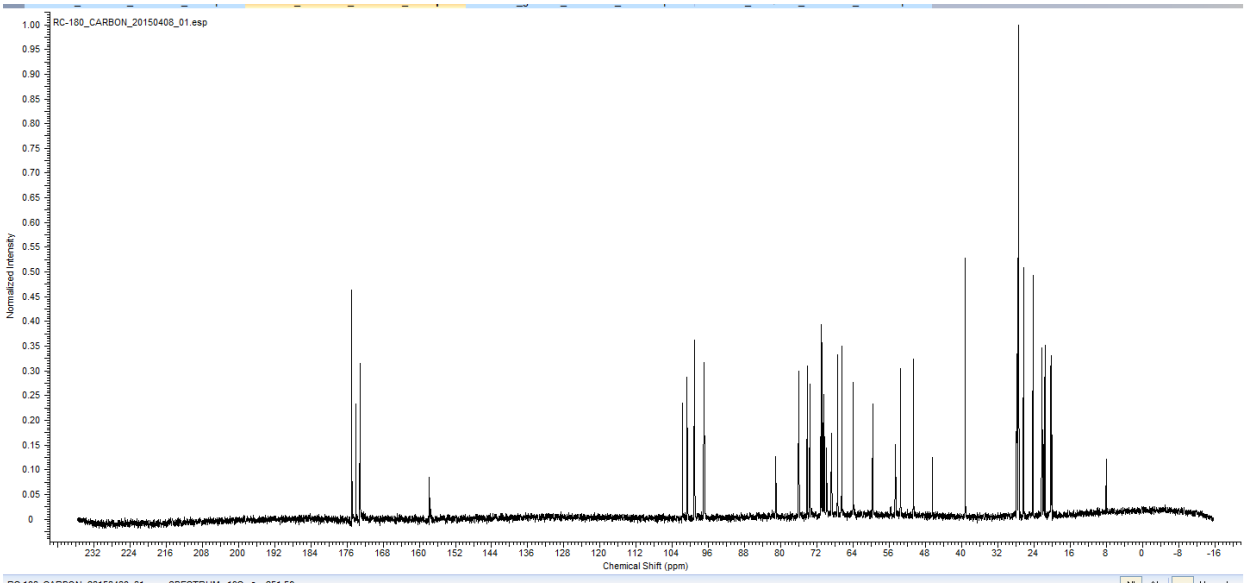


83

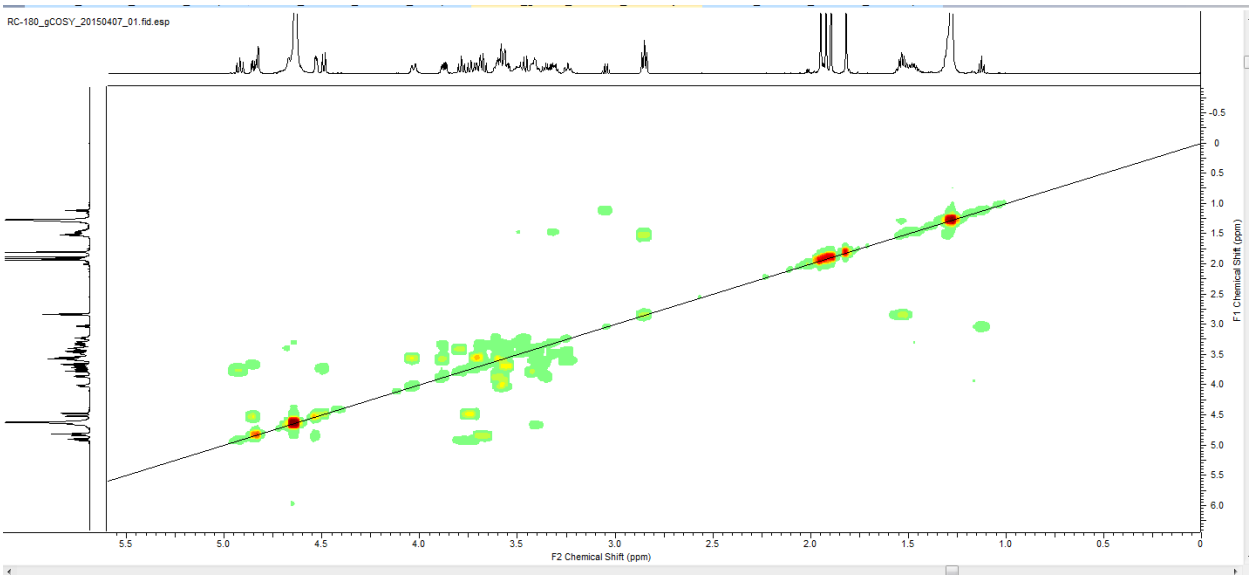
^{1}H NMR (600 MHz, D_2O)



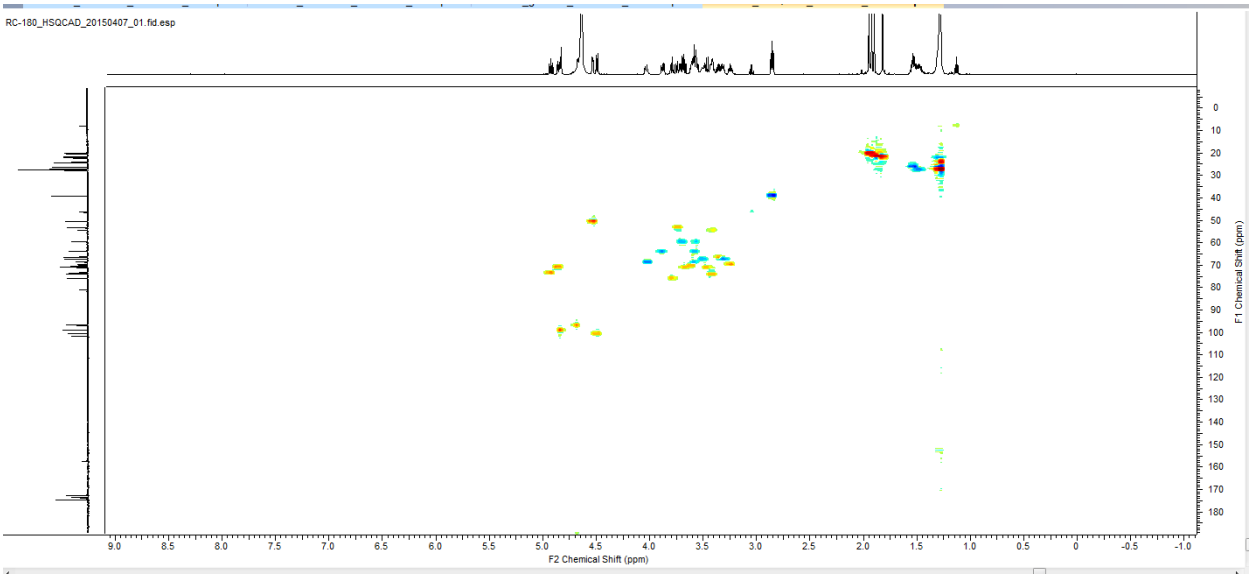
^{13}C NMR (150 MHz, D_2O)

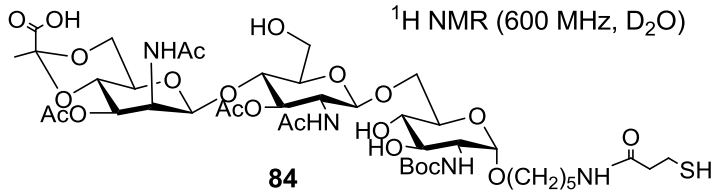


^{1}H-^{1}H COSY (600 MHz, D_2O)



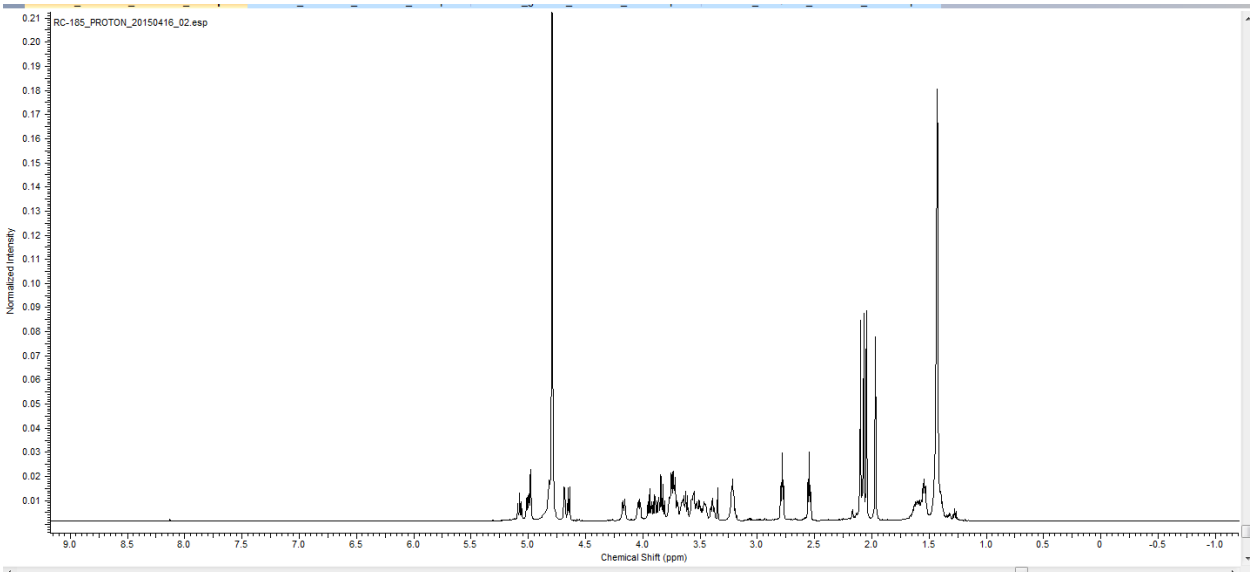
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



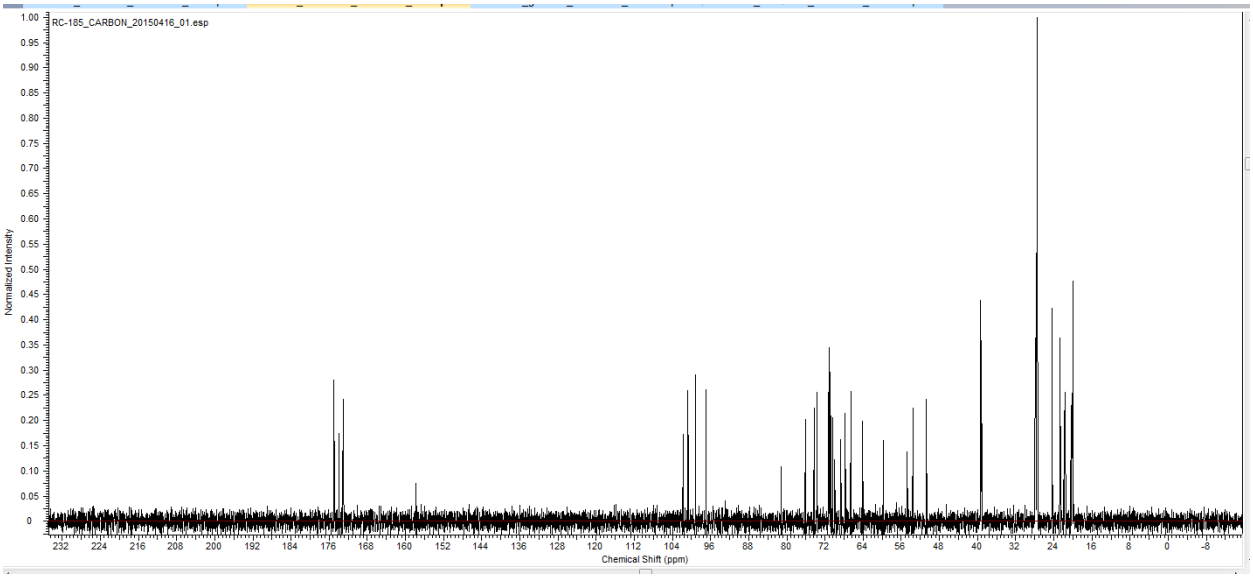


84

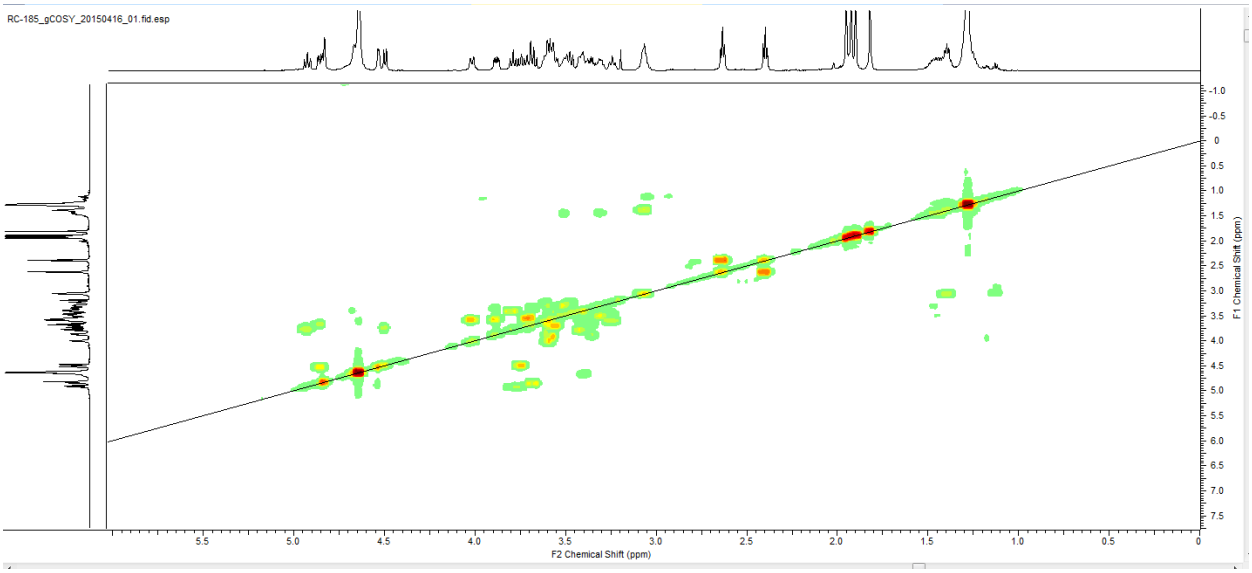
^{1}H NMR (600 MHz, D_2O)



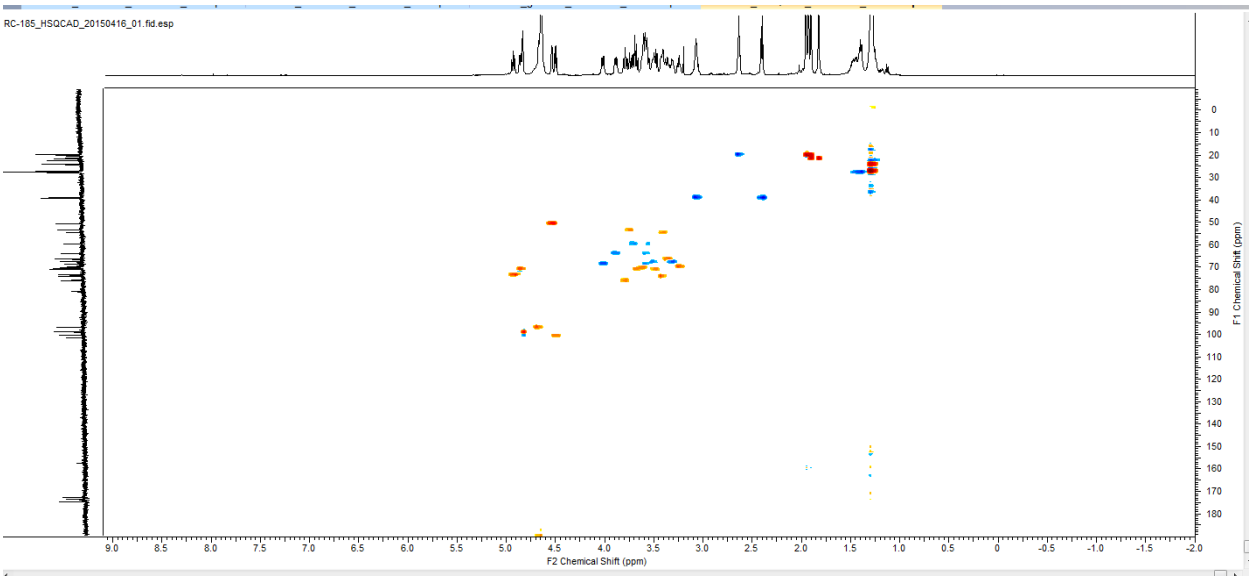
^{13}C NMR (150 MHz, D_2O)

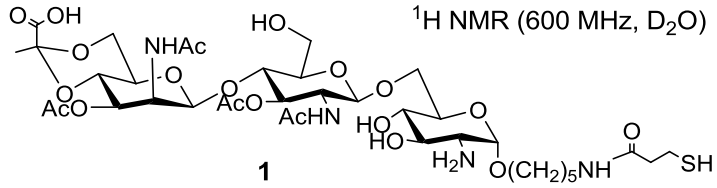


^{1}H-^{1}H COSY (600 MHz, D_2O)



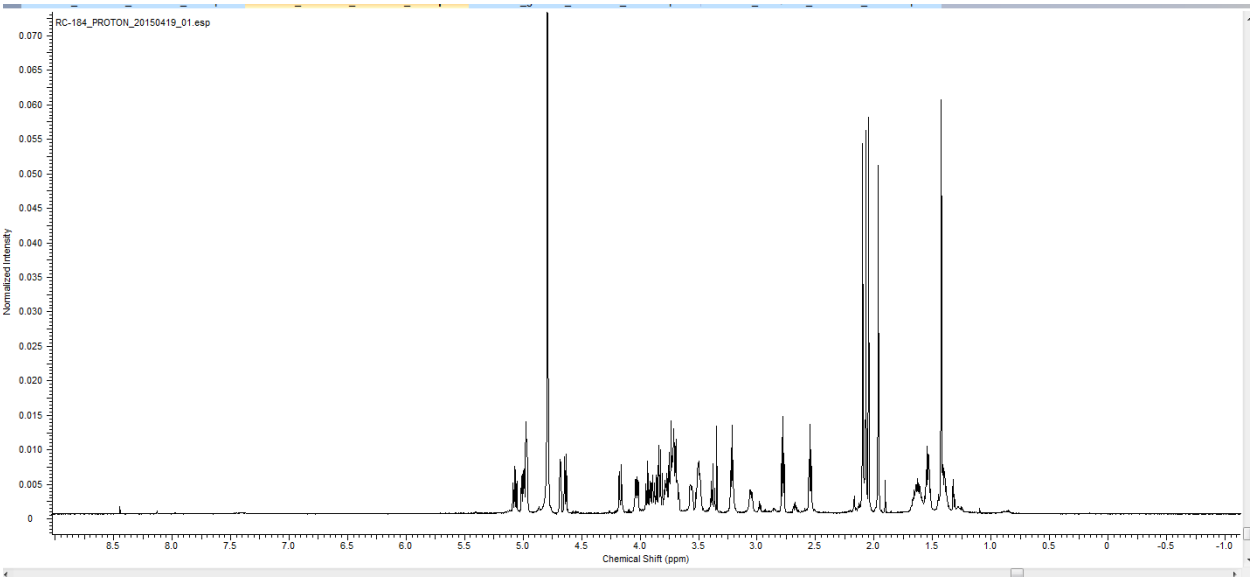
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



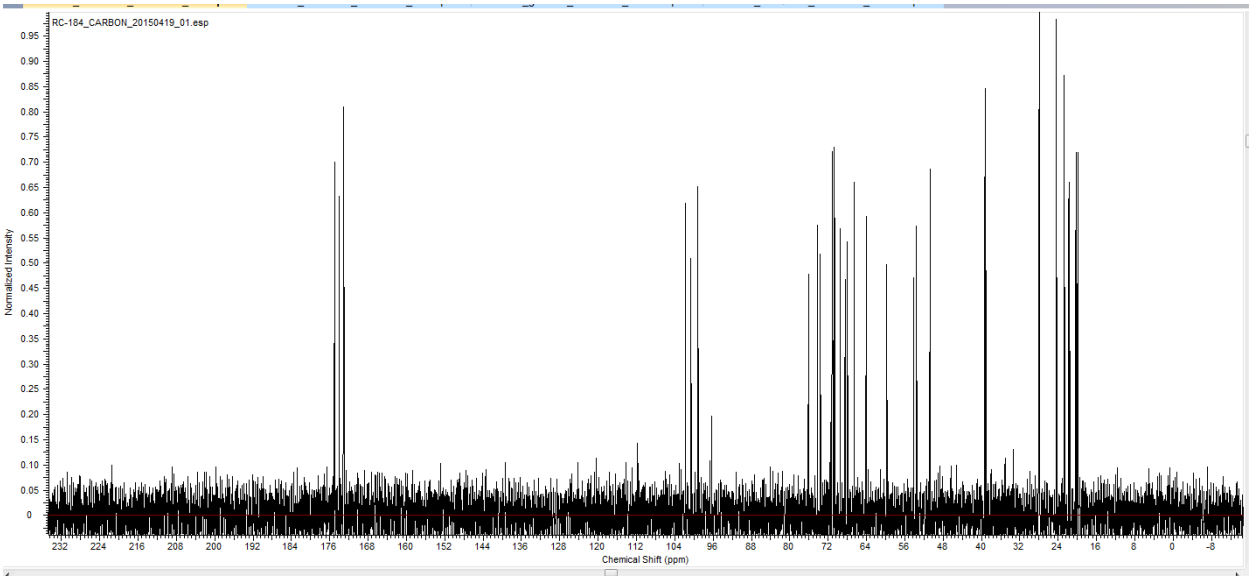


1

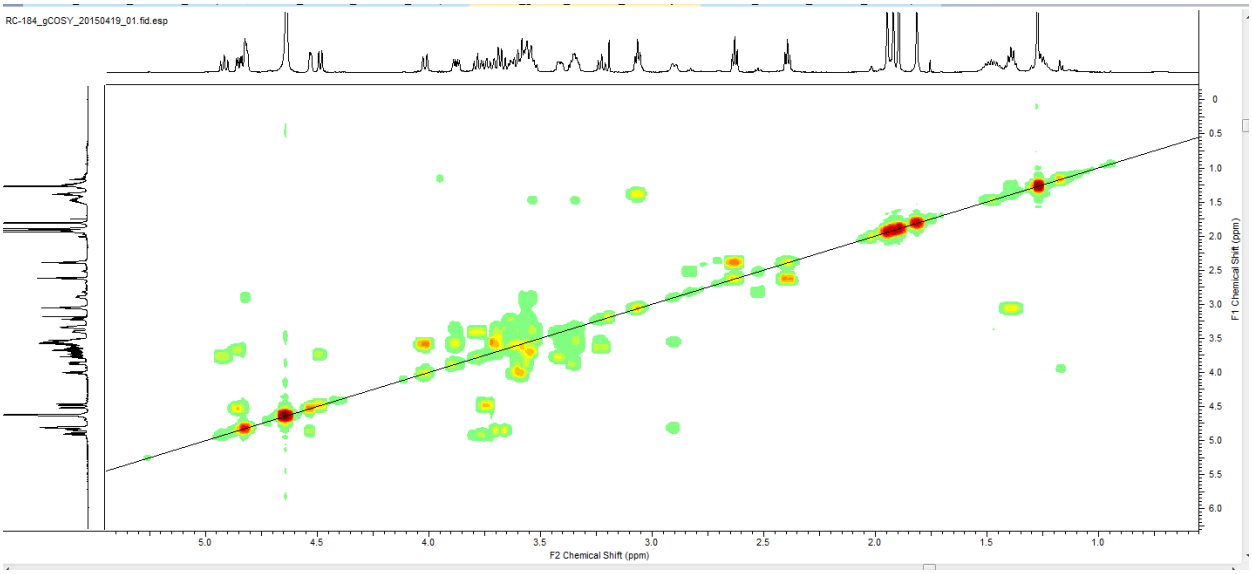
^{1}H NMR (600 MHz, D_2O)



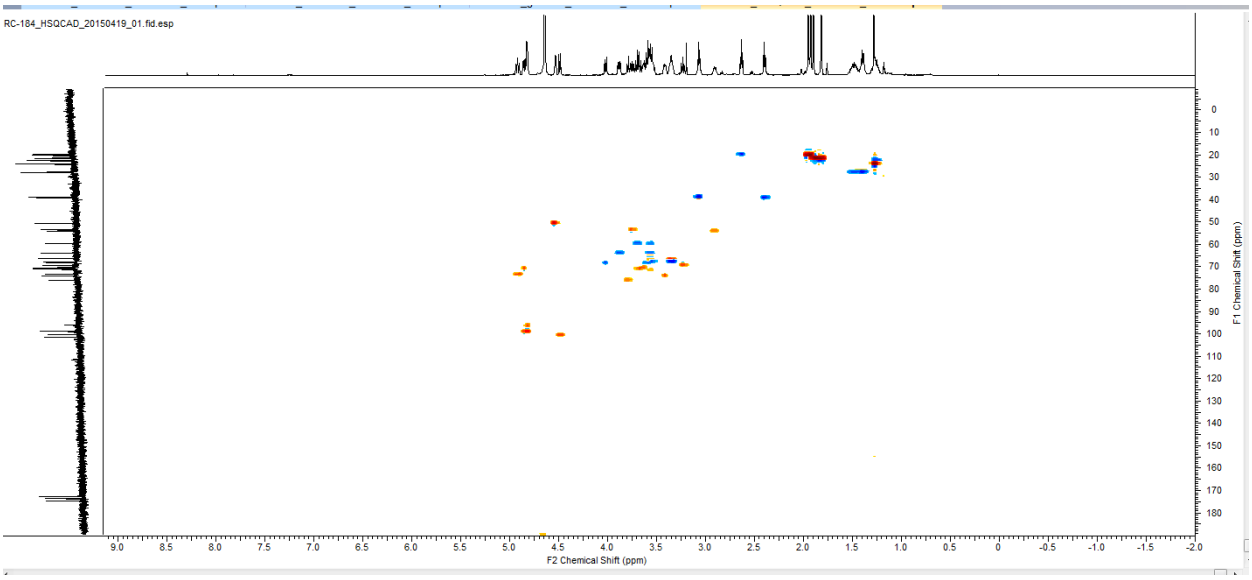
^{13}C NMR (150 MHz, D_2O)

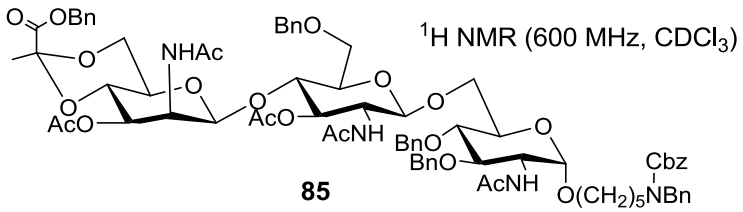


^{1}H-^{1}H COSY (600 MHz, D_2O)



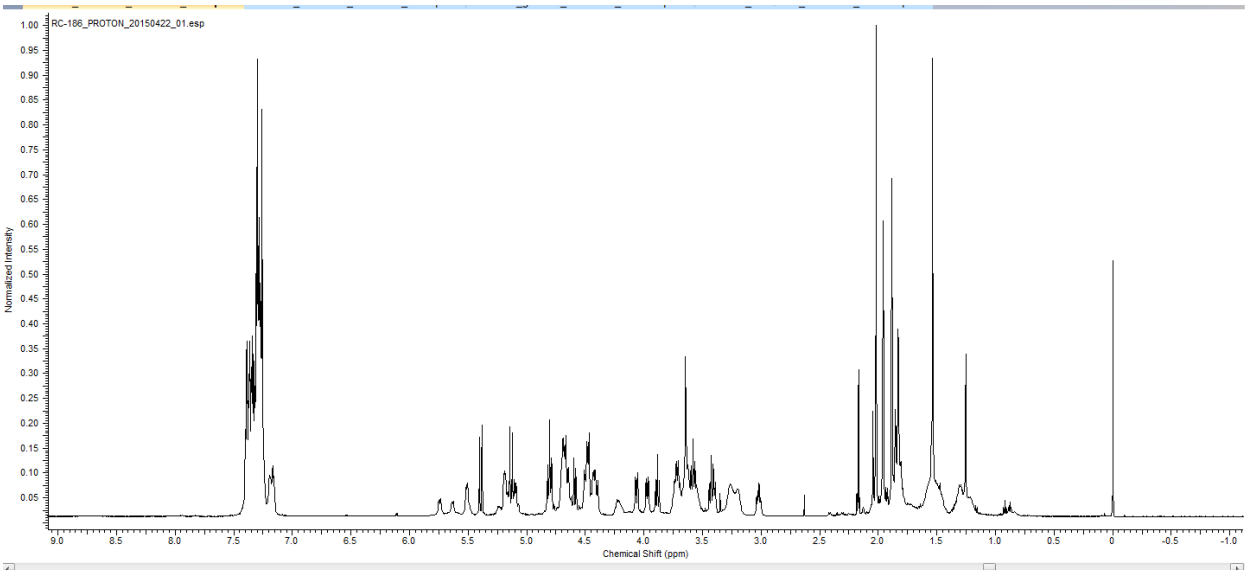
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



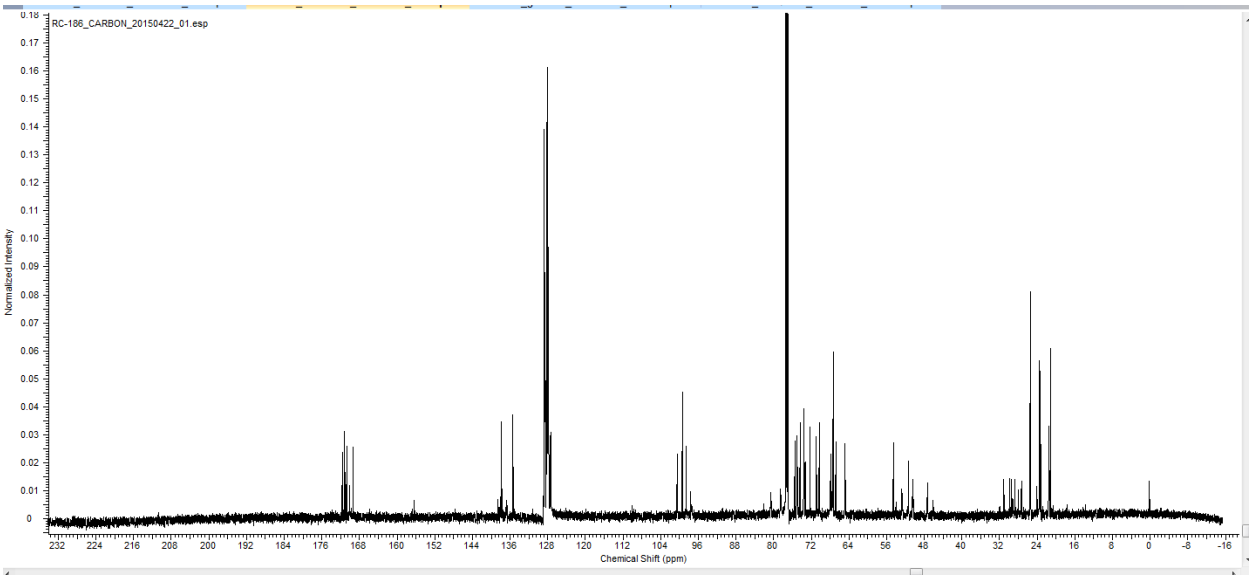


85

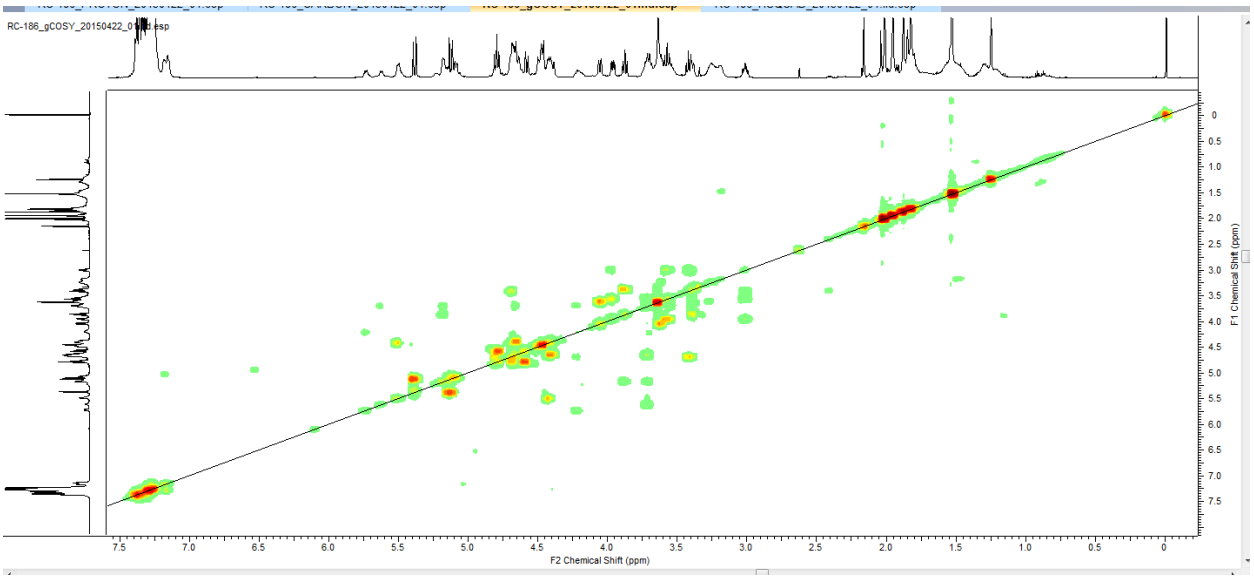
^{1}H NMR (600 MHz, $CDCl_3$)



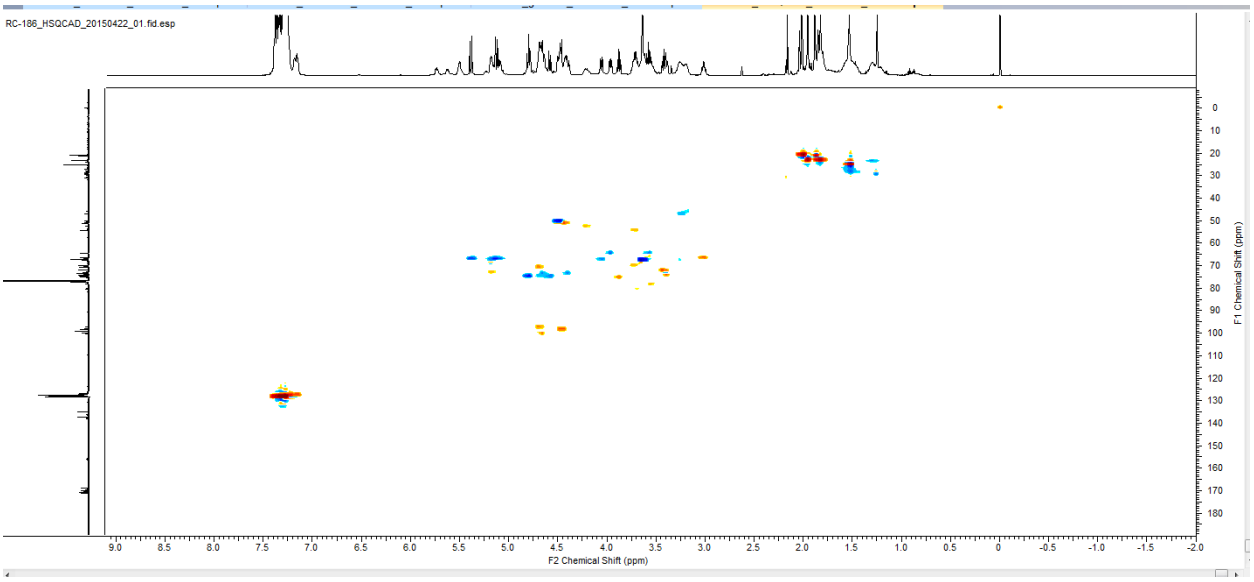
^{13}C NMR (150 MHz, $CDCl_3$)

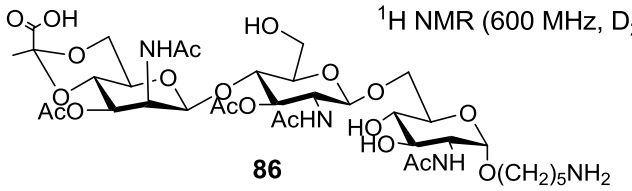


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



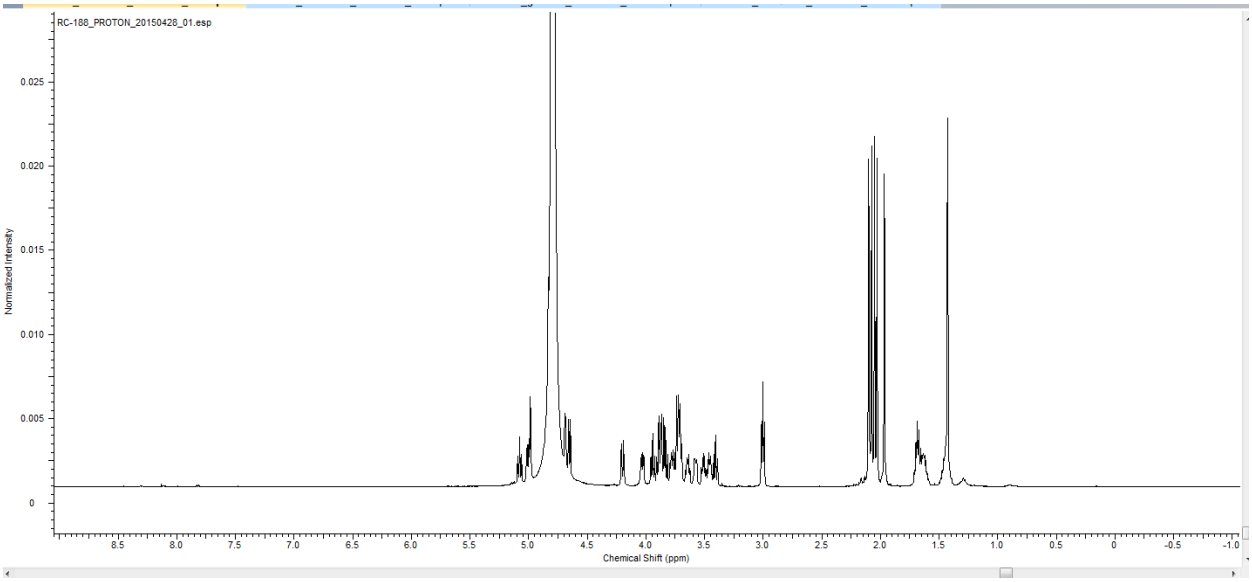
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



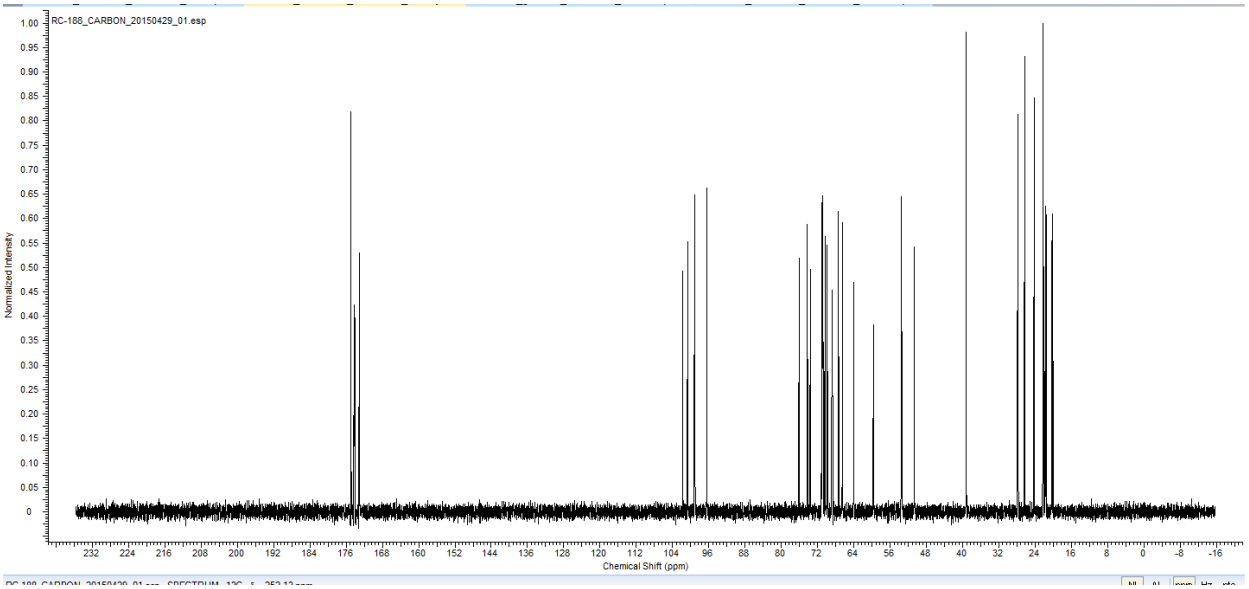


86

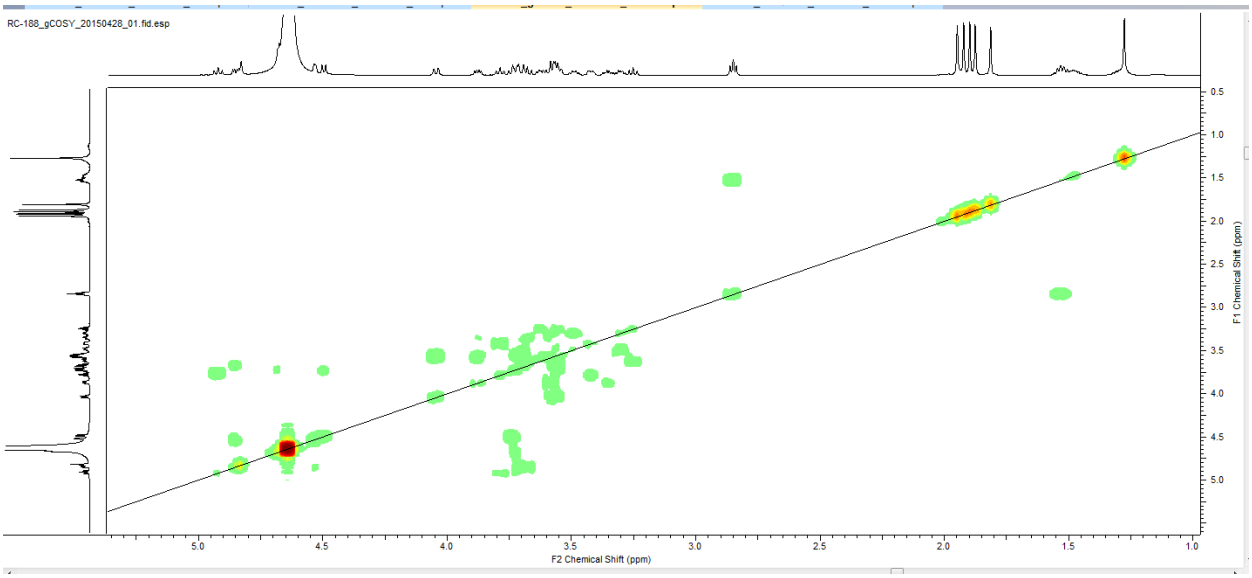
^{1}H NMR (600 MHz, D_2O)



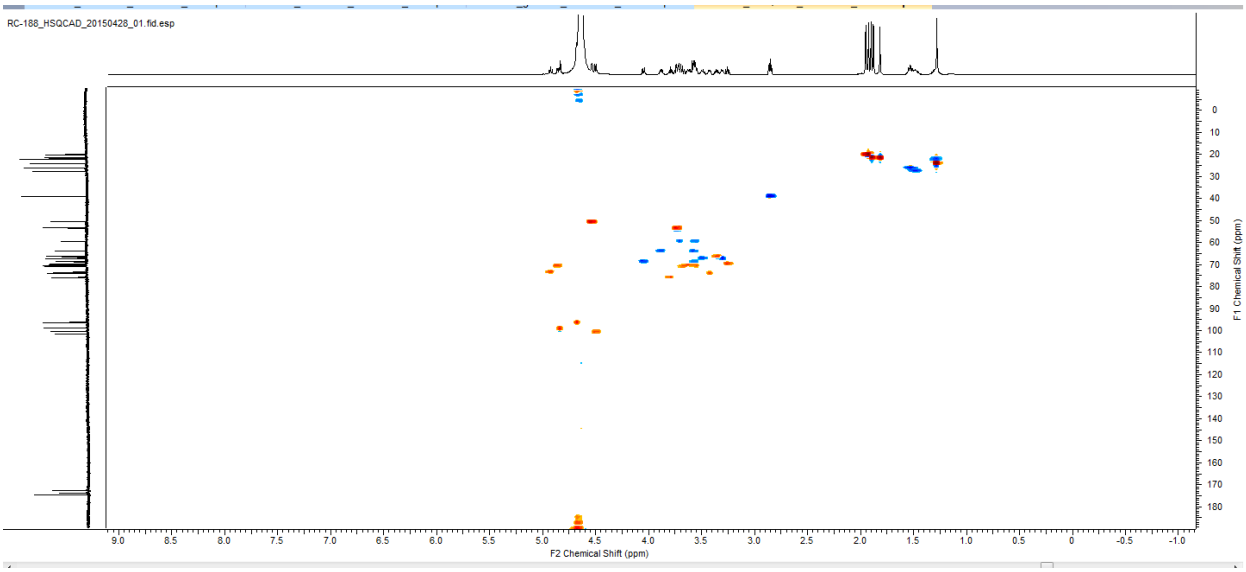
^{13}C NMR (150 MHz, D_2O)

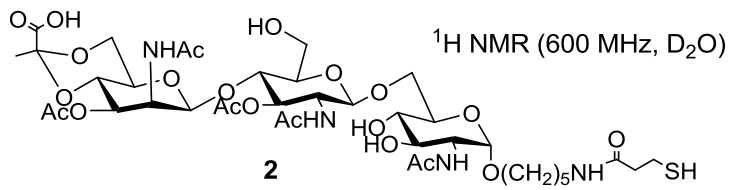


^{1}H-^{1}H COSY (600 MHz, D_2O)



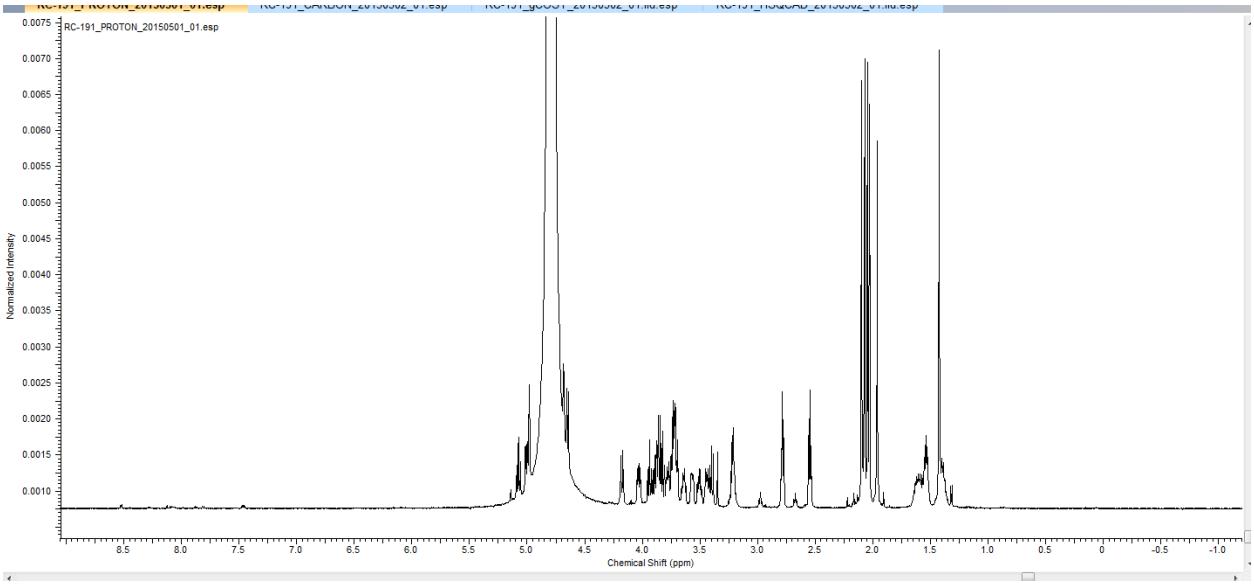
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



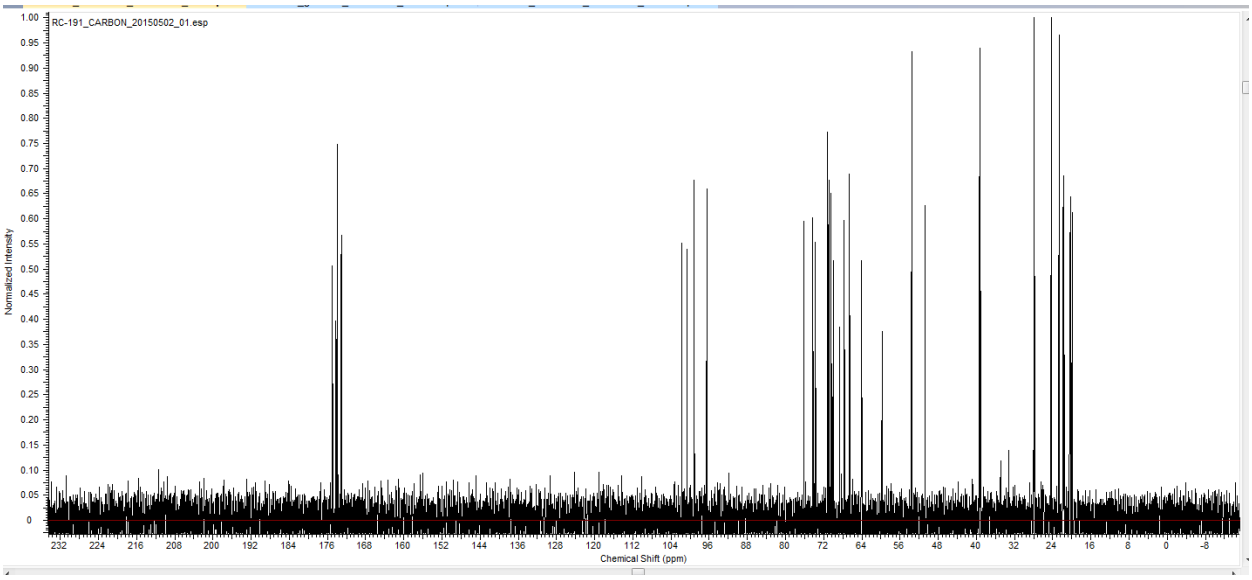


2

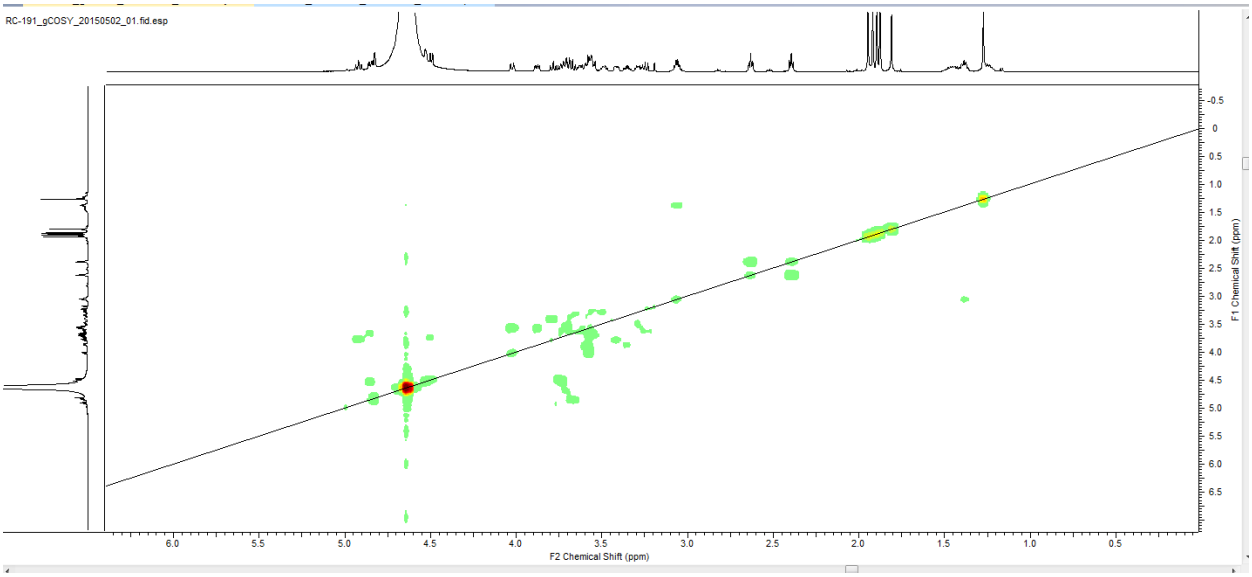
^{1}H NMR (600 MHz, D_2O)



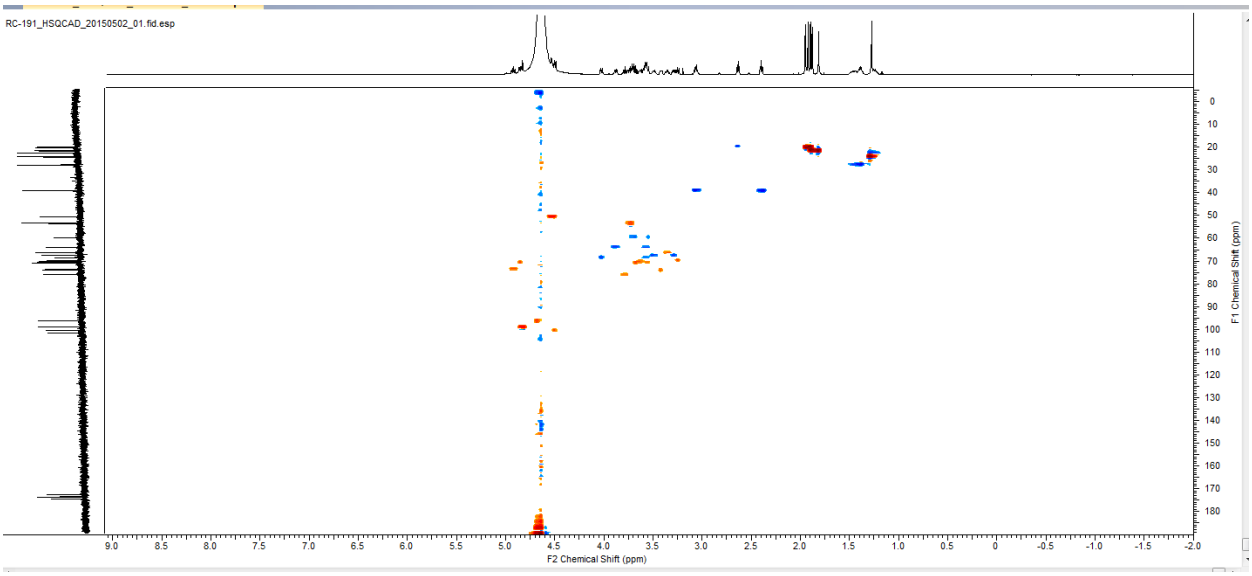
^{13}C NMR (150 MHz, D_2O)

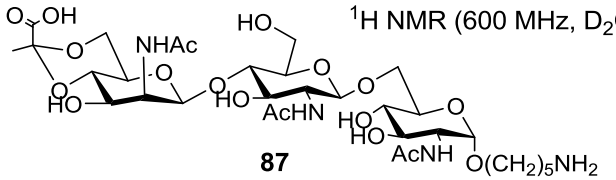


^{1}H-^{1}H COSY (600 MHz, D_2O)



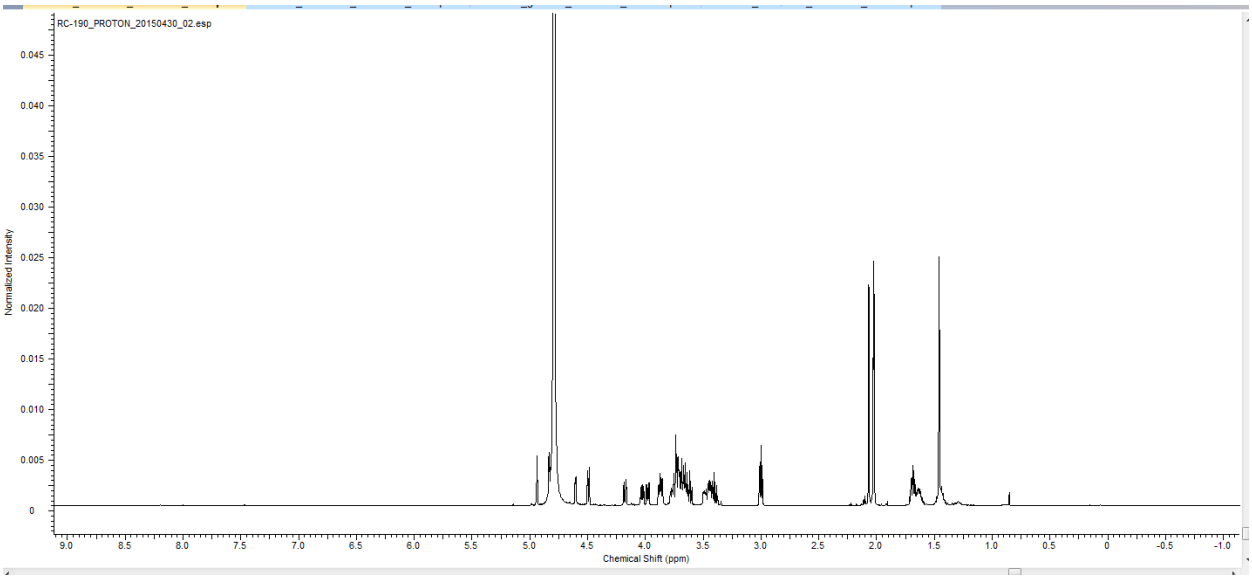
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



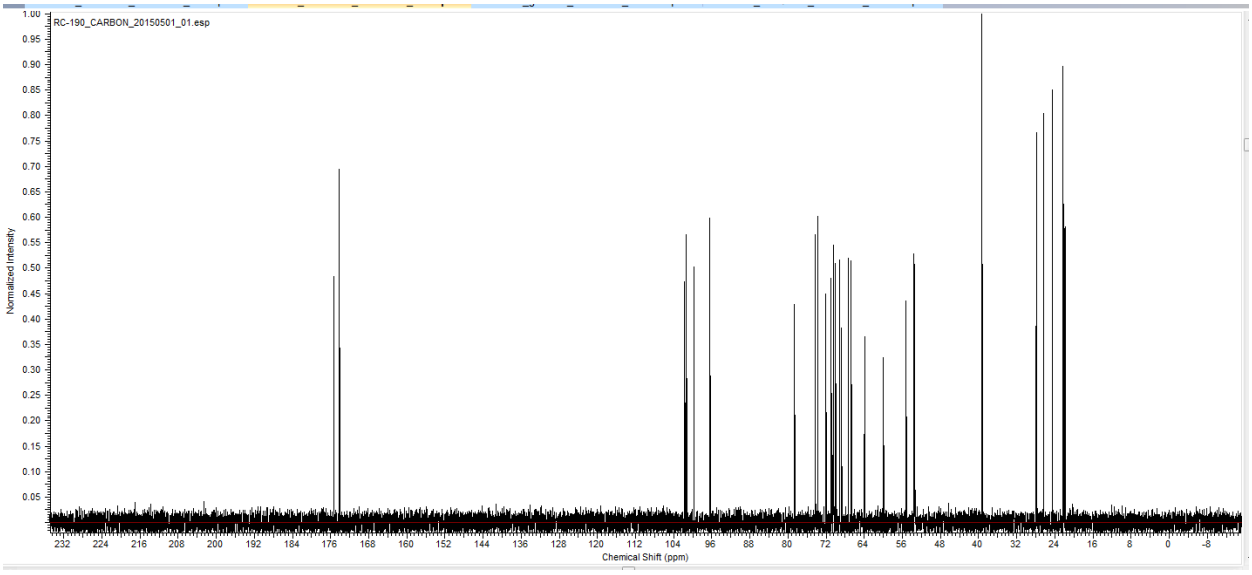


87

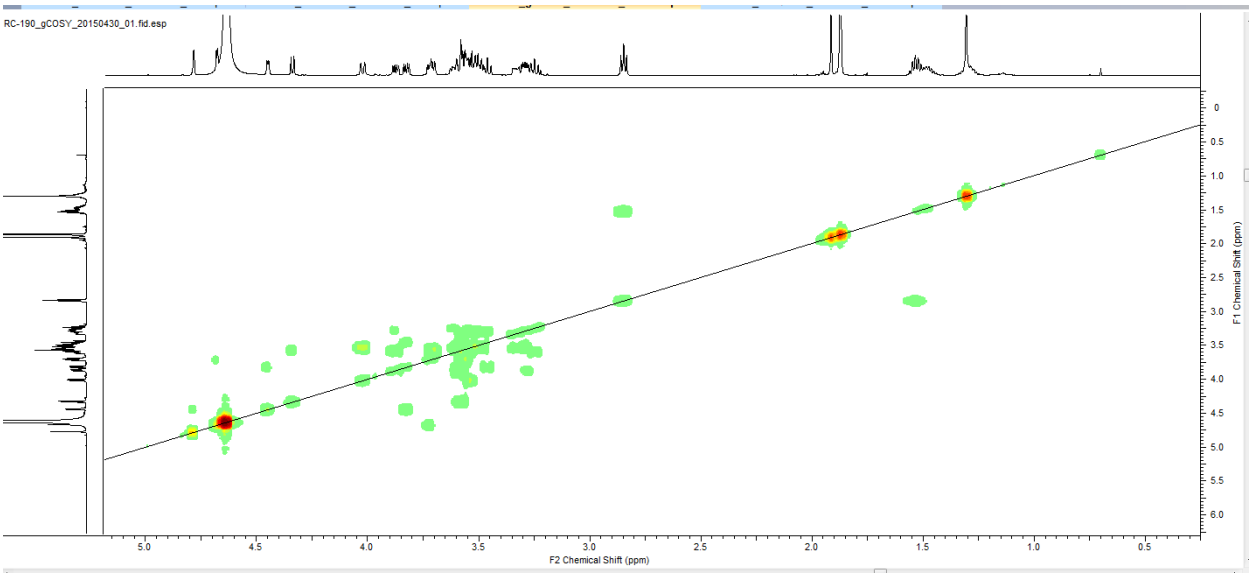
^{1}H NMR (600 MHz, D_2O)



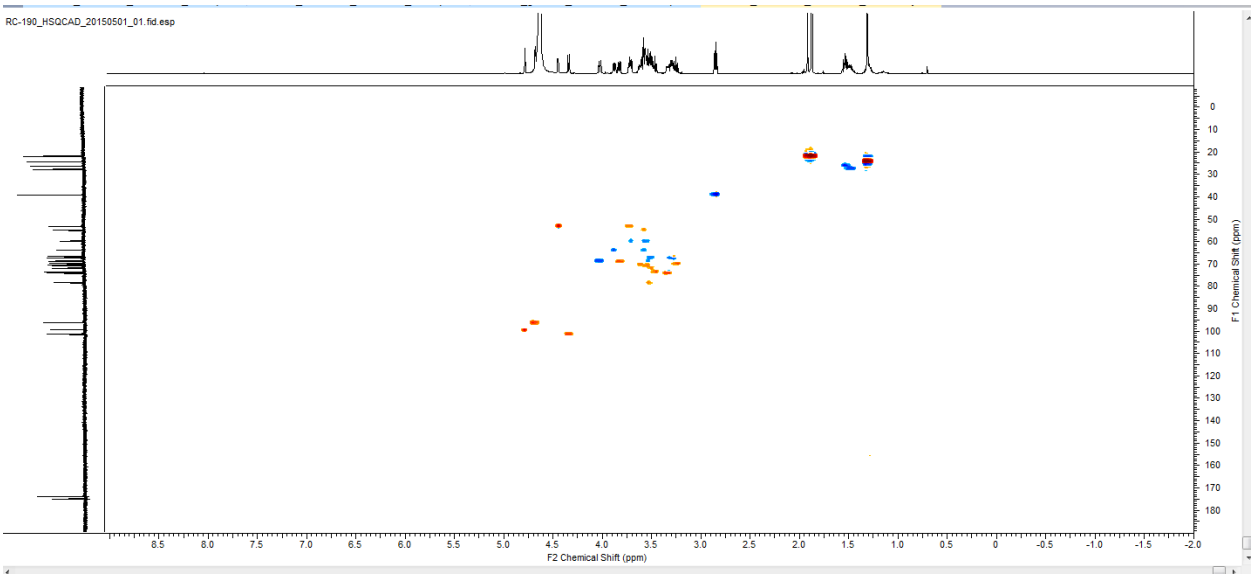
^{13}C NMR (150 MHz, D_2O)

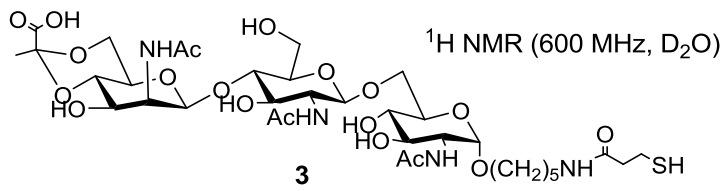


^{1}H-^{1}H COSY (600 MHz, D_2O)



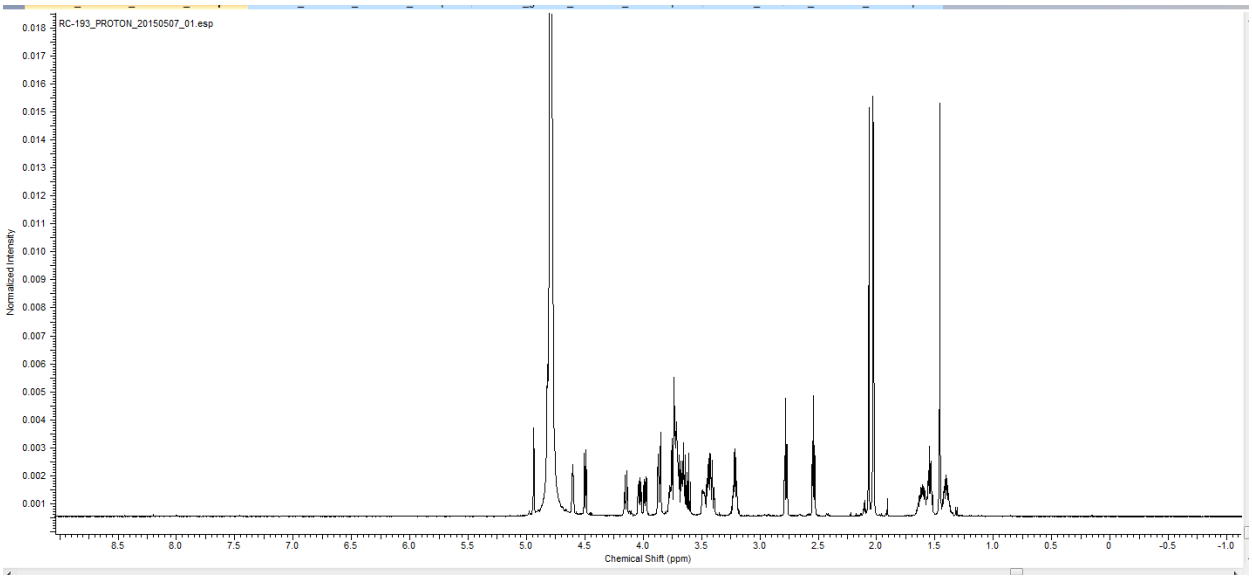
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



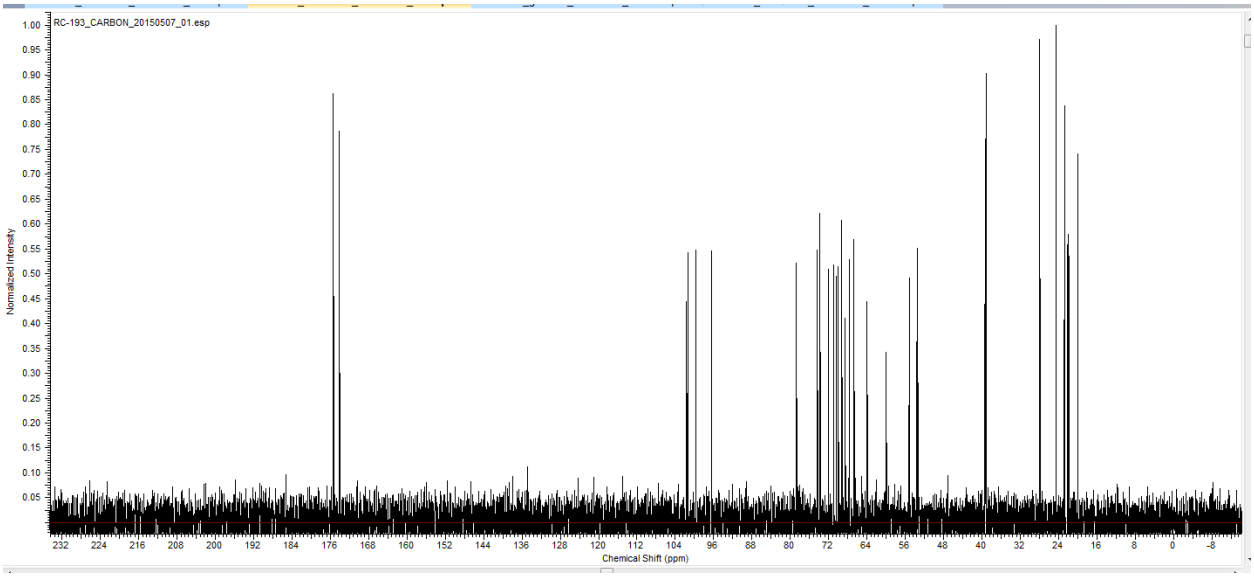


3

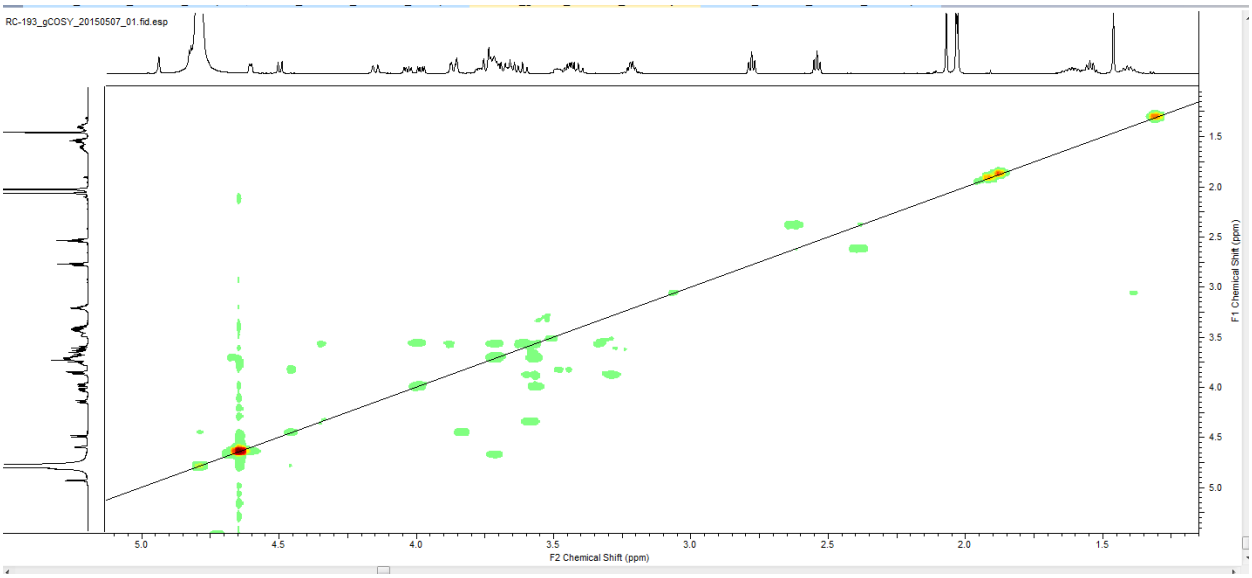
^{1}H NMR (600 MHz, D_2O)



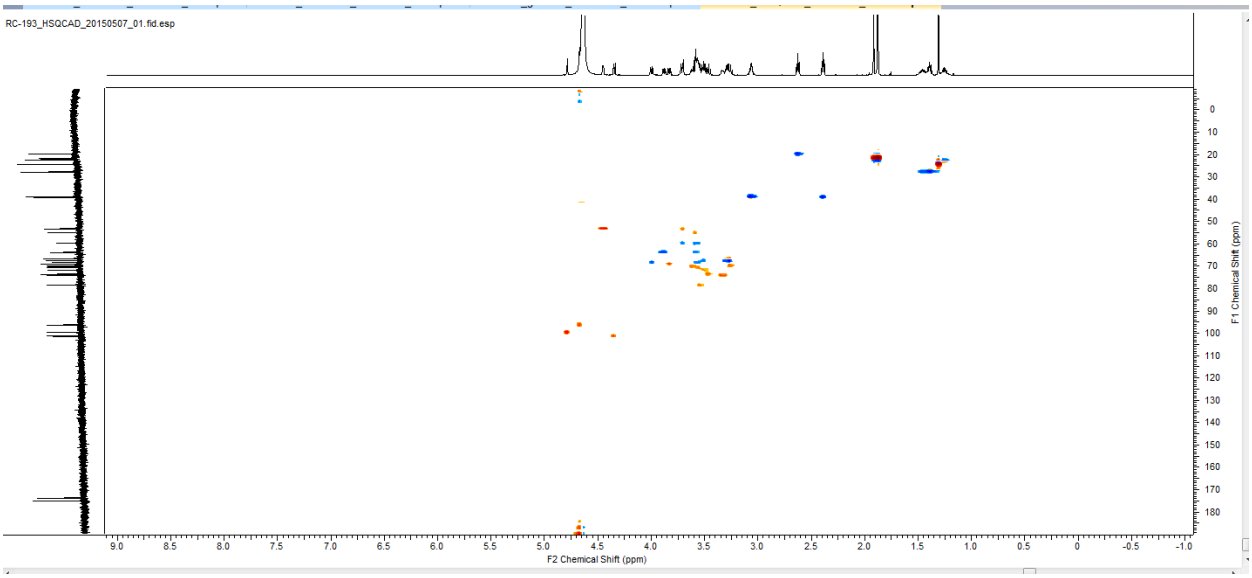
^{13}C NMR (150 MHz, D_2O)

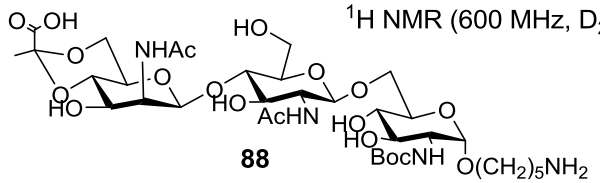


^{1}H-^{1}H COSY (600 MHz, D_2O)



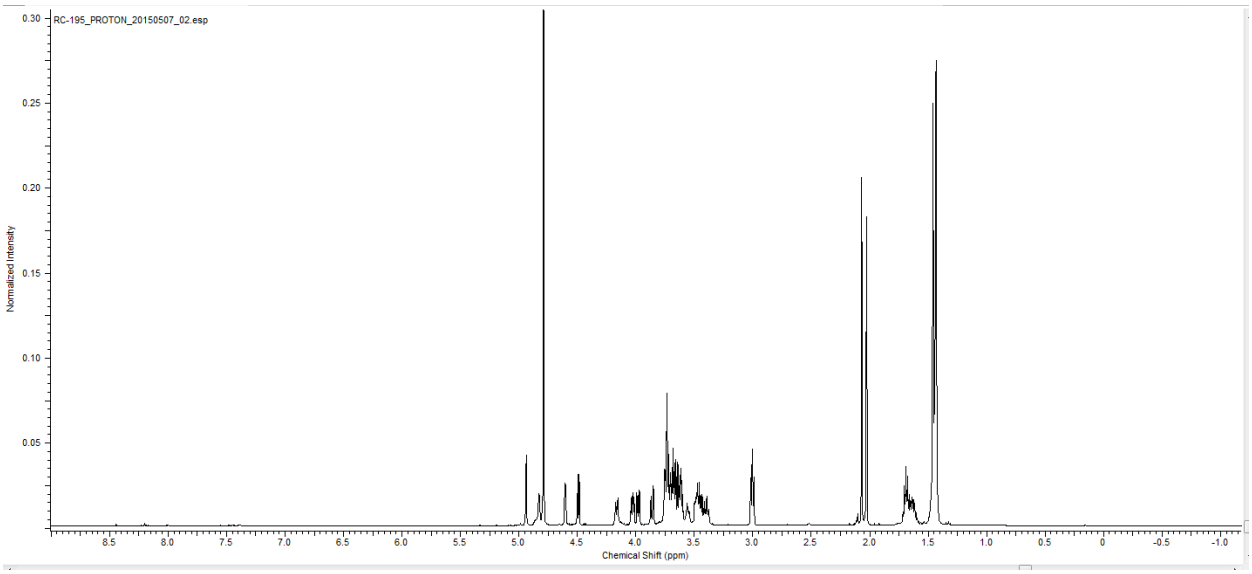
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



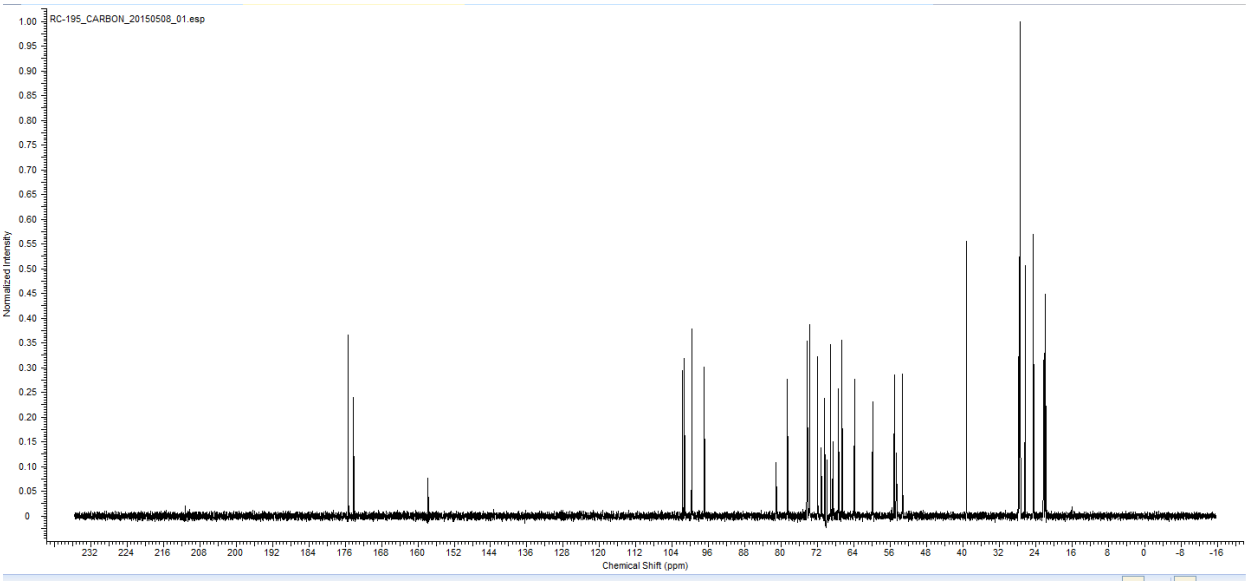


88

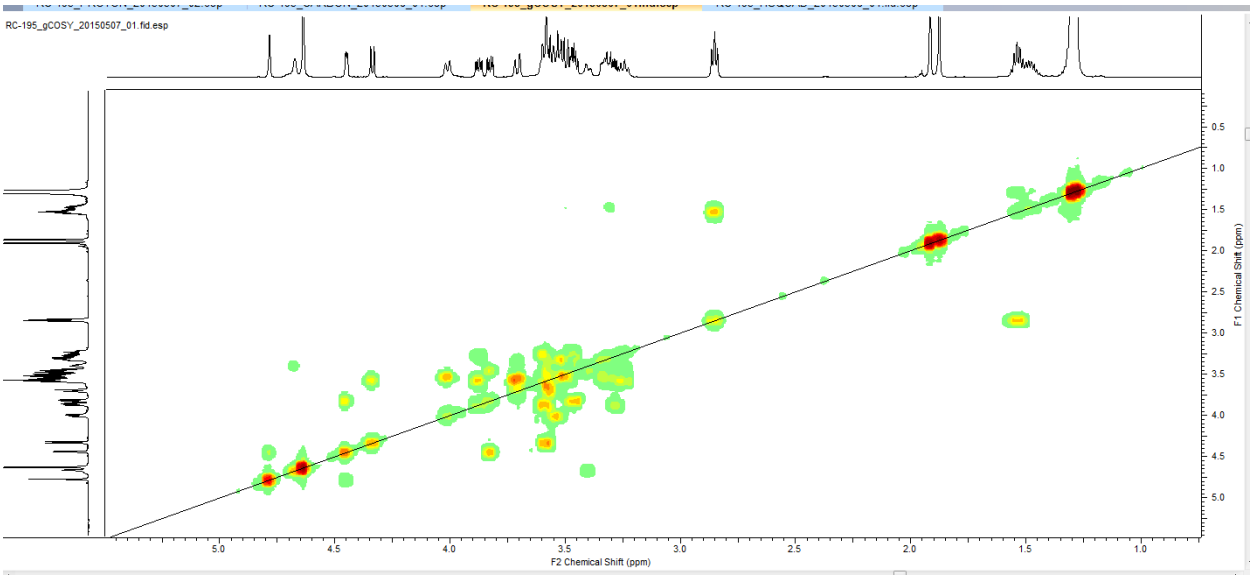
^{1}H NMR (600 MHz, D_2O)



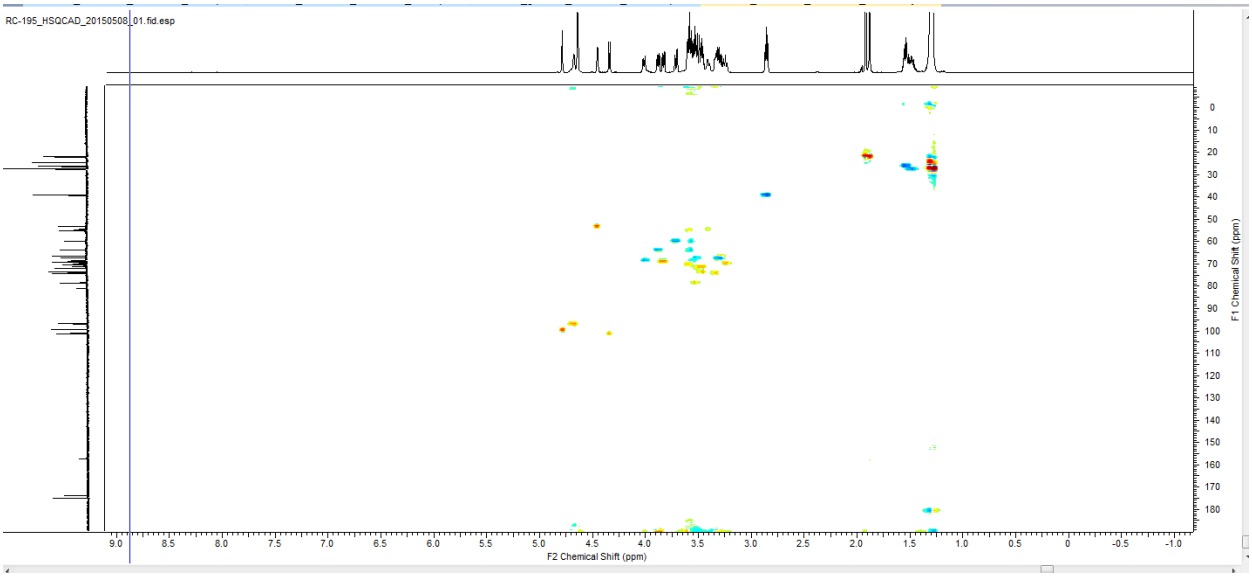
^{13}C NMR (150 MHz, D_2O)

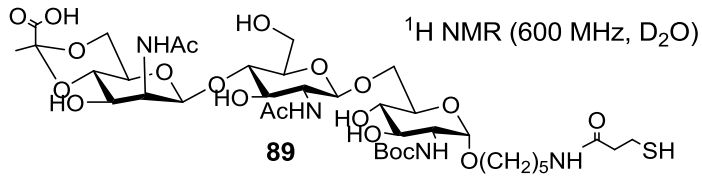


^{1}H-^{1}H COSY (600 MHz, D_2O)



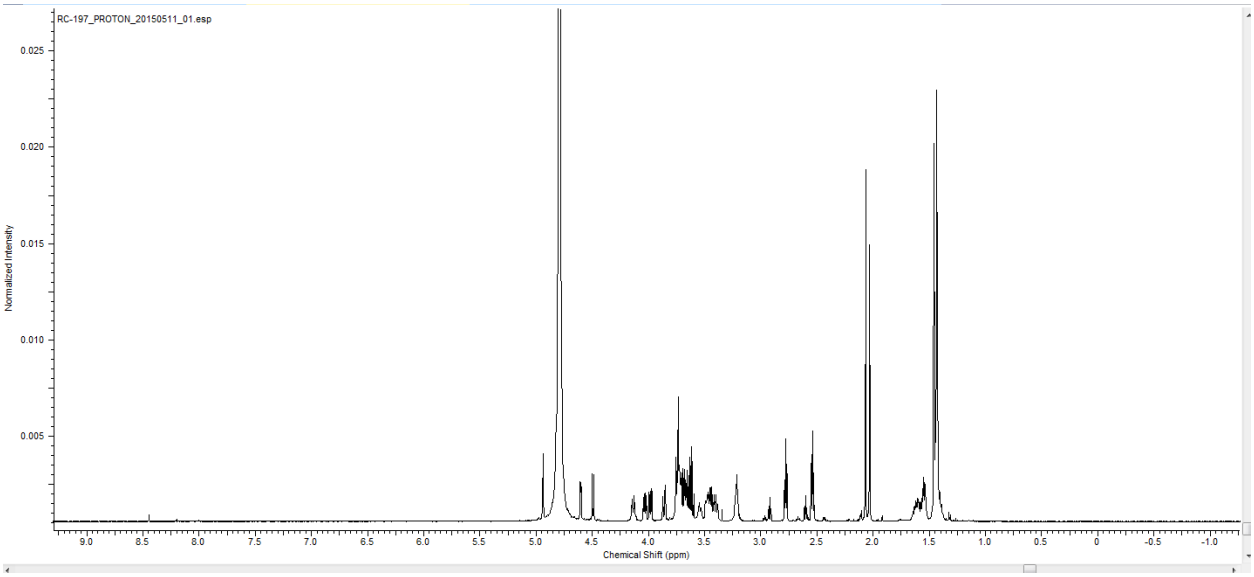
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



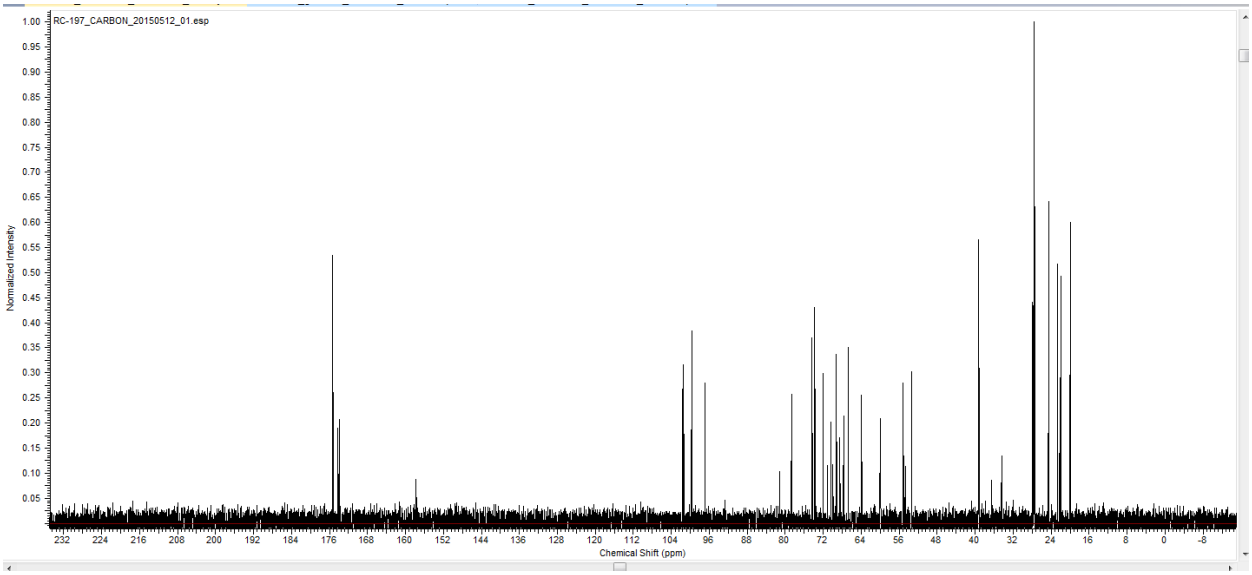


89

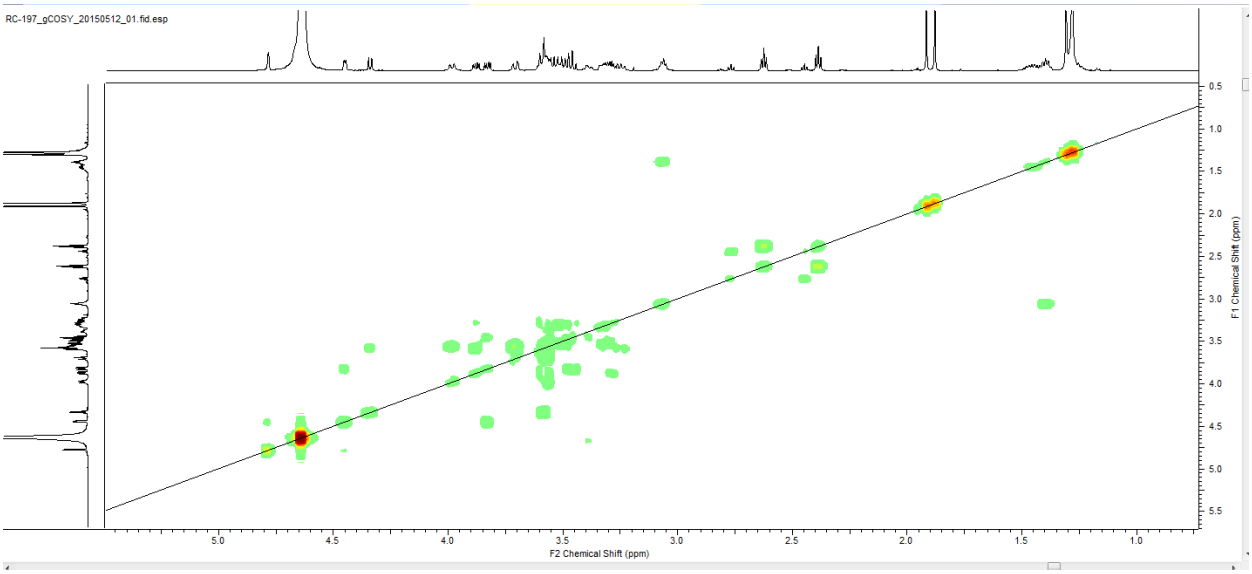
^{1}H NMR (600 MHz, D_2O)



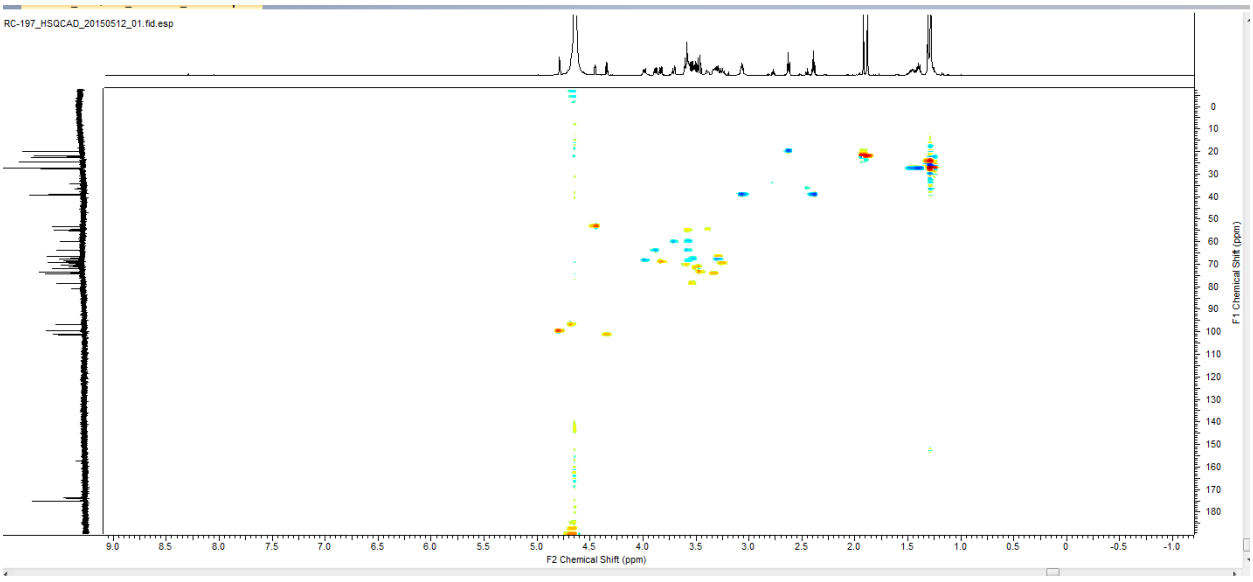
^{13}C NMR (150 MHz, D_2O)

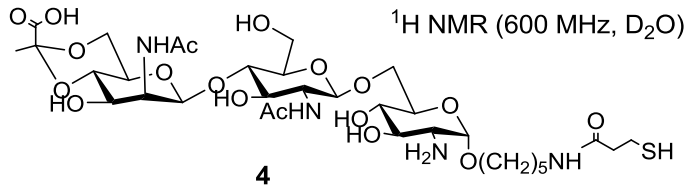


^{1}H-^{1}H COSY (600 MHz, D_2O)



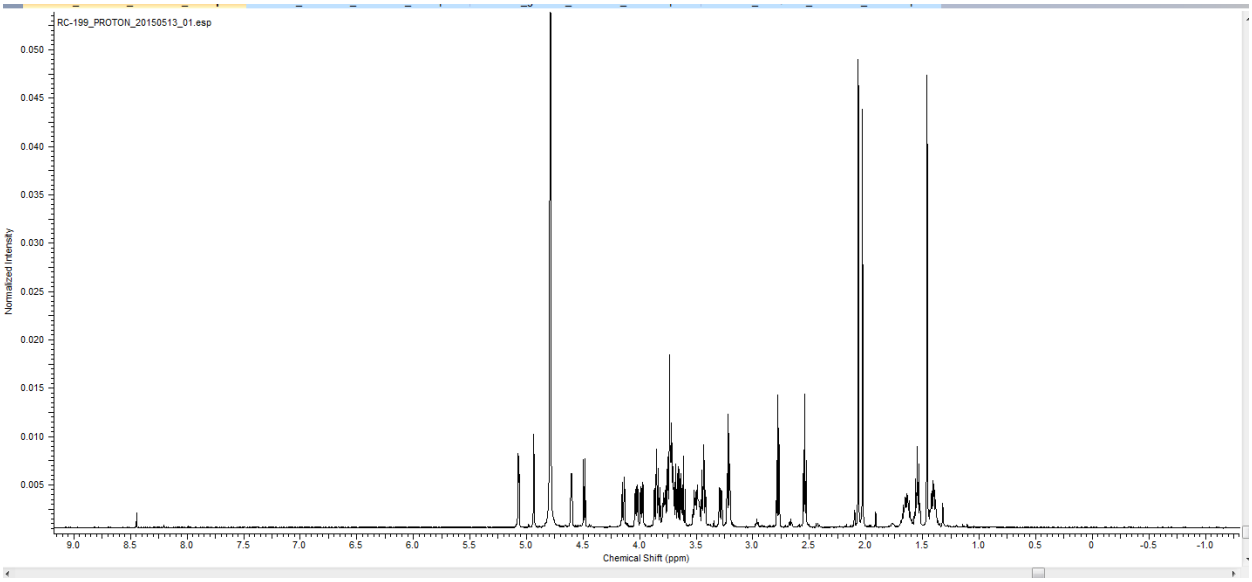
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



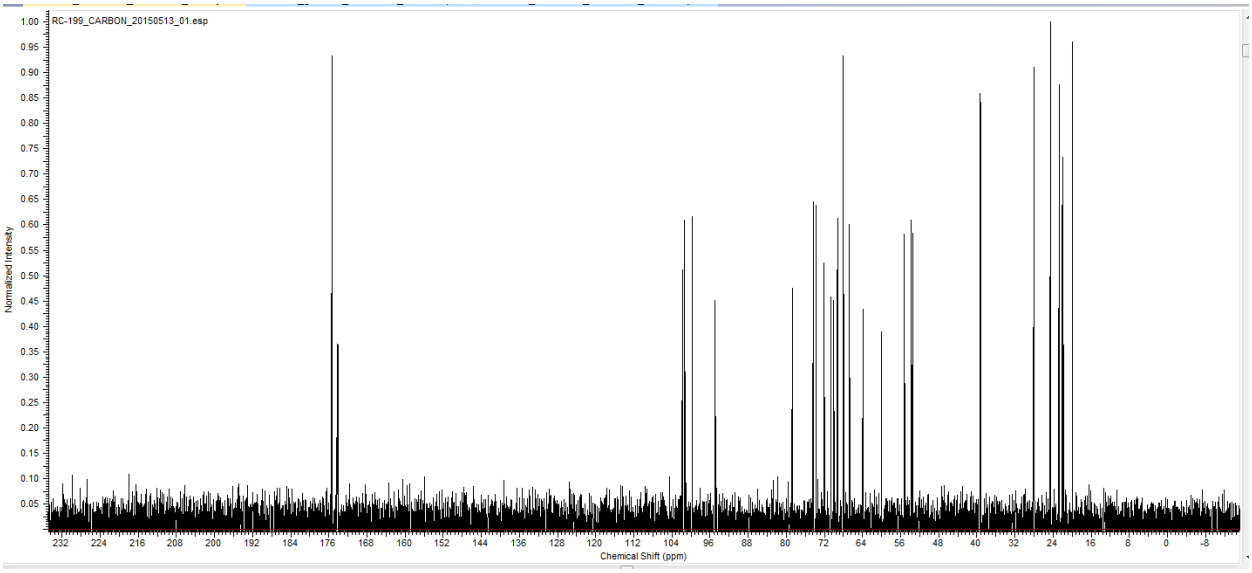


4

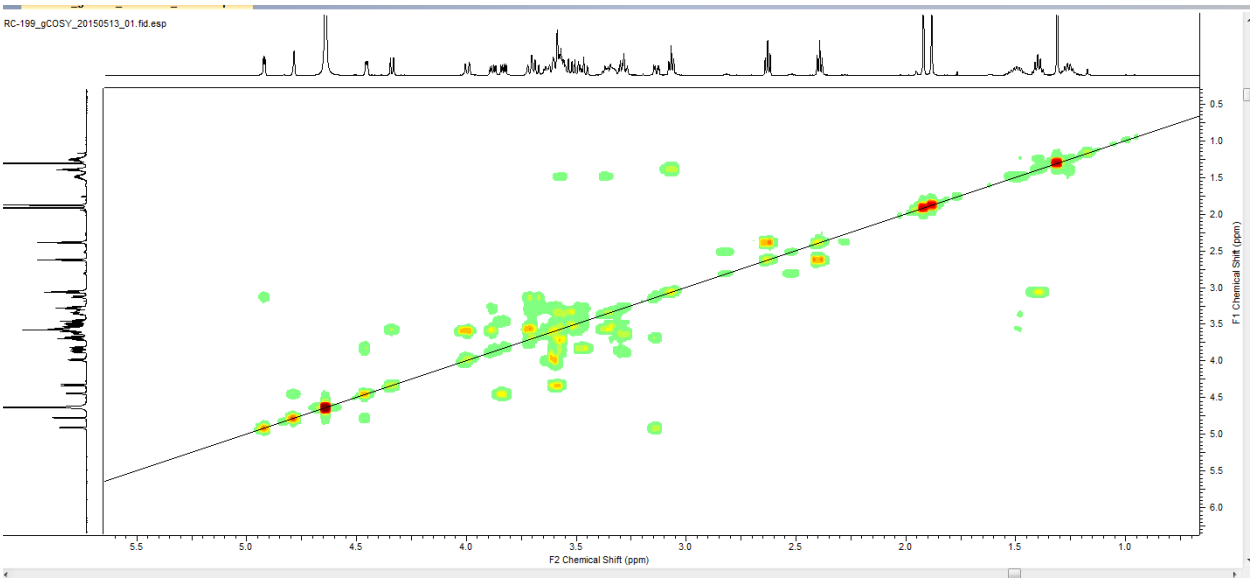
^{1}H NMR (600 MHz, D_2O)



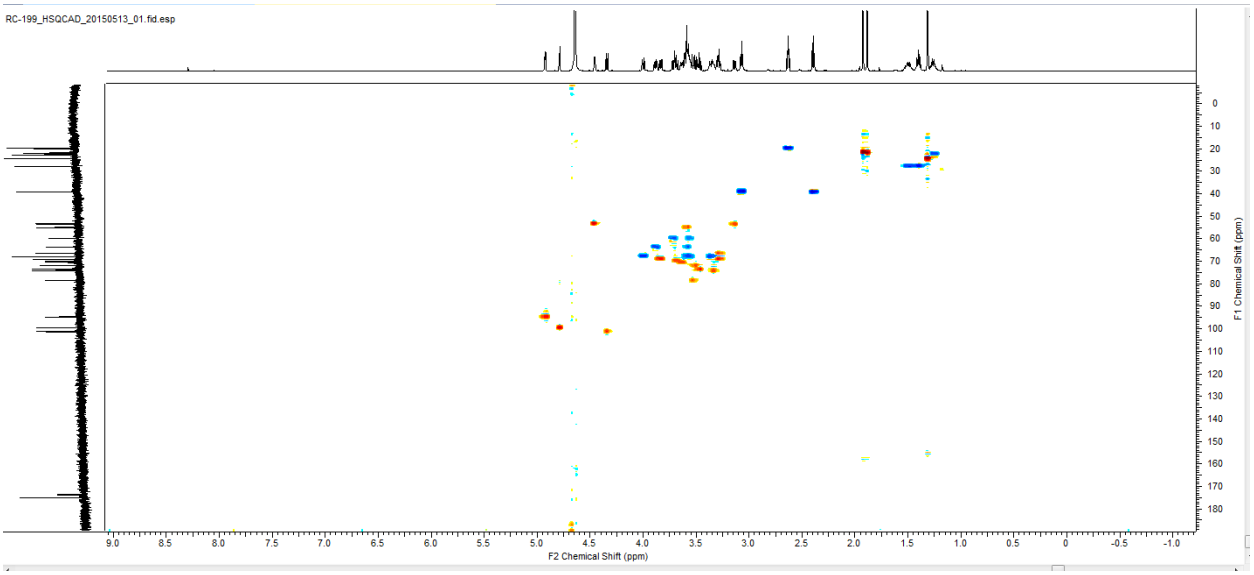
^{13}C NMR (150 MHz, D_2O)

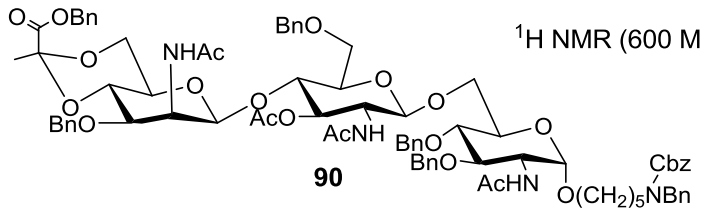


^{1}H-^{1}H COSY (600 MHz, D_2O)



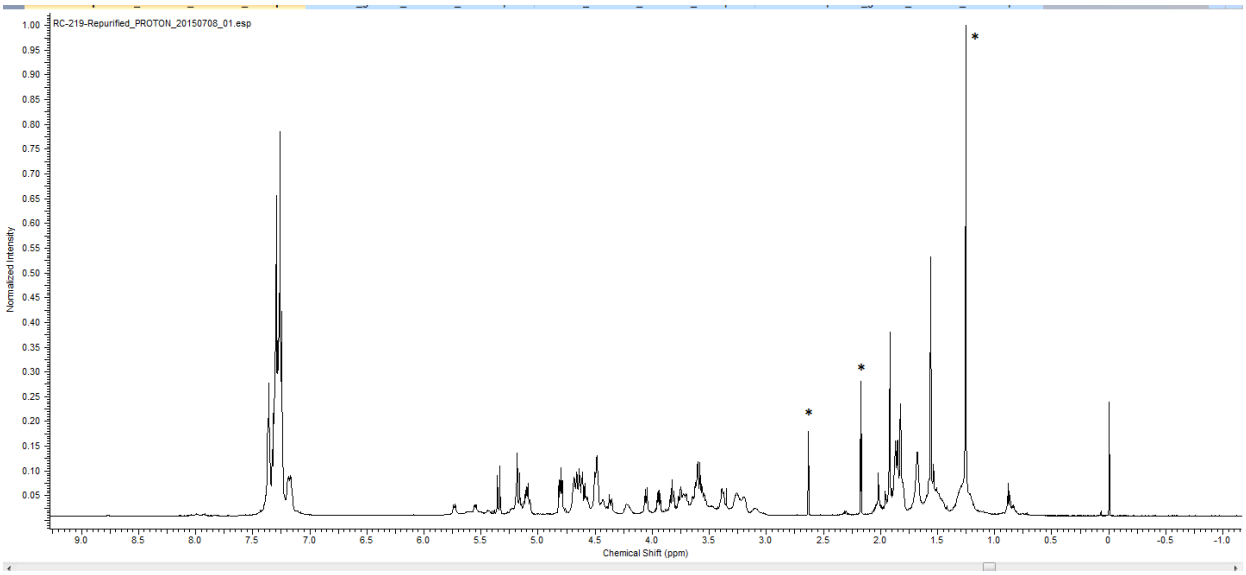
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



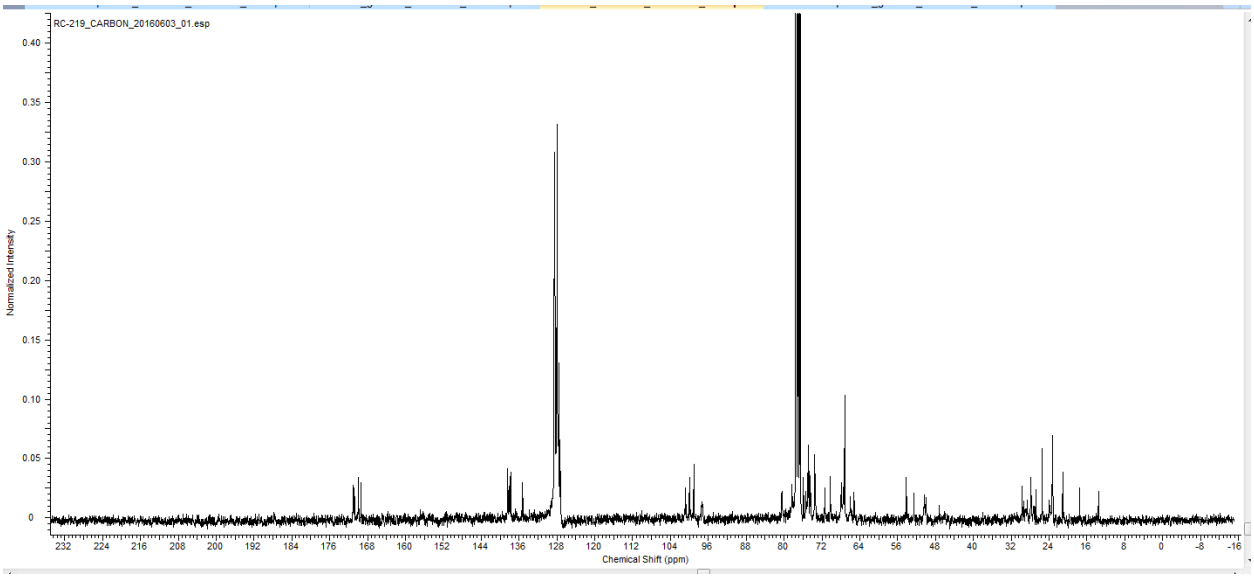


90

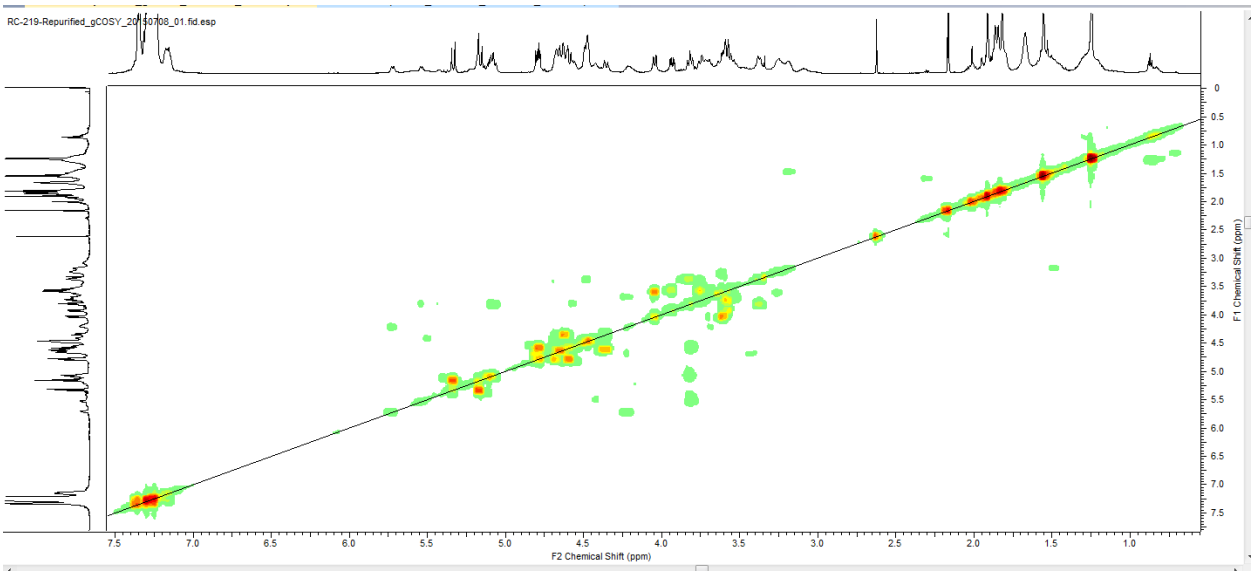
^{1}H NMR (600 MHz, D_2O)



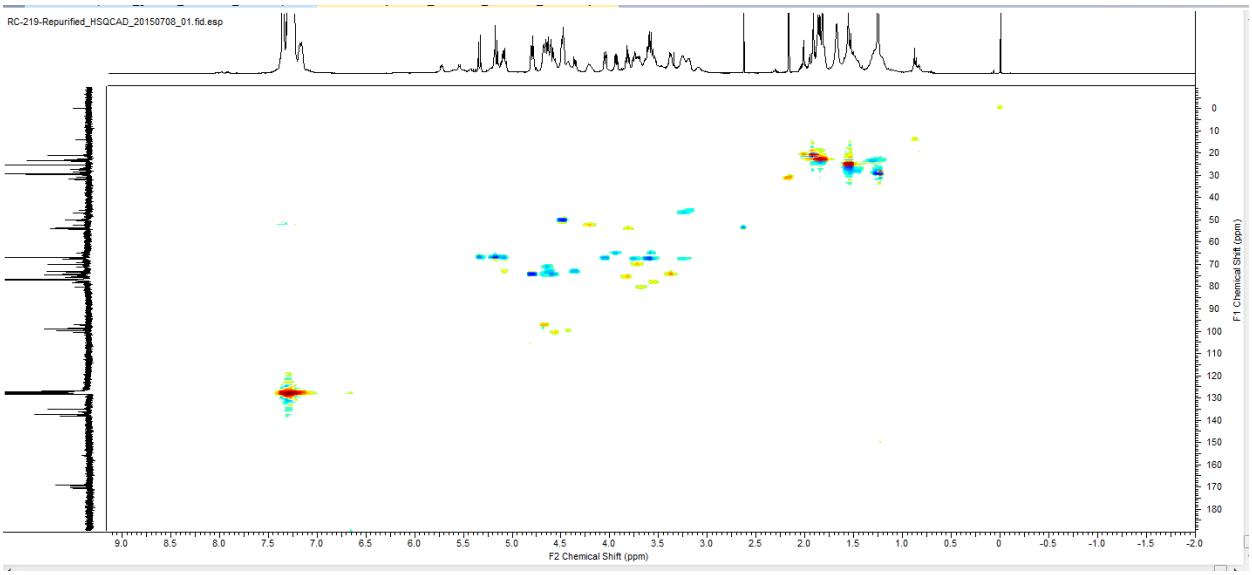
^{13}C NMR (150 MHz, D_2O)

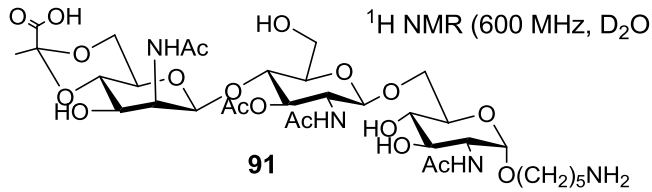


^{1}H-^{1}H COSY (600 MHz, D_2O)



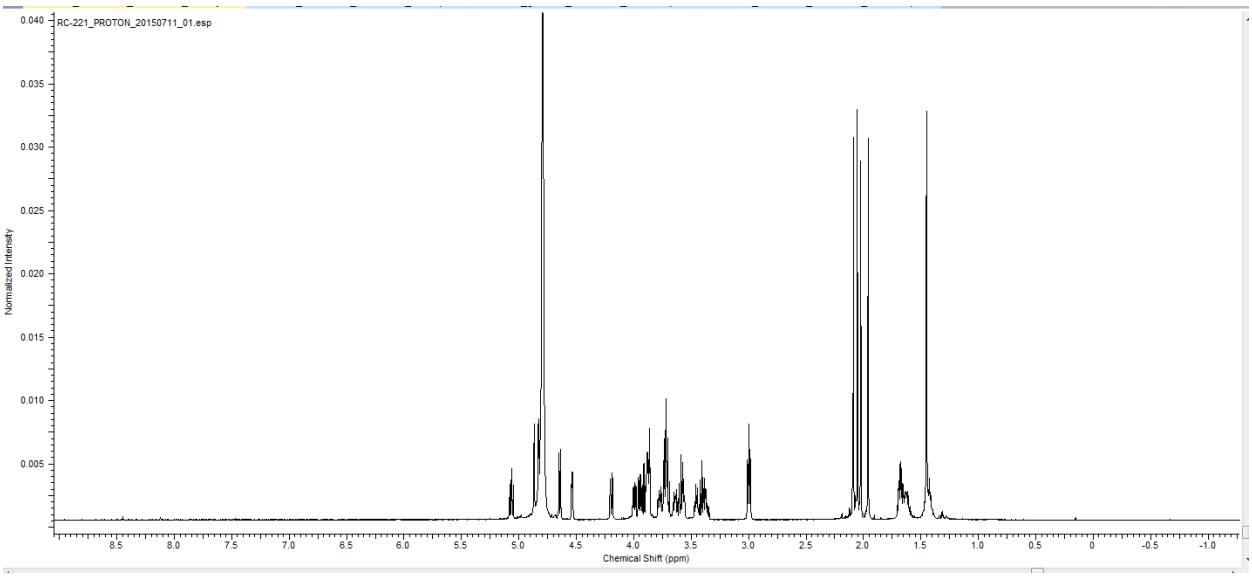
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



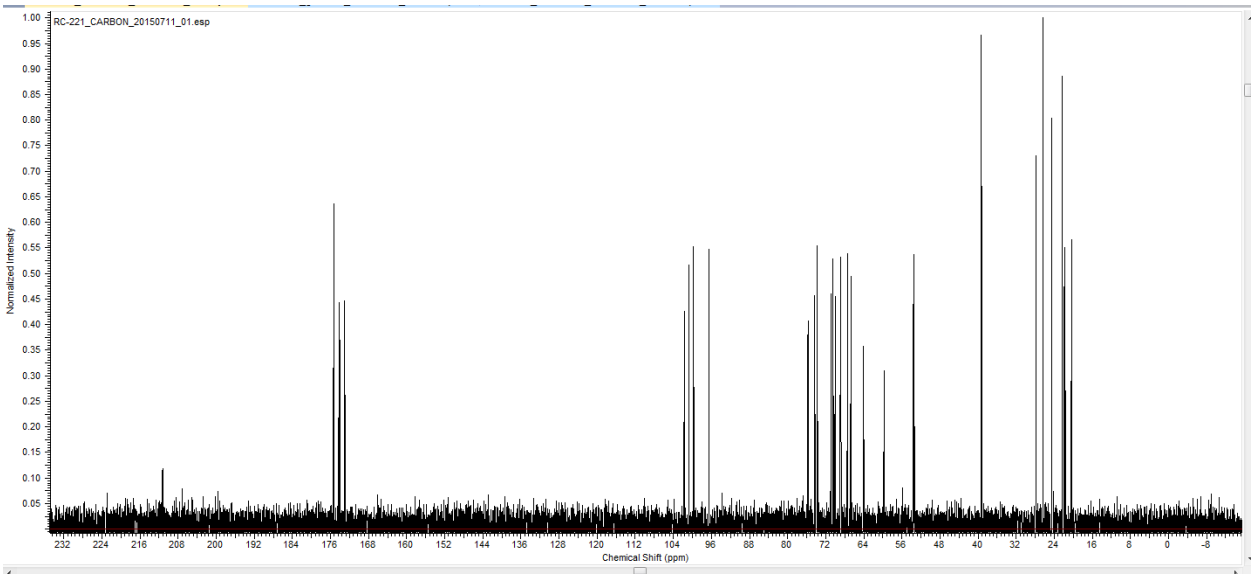


91

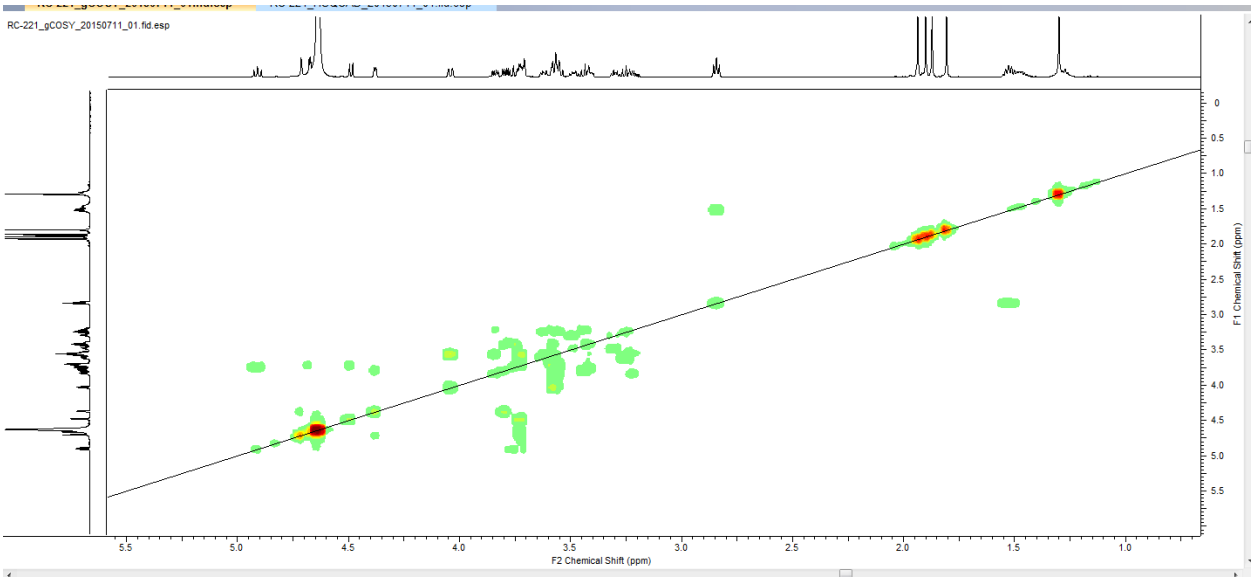
^{1}H NMR (600 MHz, D_2O)



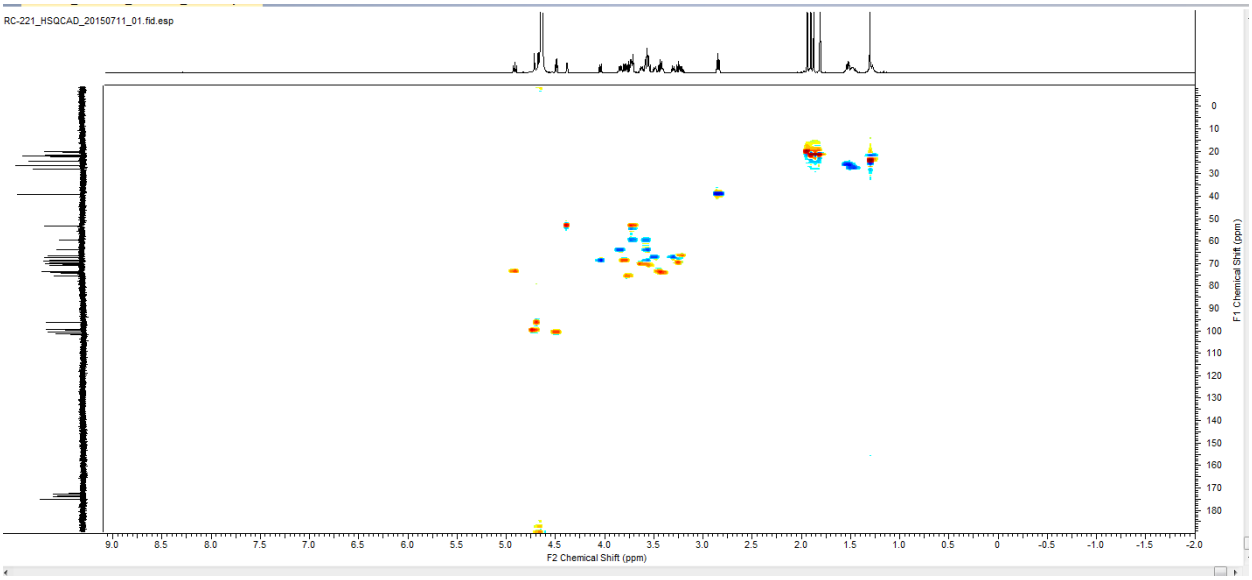
^{13}C NMR (150 MHz, D_2O)

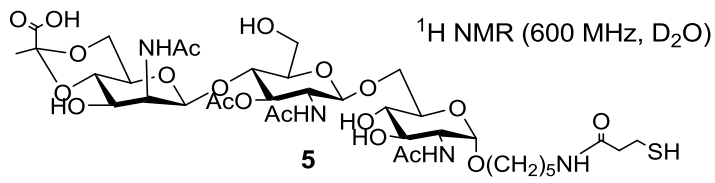


^{1}H-^{1}H COSY (600 MHz, D_2O)



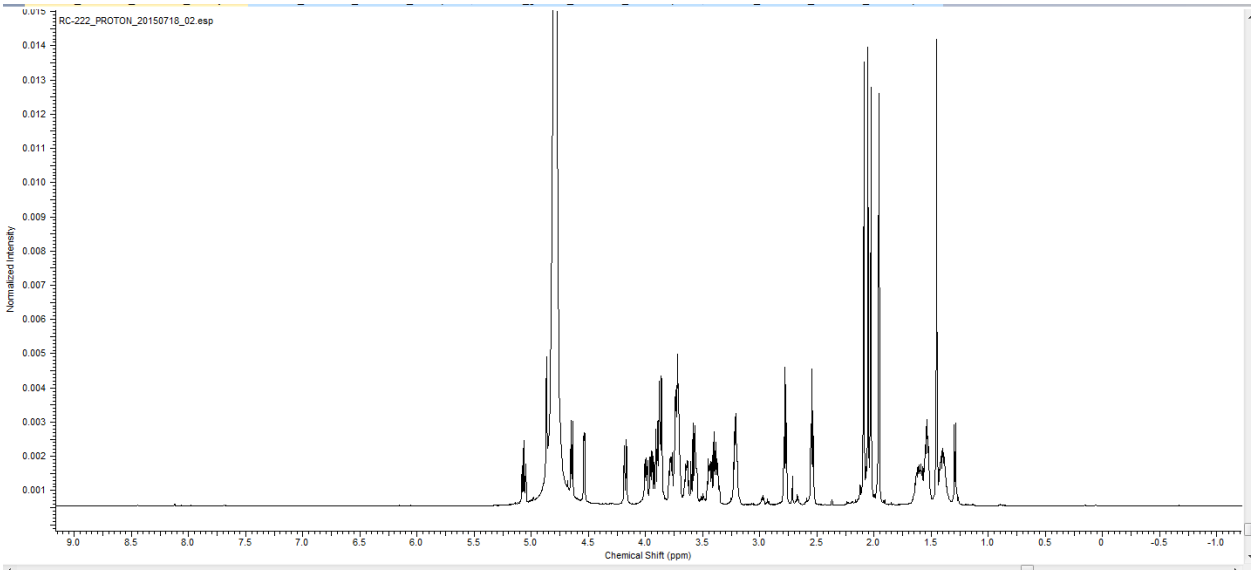
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



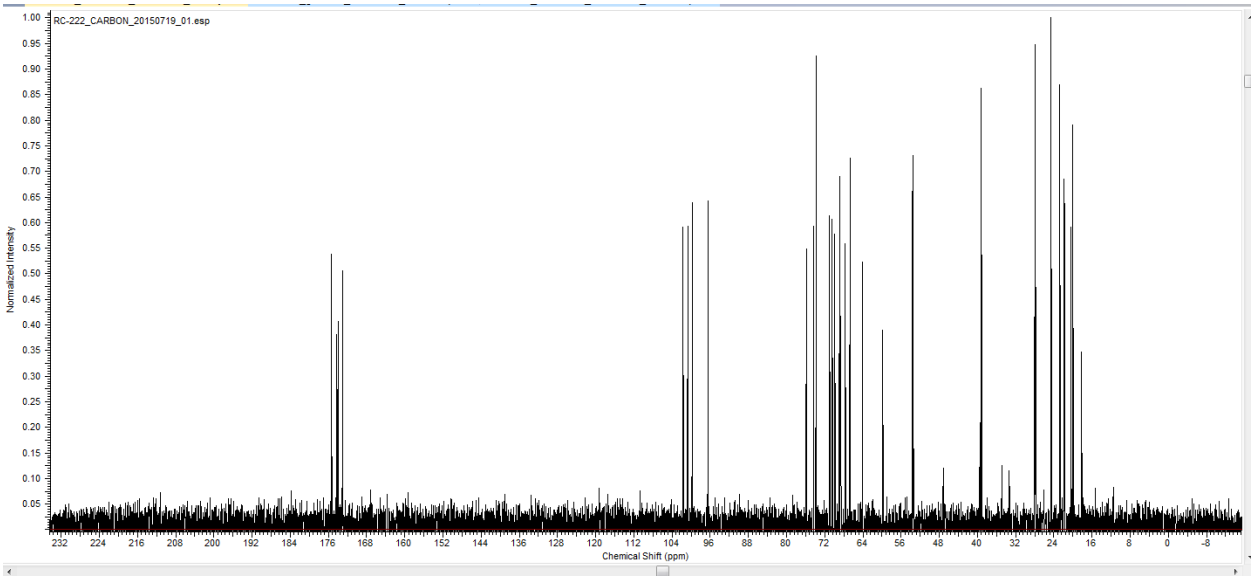


5

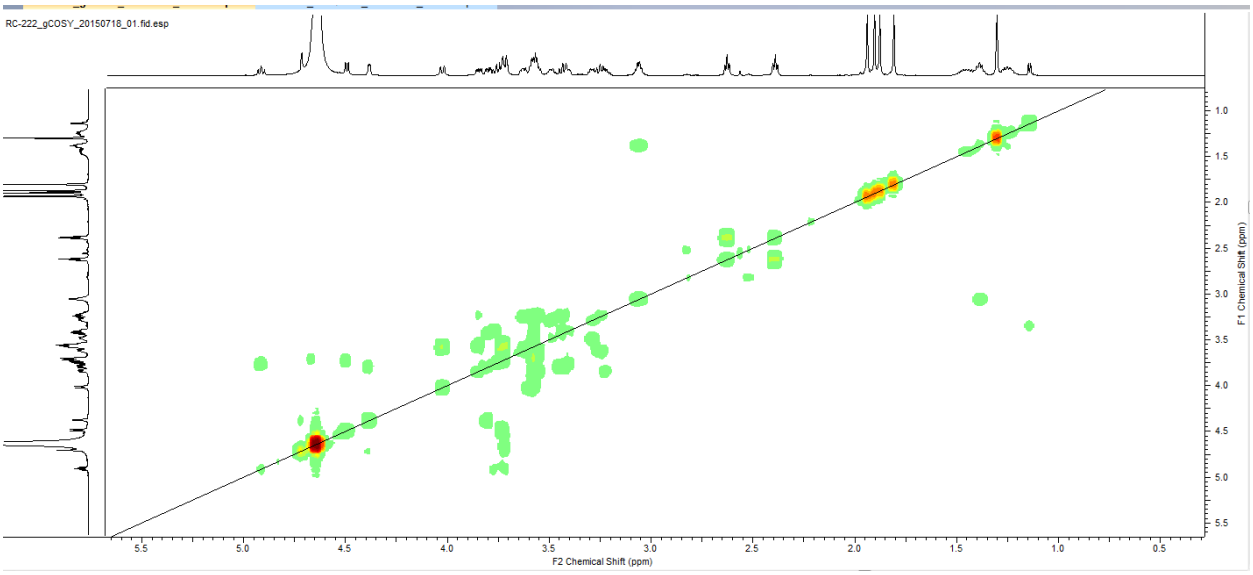
^{1}H NMR (600 MHz, D_2O)



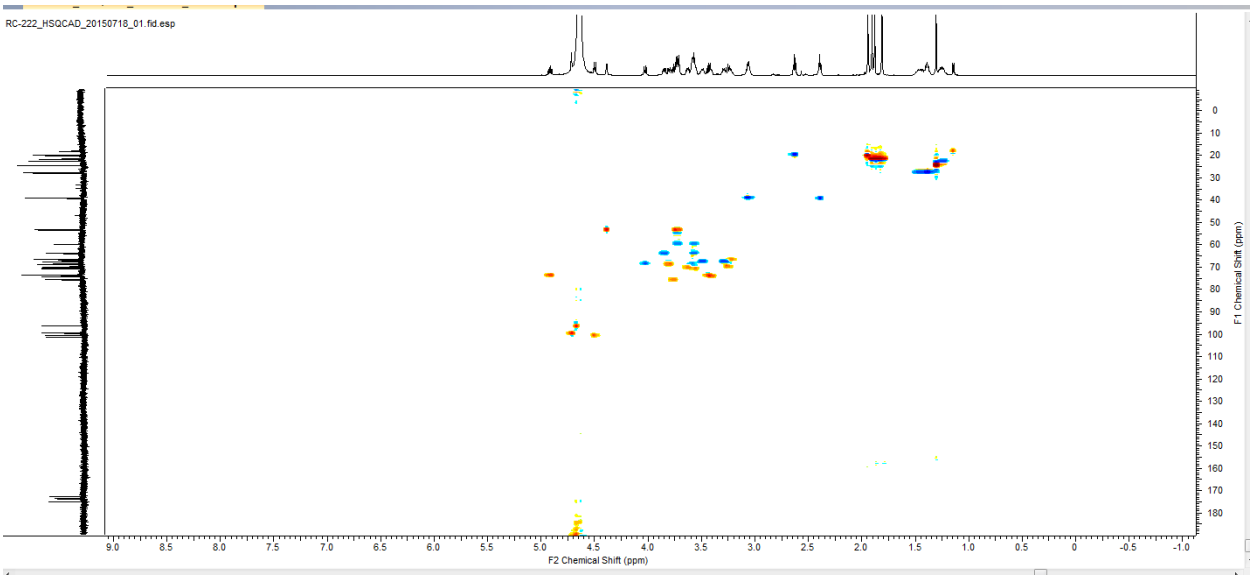
^{13}C NMR (150 MHz, D_2O)

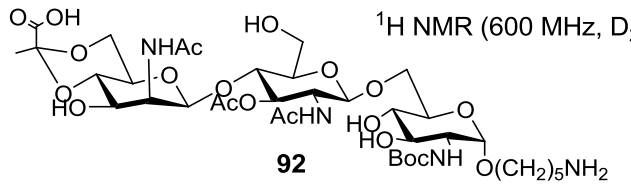


^{1}H-^{1}H COSY (600 MHz, D_2O)



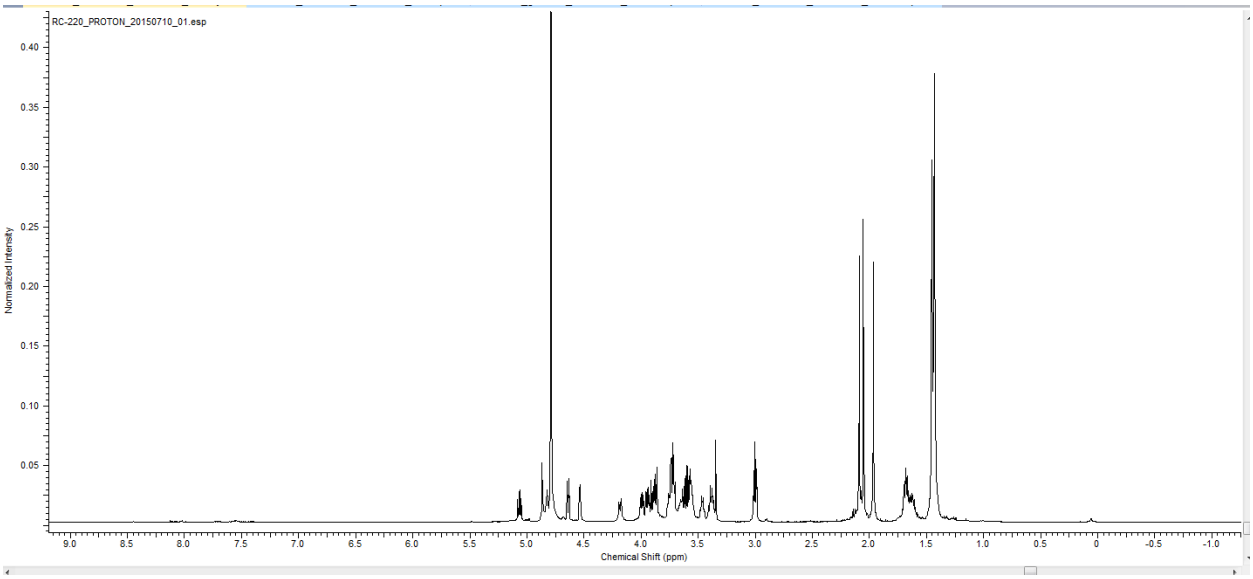
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



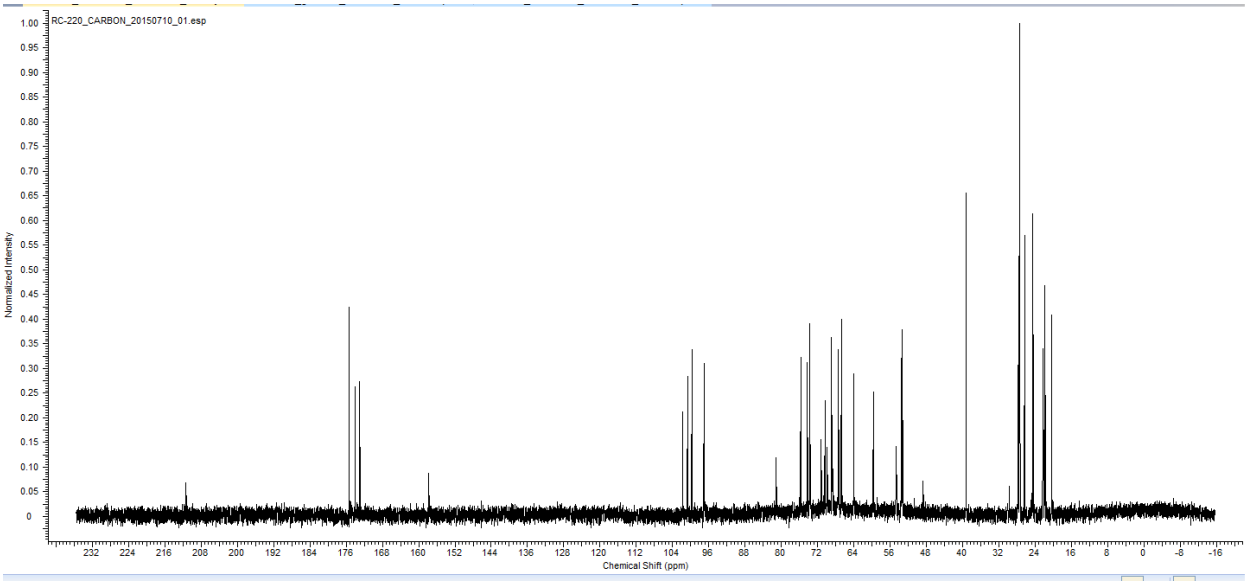


92

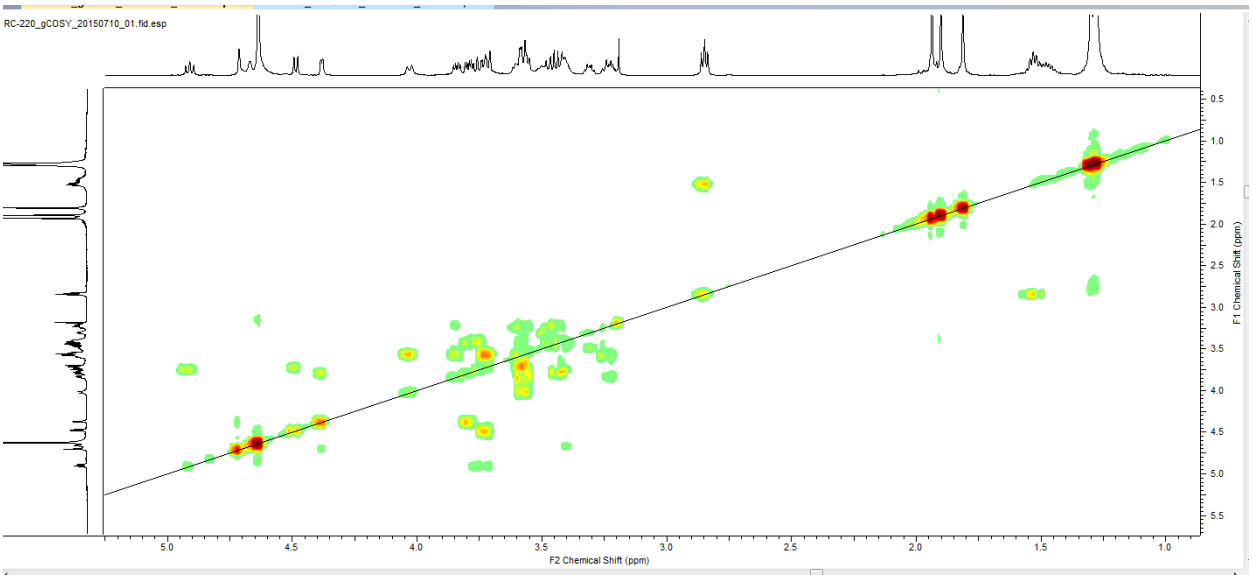
^{1}H NMR (600 MHz, D_2O)



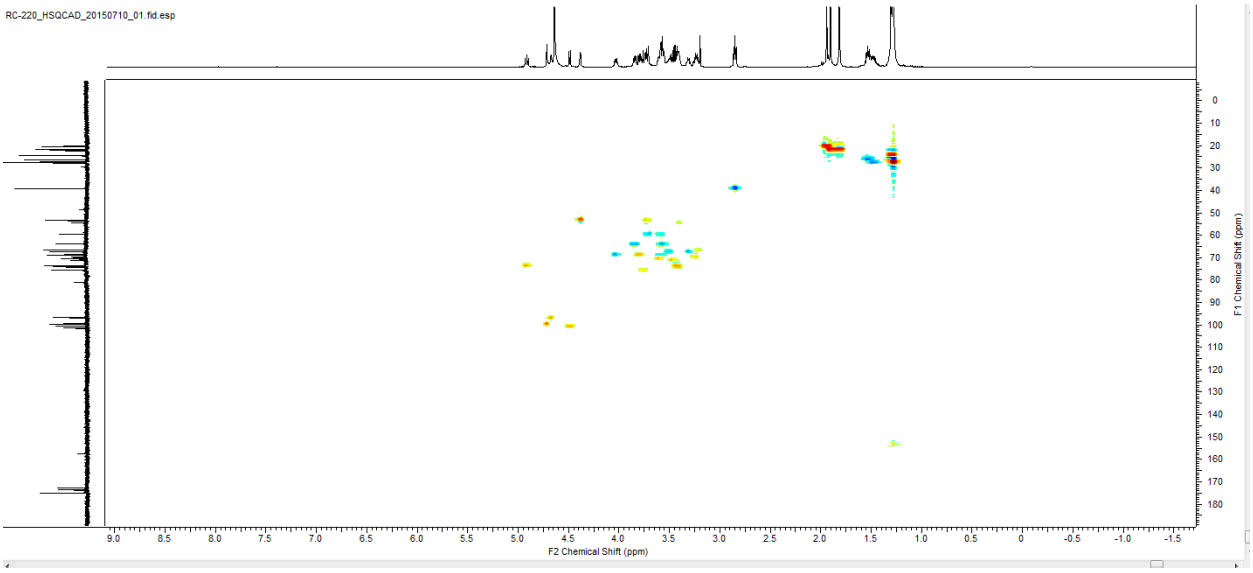
^{13}C NMR (150 MHz, D_2O)

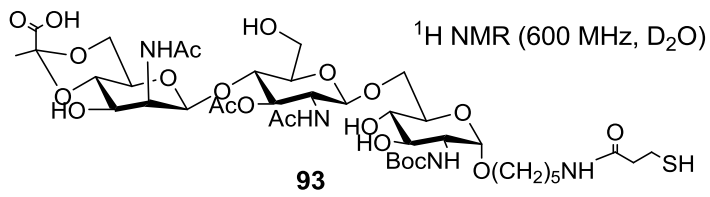


^{1}H-^{1}H COSY (600 MHz, D_2O)



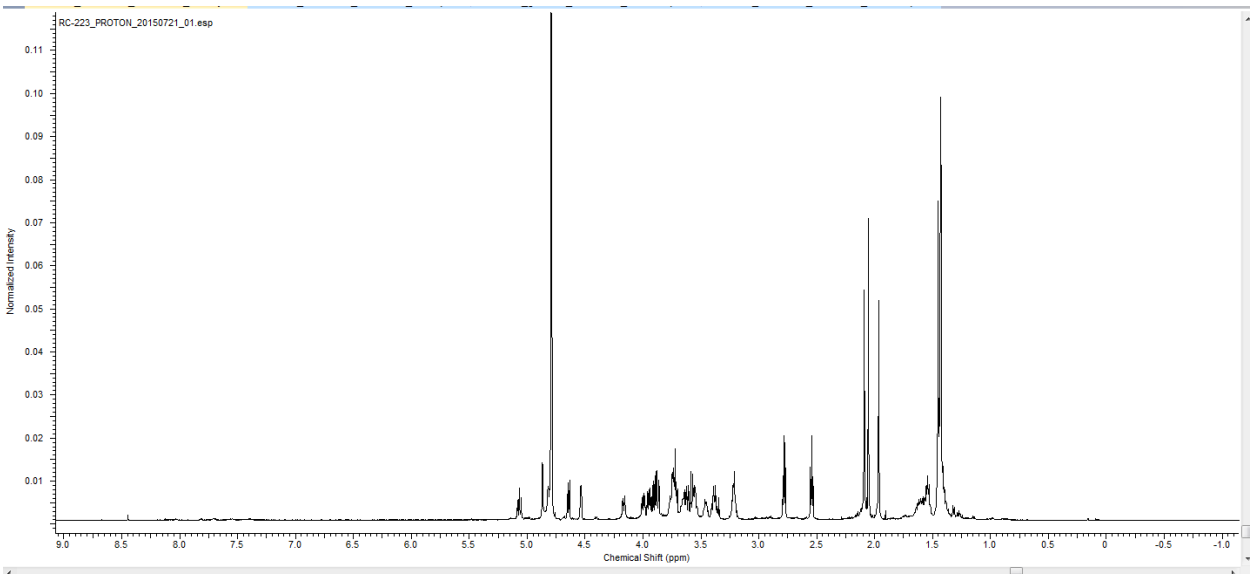
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



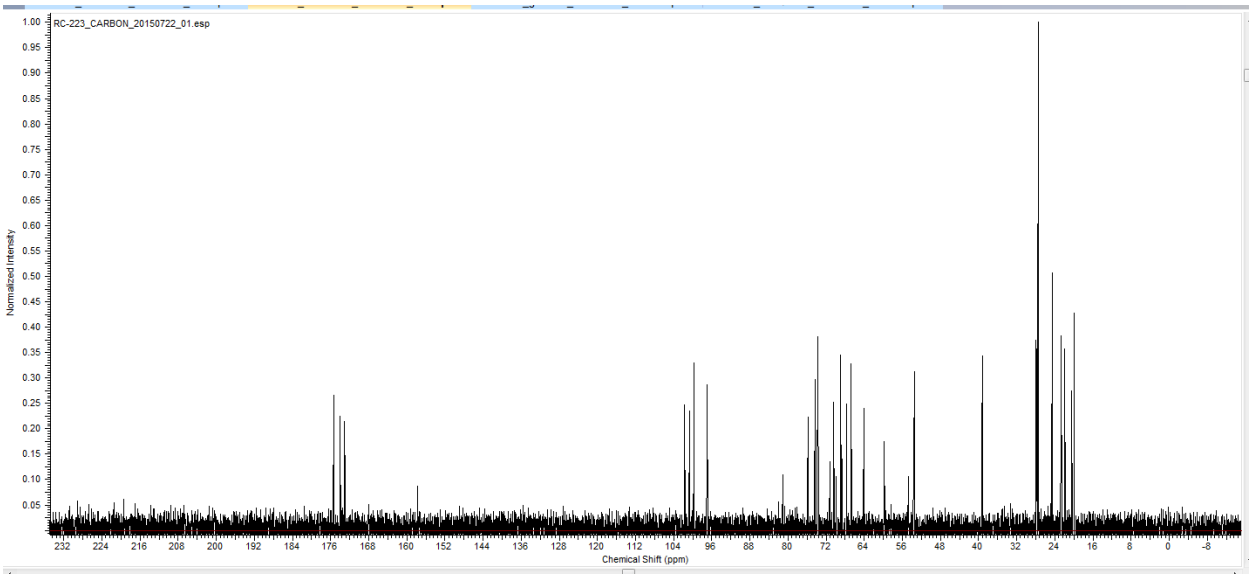


93

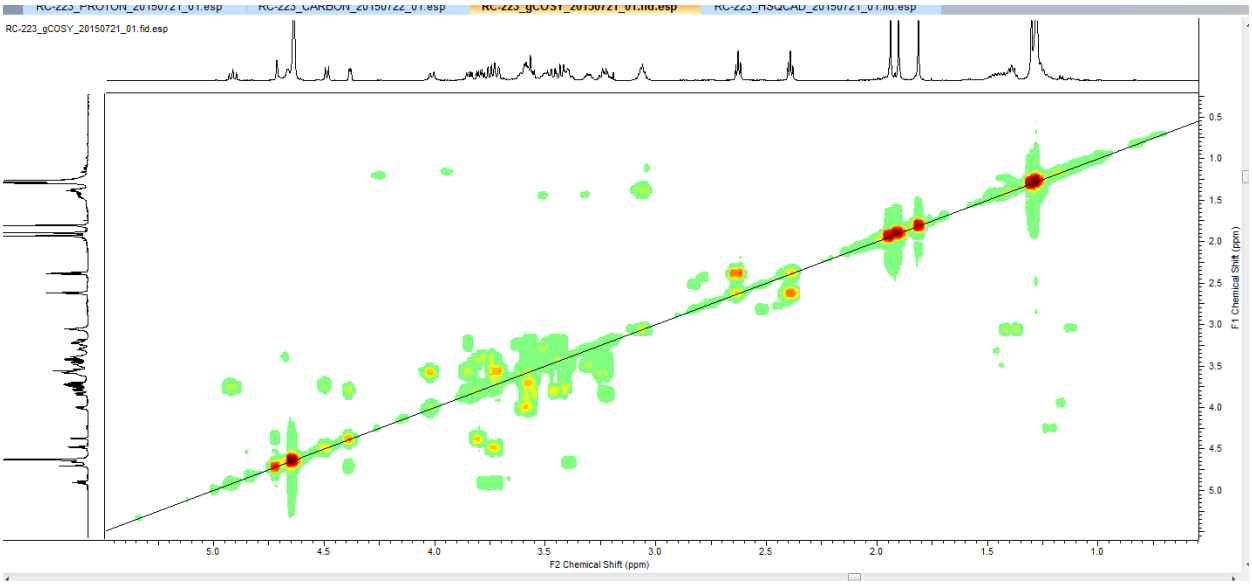
^{1}H NMR (600 MHz, D_2O)



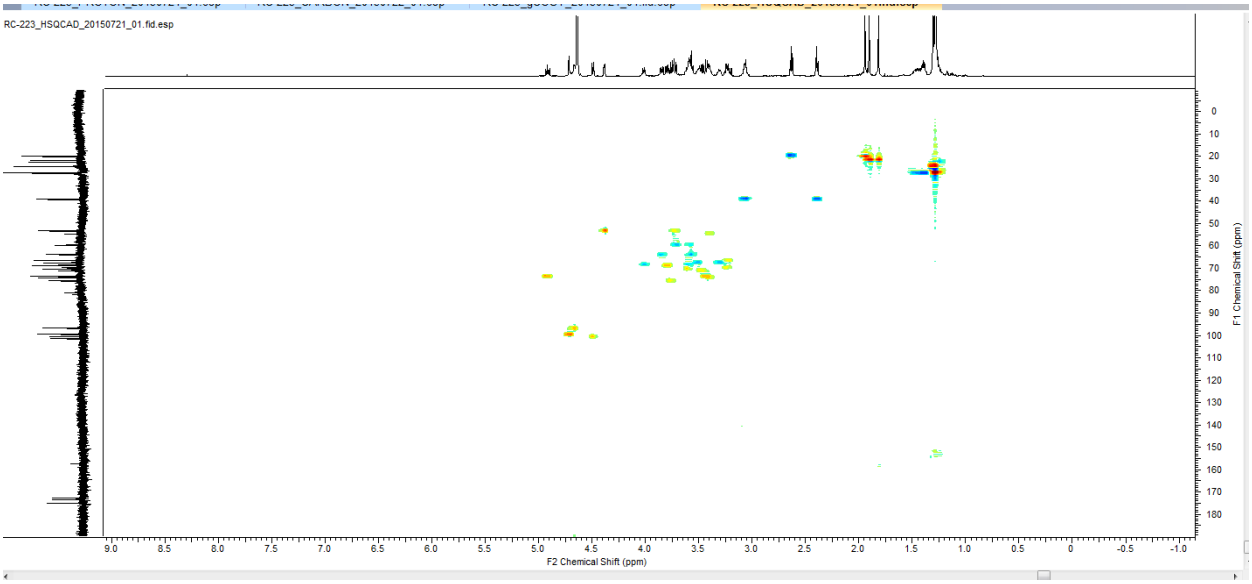
^{13}C NMR (150 MHz, D_2O)

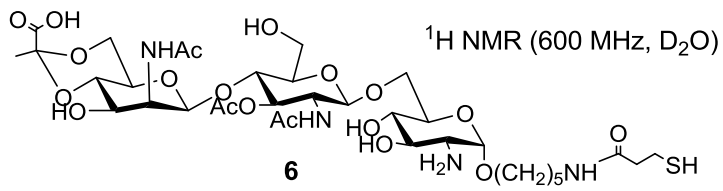


^{1}H-^{1}H COSY (600 MHz, D_2O)



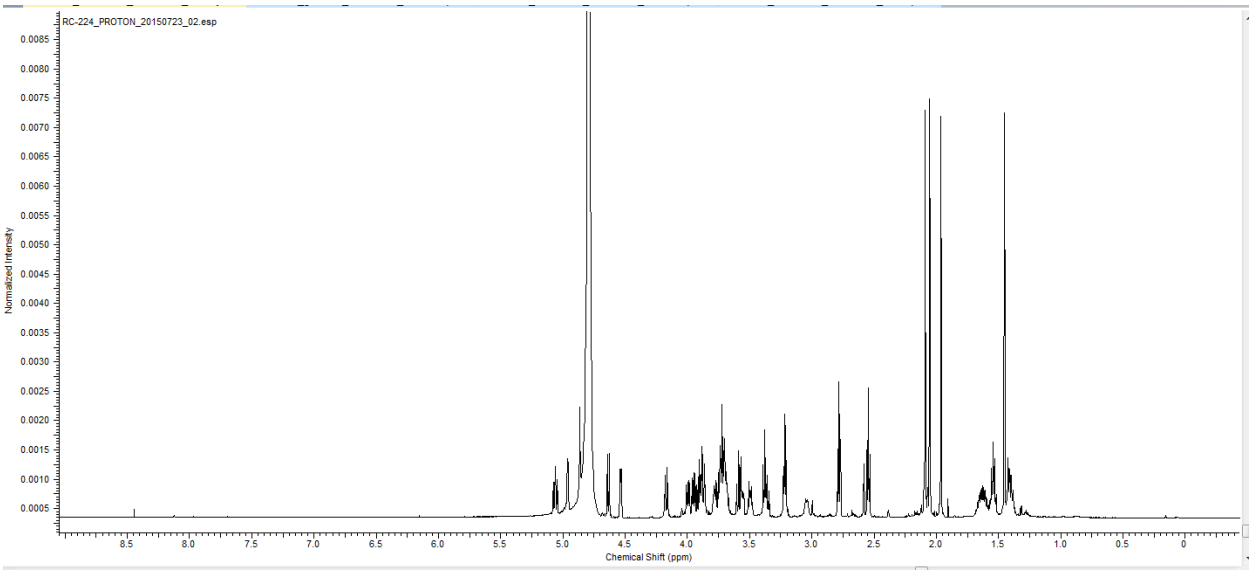
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



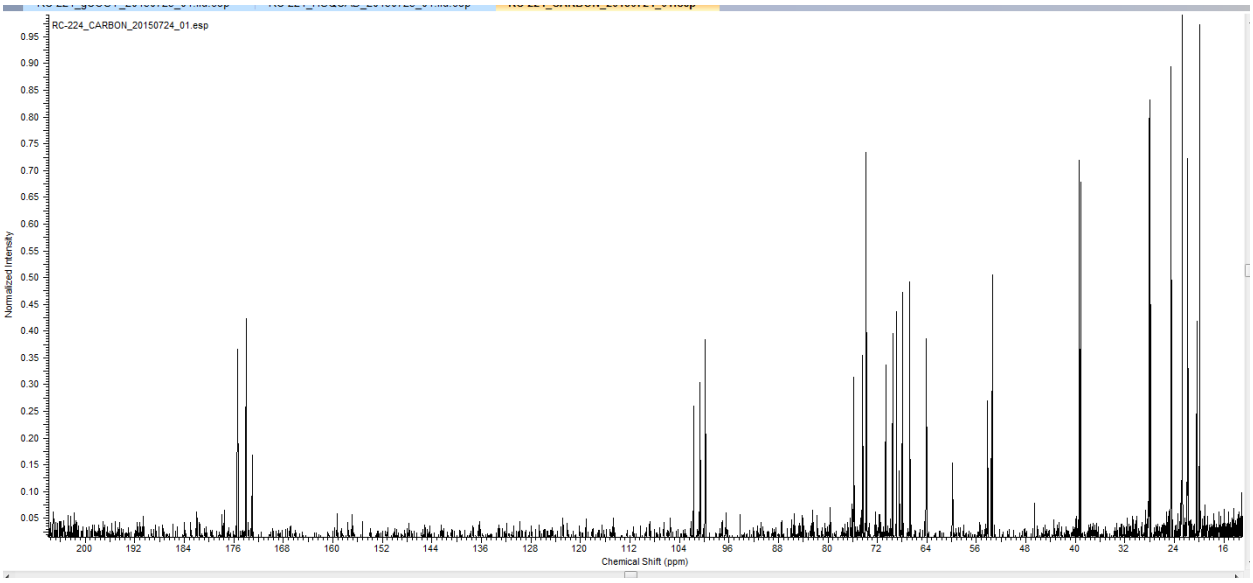


6

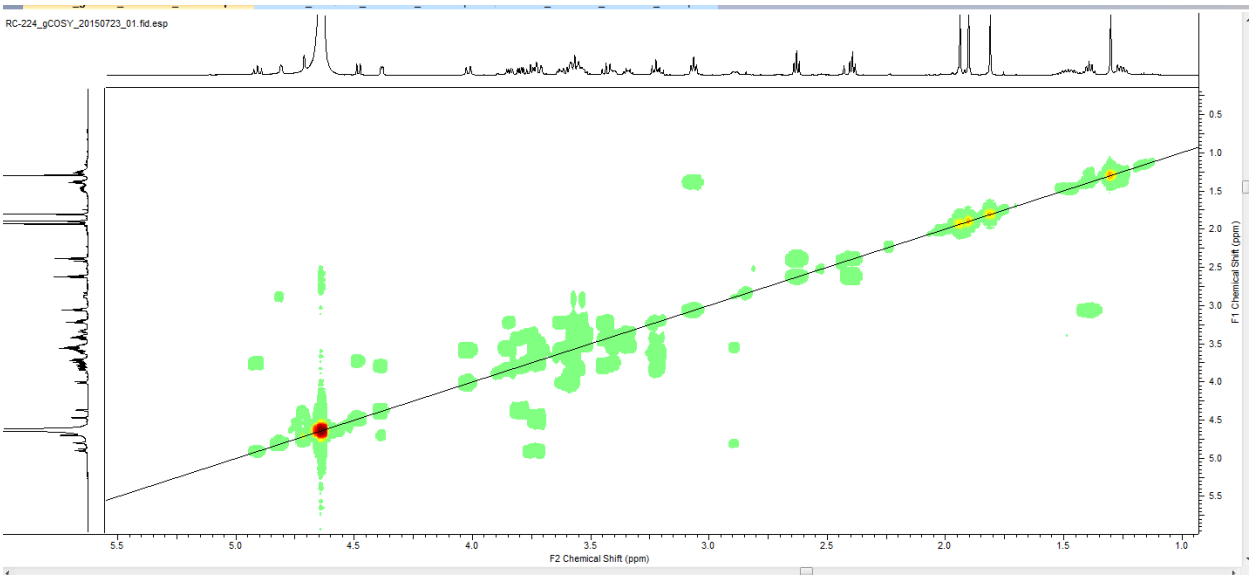
^{1}H NMR (600 MHz, D_2O)



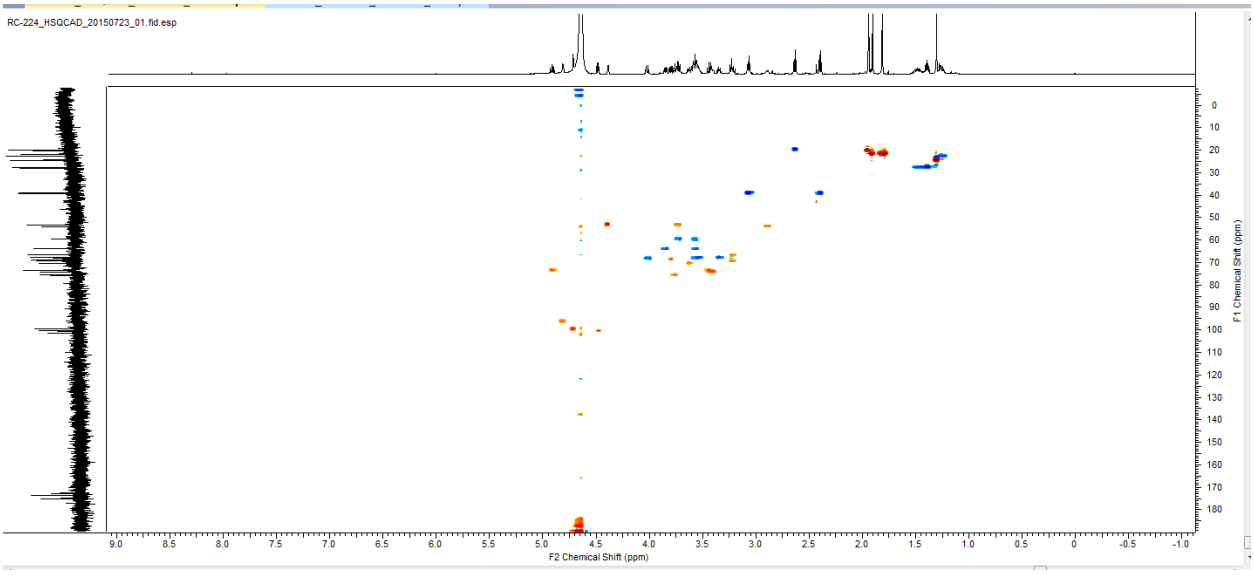
^{13}C NMR (150 MHz, D_2O)

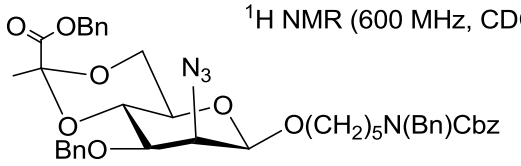


^{1}H-^{1}H COSY (600 MHz, D_2O)



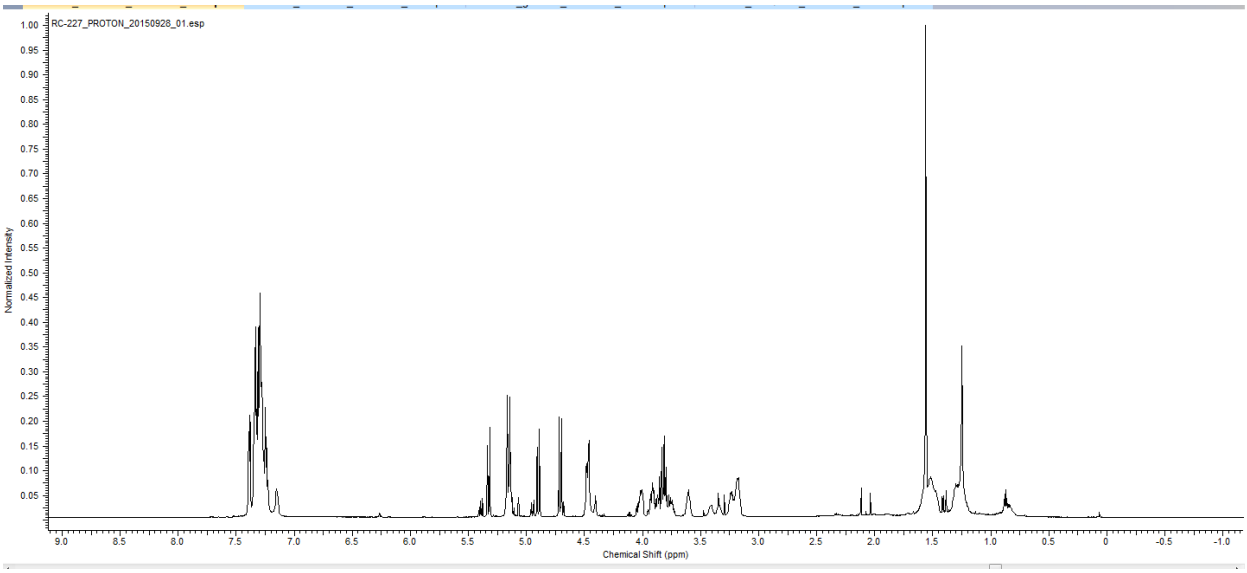
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



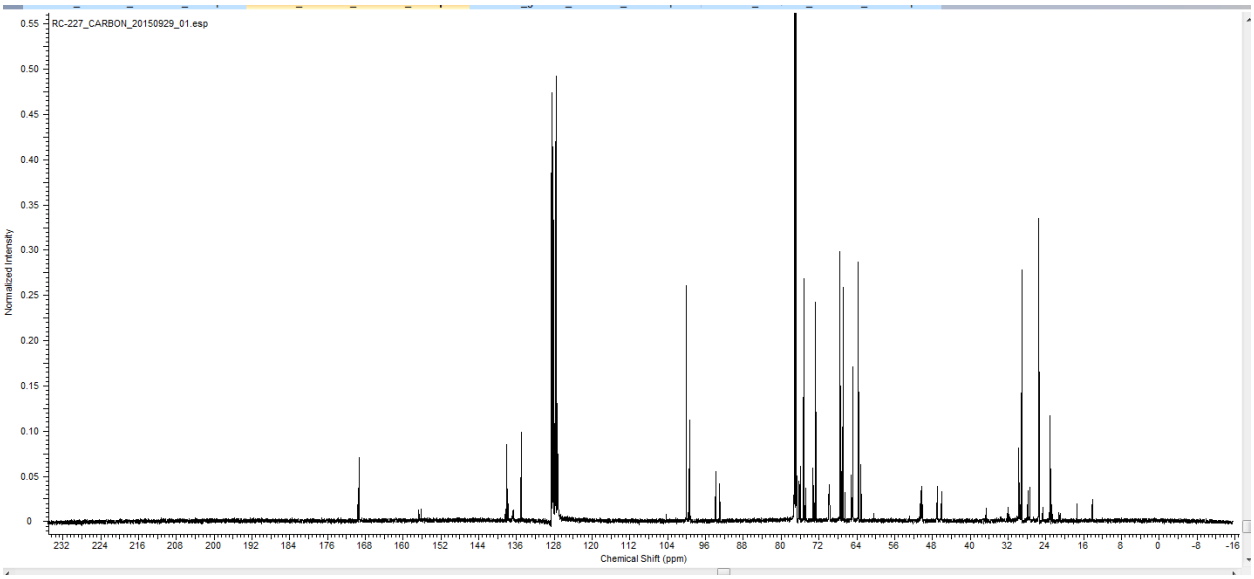


95

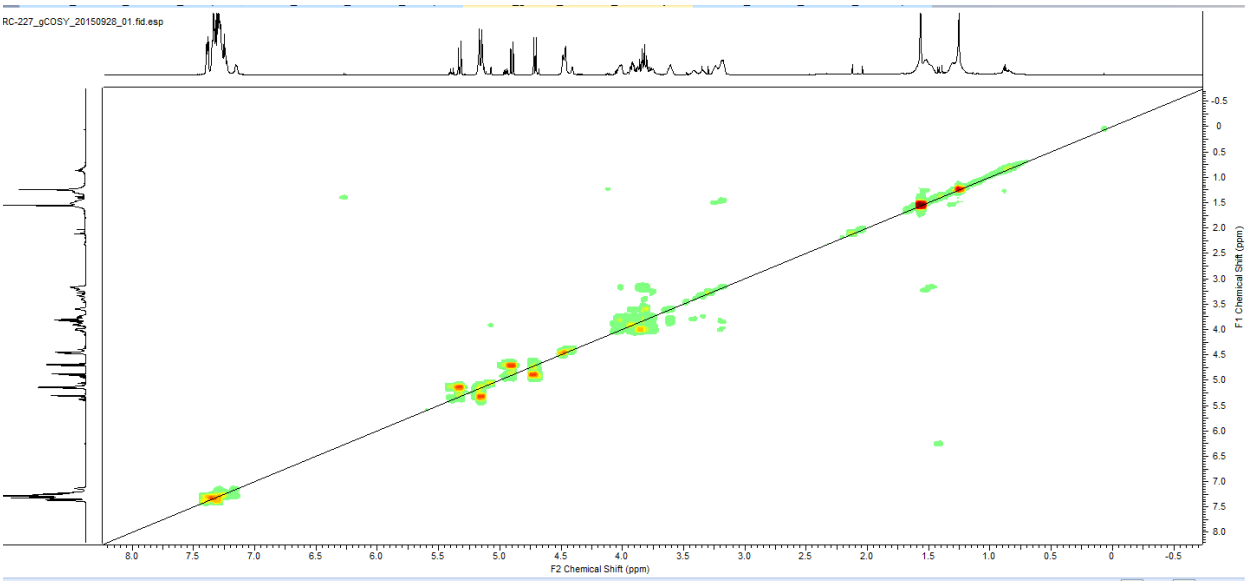
^{1}H NMR (600 MHz, $CDCl_3$)



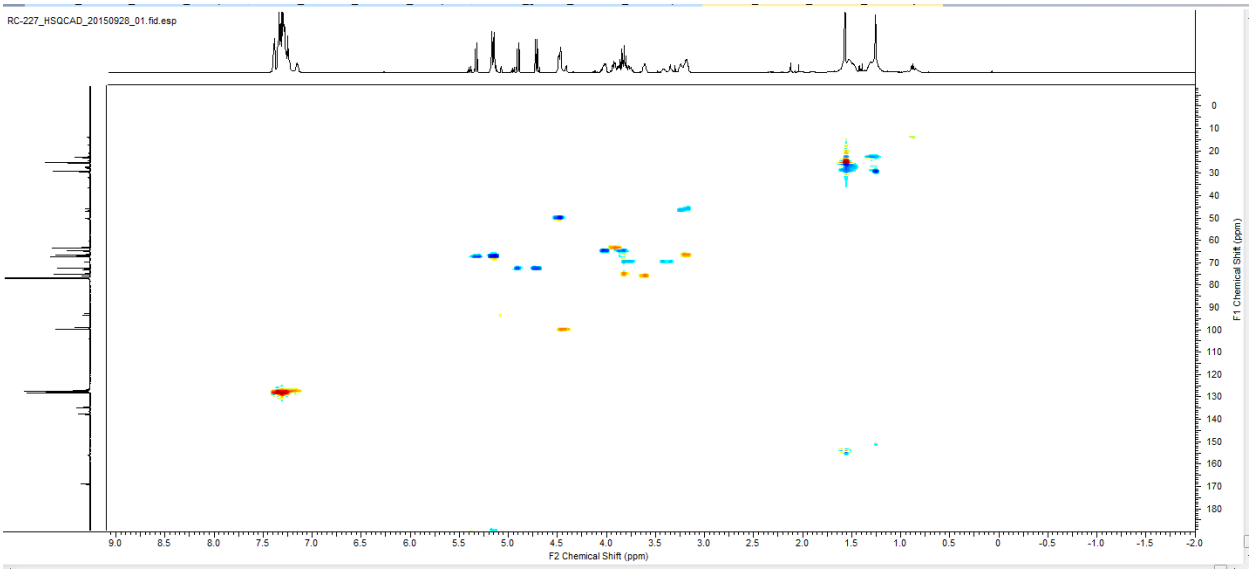
^{13}C NMR (150 MHz, $CDCl_3$)

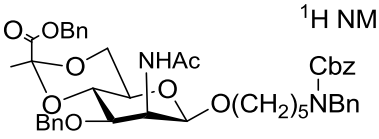


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



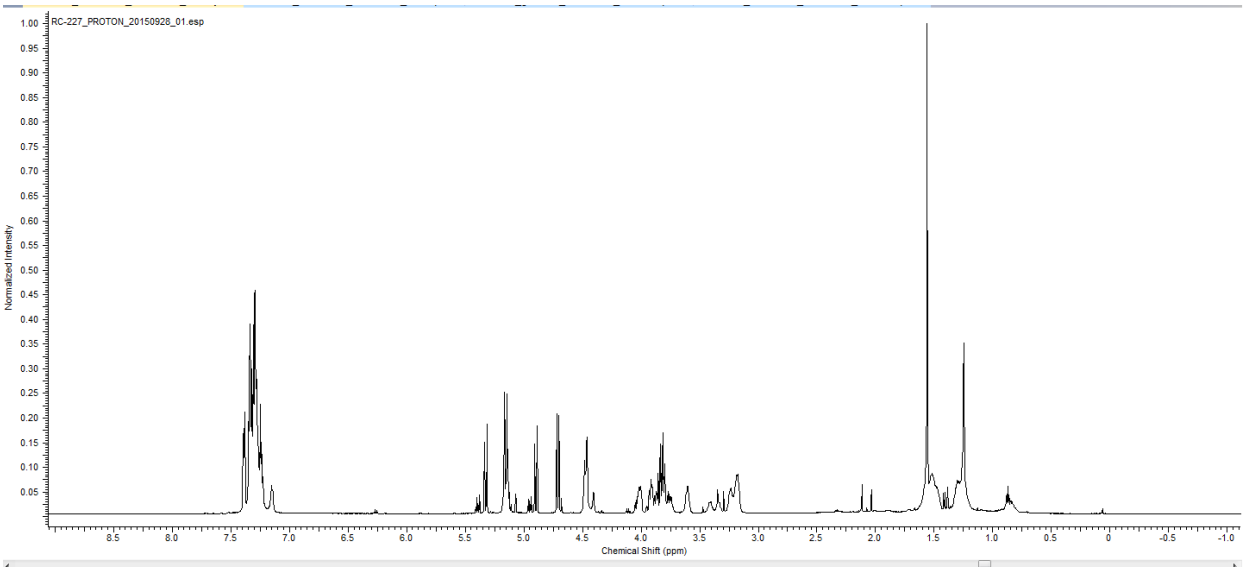
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



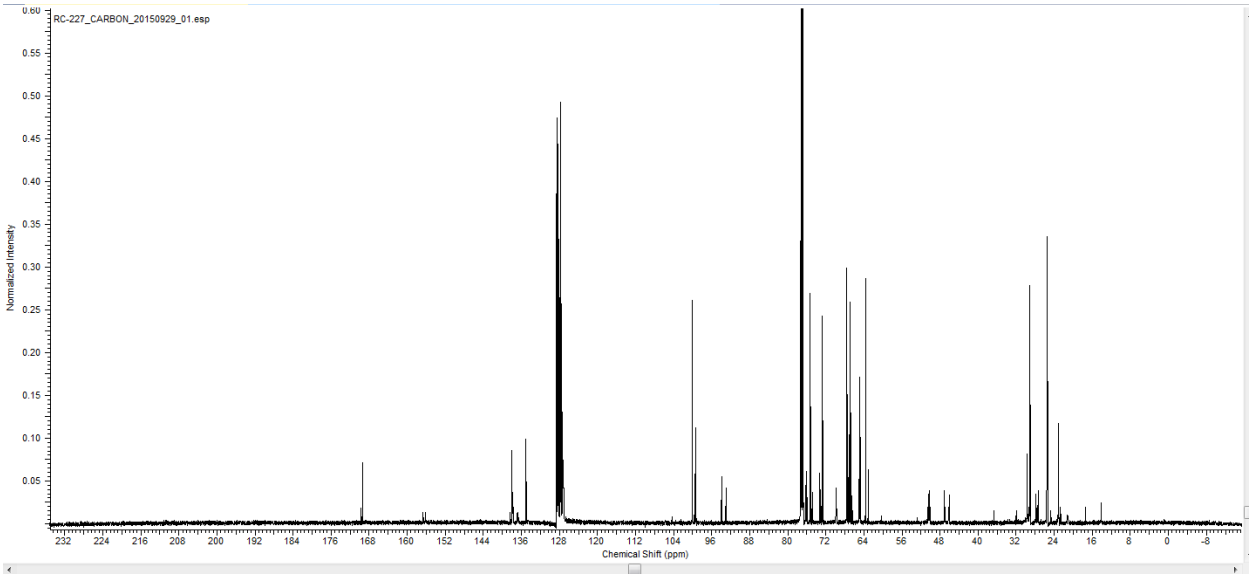


96

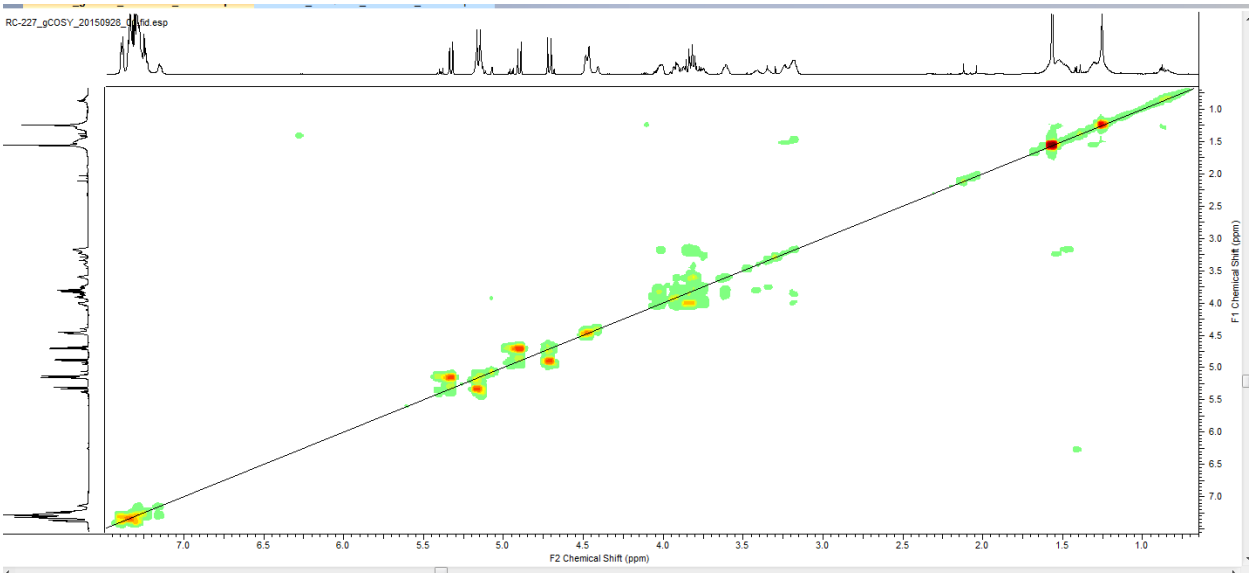
^{1}H NMR (600 MHz, $CDCl_3$)



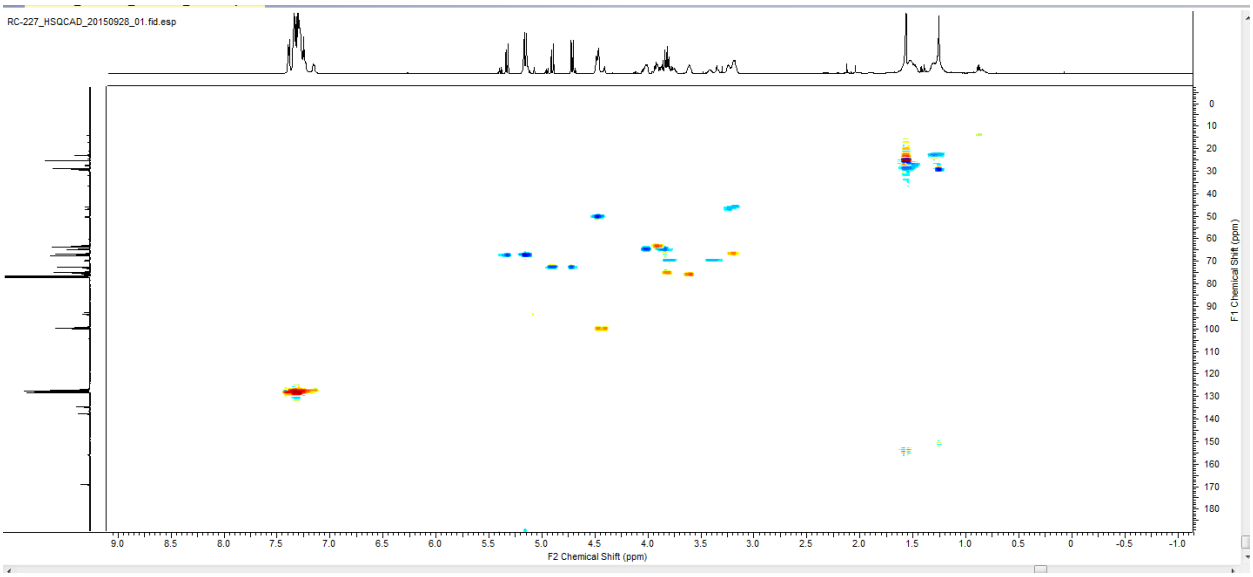
^{13}C NMR (150 MHz, $CDCl_3$)

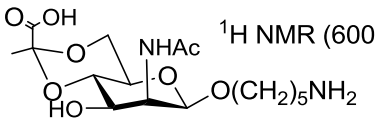


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



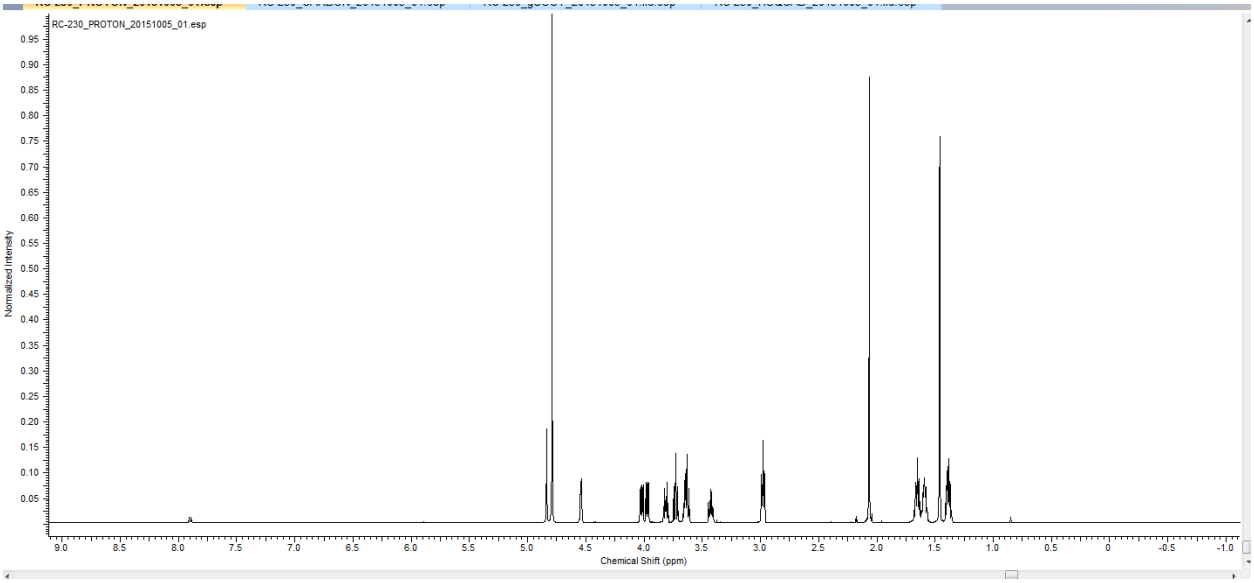
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



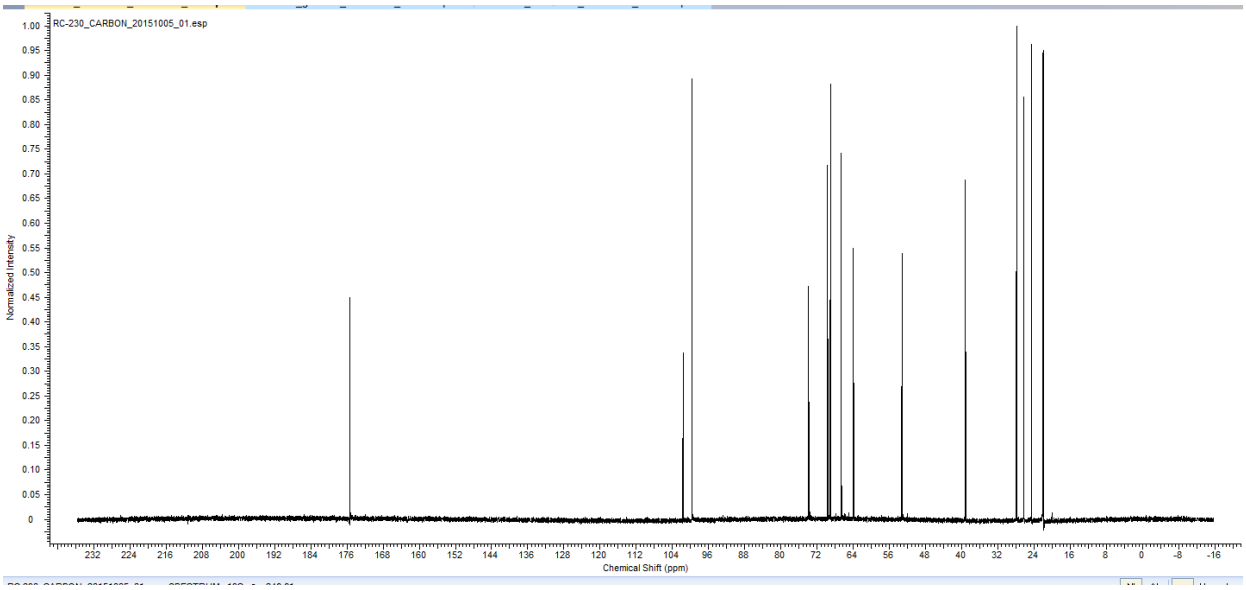


97

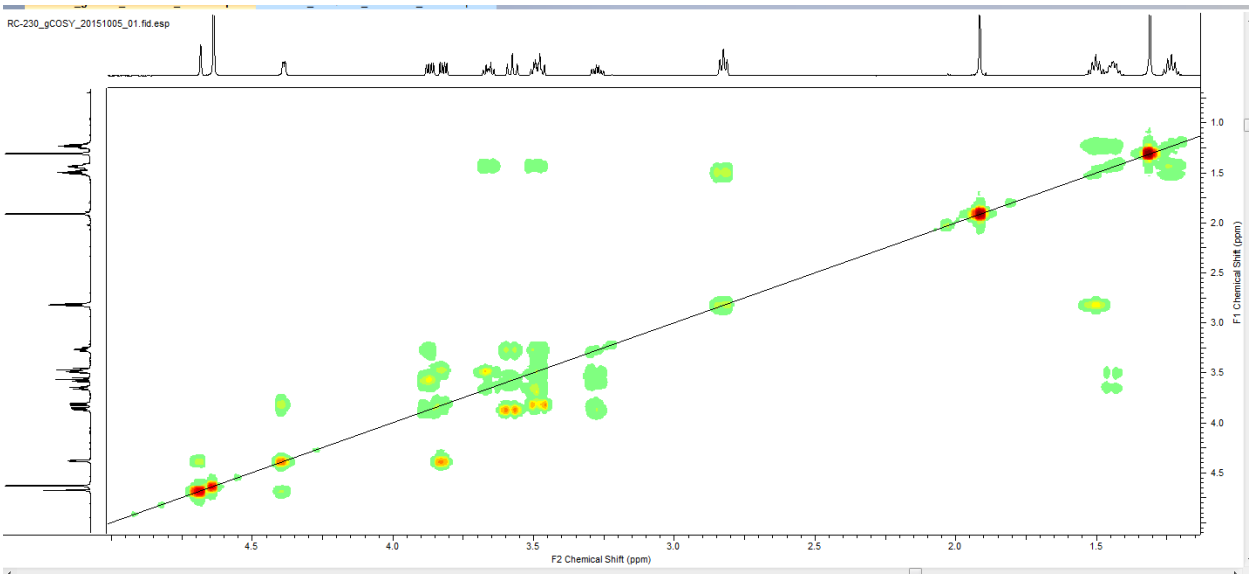
^{1}H NMR (600 MHz, D_2O)



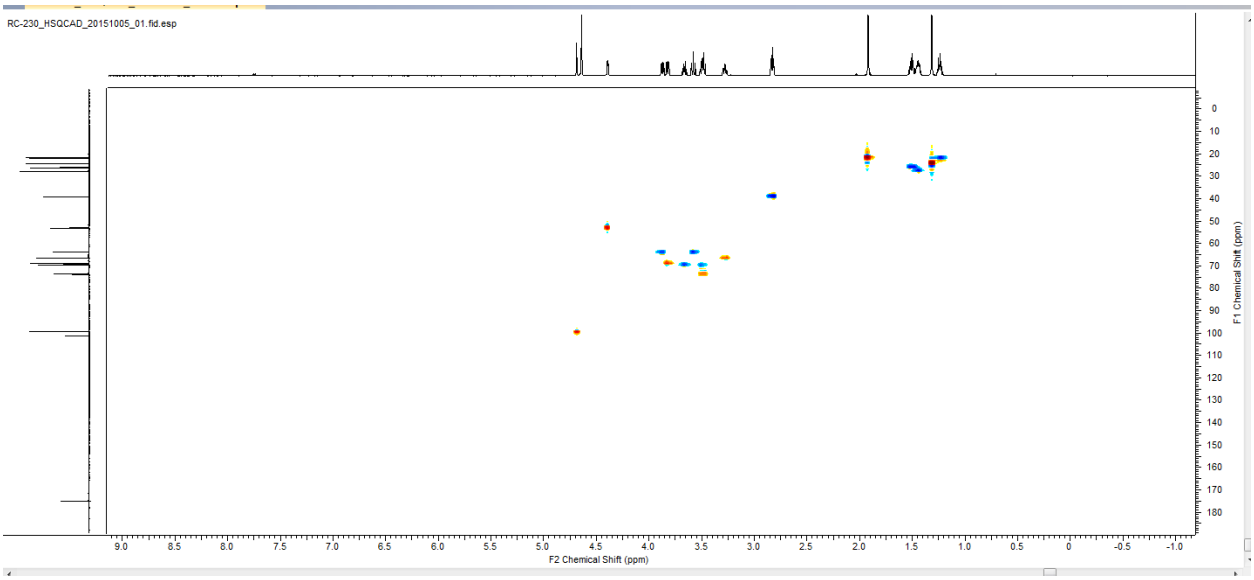
^{13}C NMR (150 MHz, D_2O)



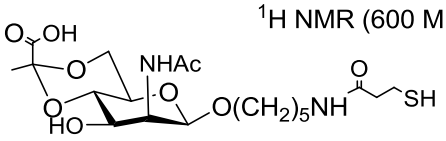
^{1}H-^{1}H COSY (600 MHz, D_2O)



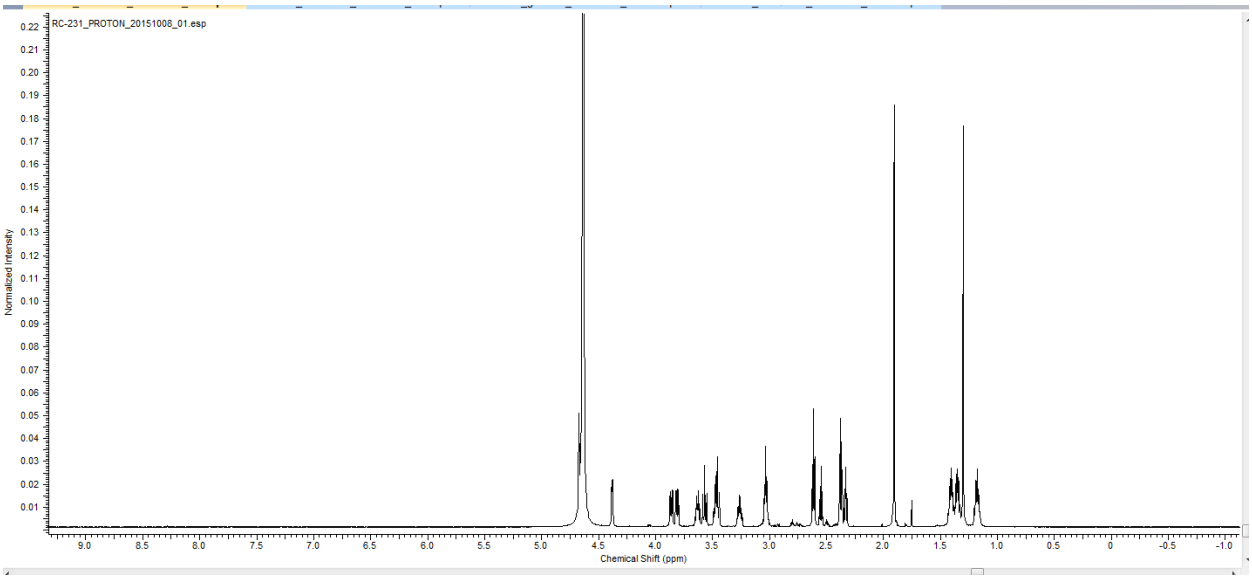
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



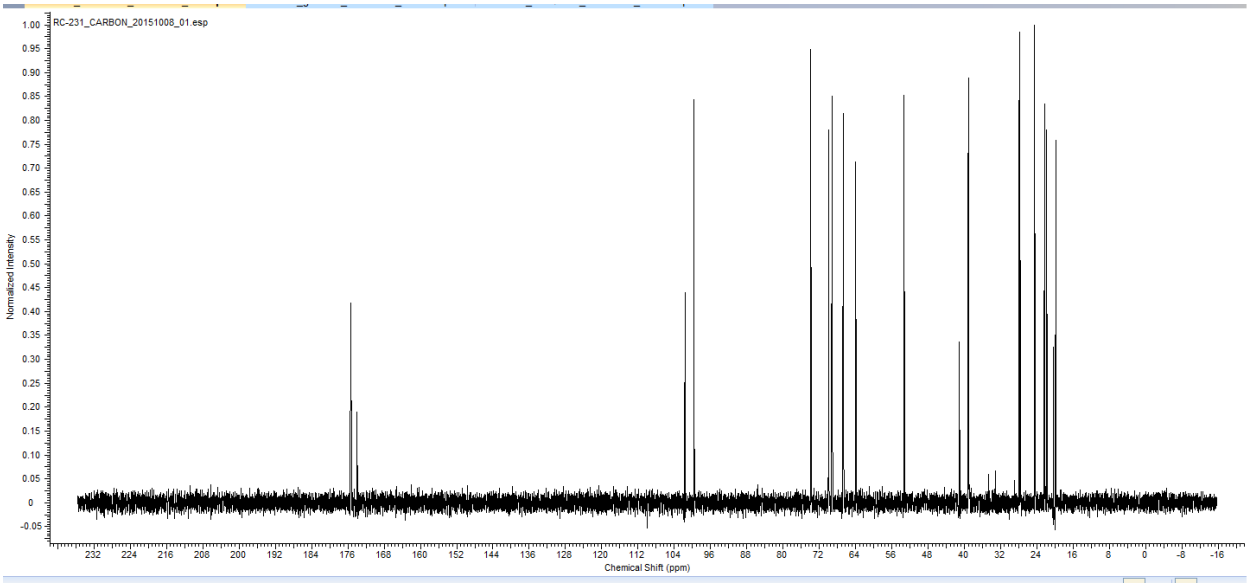
^{1}H NMR (600 MHz, D_2O)



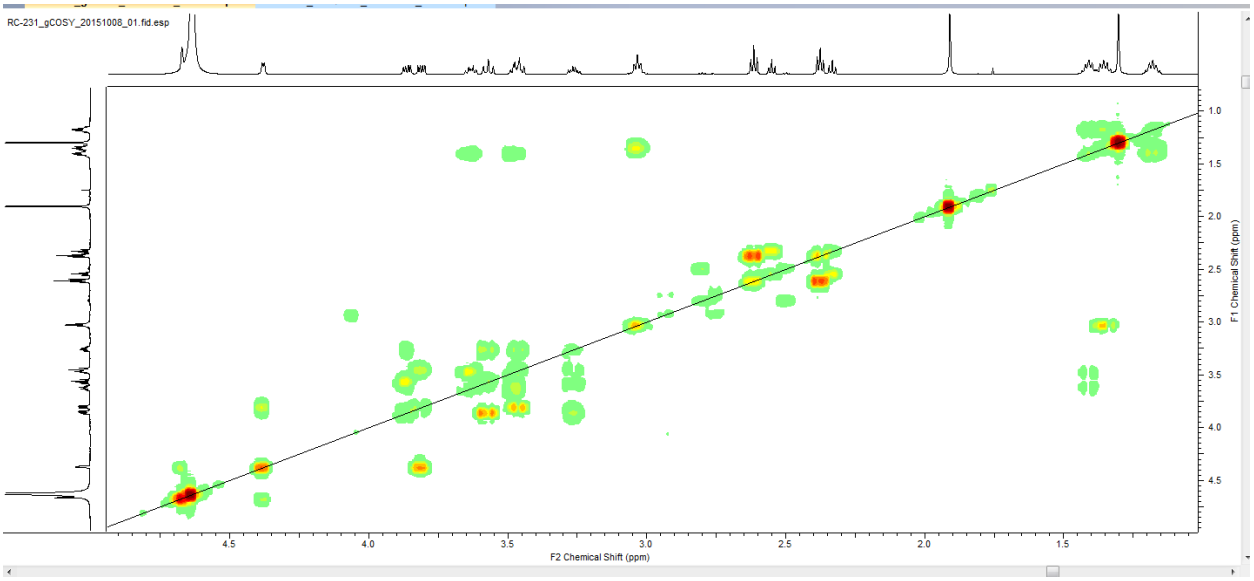
8



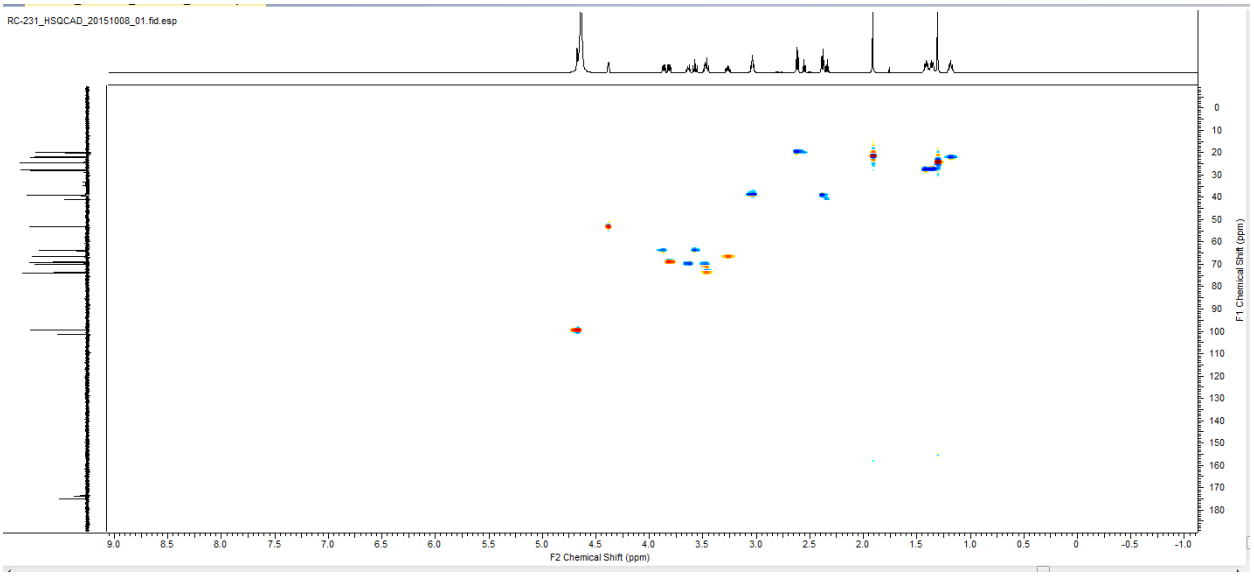
^{13}C NMR (150 MHz, D_2O)

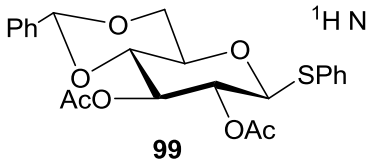


^{1}H-^{1}H COSY (600 MHz, D_2O)



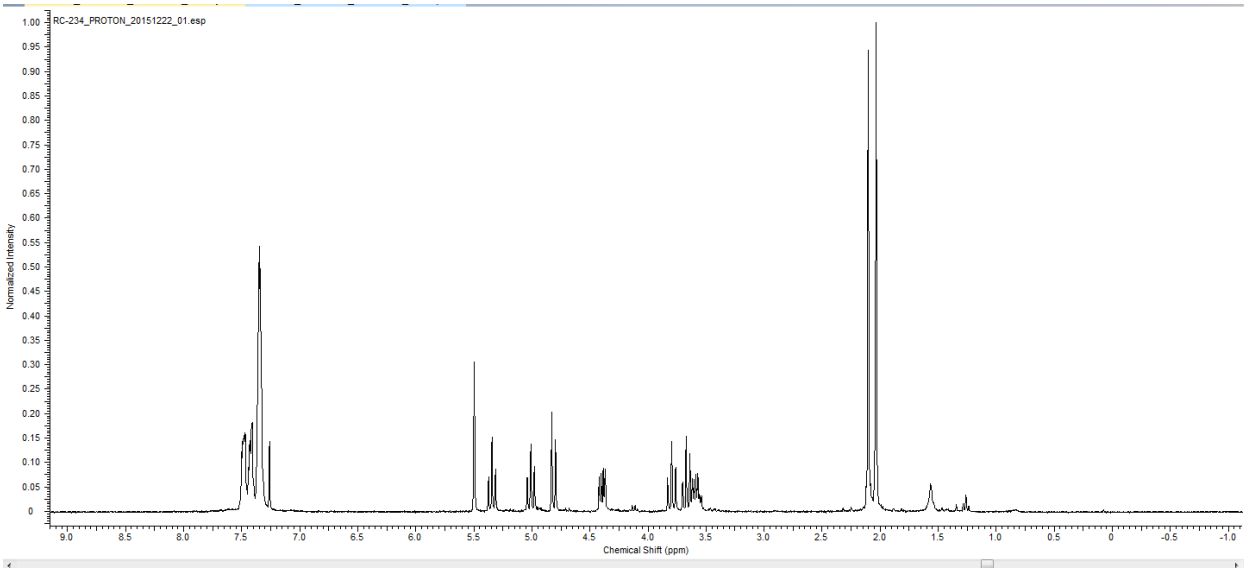
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



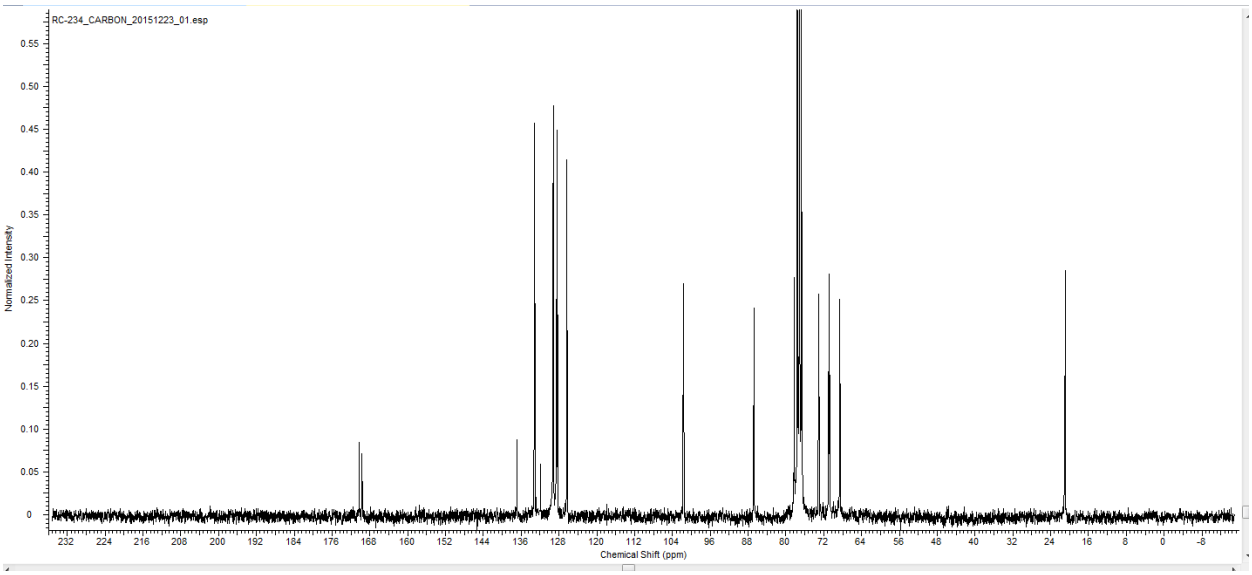


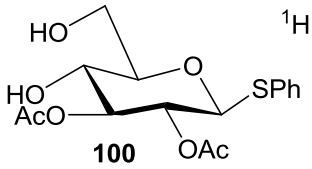
99

^{1}H NMR (300 MHz, $CDCl_3$)



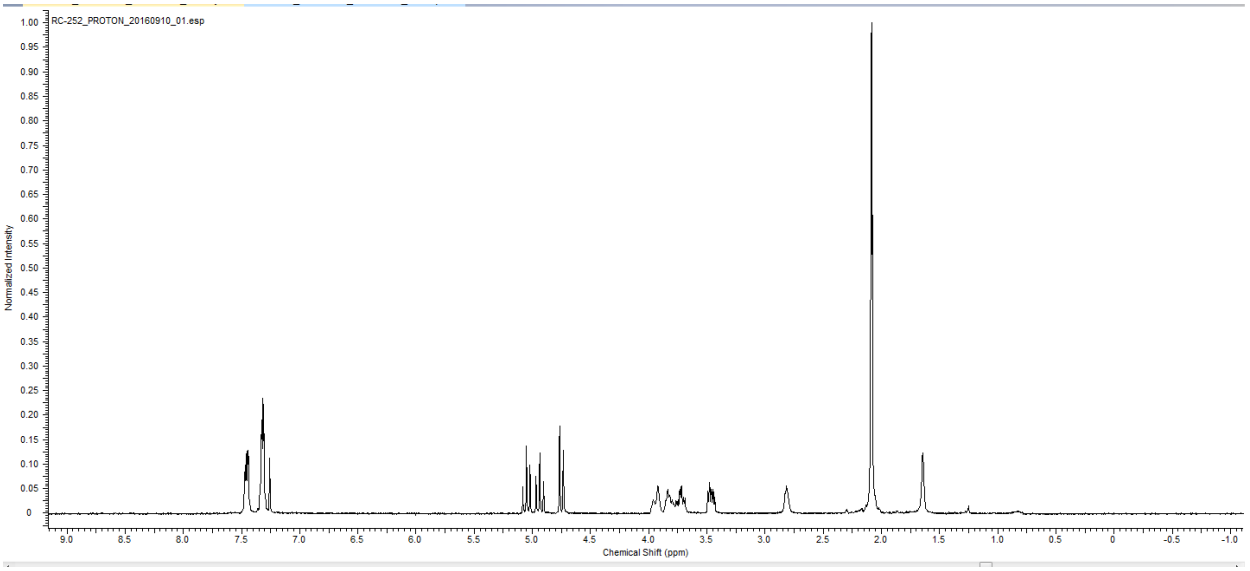
^{13}C NMR (75 MHz, $CDCl_3$)



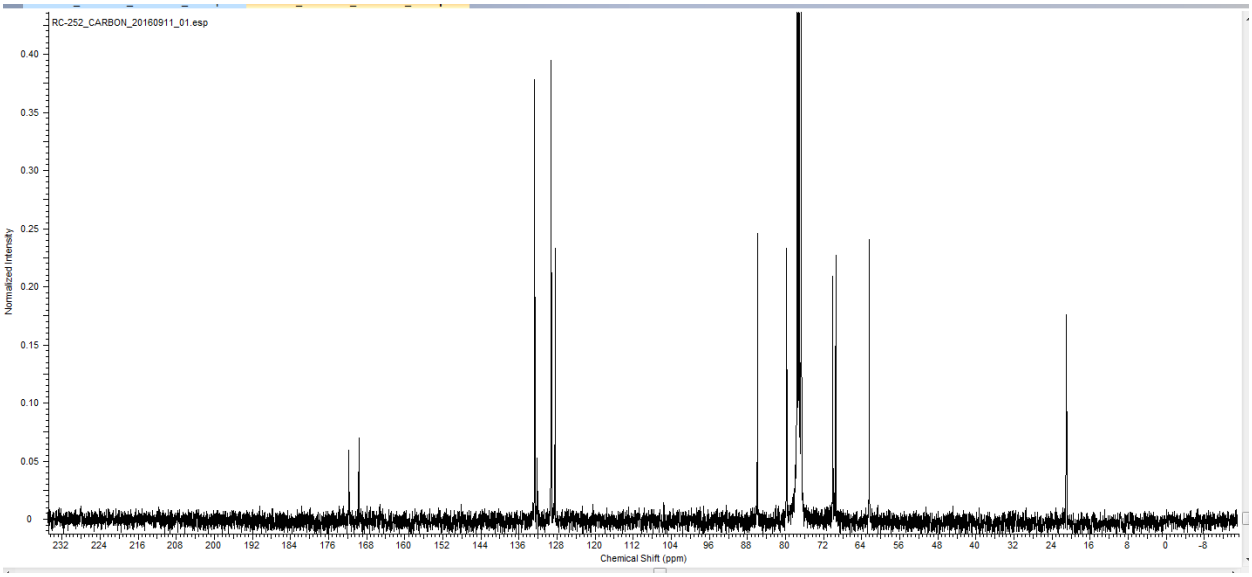


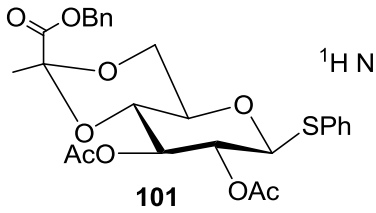
100

^{1}H NMR (300 MHz, $CDCl_3$)



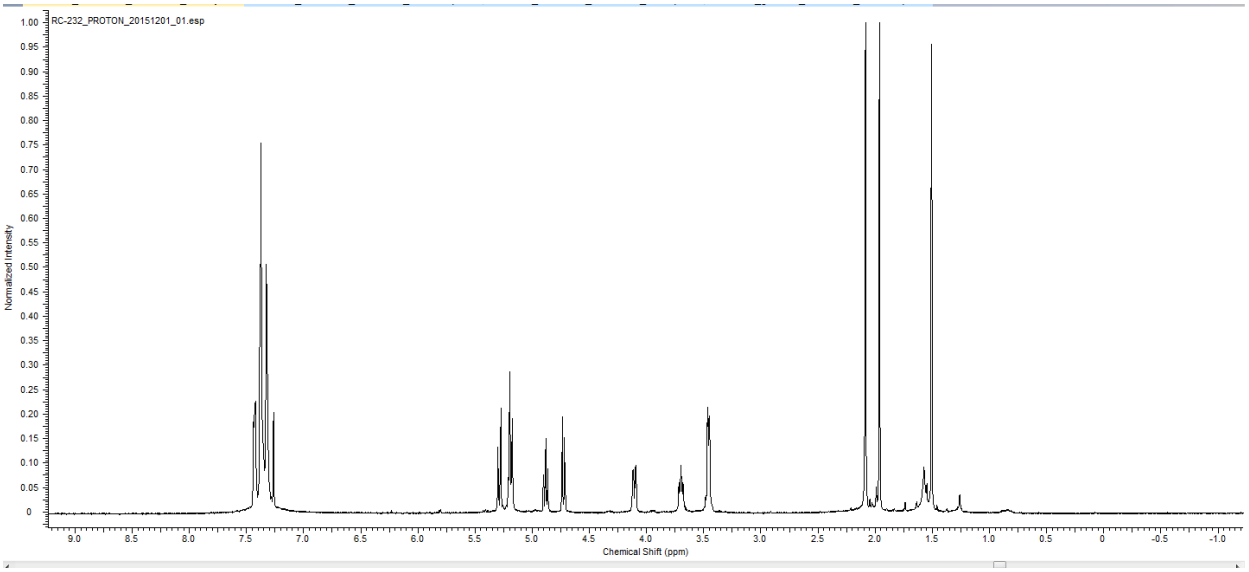
^{13}C NMR (75 MHz, $CDCl_3$)



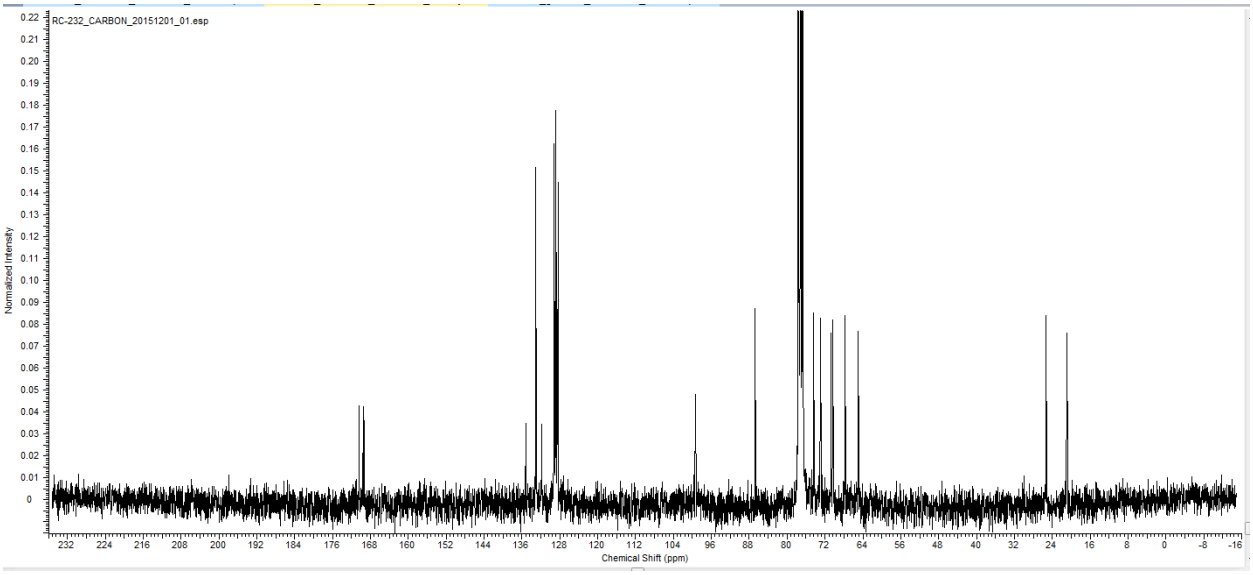


101

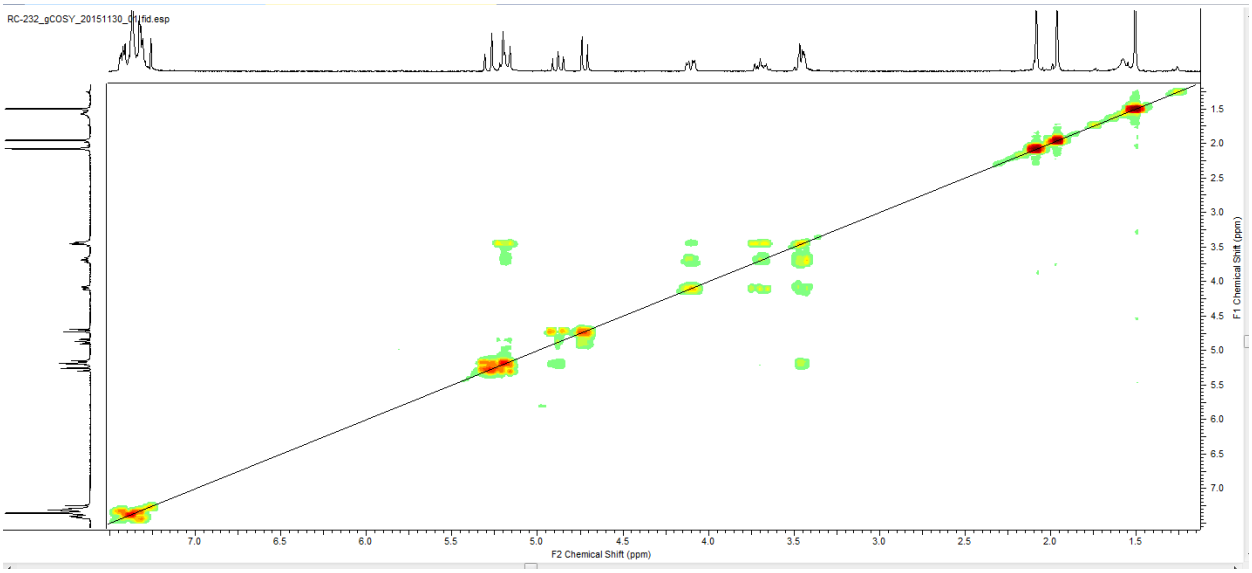
^{1}H NMR (300 MHz, $CDCl_3$)



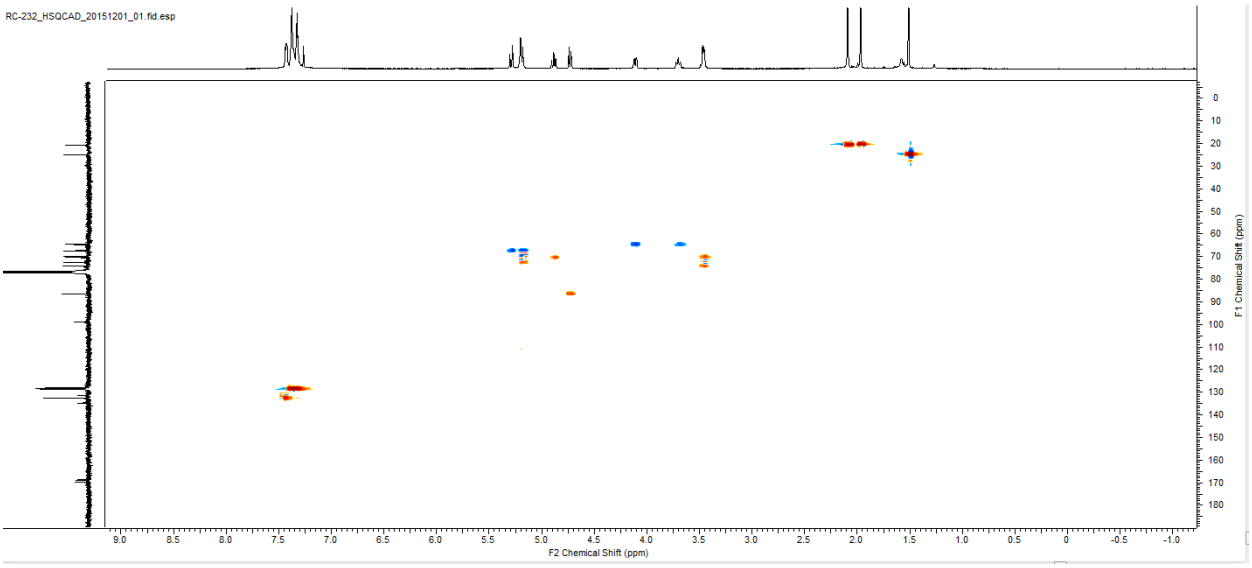
^{13}C NMR (75 MHz, $CDCl_3$)

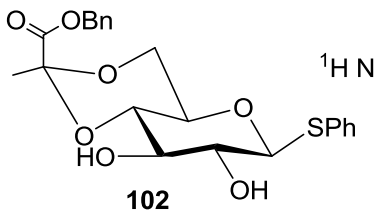


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



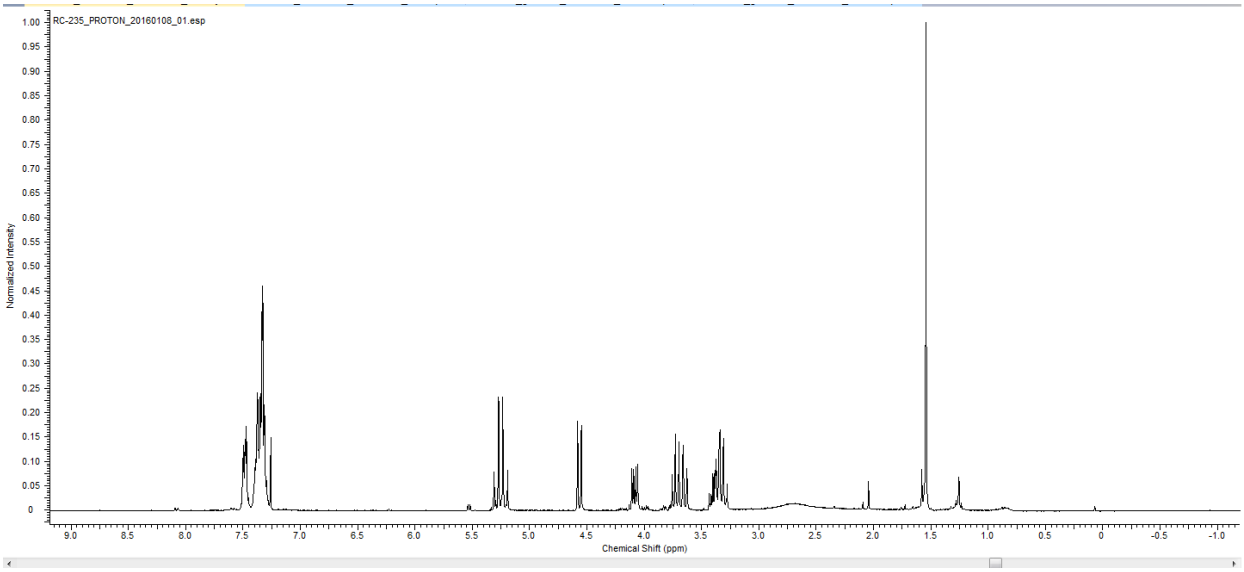
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



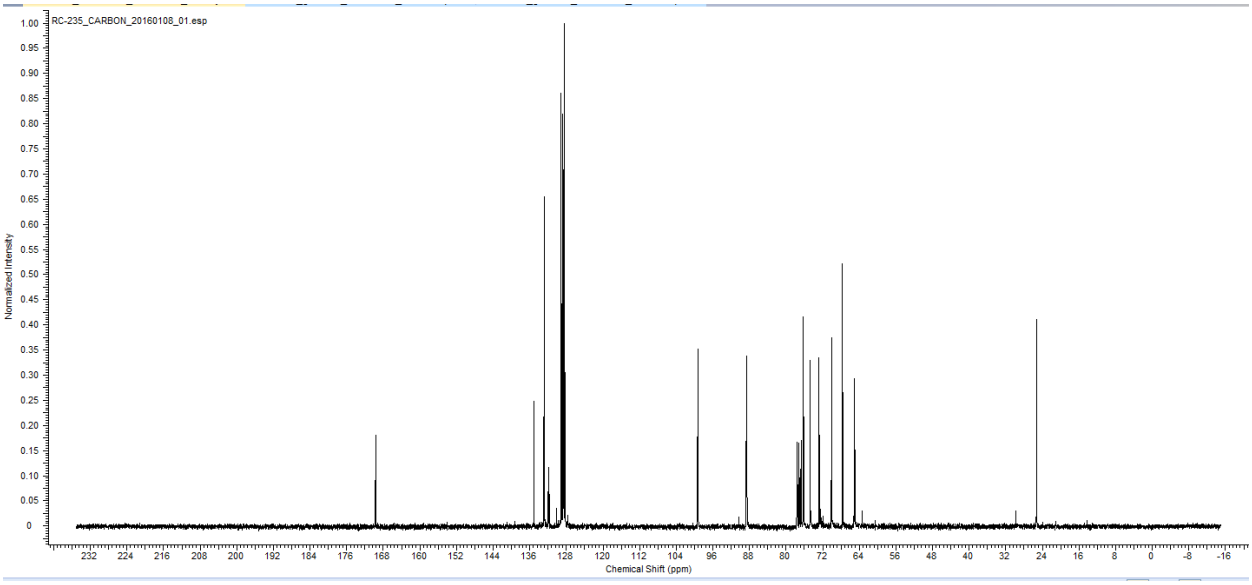


102

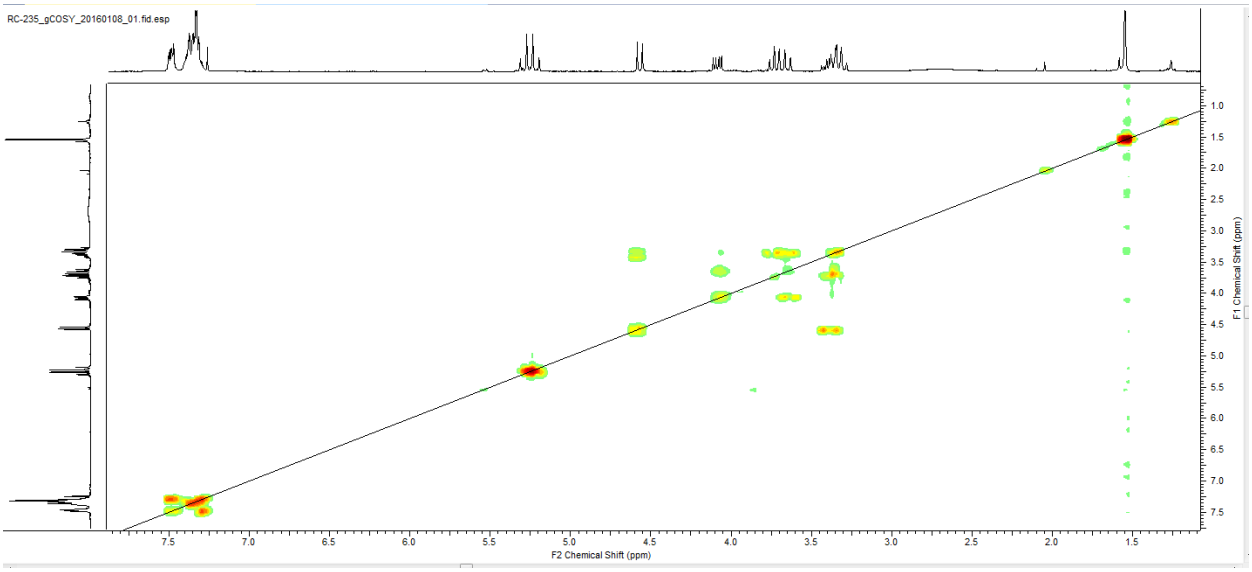
^{1}H NMR (300 MHz, $CDCl_3$)



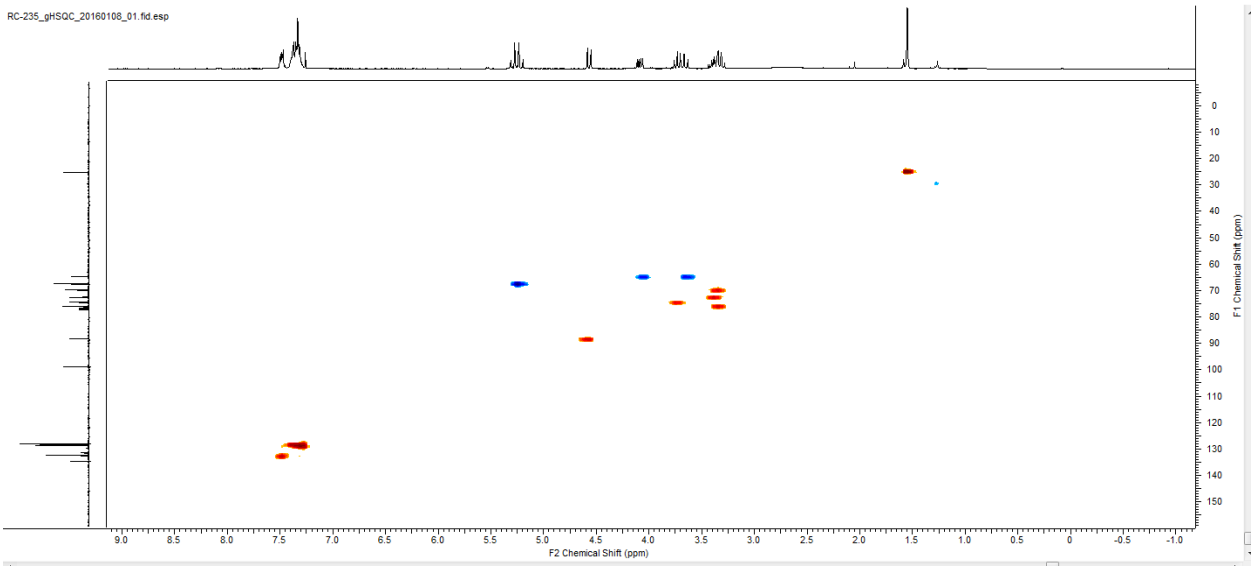
^{13}C NMR (75 MHz, $CDCl_3$)

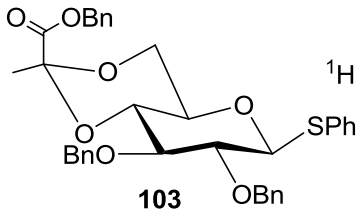


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



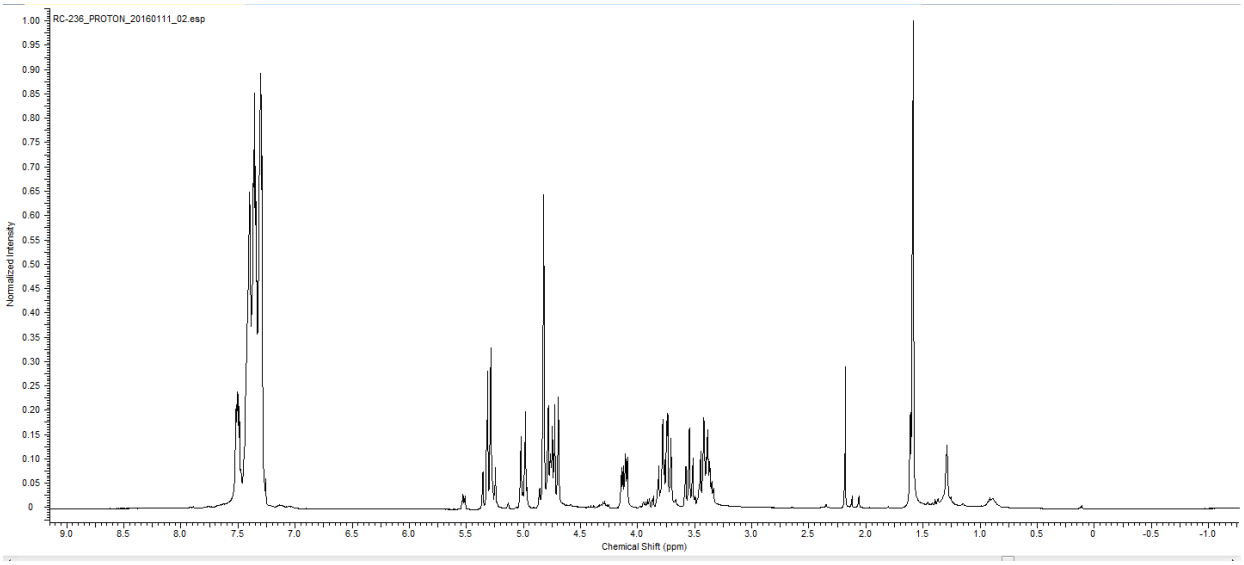
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



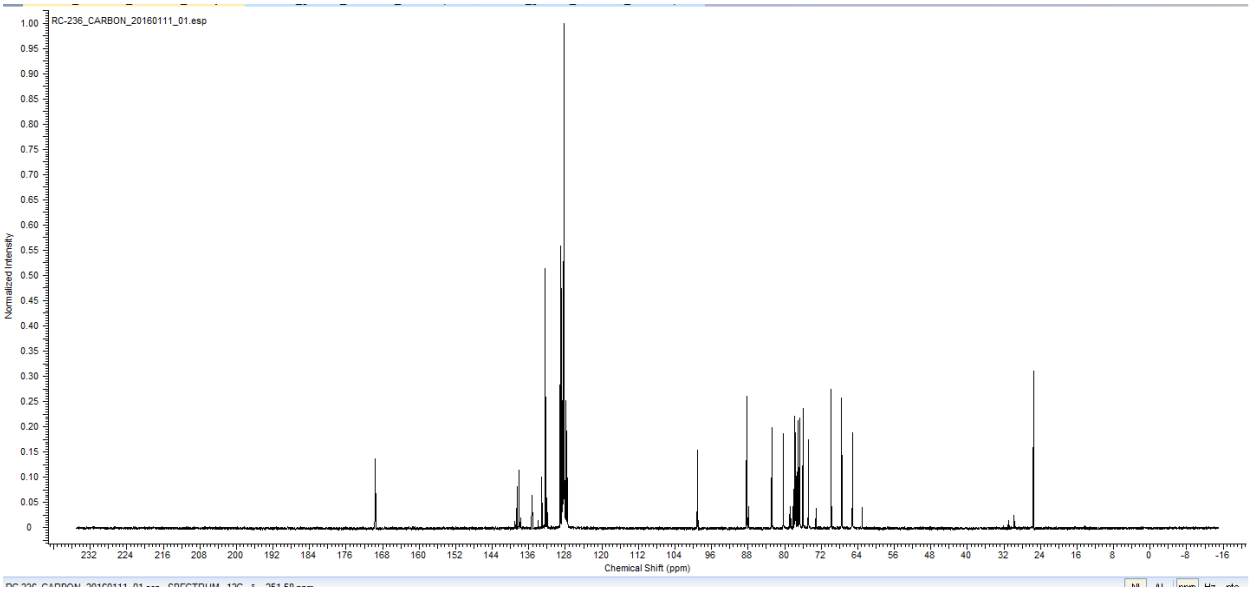


103

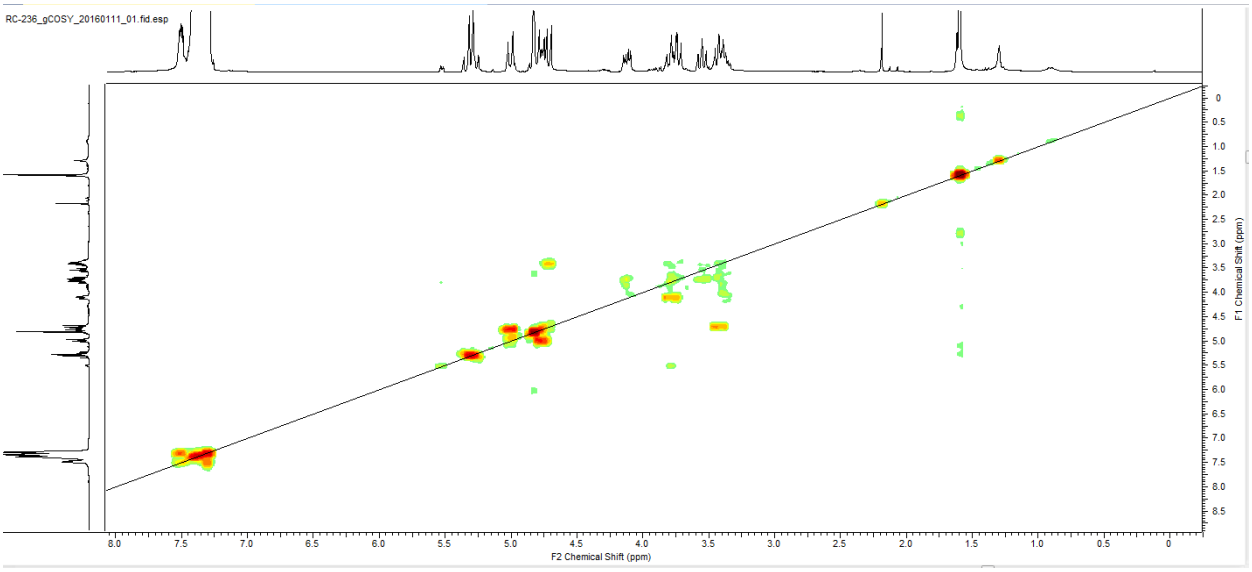
^{1}H NMR (300 MHz, $CDCl_3$)



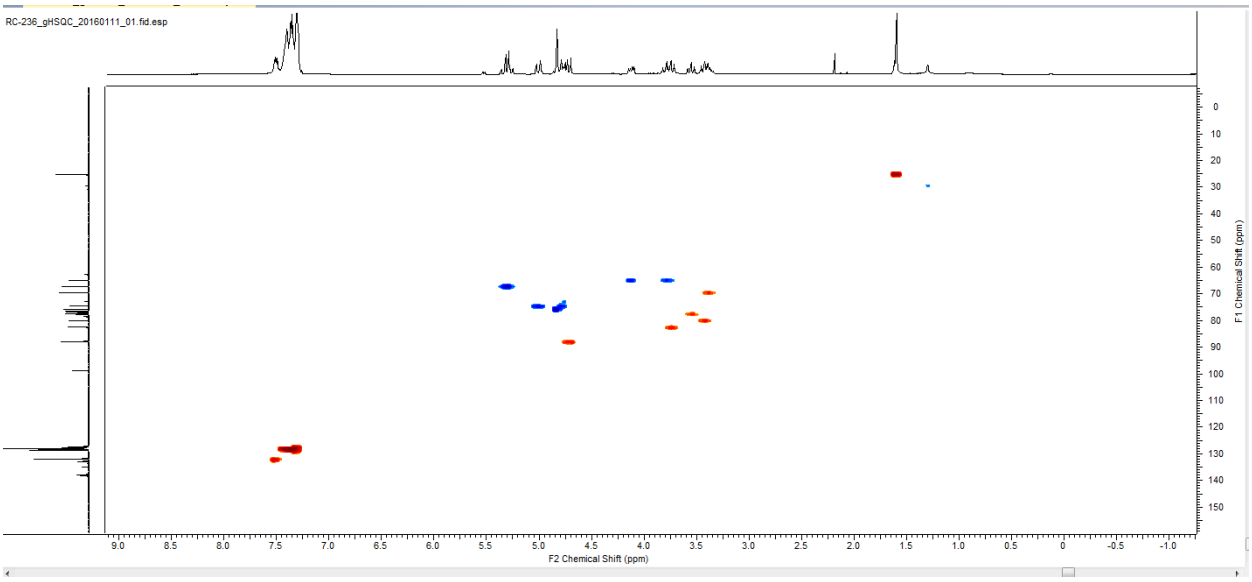
^{13}C NMR (75 MHz, $CDCl_3$)

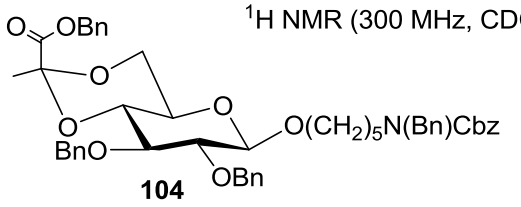


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



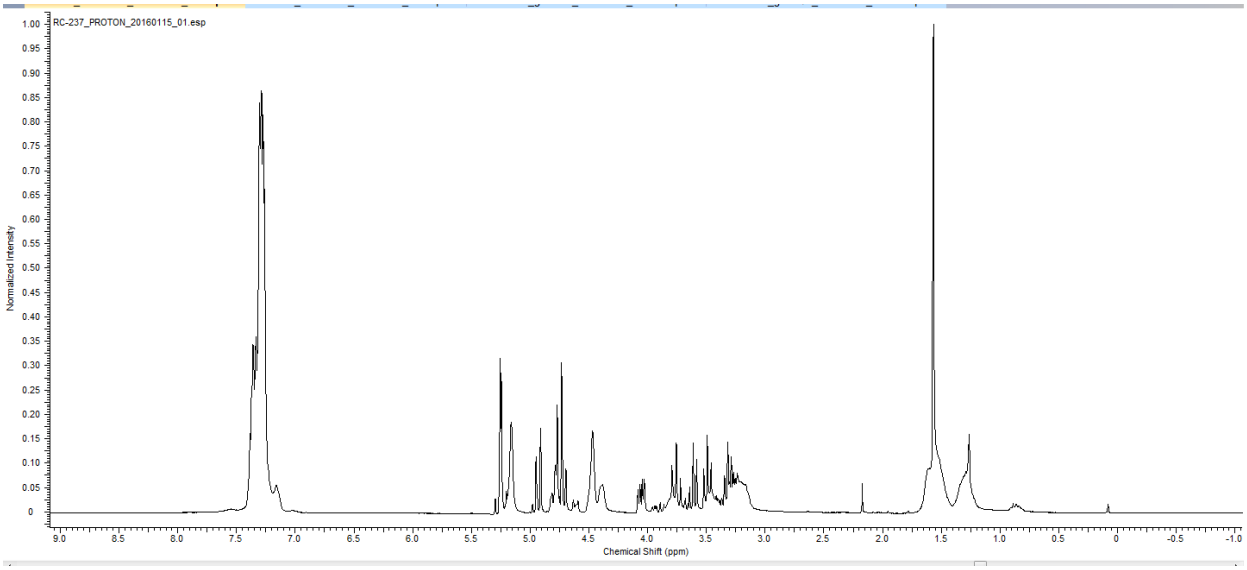
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



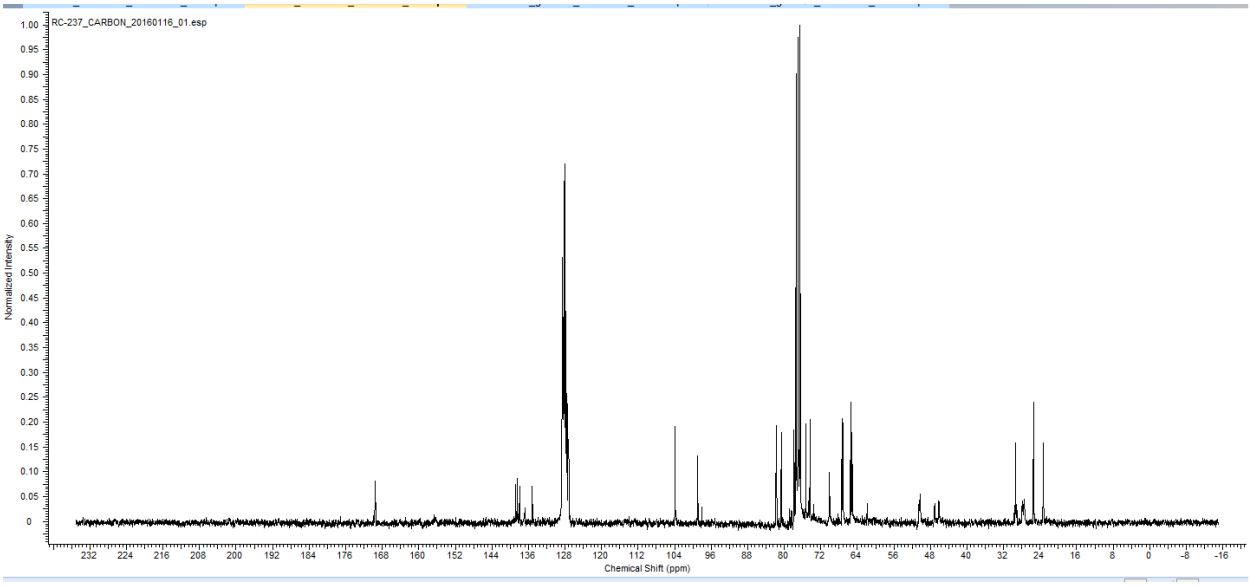


104

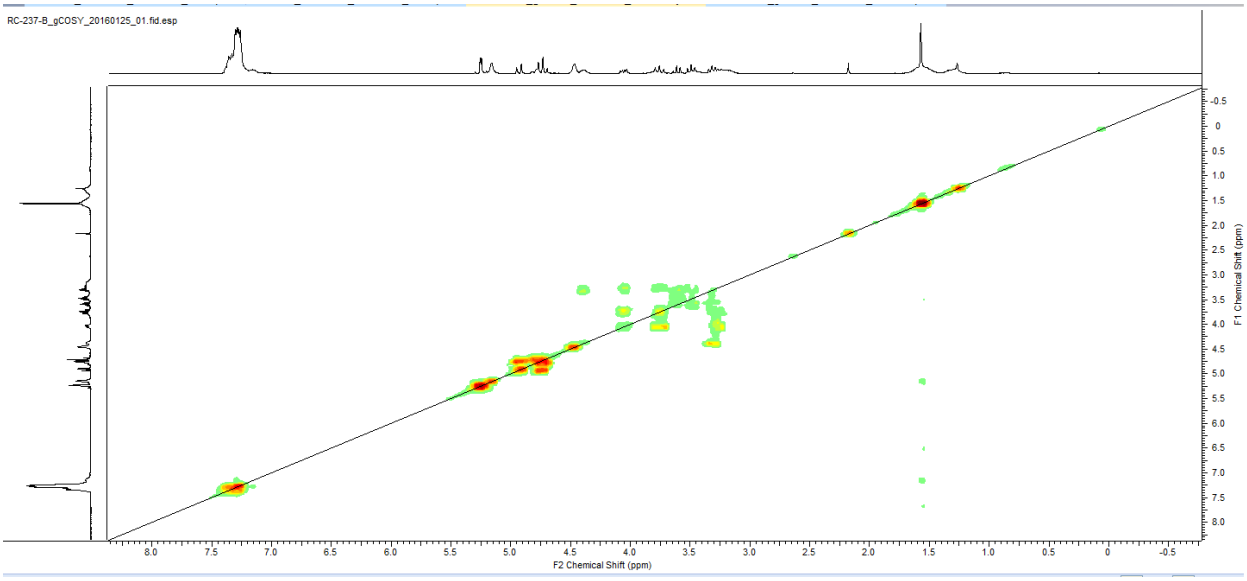
^{1}H NMR (300 MHz, $CDCl_3$)



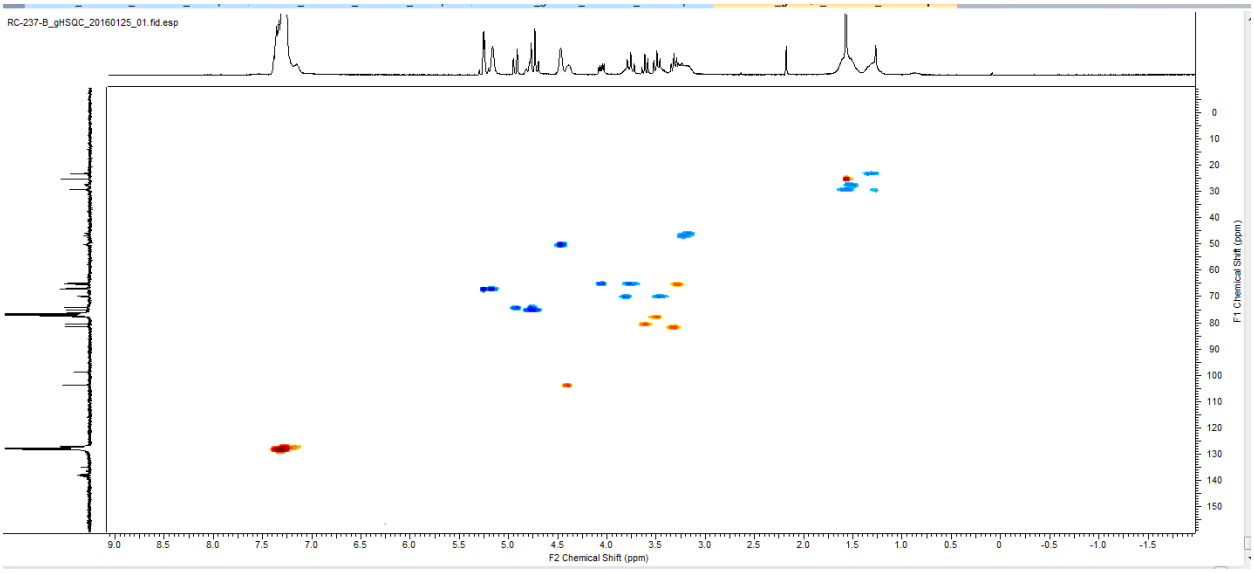
^{13}C NMR (75 MHz, $CDCl_3$)

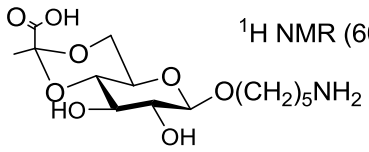


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



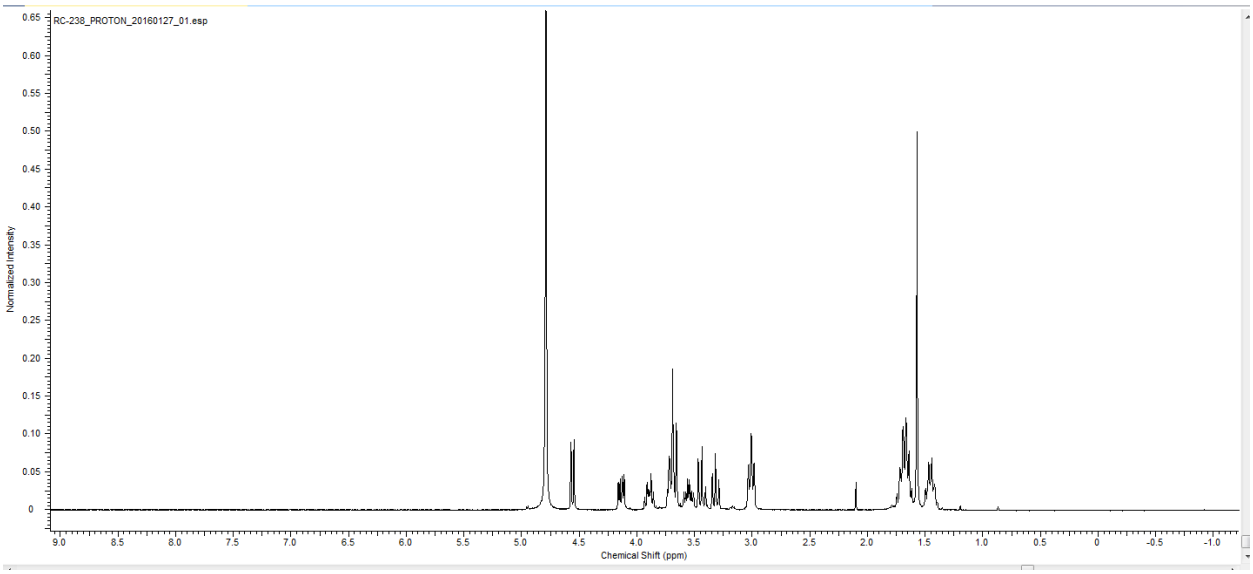
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



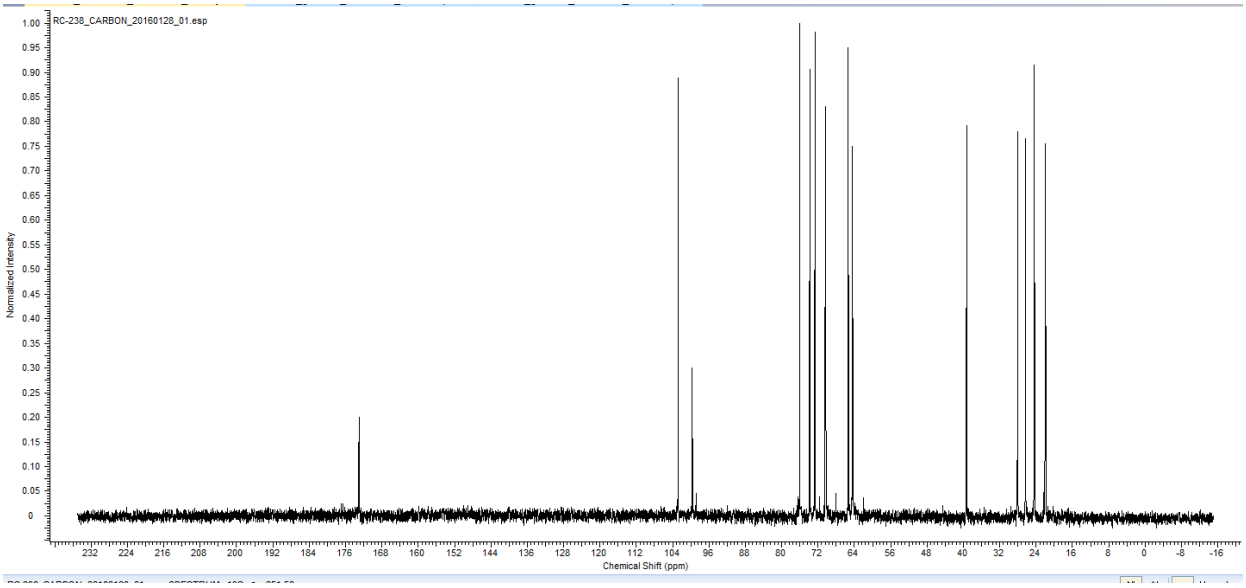


105

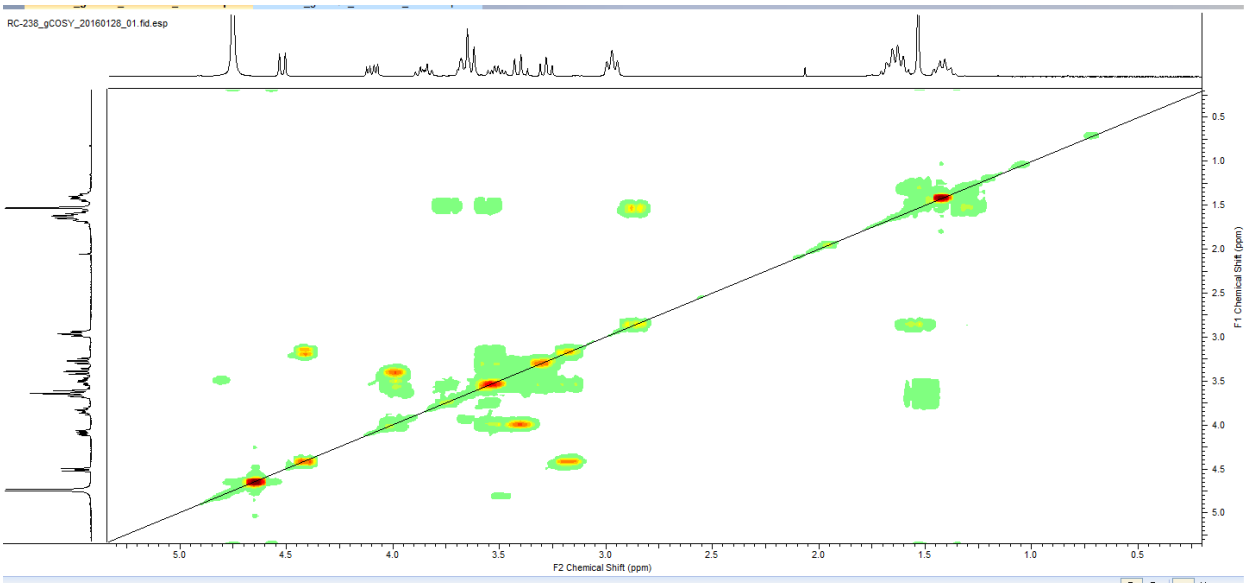
^{1}H NMR (600 MHz, D_2O)



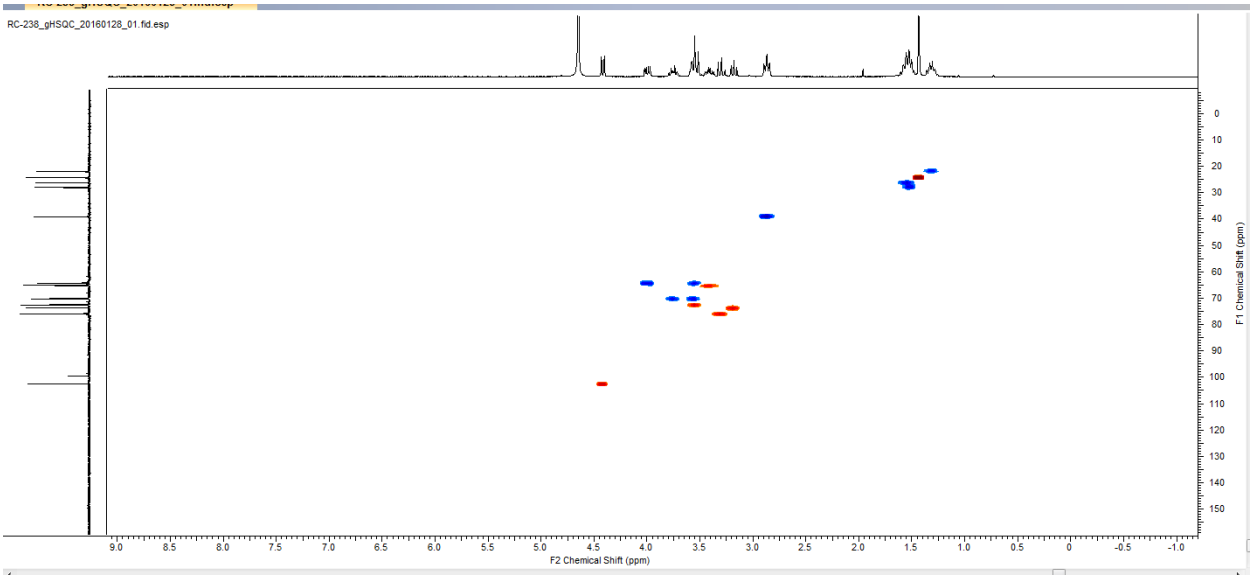
^{13}C NMR (150 MHz, D_2O)

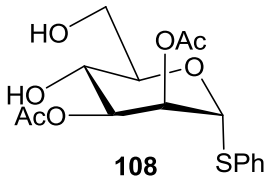


^{1}H-^{1}H COSY (600 MHz, D_2O)



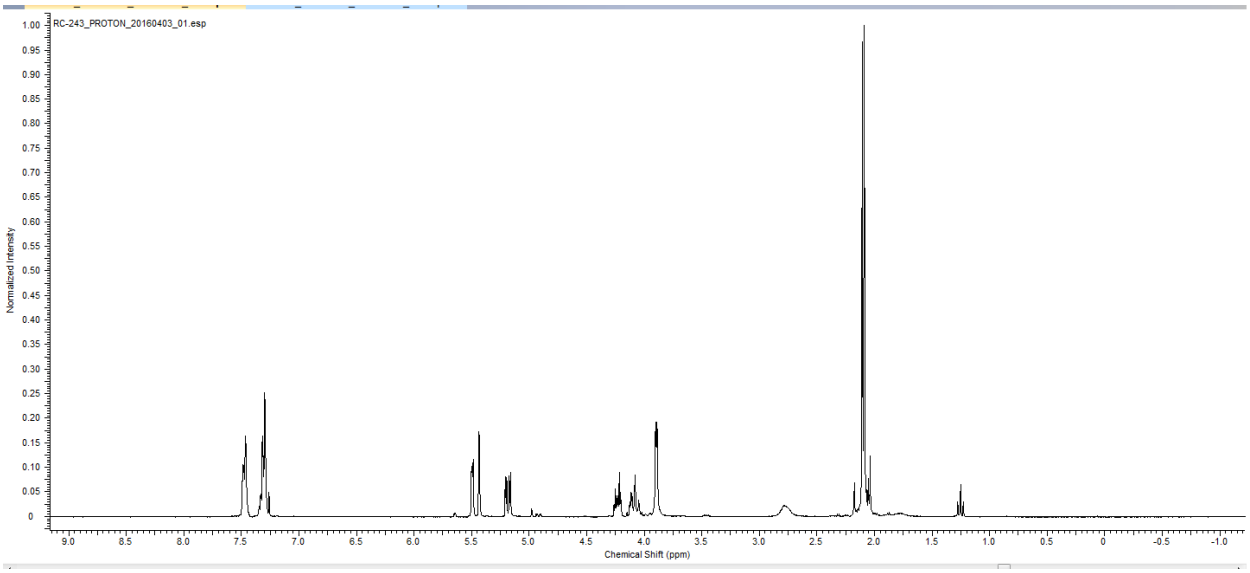
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



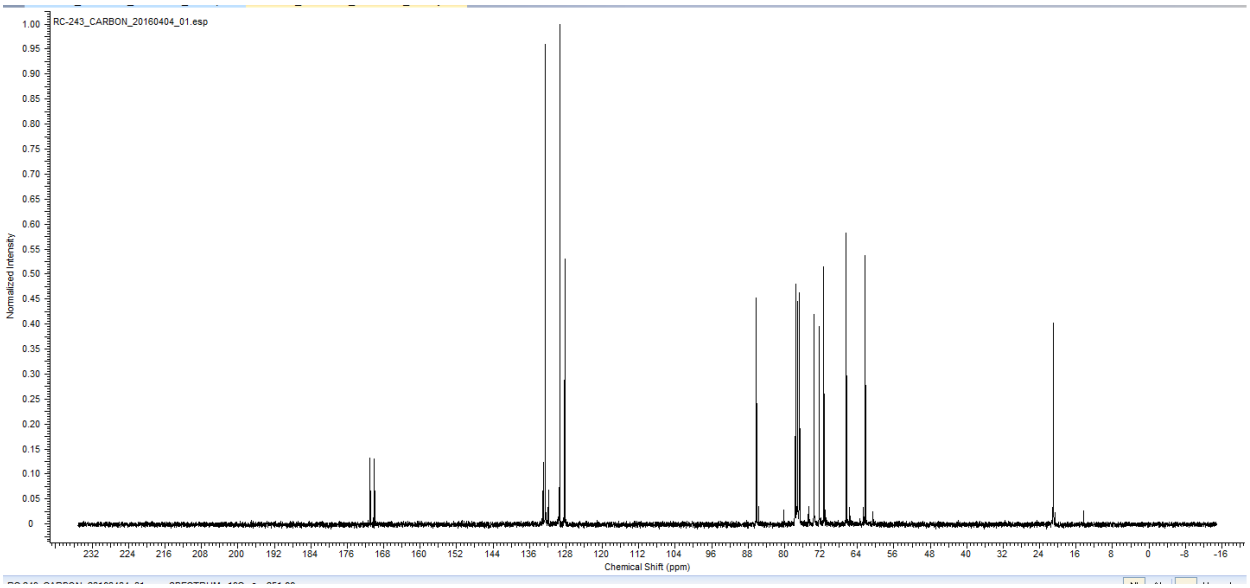


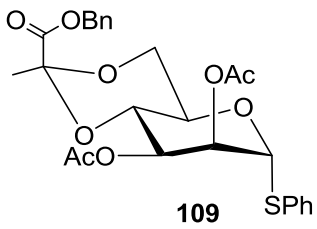
108

^{1}H NMR (300 MHz, $CDCl_3$)



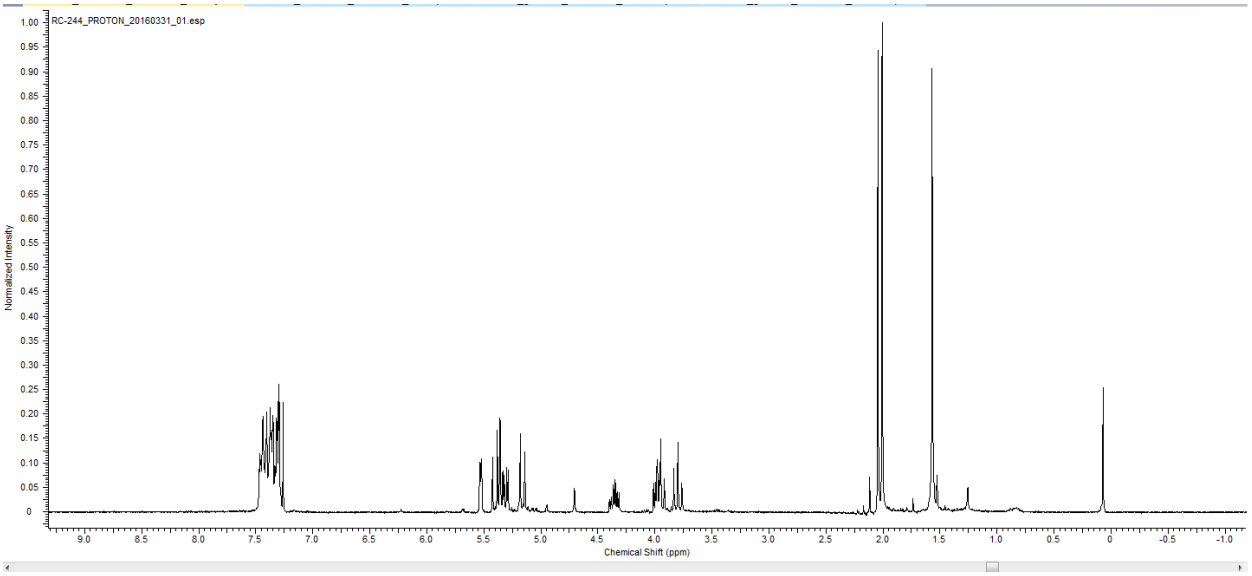
^{13}C NMR (75 MHz, $CDCl_3$)



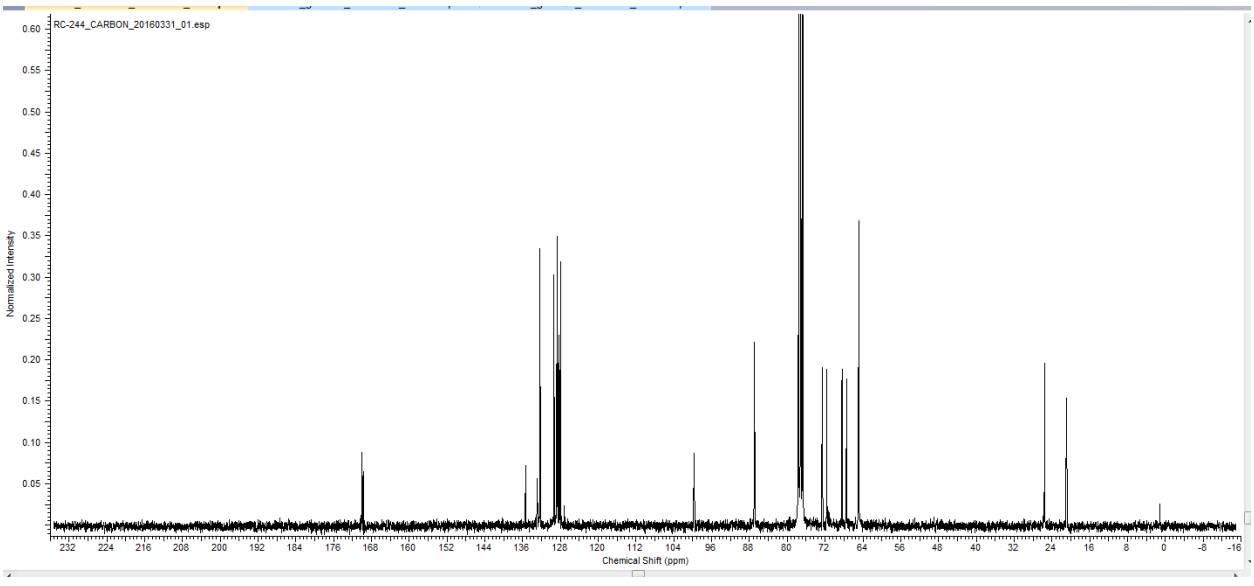


109

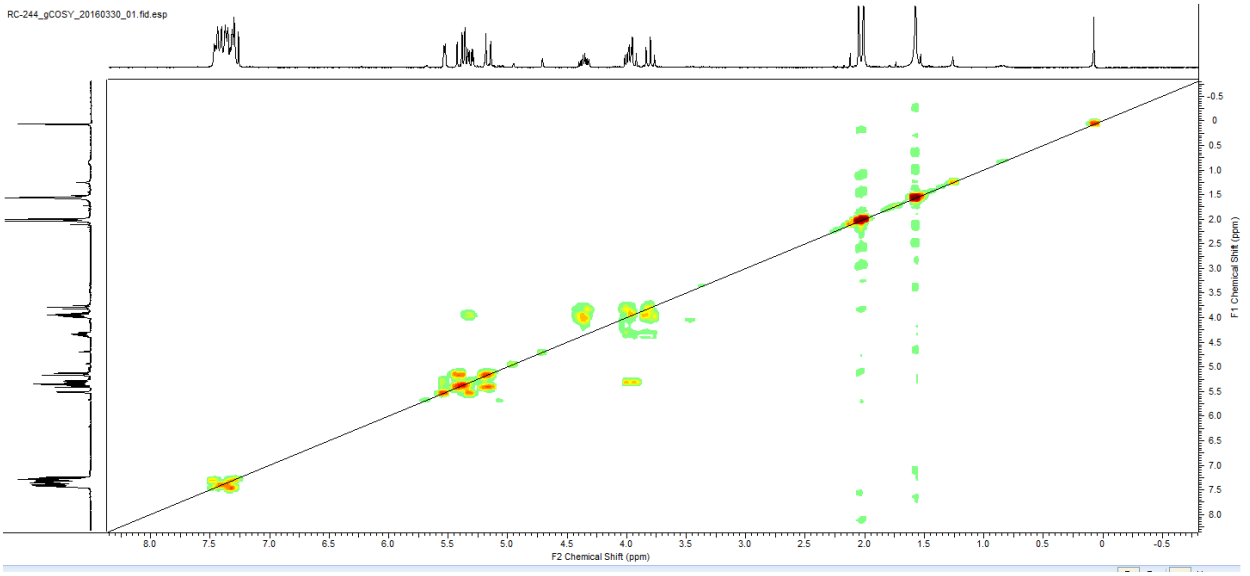
^{1}H NMR (300 MHz, $CDCl_3$)



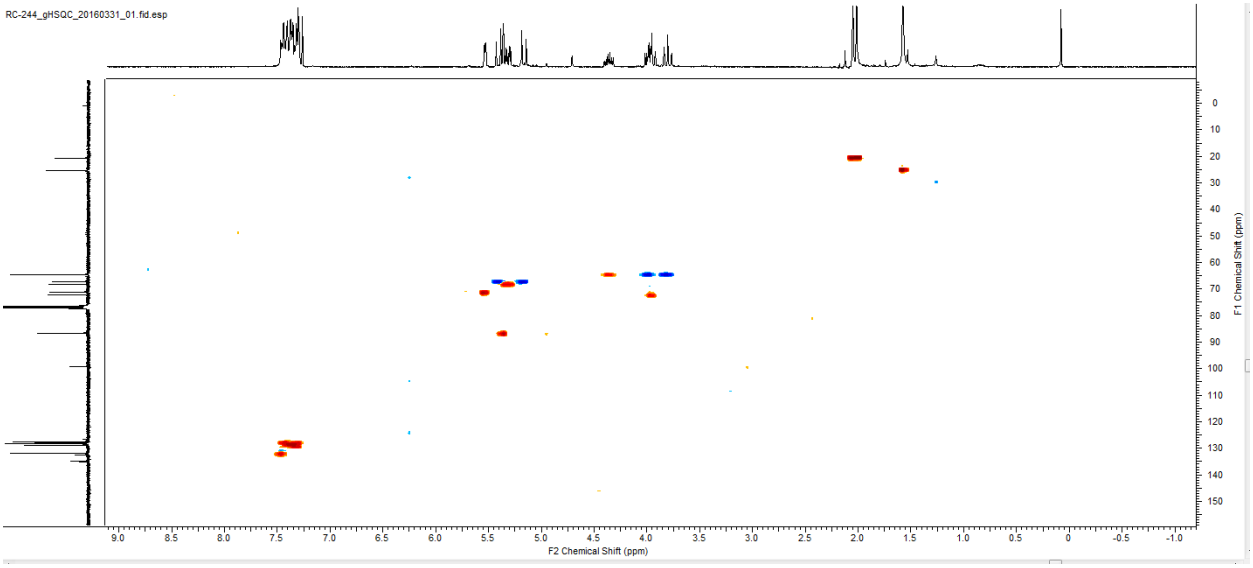
^{13}C NMR (75 MHz, $CDCl_3$)

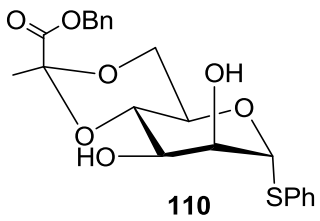


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



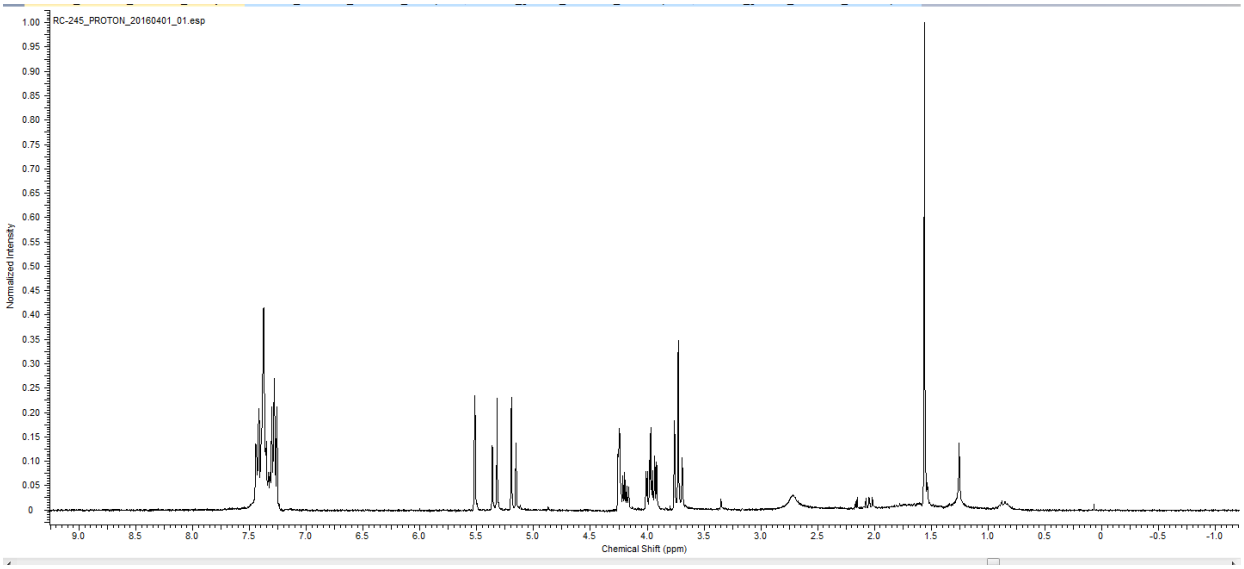
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



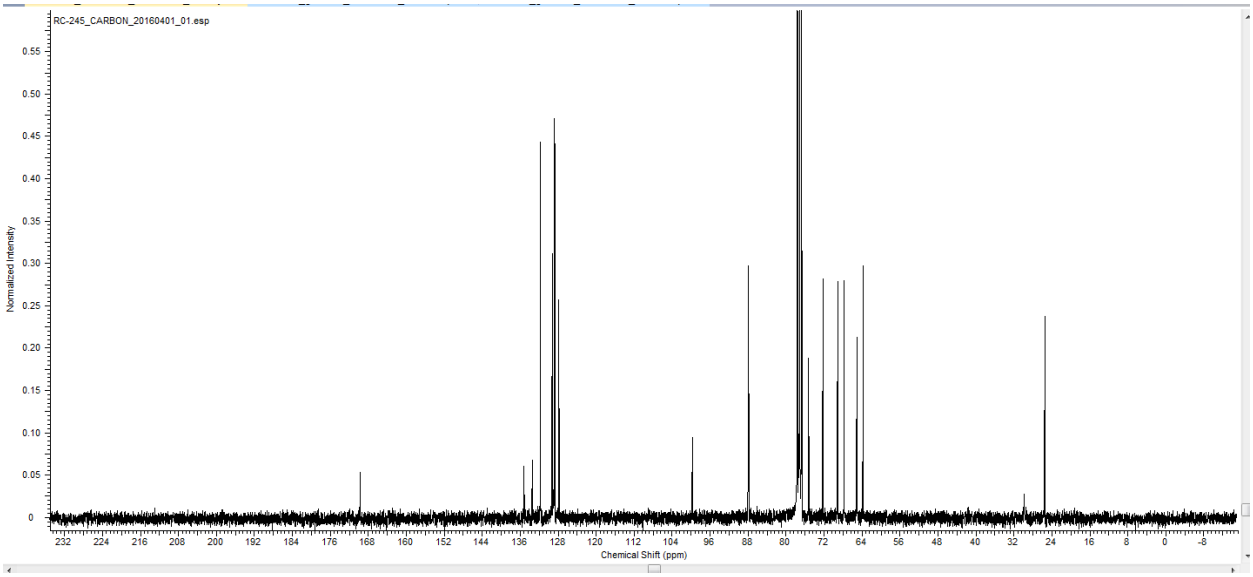


110

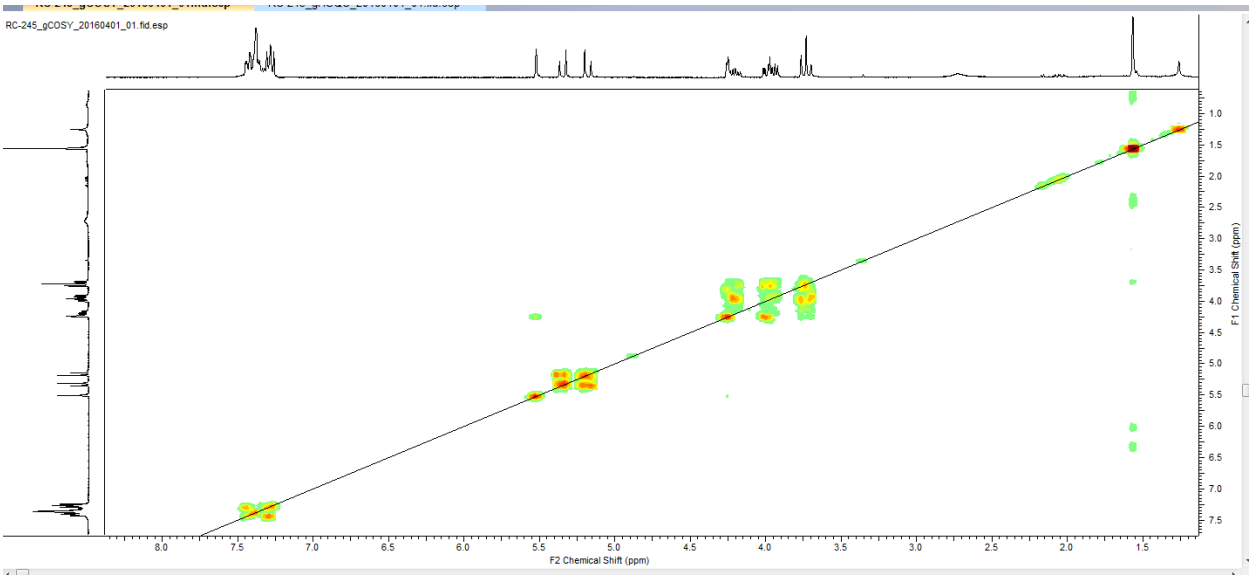
^{1}H NMR (300 MHz, $CDCl_3$)



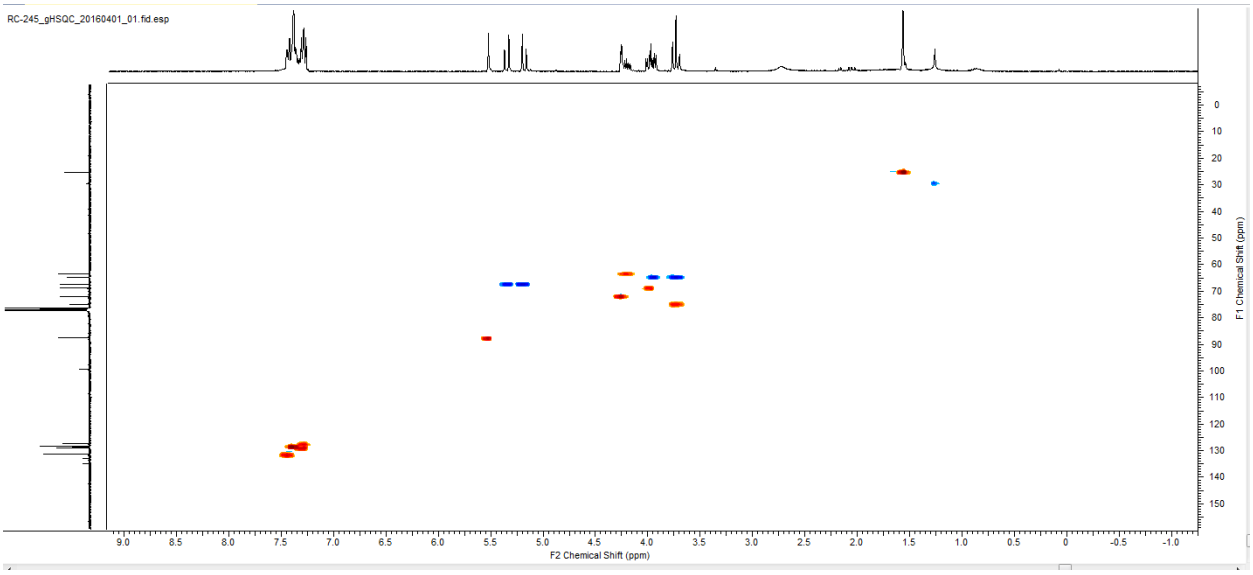
^{13}C NMR (75 MHz, $CDCl_3$)

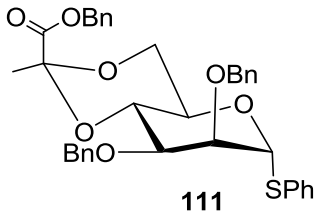


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



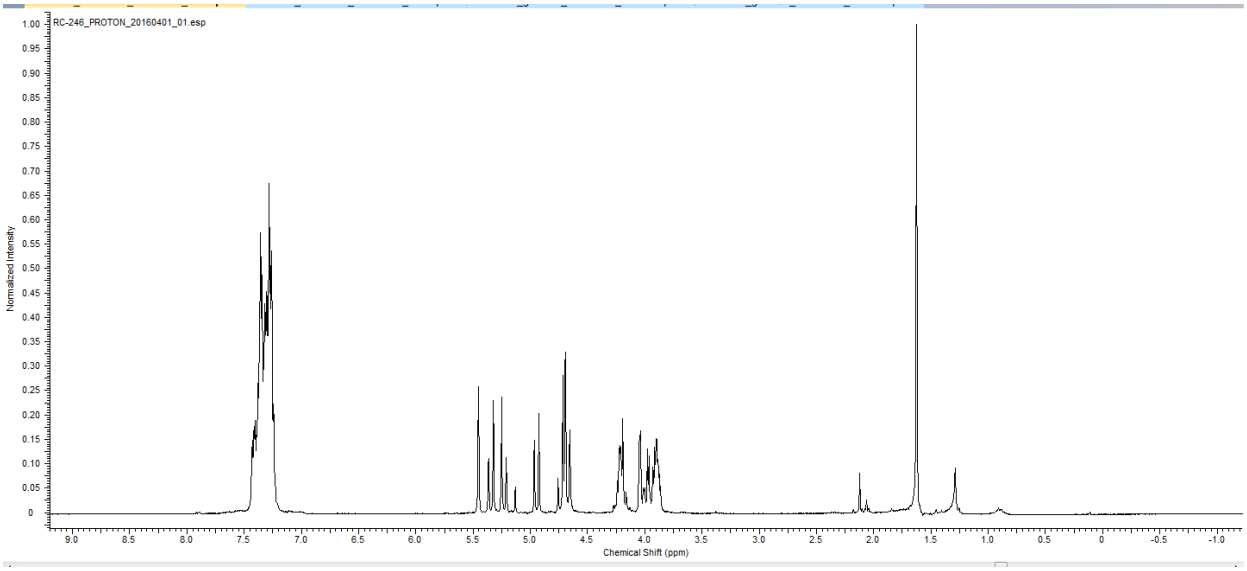
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



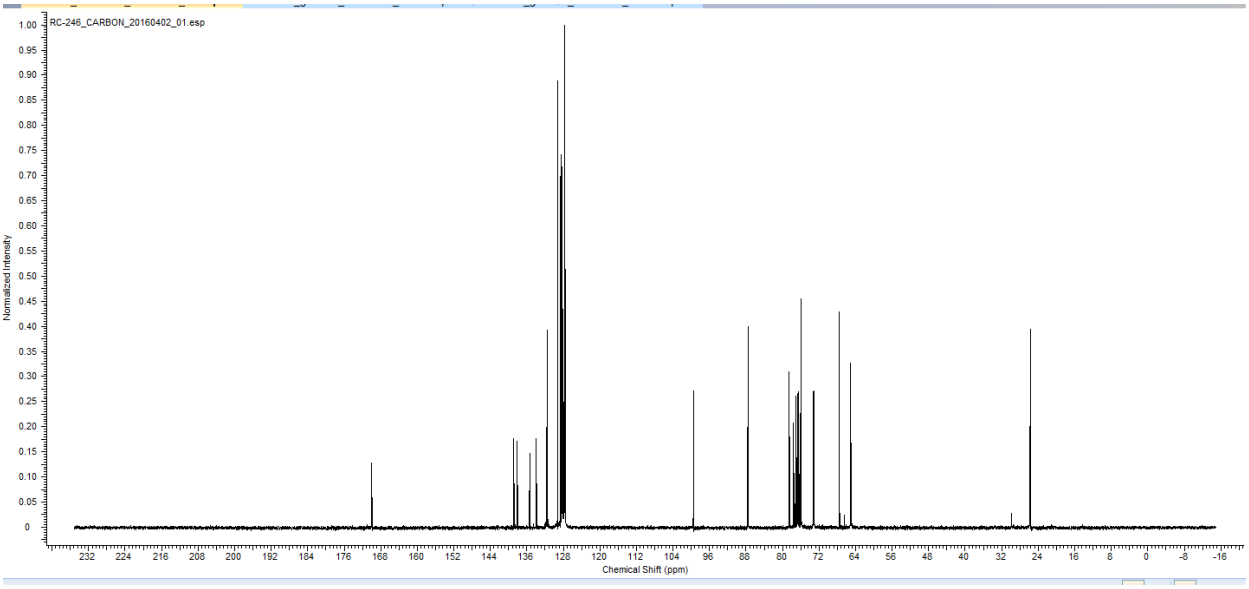


111

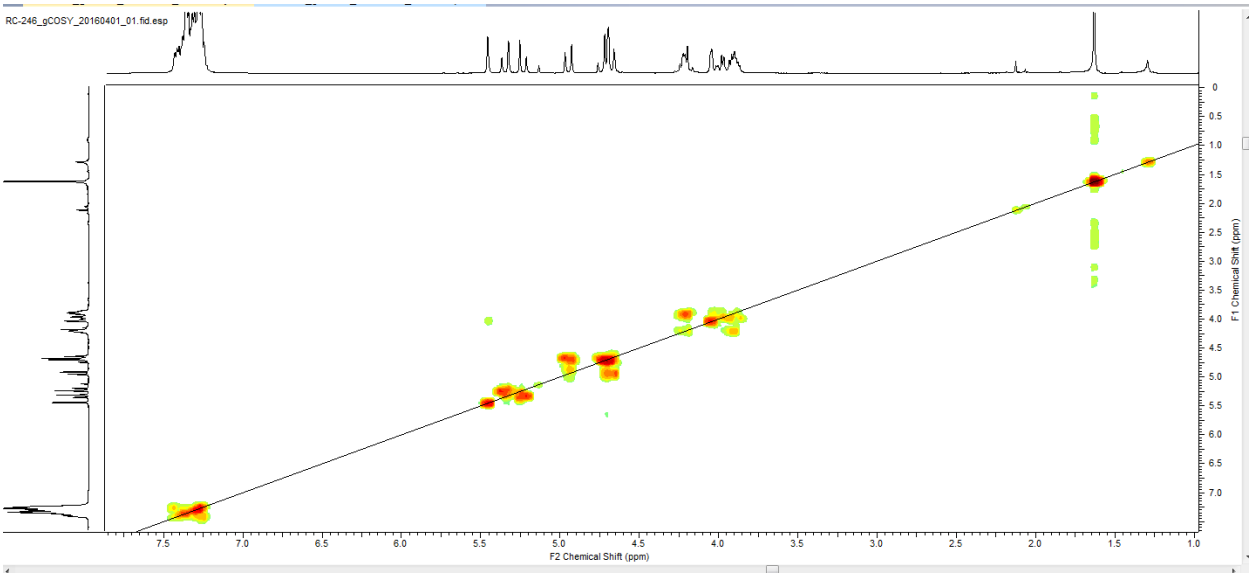
^{1}H NMR (300 MHz, $CDCl_3$)



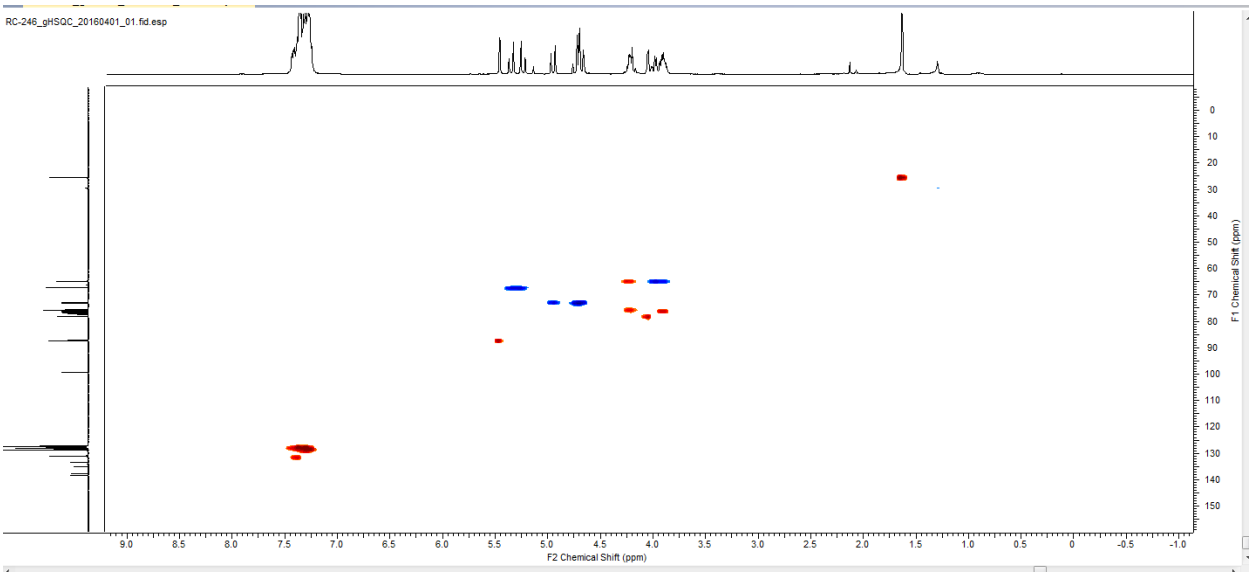
^{13}C NMR (75 MHz, $CDCl_3$)

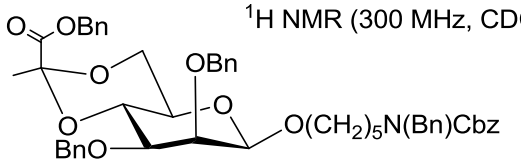


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



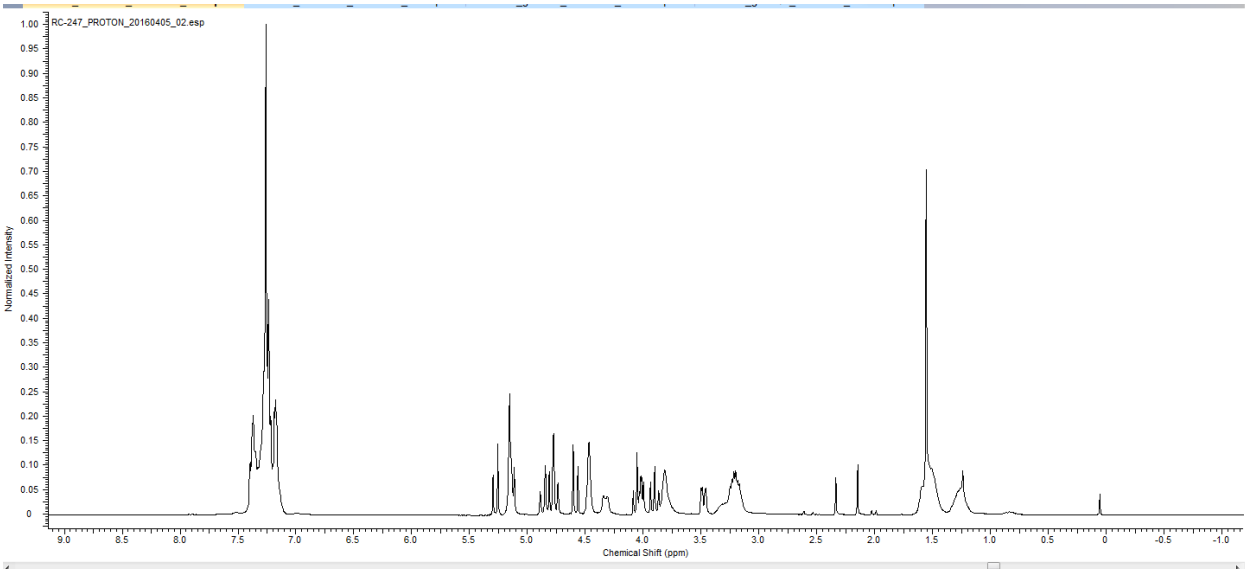
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



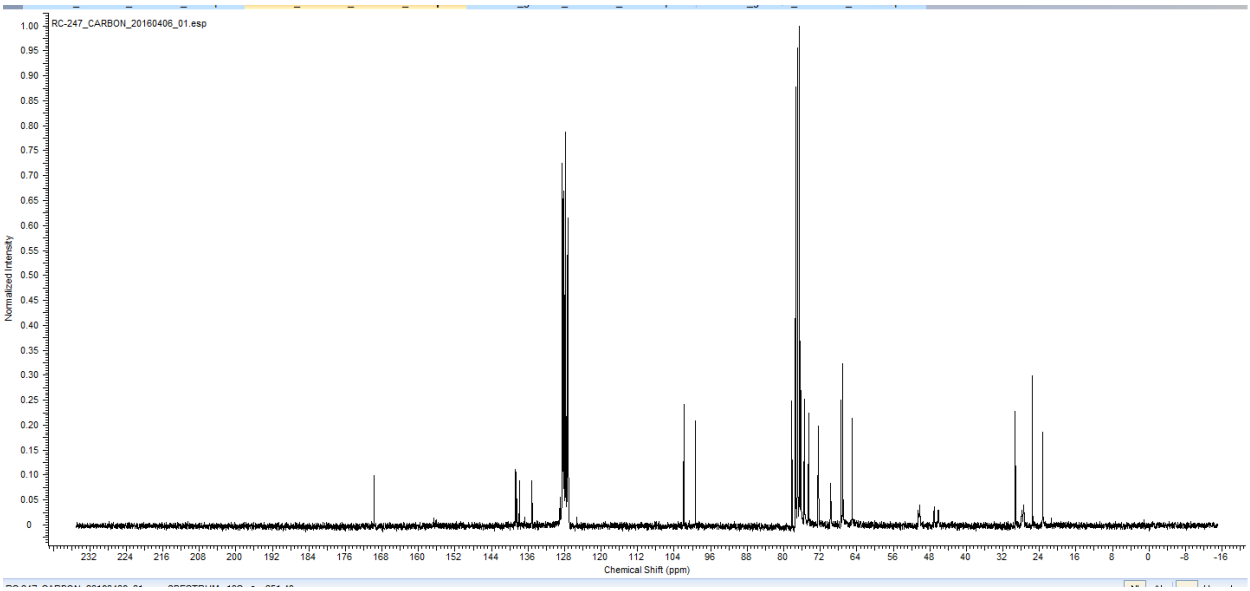


112

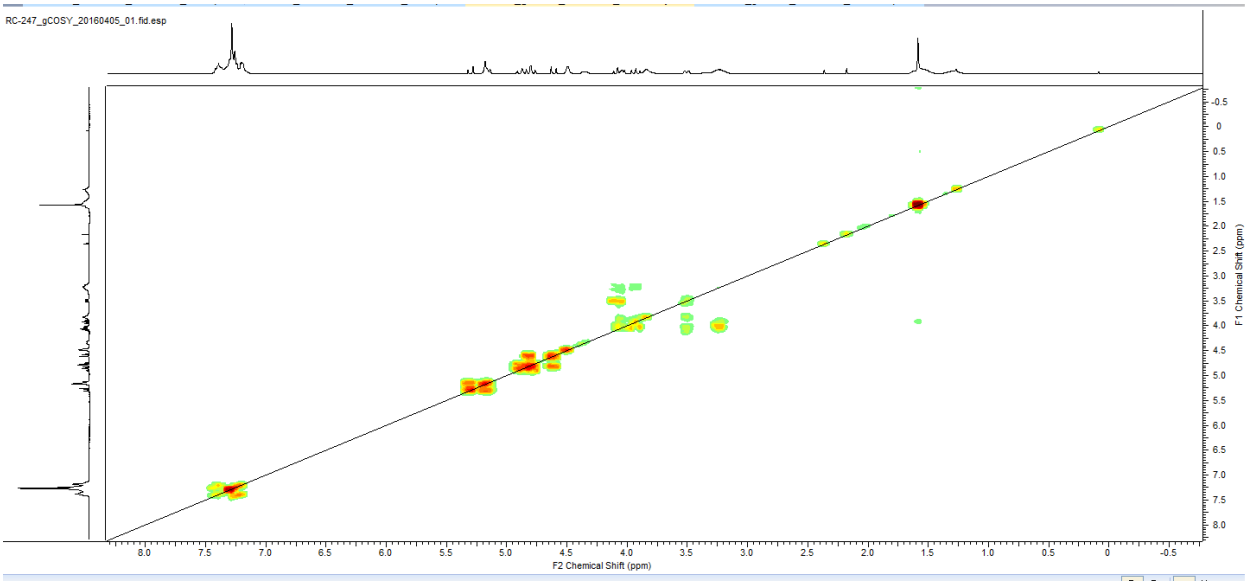
^{1}H NMR (300 MHz, $CDCl_3$)



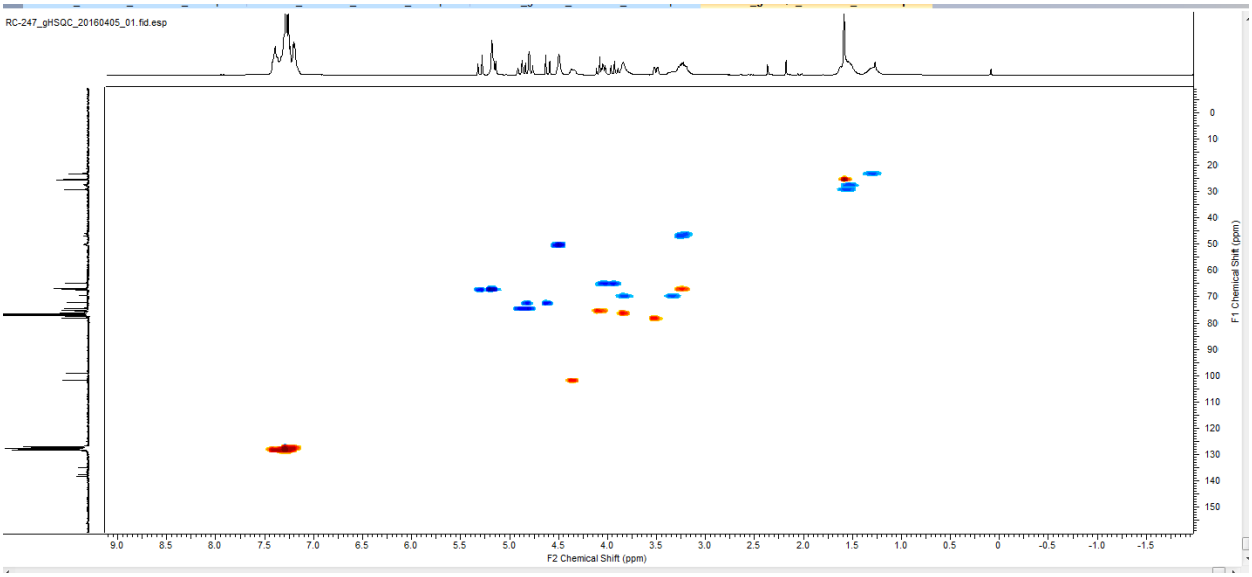
^{13}C NMR (75 MHz, $CDCl_3$)

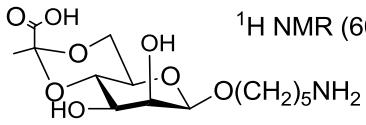


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



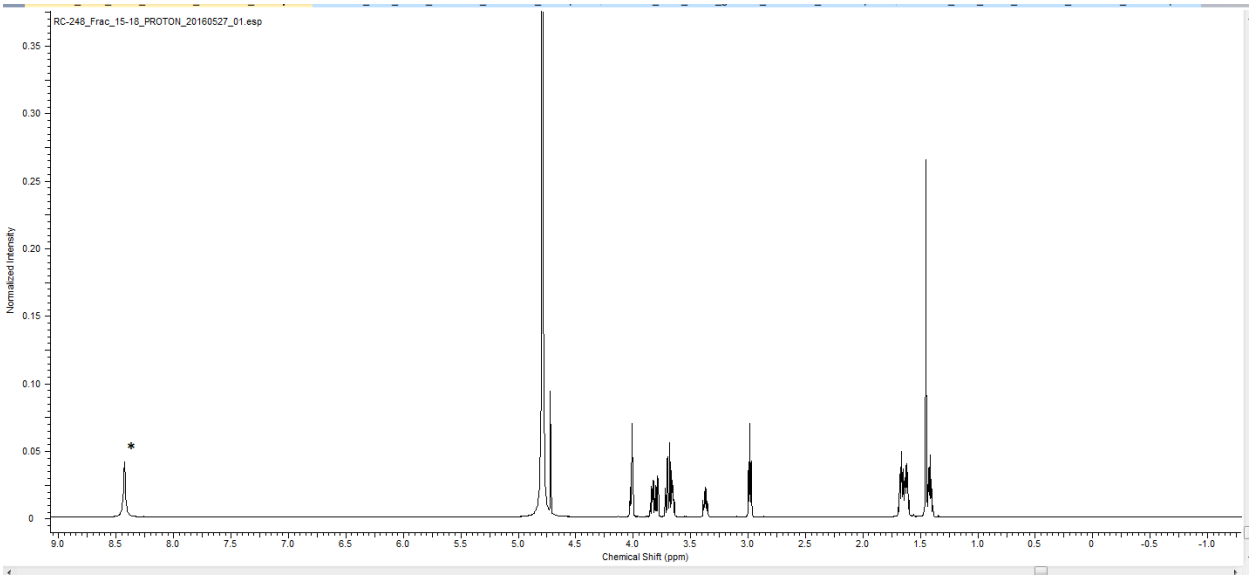
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



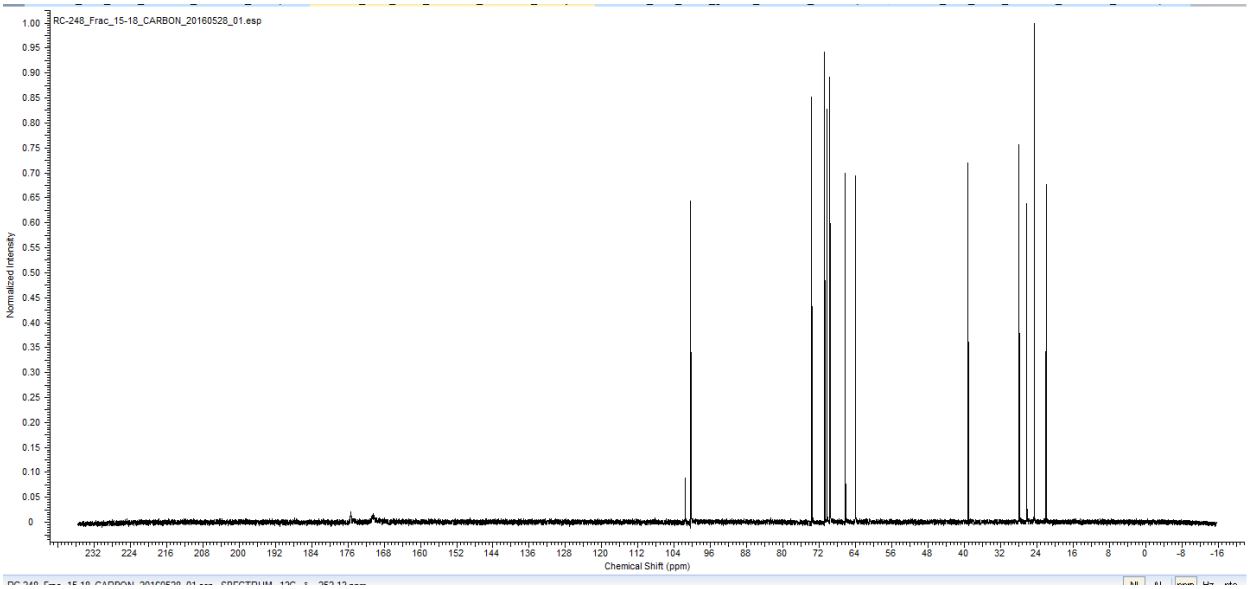


113

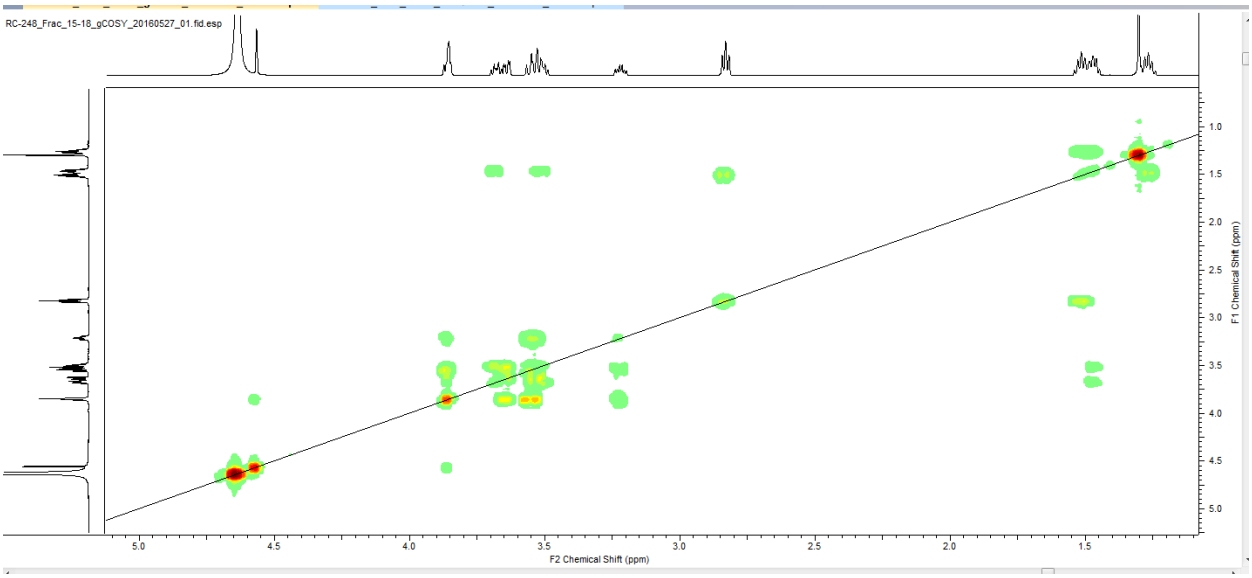
^{1}H NMR (600 MHz, D_2O)



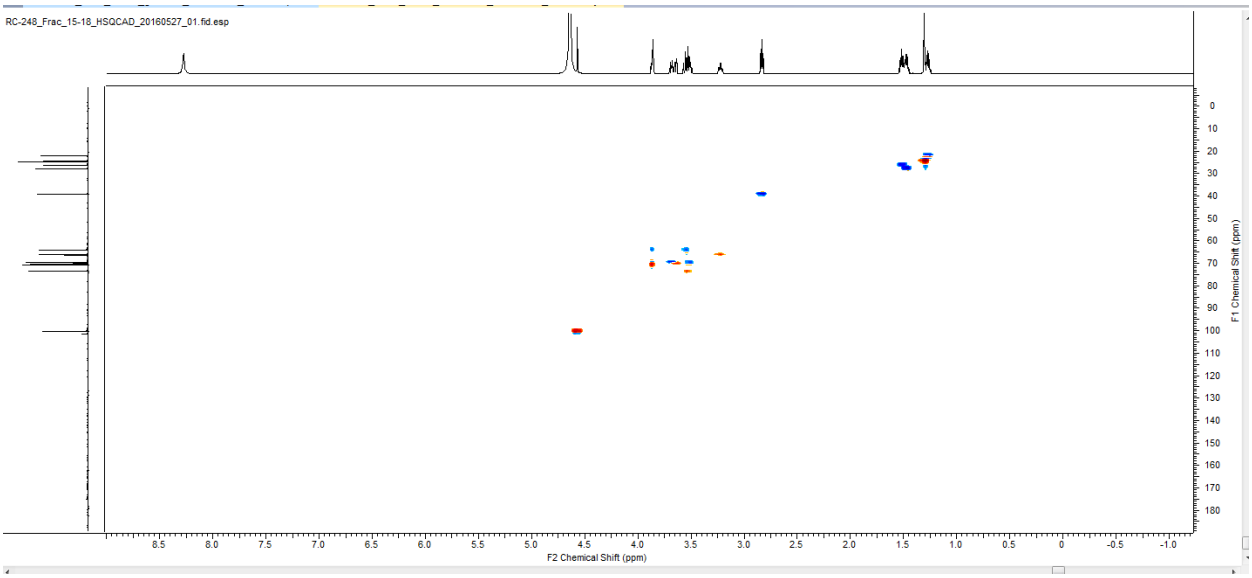
^{13}C NMR (150 MHz, D_2O)

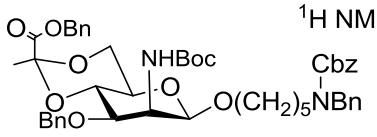


^{1}H-^{1}H COSY (600 MHz, D_2O)



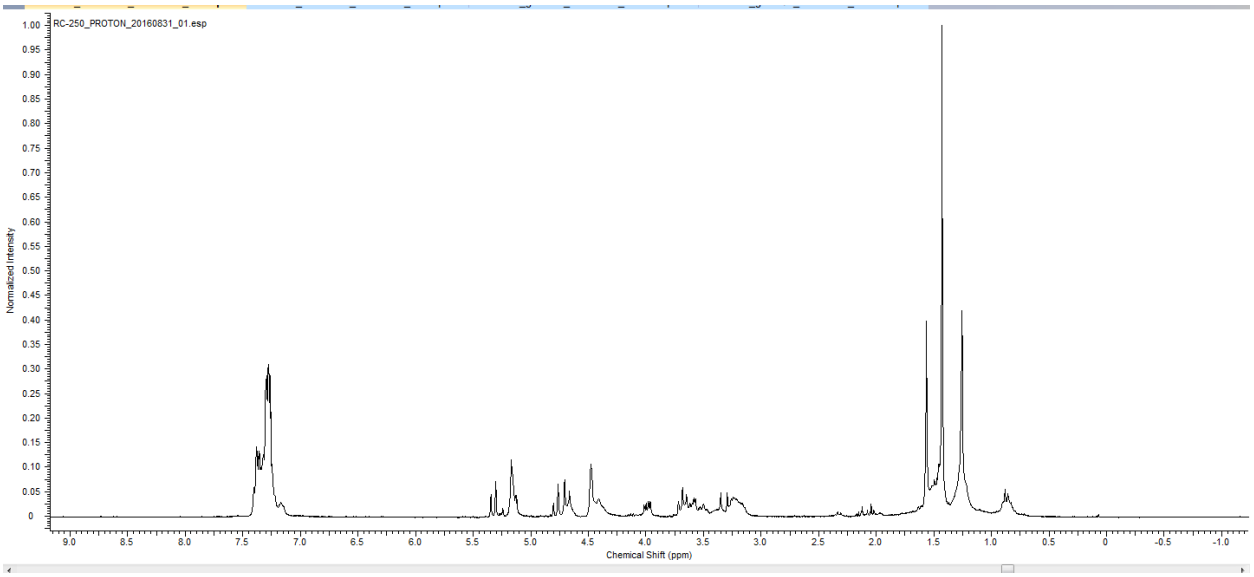
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



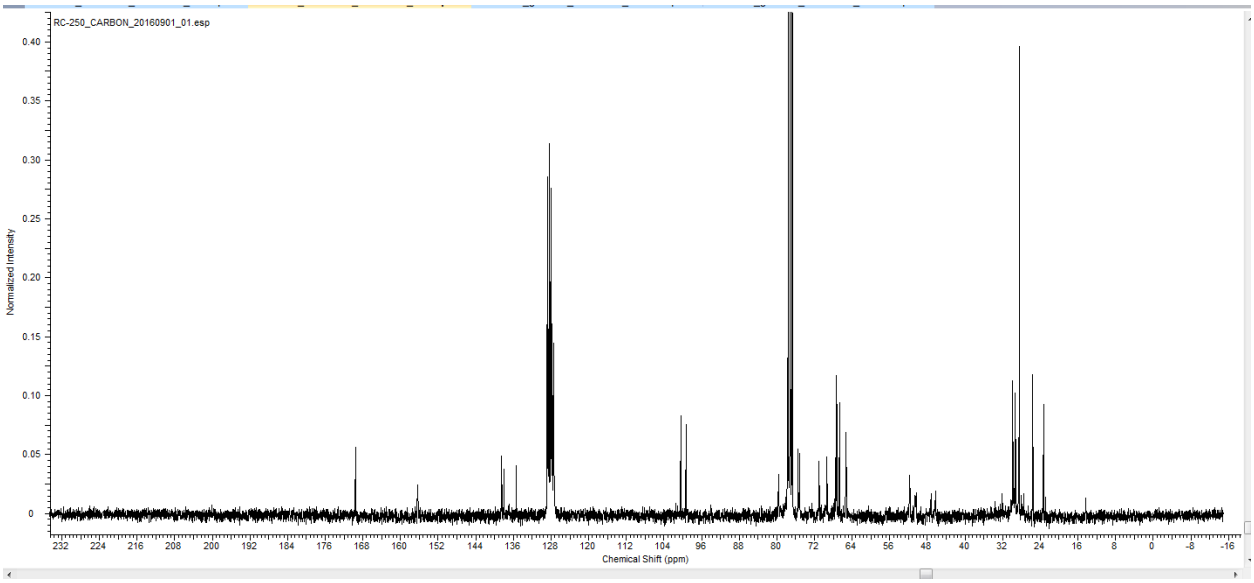


114

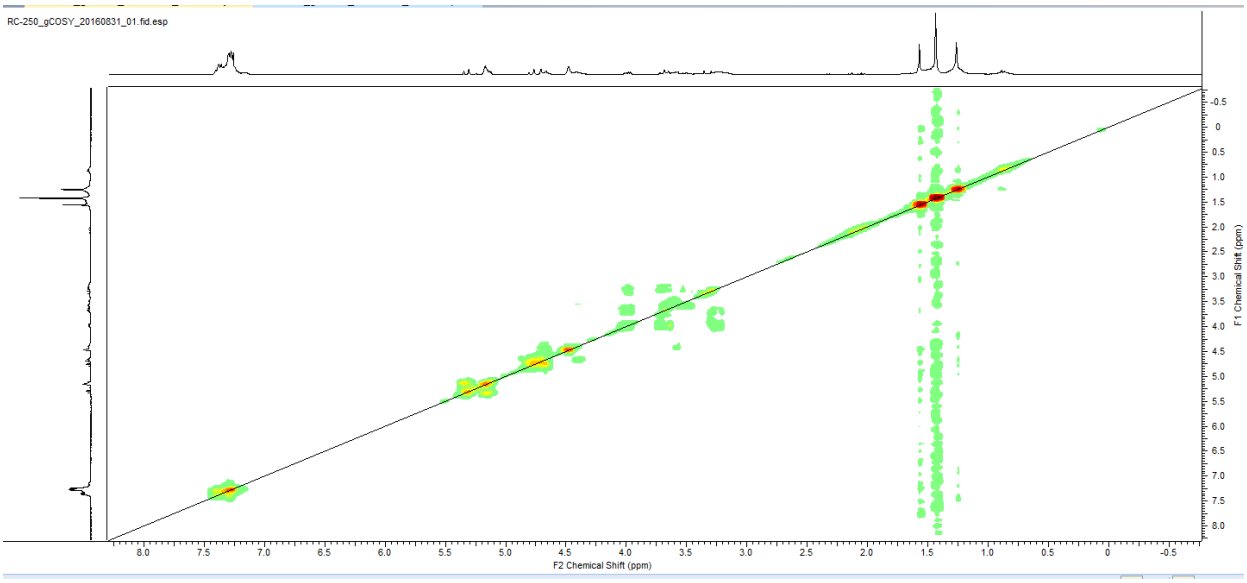
^{1}H NMR (300 MHz, $CDCl_3$)



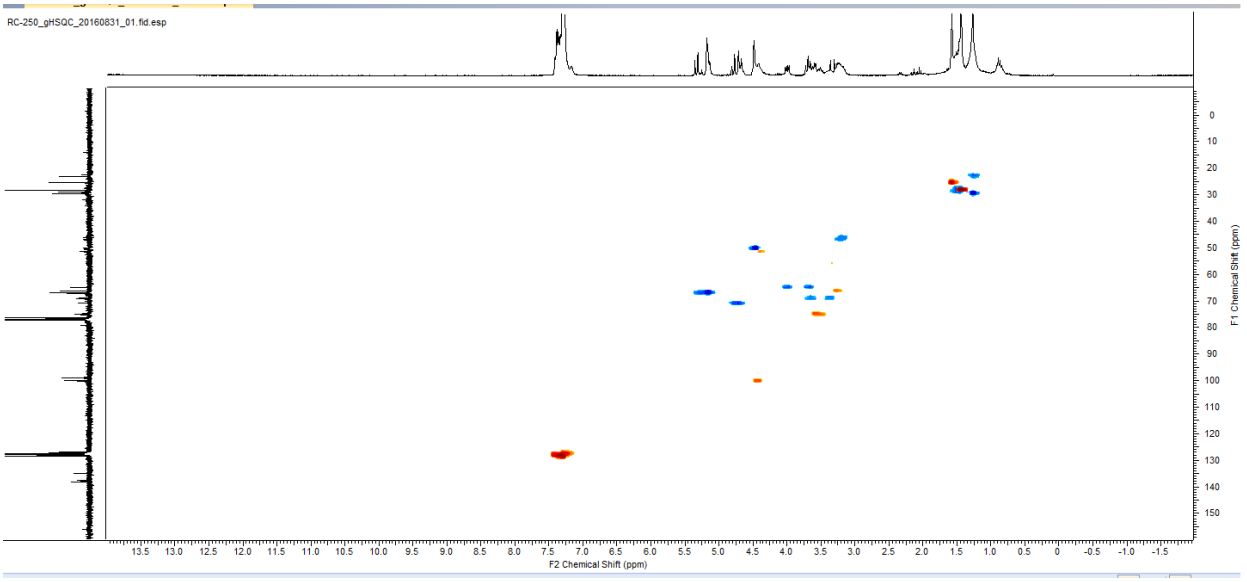
^{13}C NMR (75 MHz, $CDCl_3$)

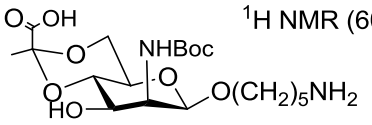


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



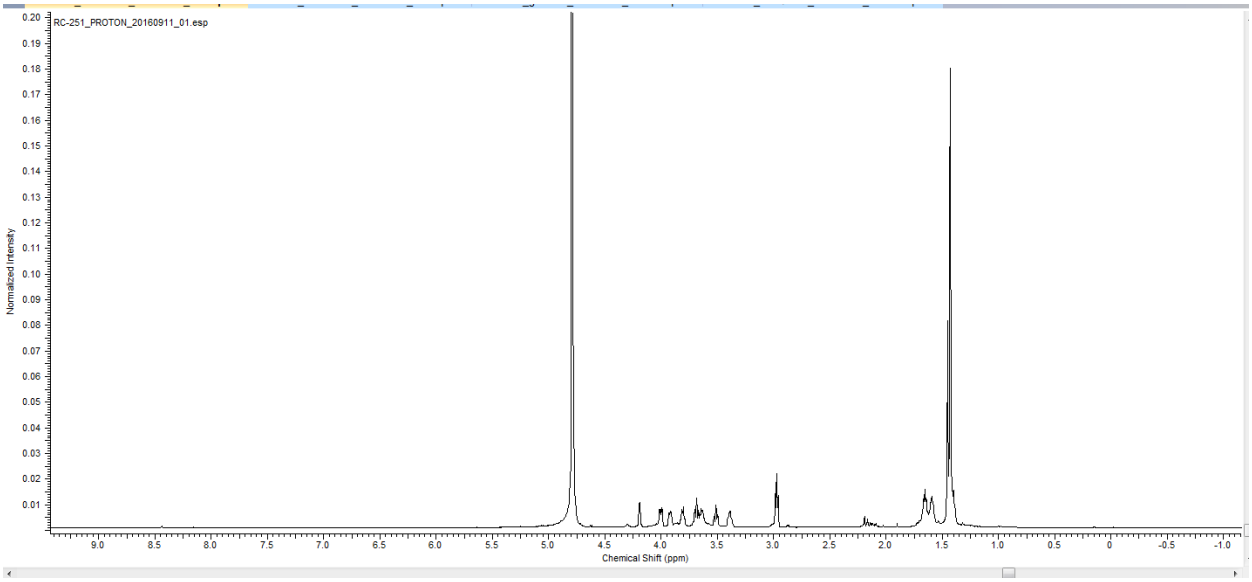
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



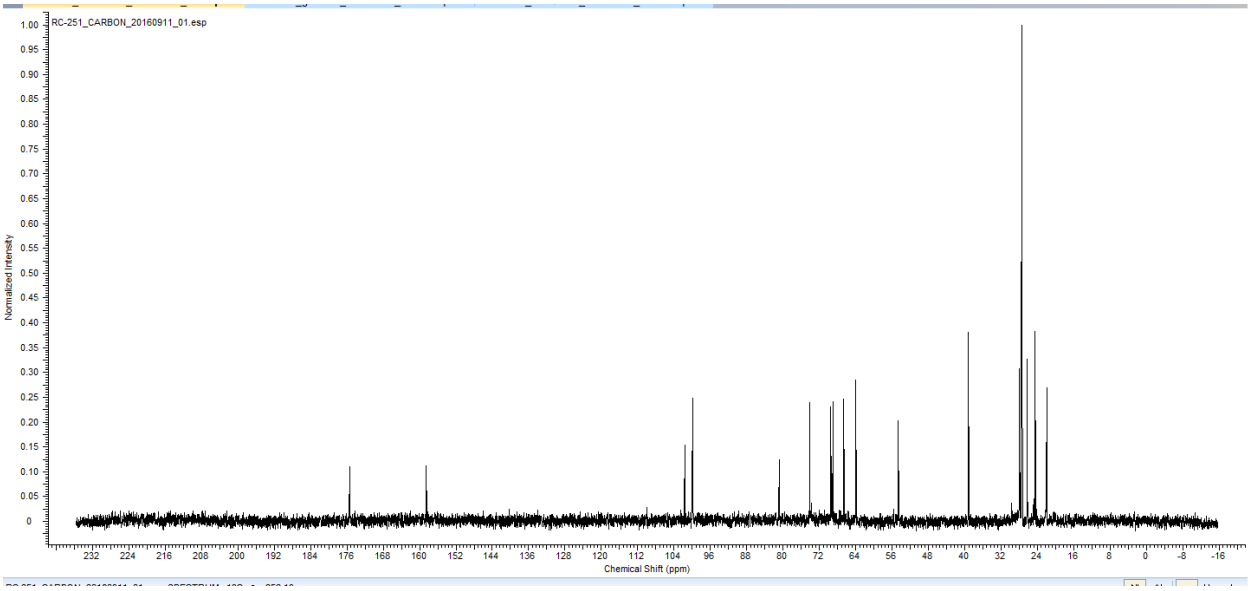


115

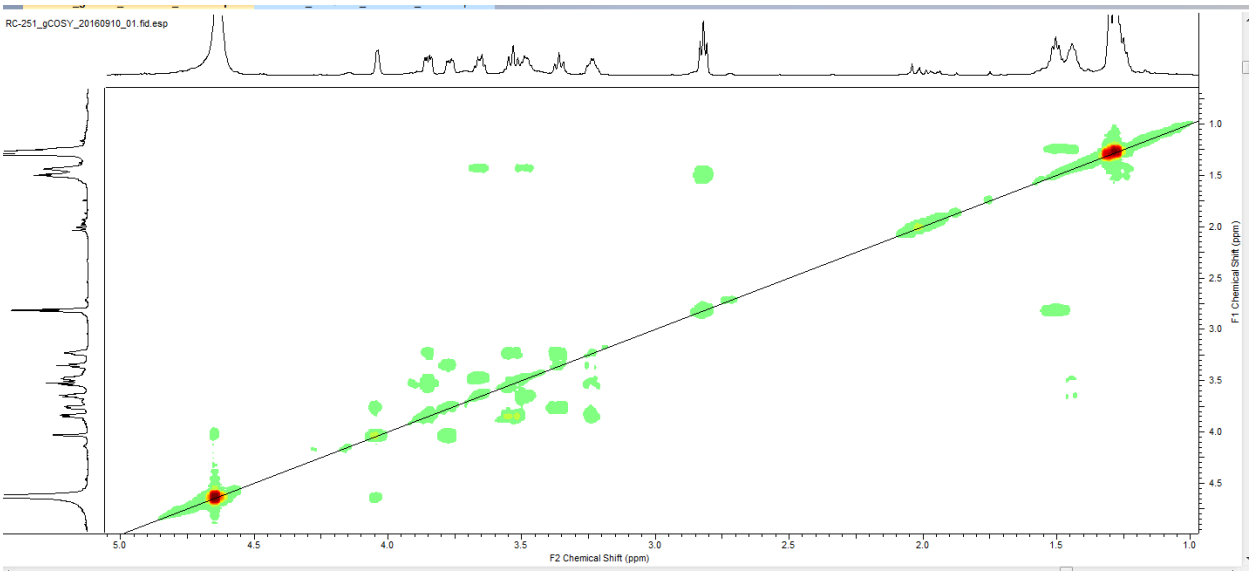
^{1}H NMR (600 MHz, D_2O)



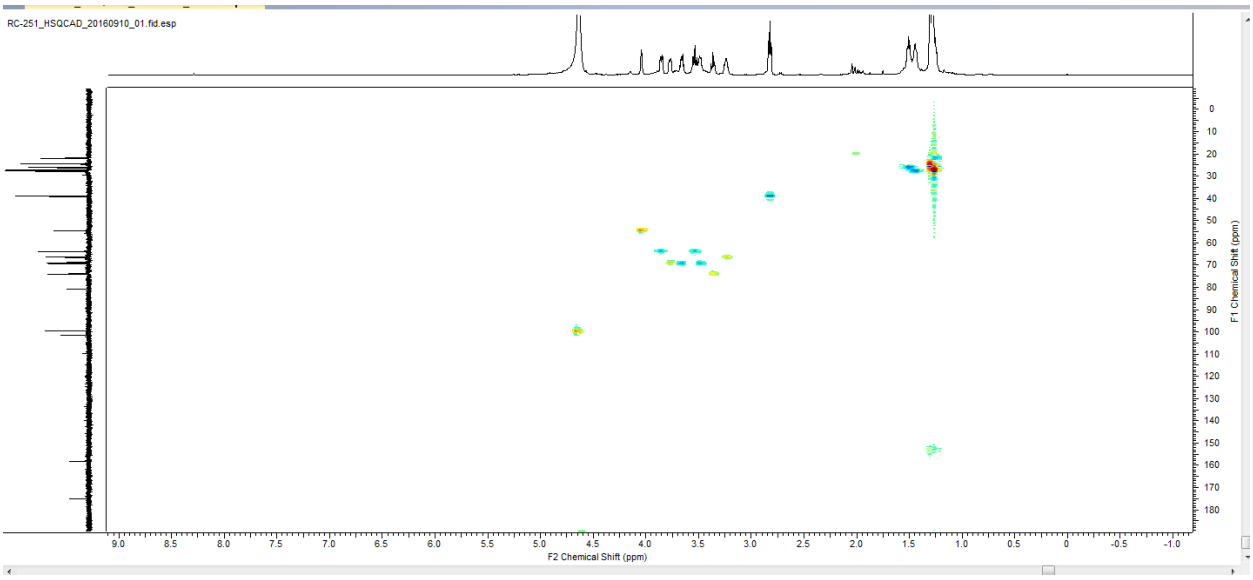
^{13}C NMR (150 MHz, D_2O)



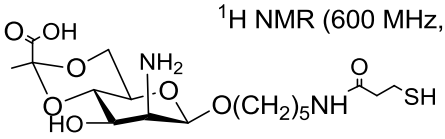
^{1}H-^{1}H COSY (600 MHz, D_2O)



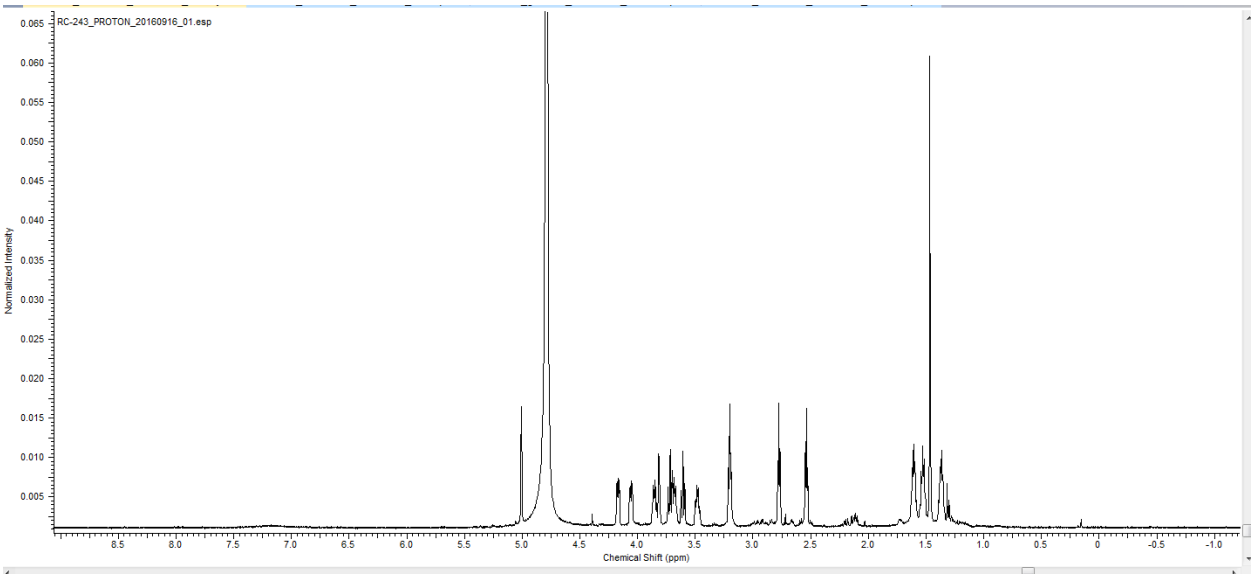
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



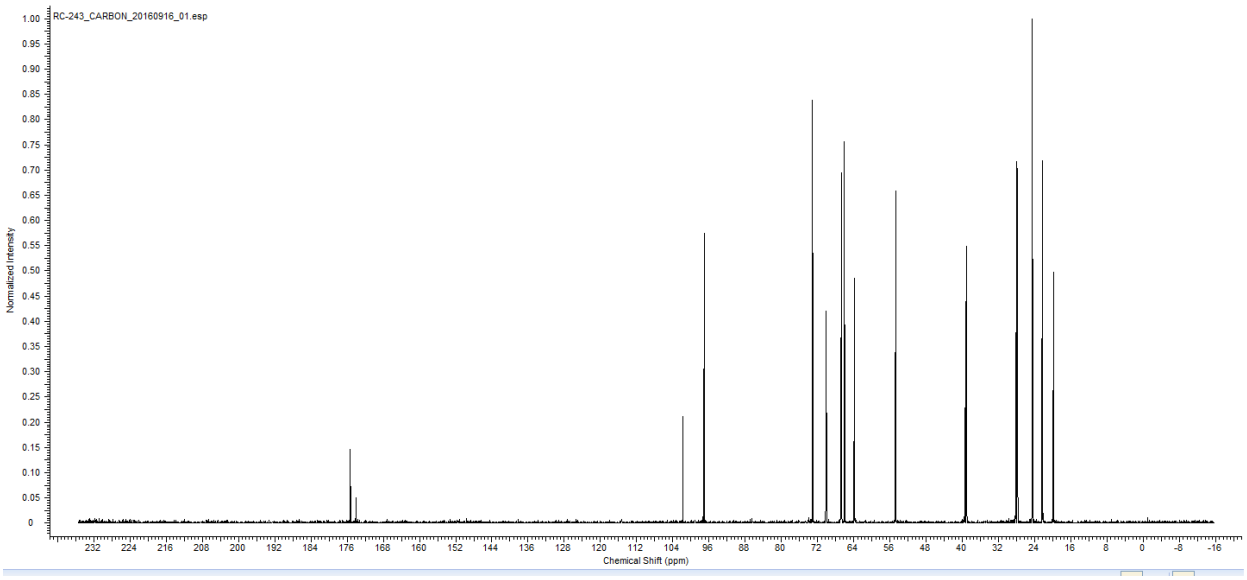
^{1}H NMR (600 MHz, D_2O)



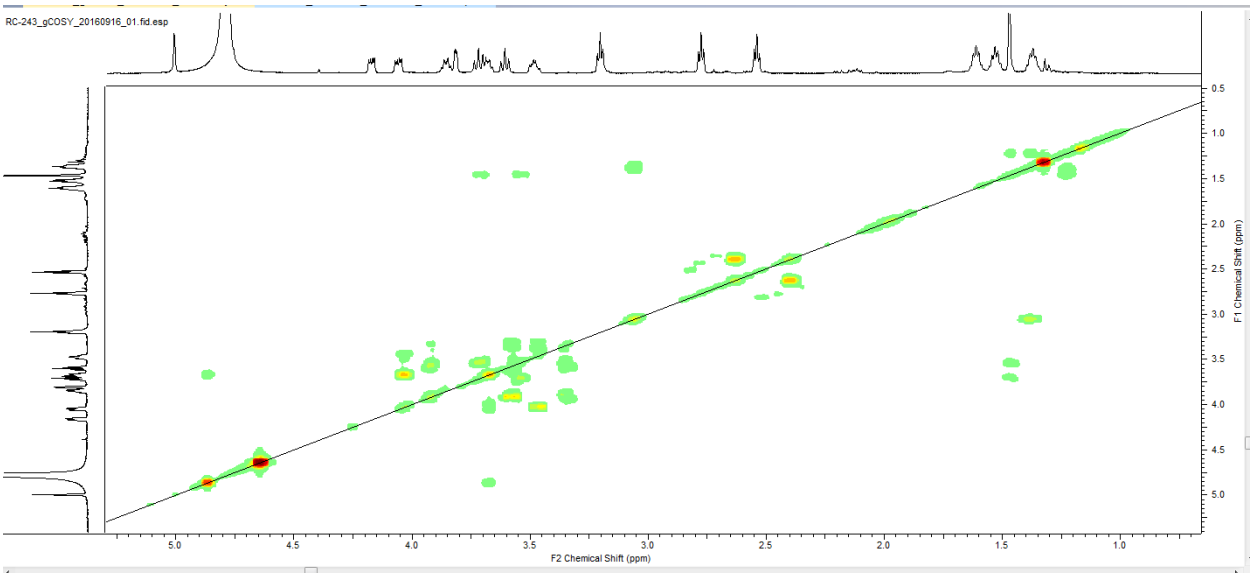
11



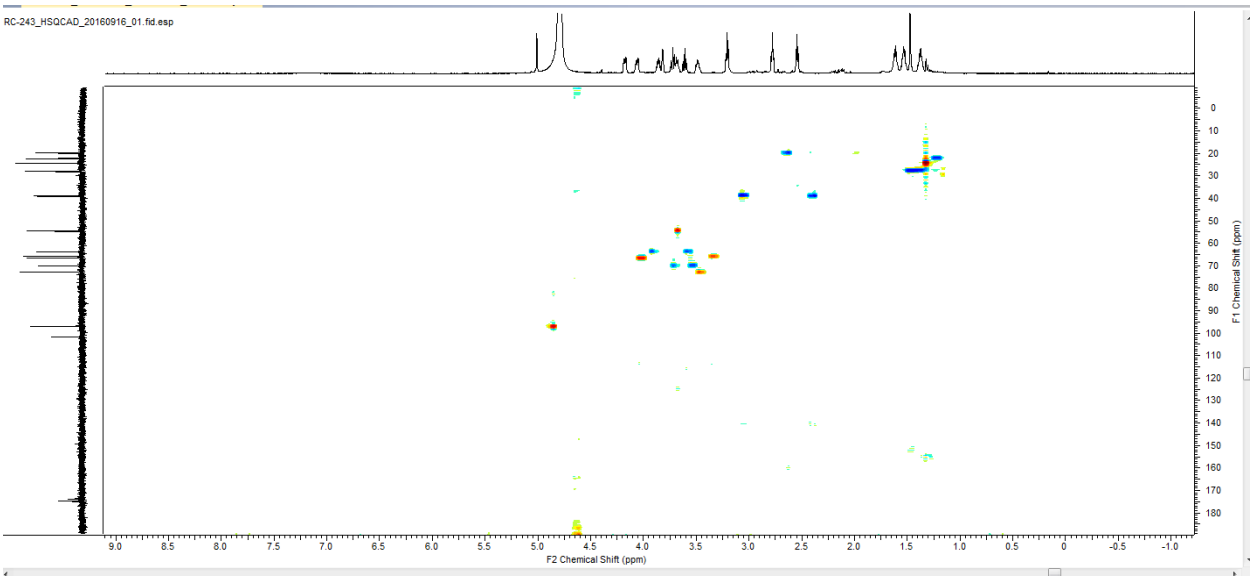
^{13}C NMR (150 MHz, D_2O)



^{1}H-^{1}H COSY (600 MHz, D_2O)



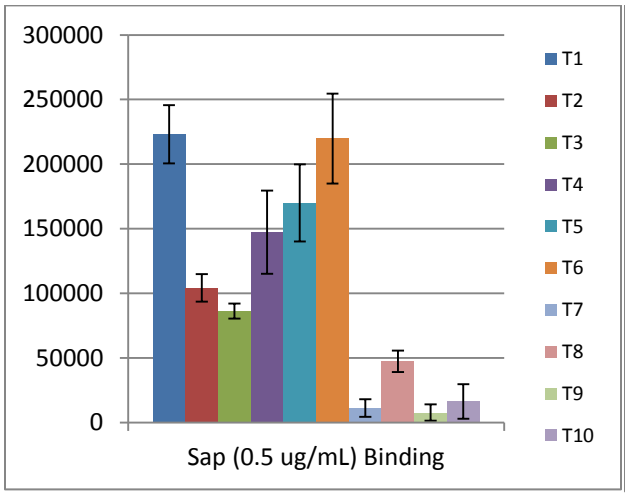
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)

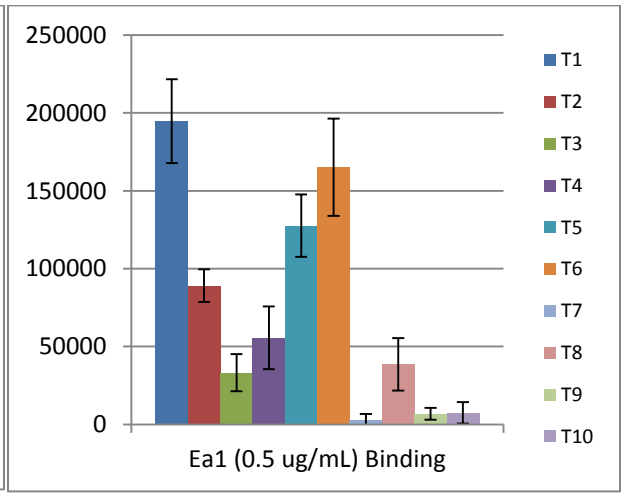


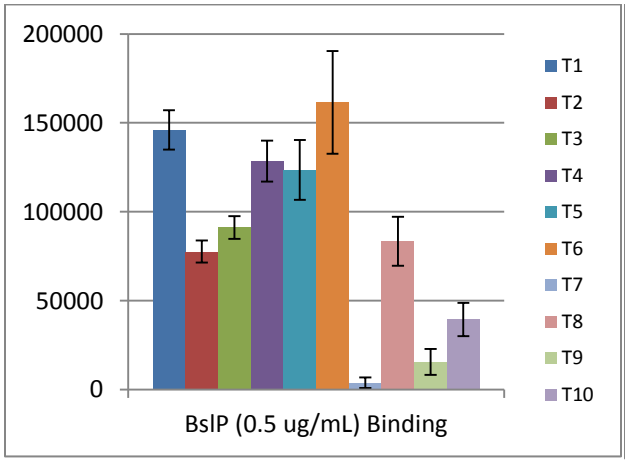
Determination of mCherry Fusion Protein Concentration:

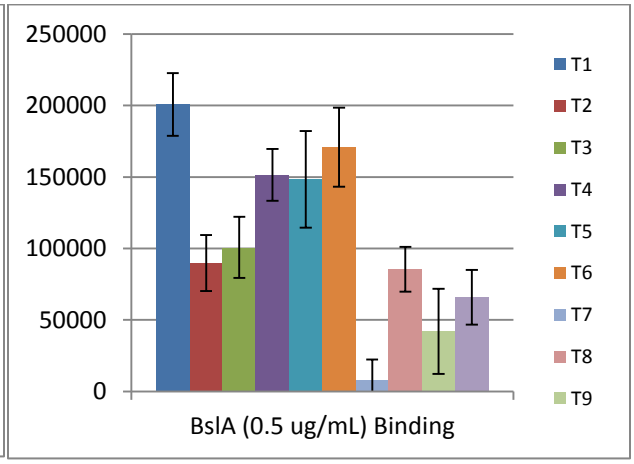
Protein Concentration:		
mCherry protein	*Fluor (mg/mL)*	*BCA (mg/mL)*
I	3.4	3.3
M	9.5	9.4
K	1.5	3.2
O	14.4	14.4
SAP	14.9	14.2
A	0.1	-0.1
Ply6	20.0	13.5
K'	1.8	4.1
R	2.3	2.2
P	2.2	1.7
A 2nd	0.1	2.7
U	0.2	2.2
EA1	0.4	3.0
Control	2.5	2.6
T	1.2	2.6
S	0.2	4.4

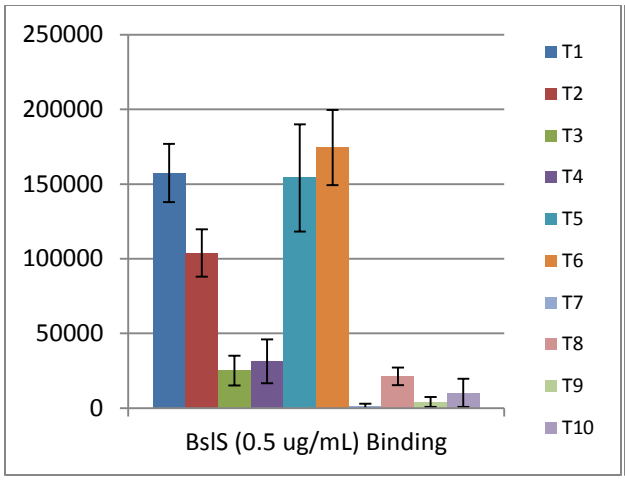
Microarray Results:

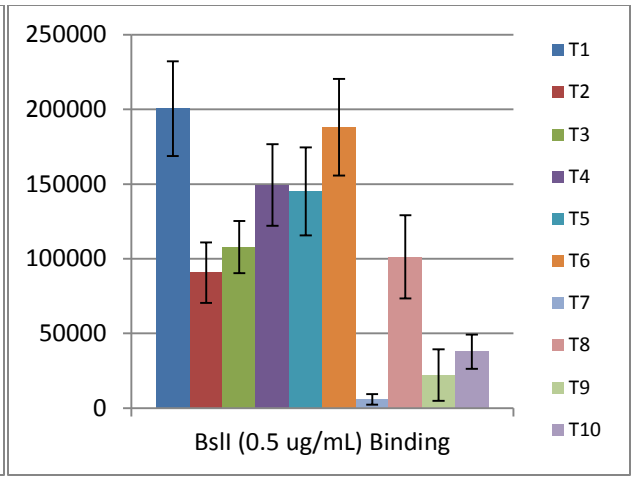


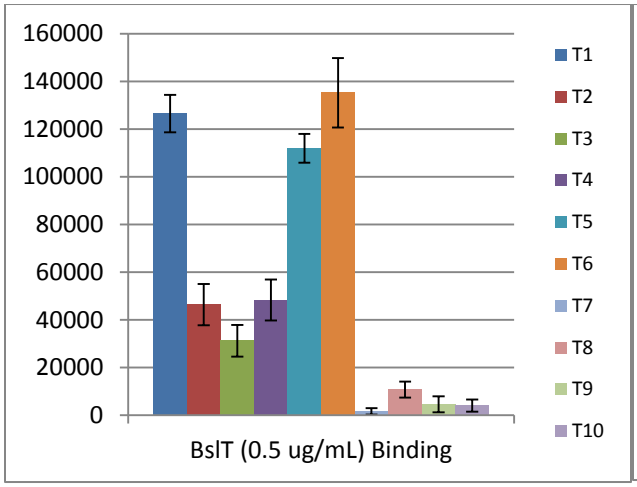


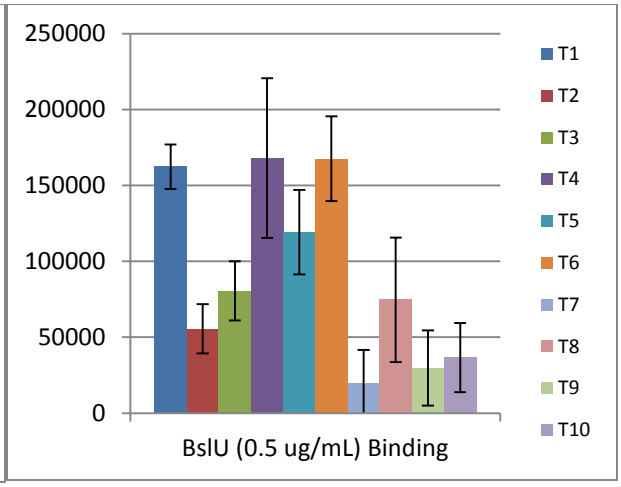


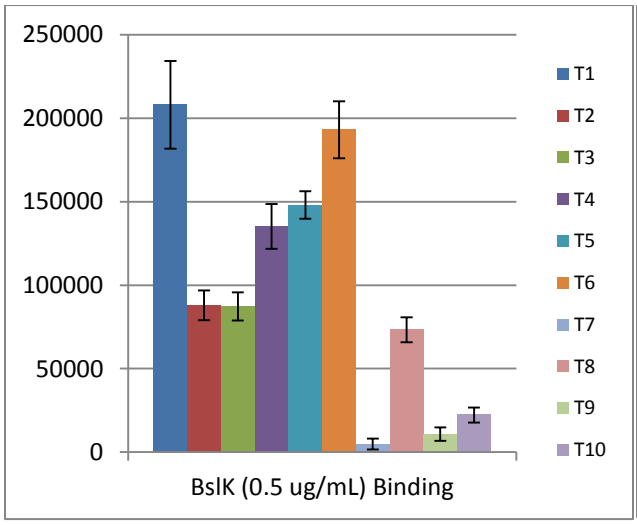


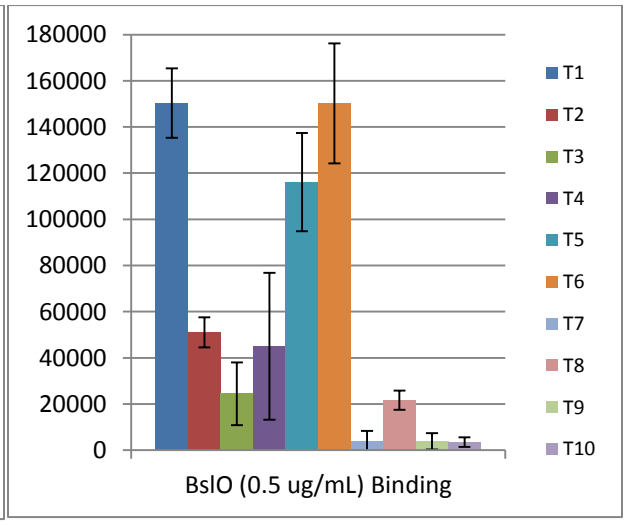

250000
200000
150000
100000
50000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslS (0.5 ug/mL) Binding

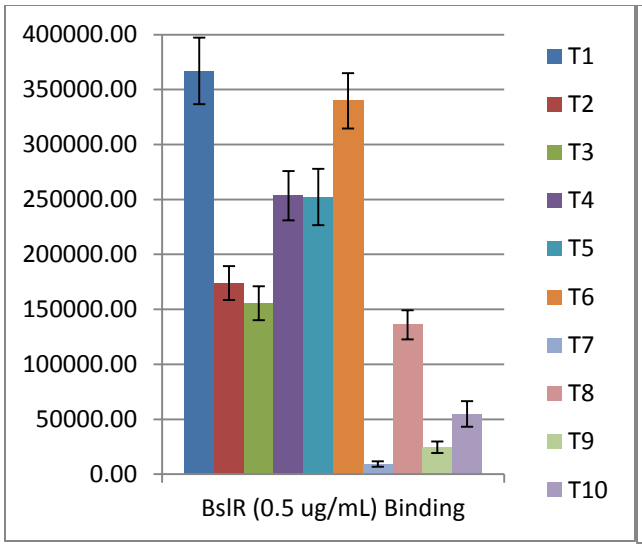

250000
200000
150000
100000
50000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslI (0.5 ug/mL) Binding

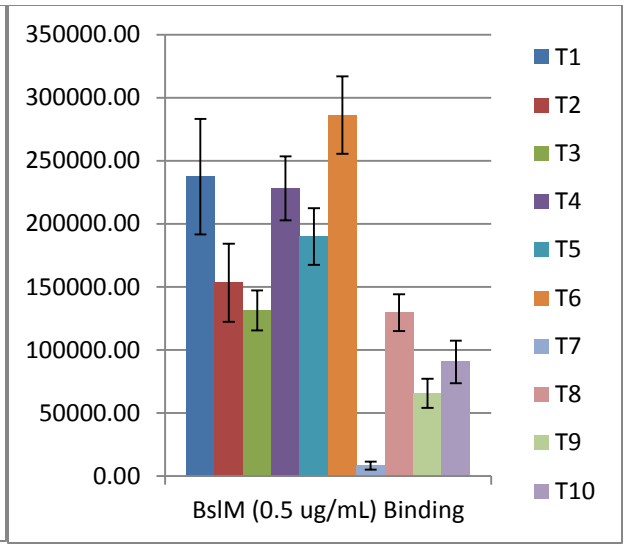

160000
140000
120000
100000
80000
60000
40000
20000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslT (0.5 ug/mL) Binding

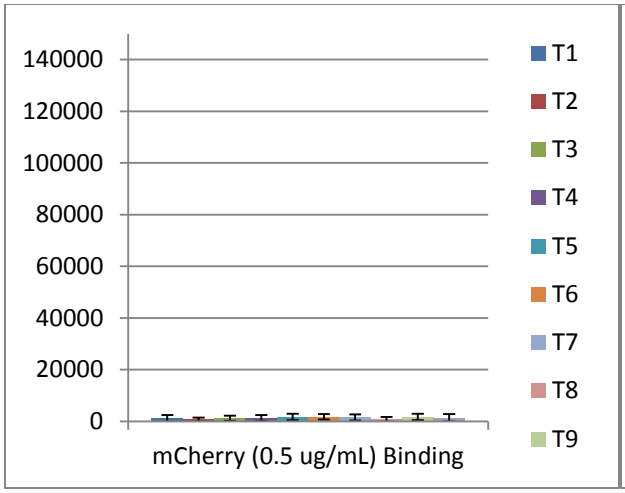

250000
200000
150000
100000
50000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslU (0.5 ug/mL) Binding

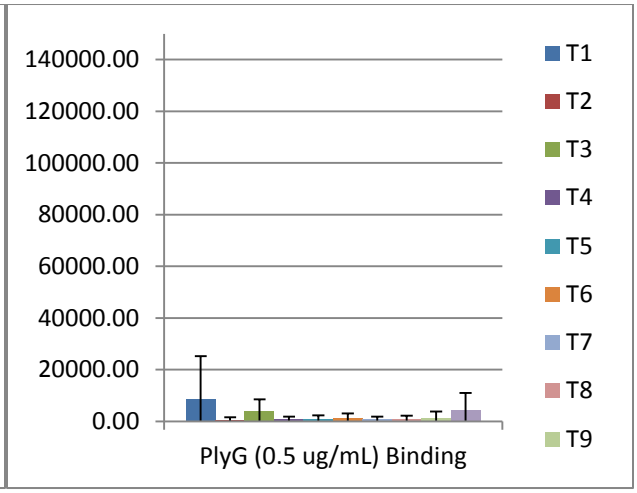

250000
200000
150000
100000
50000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslK (0.5 ug/mL) Binding


180000
160000
140000
120000
100000
80000
60000
40000
20000
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
BslO (0.5 ug/mL) Binding

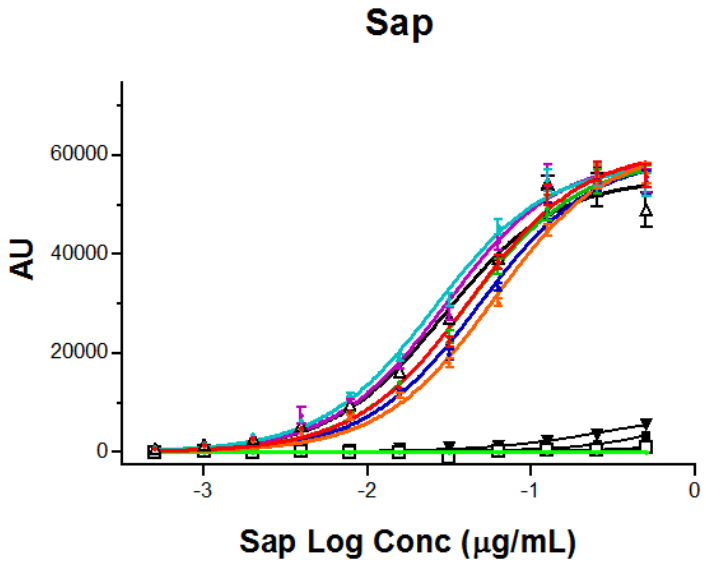

400000.00
350000.00
300000.00
250000.00
200000.00
150000.00
100000.00
50000.00
0.00
BslR (0.5 ug/mL) Binding
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10


350000.00
300000.00
250000.00
200000.00
150000.00
100000.00
50000.00
0.00
BslM (0.5 ug/mL) Binding
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10

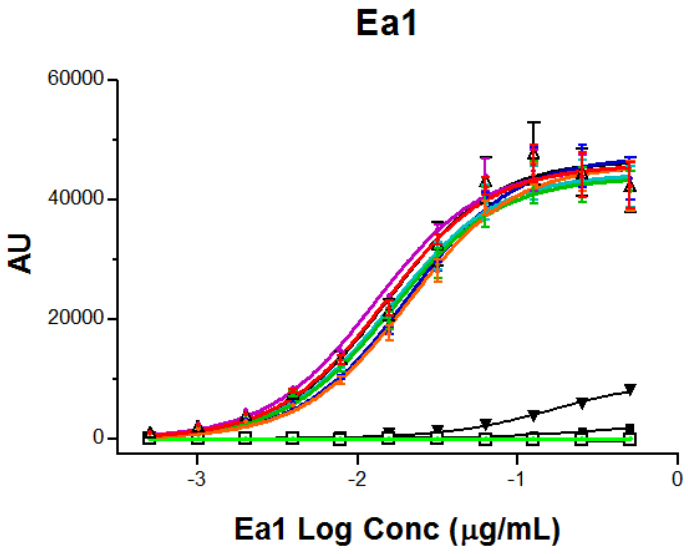

140000
120000
100000
80000
60000
40000
20000
0
mCherry (0.5 ug/mL) Binding
T1
T2
T3
T4
T5
T6
T7
T8
T9


140000.00
120000.00
100000.00
80000.00
60000.00
40000.00
20000.00
0.00
PlyG (0.5 ug/mL) Binding
T1
T2
T3
T4
T5
T6
T7
T8
T9

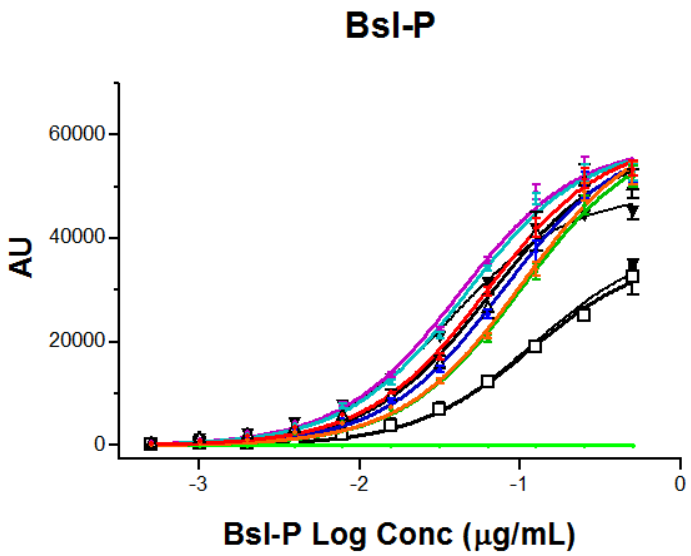
ELISA Dose Response Curves:



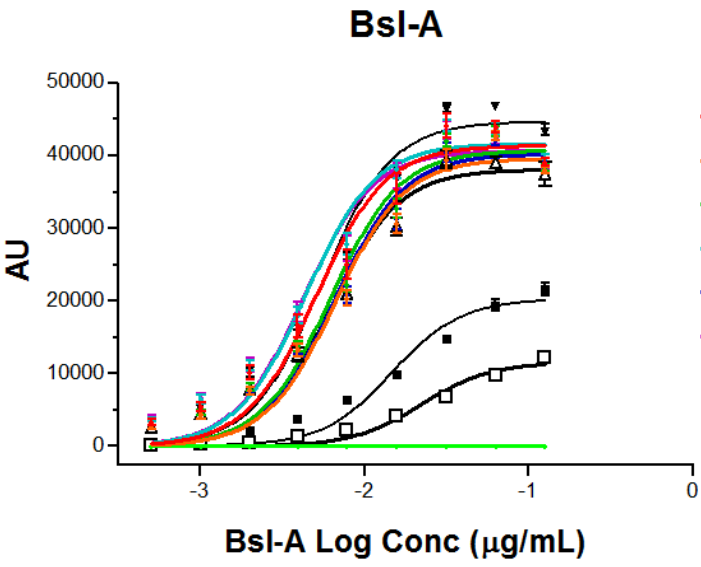
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.4170	0.037	0.047
TGT-2	0.0589	0.053	0.066
TGT-3	0.0402	0.036	0.045
TGT-4	0.0256	0.023	0.029
TGT-5	0.0482	0.043	0.055
TGT-6	0.0298	0.026	0.034
TGT-7	--	--	--
TGT-8	0.0291	0.024	0.035
TGT-9	NA	NA	NA
TGT-10	NA	NA	NA
TGT-11	NA	NA	NA



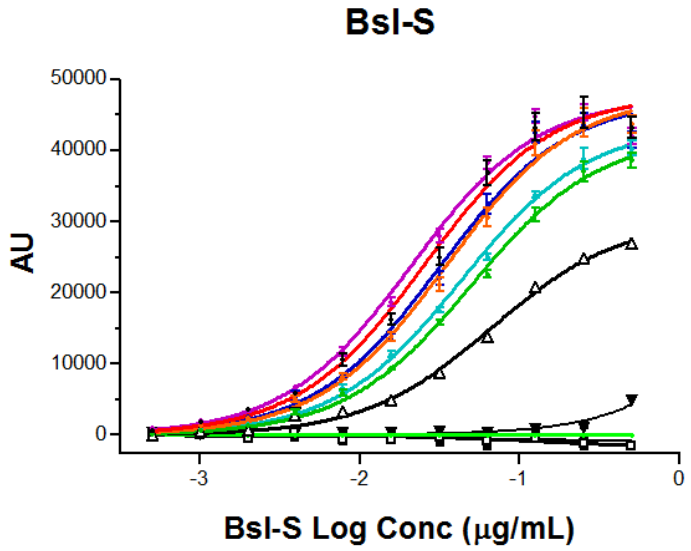
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.0147	0.012	0.018
TGT-2	0.0207	0.017	0.025
TGT-3	0.0165	0.013	0.020
TGT-4	0.0160	0.013	0.020
TGT-5	0.0204	0.017	0.024
TGT-6	0.0127	0.010	0.015
TGT-7	--	--	--
TGT-8	0.0154	0.012	0.020
TGT-9	NA	NA	NA
TGT-10	NA	NA	NA
TGT-11	NA	NA	NA



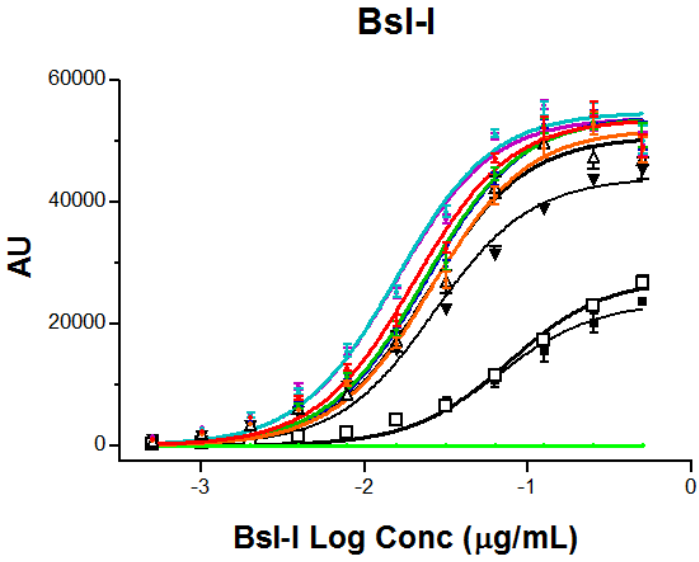
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.0611	0.055	0.068
TGT-2	0.1009	0.090	0.113
TGT-3	0.1013	0.090	0.114
TGT-4	0.0451	0.041	0.050
TGT-5	0.0767	0.068	0.087
TGT-6	0.0410	0.037	0.046
TGT-7	--	--	--
TGT-8	0.0634	0.051	0.078
TGT-9	0.1164	0.095	0.142
TGT-10	0.1208	0.107	0.136
TGT-11	0.0360	0.033	0.039



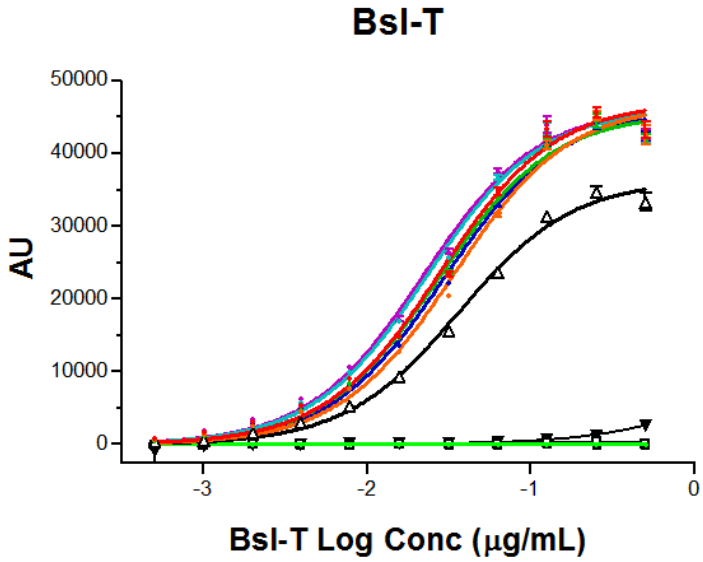
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.00505	0.0044	0.0059
TGT-2	0.0066	0.0058	0.0075
TGT-3	0.00617	0.0055	0.0069
TGT-4	0.00437	0.0039	0.0049
TGT-5	0.00653	0.0058	0.0074
TGT-6	0.00423	0.0037	0.0048
TGT-7	--	--	--
TGT-8	0.0062	0.0053	0.0073
TGT-9	0.0223	0.0186	0.0268
TGT-10	0.0146	0.0121	0.0175
TGT-11	0.0055	0.0047	0.0064



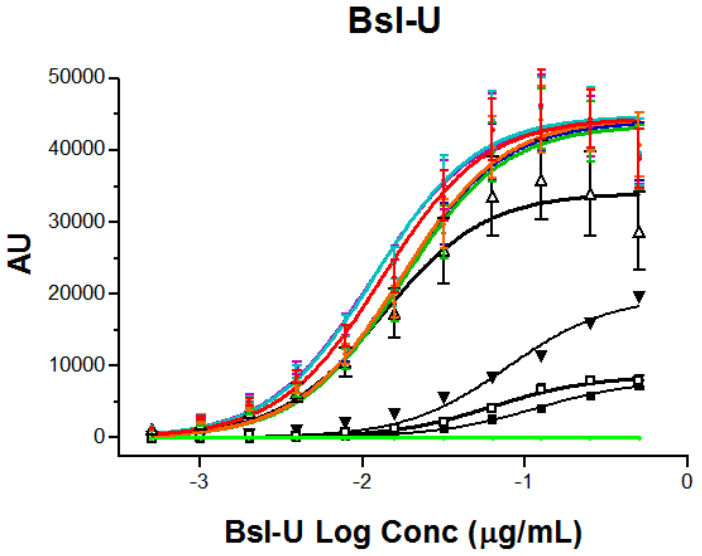
	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0254	0.022	0.030
TGT-2	0.0350	0.031	0.040
TGT-3	0.0498	0.045	0.055
TGT-4	0.0431	0.039	0.047
TGT-5	0.0315	0.027	0.036
TGT-6	0.0209	0.019	0.023
TGT-7	--	--	--
TGT-8	0.0670	0.059	0.076
TGT-9	NA	NA	NA
TGT-10	NA	NA	NA
TGT-11	NA	NA	NA



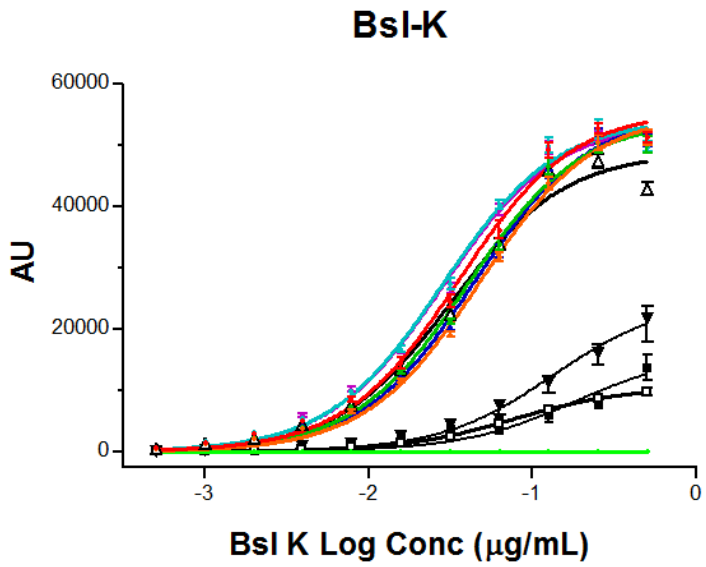
	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0199	0.018	0.022
TGT-2	0.0256	0.023	0.029
TGT-3	0.0229	0.021	0.025
TGT-4	0.0155	0.014	0.017
TGT-5	0.0237	0.021	0.026
TGT-6	0.0154	0.014	0.017
TGT-7	--	--	
TGT-8	0.0245	0.021	0.029
TGT-9	0.0760	0.066	0.088
TGT-10	0.0656	0.053	0.081
TGT-11	0.0266	0.023	0.030



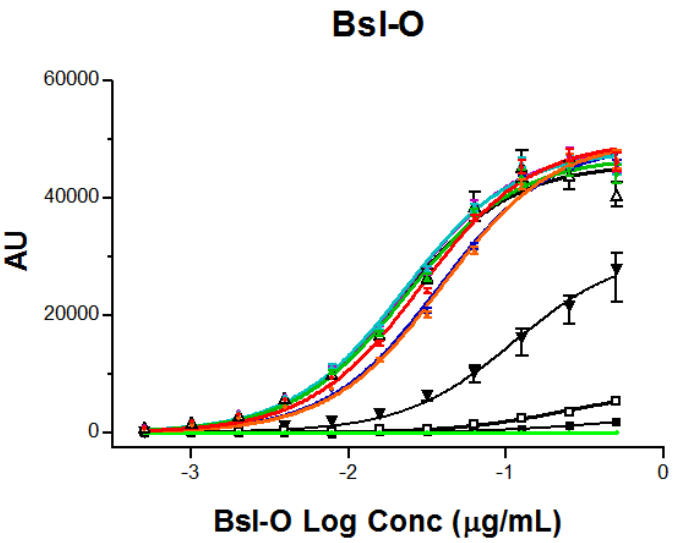
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.0276	0.025	0.030
TGT-2	0.0338	0.031	0.037
TGT-3	0.0272	0.025	0.029
TGT-4	0.0231	0.021	0.025
TGT-5	0.0297	0.028	0.032
TGT-6	0.0219	0.020	0.024
TGT-7	--	--	--
TGT-8	0.0363	0.033	0.040
TGT-9	NA	NA	NA
TGT-10	NA	NA	NA
TGT-11	NA	NA	NA



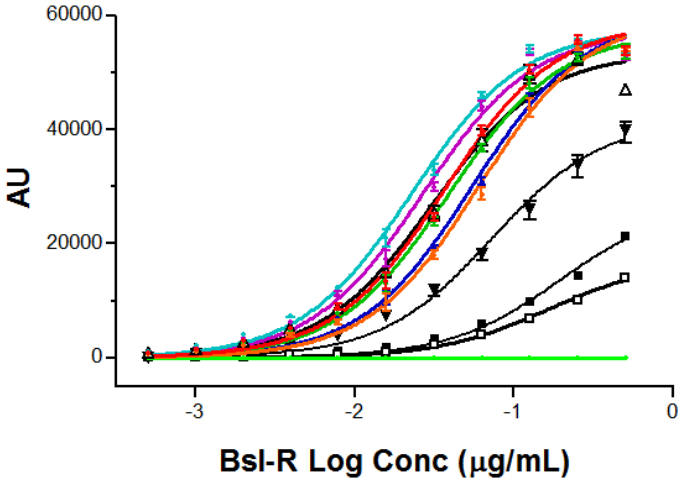
	EC50 (ug/mL)	95% Confidence +	95% Confidence -
TGT-1	0.0131	0.010	0.017
TGT-2	0.0171	0.013	0.023
TGT-3	0.0174	0.013	0.023
TGT-4	0.0117	0.009	0.015
TGT-5	0.0169	0.013	0.022
TGT-6	0.0114	0.009	0.015
TGT-7	--	--	
TGT-8	0.0131	0.008	0.020
TGT-9	0.0625	0.051	0.077
TGT-10	0.1122	0.096	0.132
TGT-11	0.0772	0.066	0.091



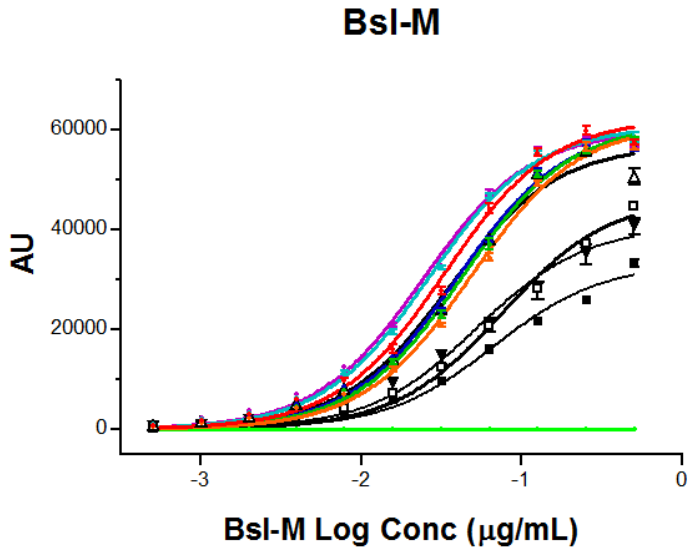
	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0352	0.032	0.039
TGT-2	0.0476	0.044	0.051
TGT-3	0.0399	0.036	0.044
TGT-4	0.0280	0.026	0.031
TGT-5	0.0439	0.041	0.048
TGT-6	0.0281	0.026	0.031
TGT-7	--	--	
TGT-8	0.0319	0.028	0.037
TGT-9	0.0765	0.062	0.095
TGT-10	0.1766	0.105	0.296
TGT-11	0.1319	0.105	0.166



	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0286	0.026	0.031
TGT-2	0.0392	0.036	0.043
TGT-3	0.0232	0.021	0.025
TGT-4	0.0221	0.020	0.024
TGT-5	0.0368	0.033	0.041
TGT-6	0.0228	0.021	0.025
TGT-7	--	--	
TGT-8	0.0214	0.018	0.026
TGT-9	NA	NA	NA
TGT-10	NA	NA	NA
TGT-11	0.1112	0.088	0.140

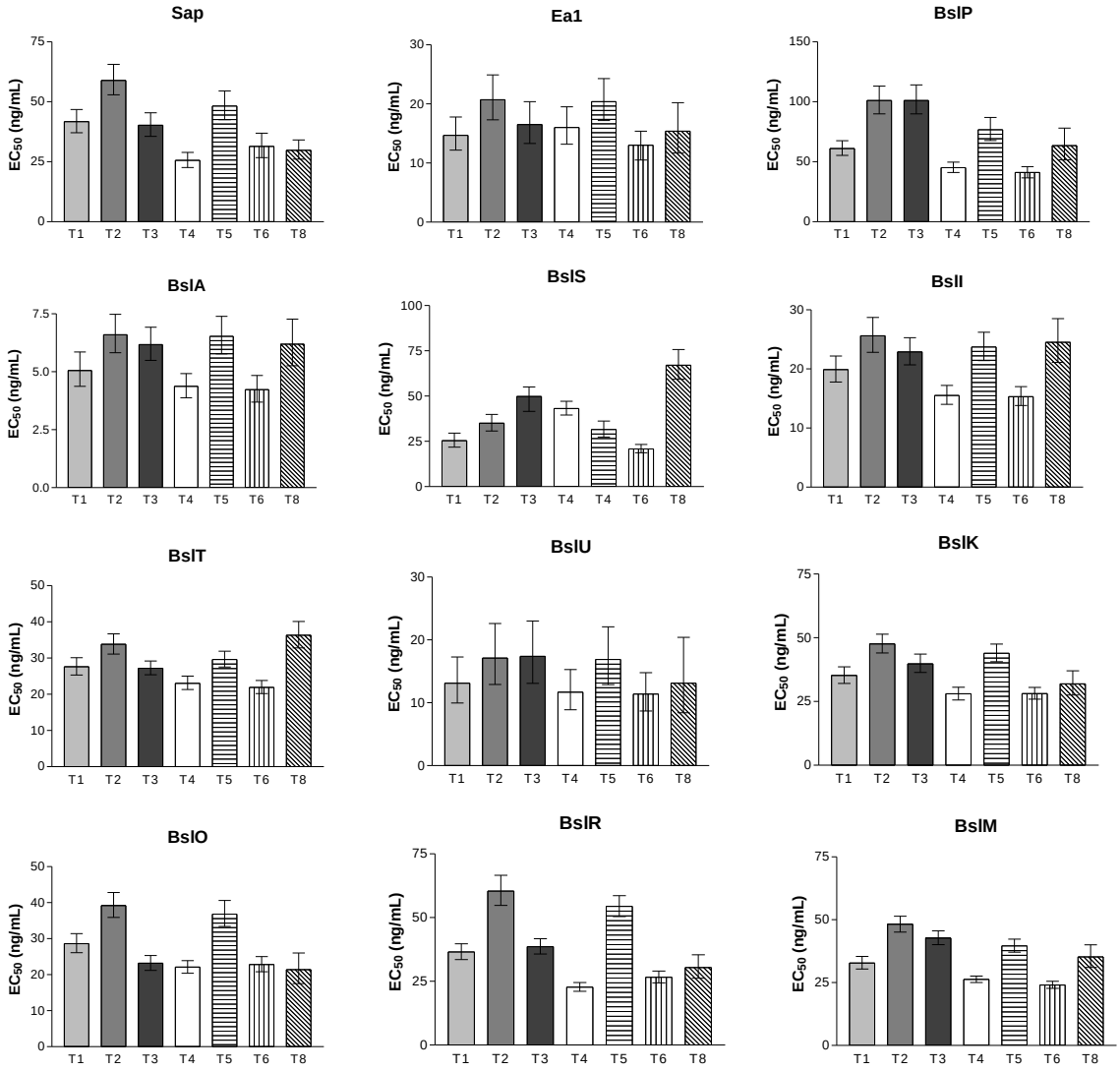


	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0365	0.034	0.040
TGT-2	0.0604	0.055	0.067
TGT-3	0.0386	0.036	0.042
TGT-4	0.0227	0.021	0.024
TGT-5	0.0544	0.050	0.059
TGT-6	0.0266	0.024	0.029
TGT-7	--	--	
TGT-8	0.0305	0.026	0.035
TGT-9	0.1663	0.144	0.192
TGT-10	0.1915	0.166	0.221
TGT-11	0.0725	0.064	0.082



	EC50 (ug/mL)	95% Confidence +	-
TGT-1	0.0328	0.030	0.035
TGT-2	0.0482	0.045	0.051
TGT-3	0.0428	0.040	0.046
TGT-4	0.0263	0.025	0.028
TGT-5	0.0396	0.037	0.042
TGT-6	0.0241	0.023	0.025
TGT-7	--	--	
TGT-8	0.0352	0.031	0.040
TGT-9	0.0753	0.068	0.084
TGT-10	0.0662	0.058	0.076
TGT-11	0.0523	0.045	0.061

Table 1: ELISA EC_{50} Results (ng/mL) for SLP and BSL mCherry Fusion Proteins:

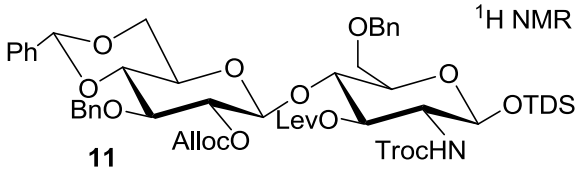


Error displayed as 95% Confidence Intervals, n=6 replicates for TGTs 1-6, n=3 replicates for TGT-8

APPENDIX B

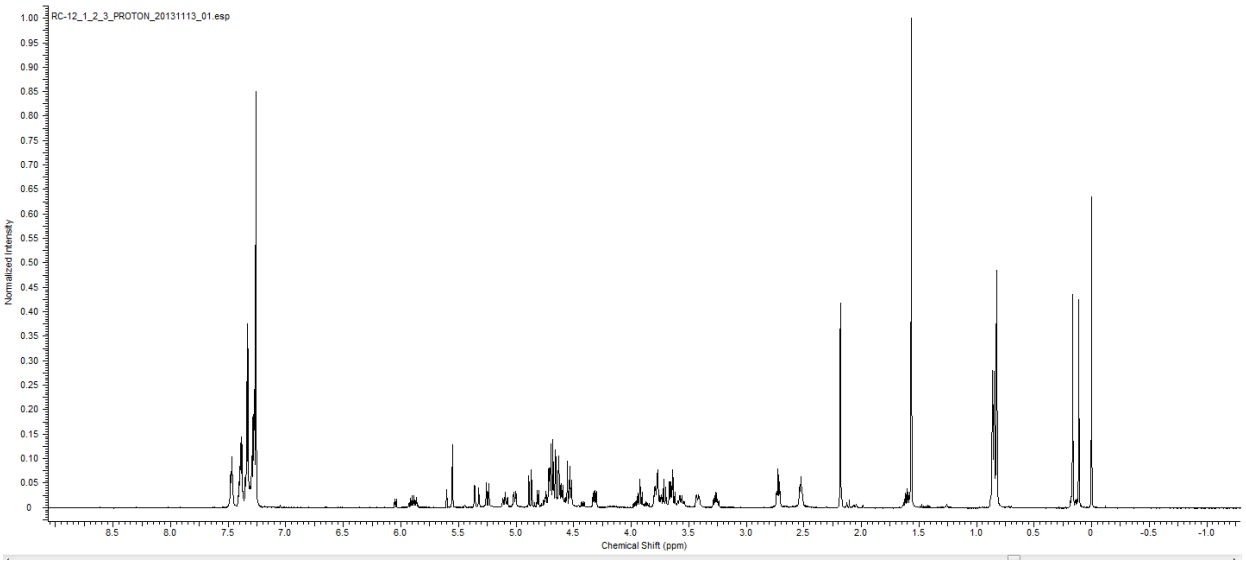
SUPPLEMENTARY DATA FOR CHAPTER 3

^{1}H and ^{13}C NMR Data: p 463-506

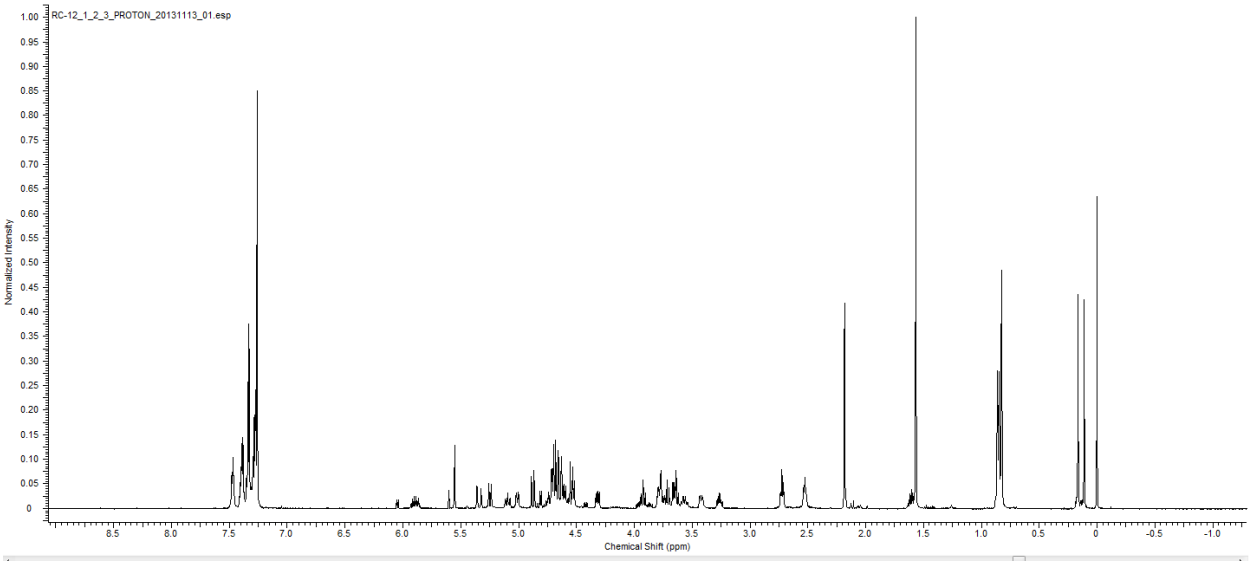


11

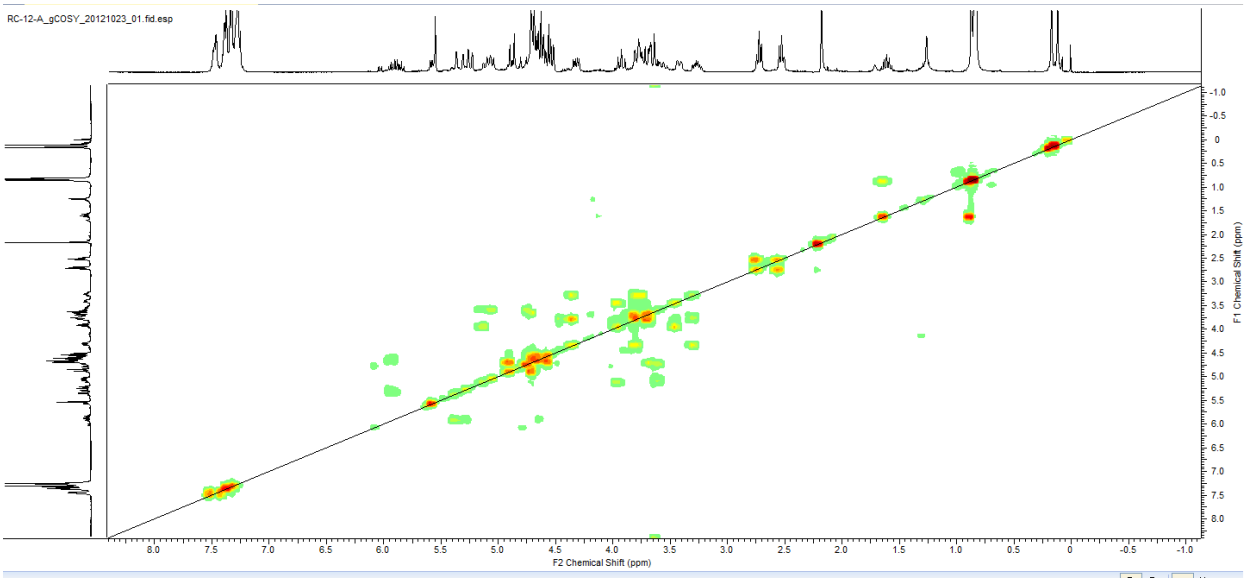
^{1}H NMR (500 MHz, $CDCl_3$)



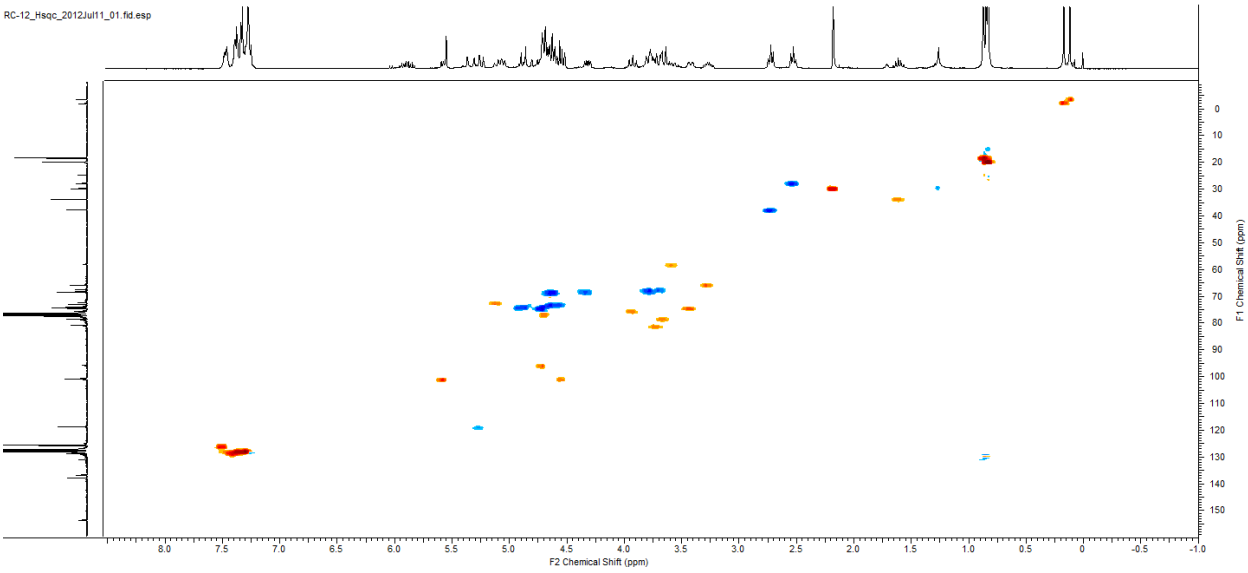
^{13}C NMR (150 MHz, $CDCl_3$)

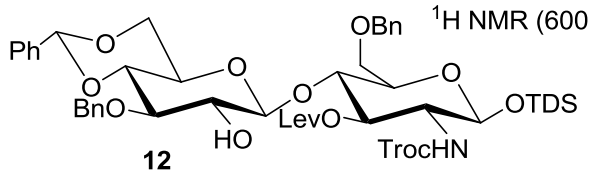


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



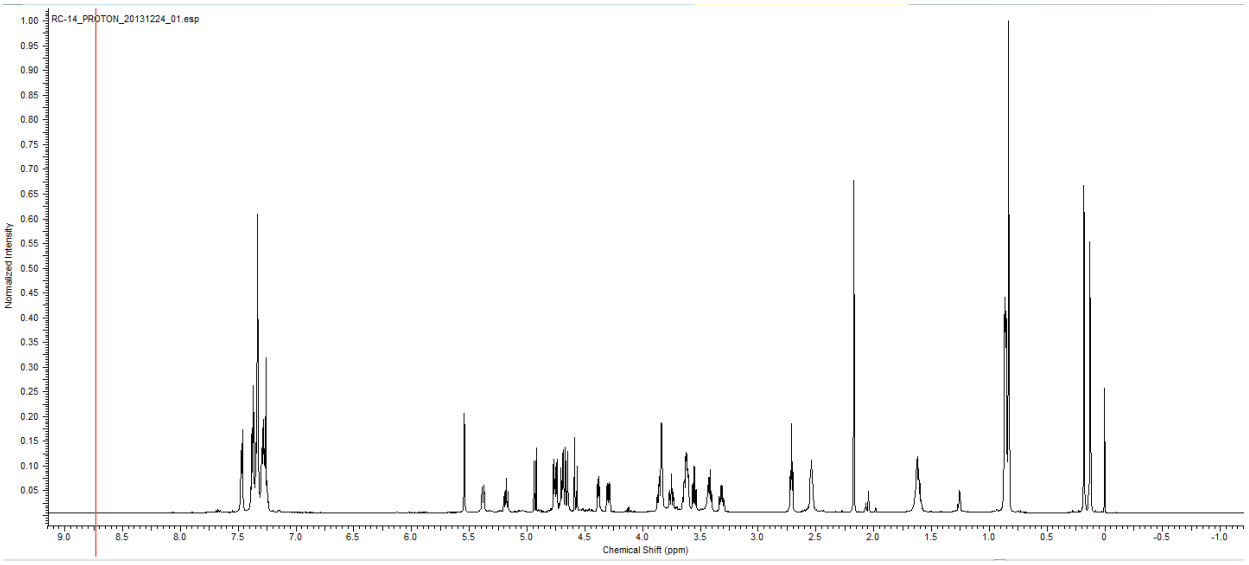
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



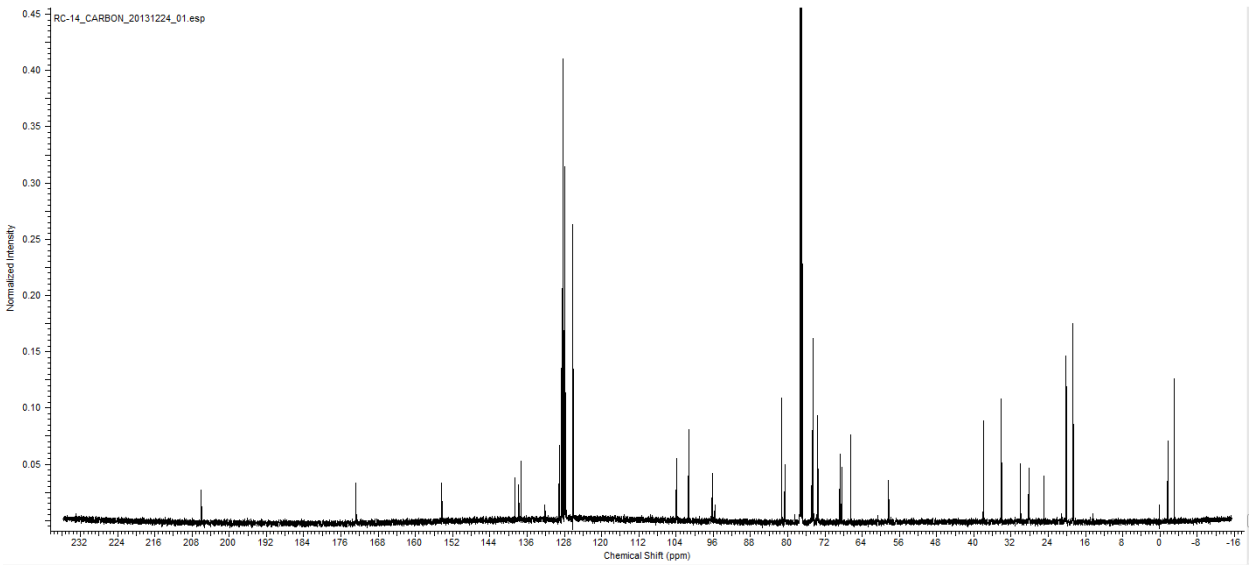


12

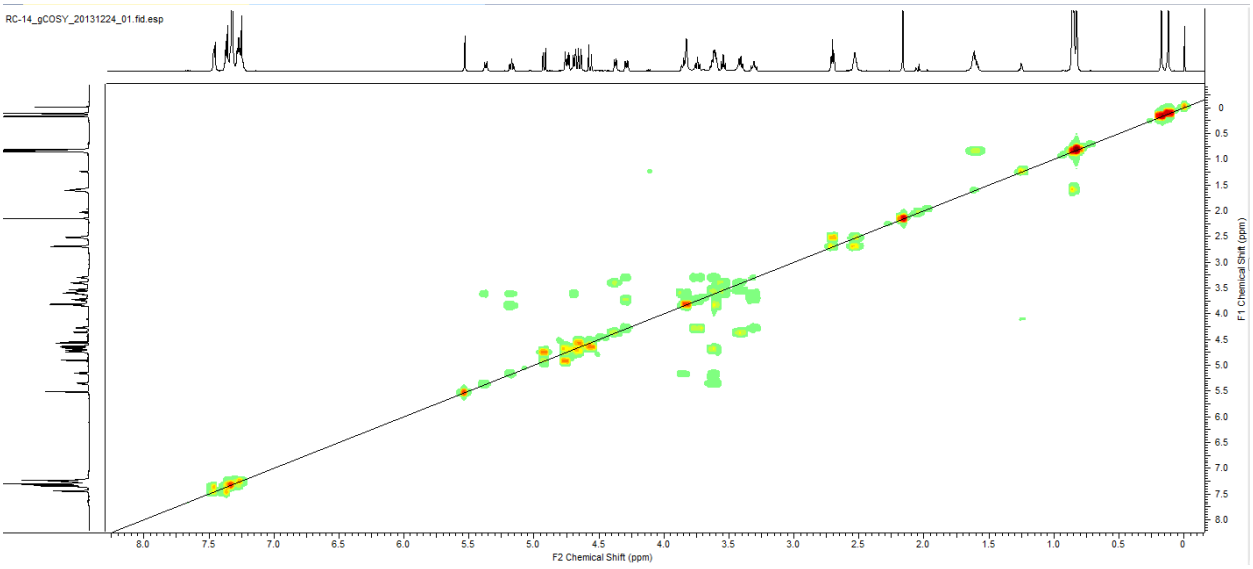
^{1}H NMR (600 MHz, $CDCl_3$)



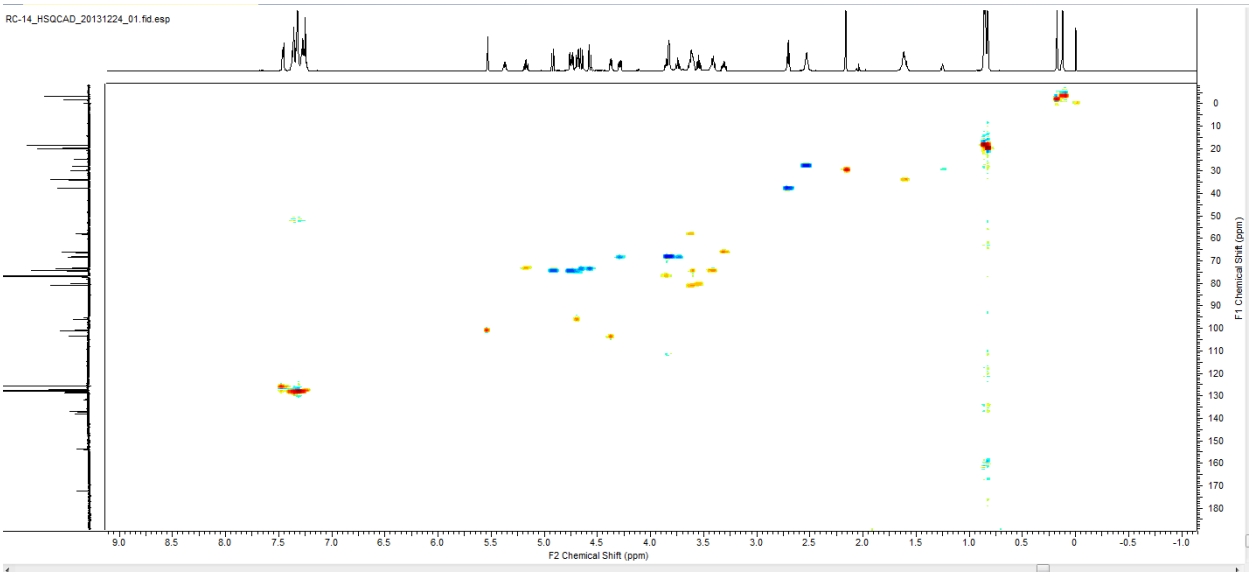
^{13}C NMR (150 MHz, $CDCl_3$)

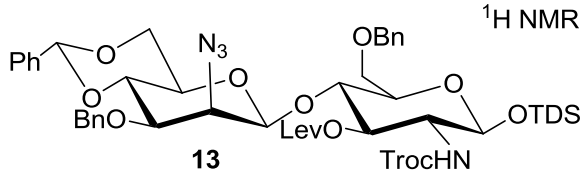


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



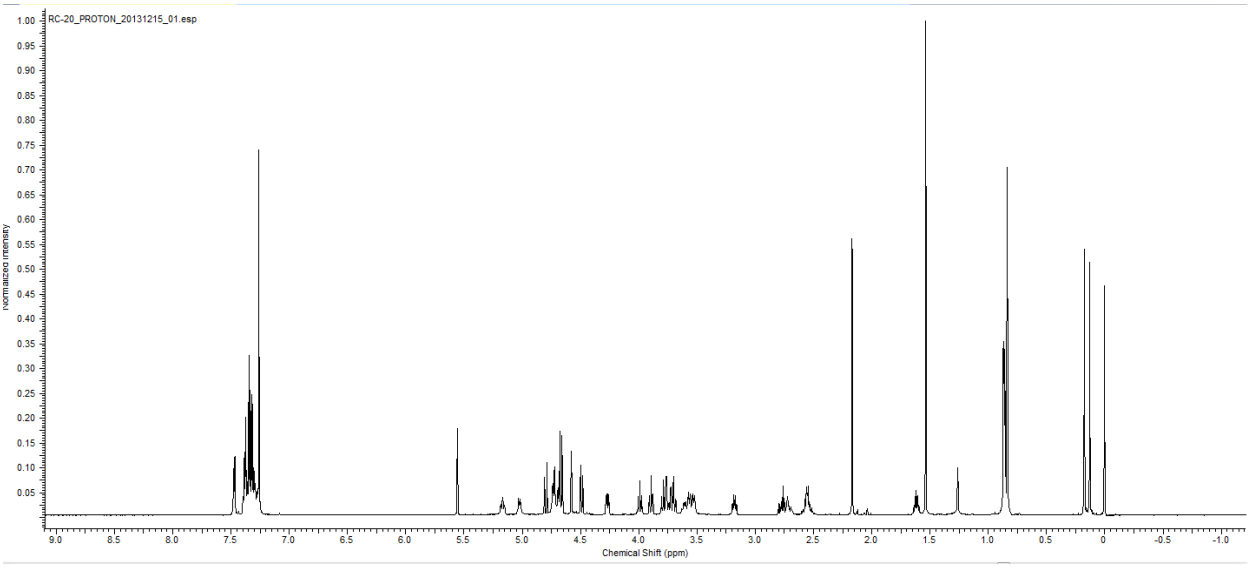
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



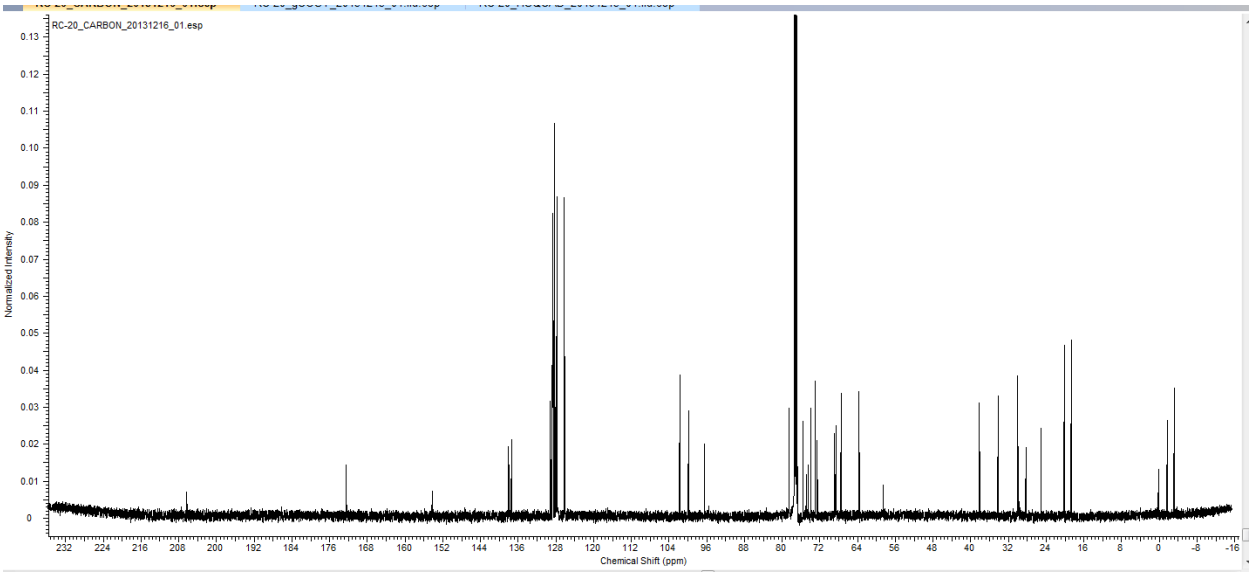


13

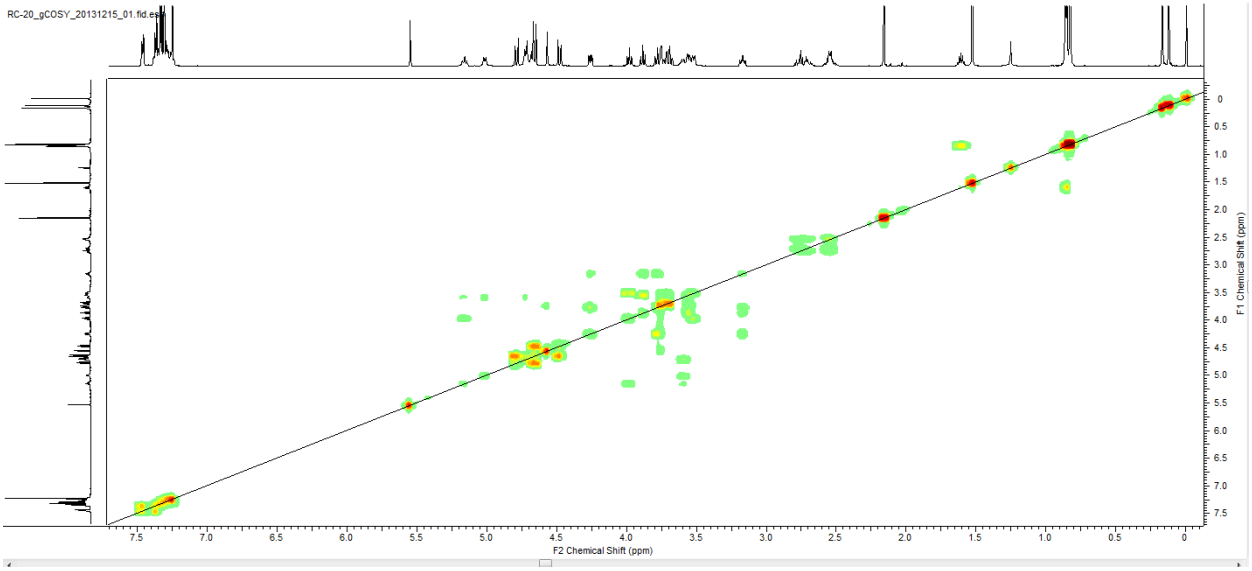
^{1}H NMR (600 MHz, $CDCl_3$)



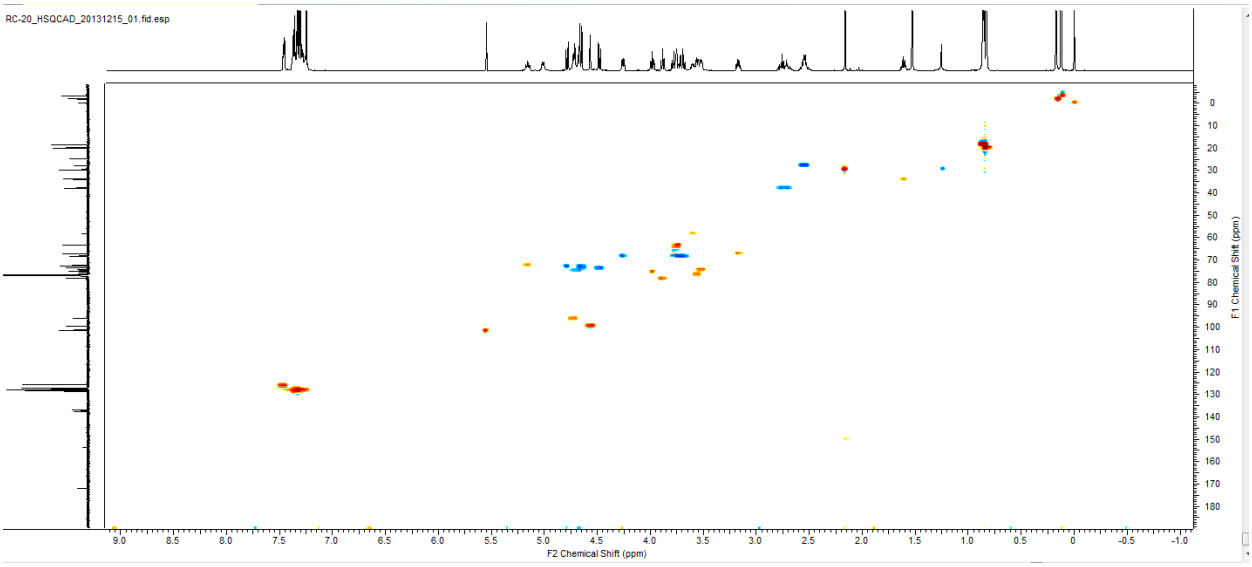
^{13}C NMR (150 MHz, $CDCl_3$)

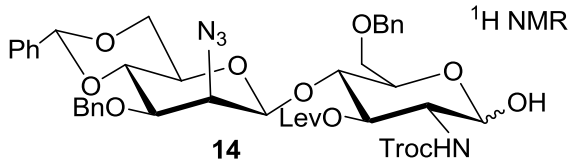


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



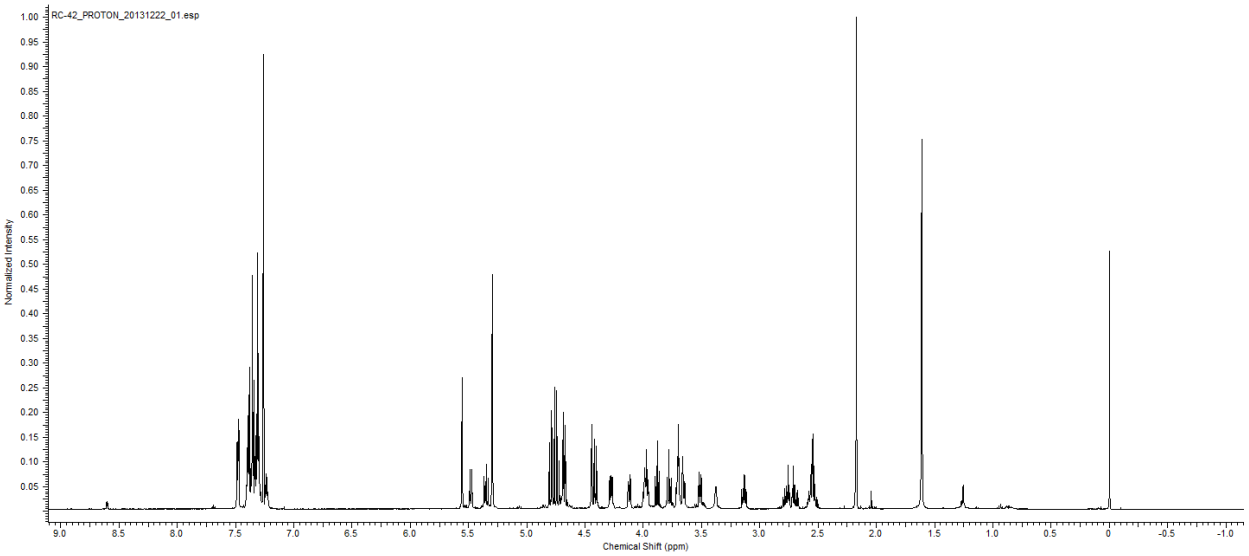
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



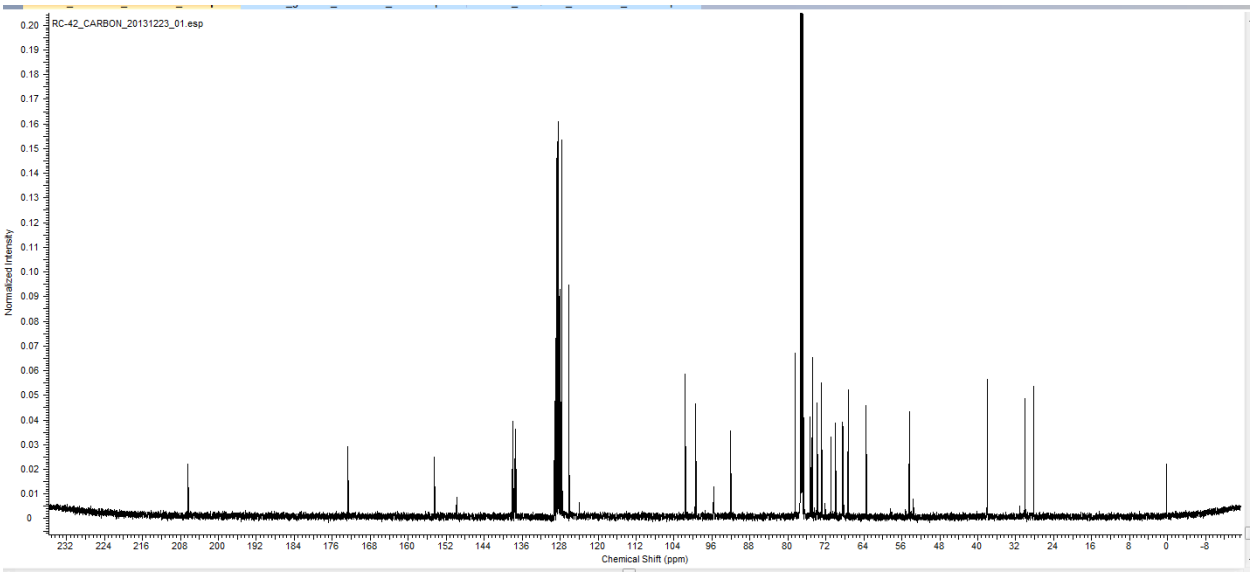


14

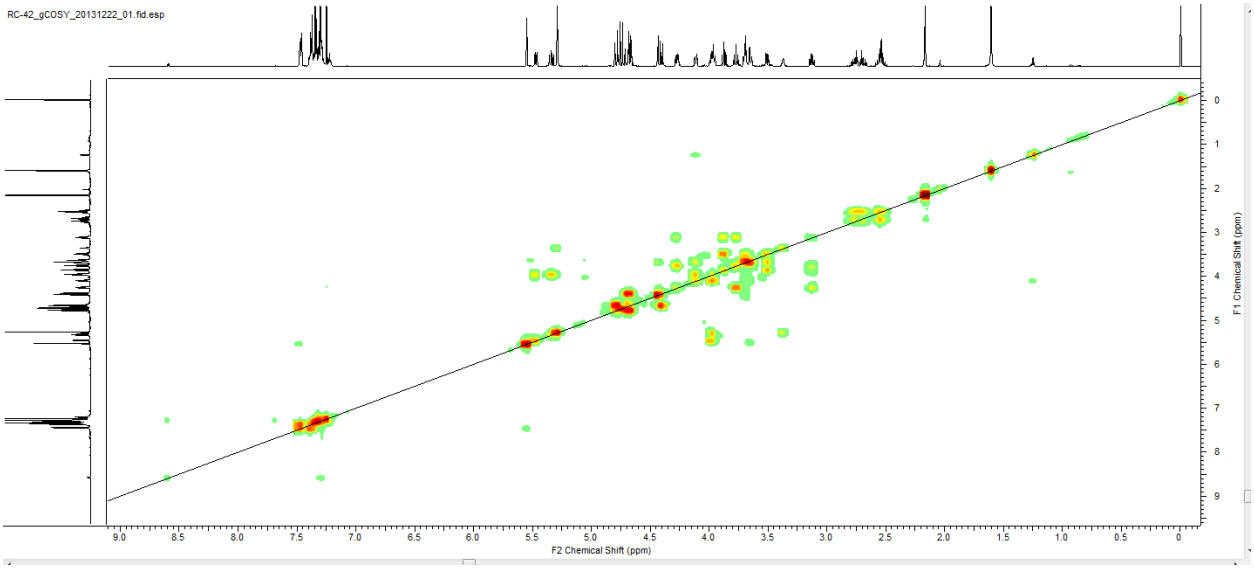
^{1}H NMR (600 MHz, $CDCl_3$)



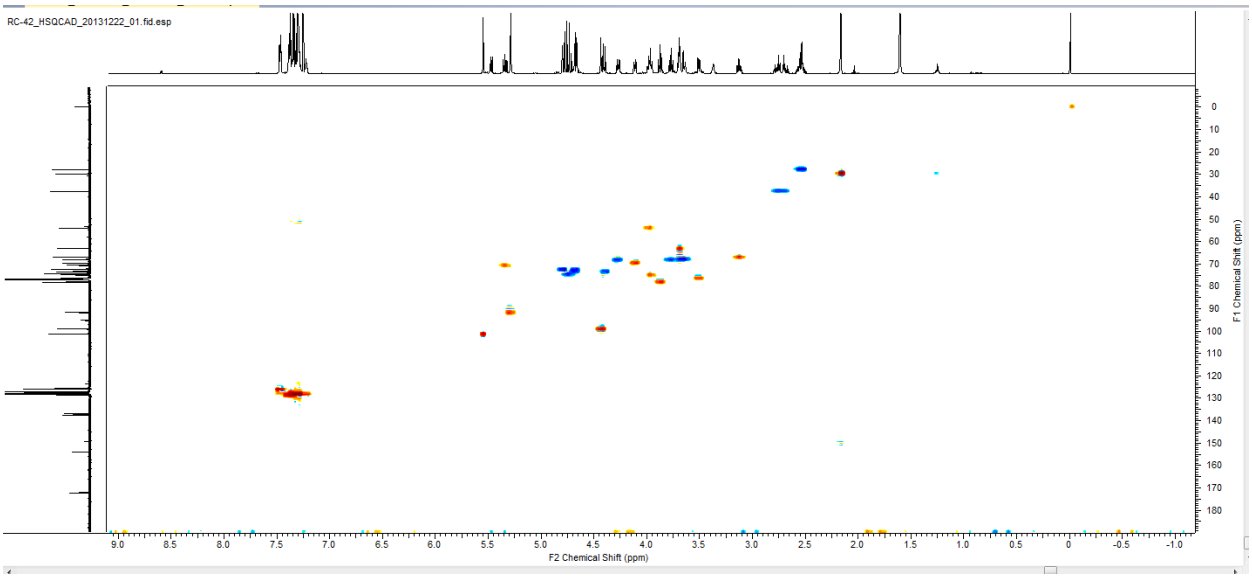
^{13}C NMR (150 MHz, $CDCl_3$)

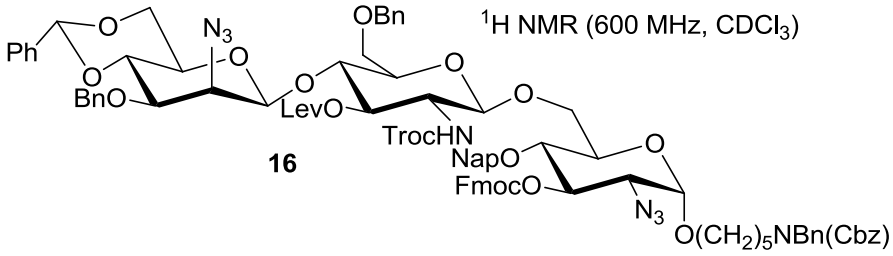


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



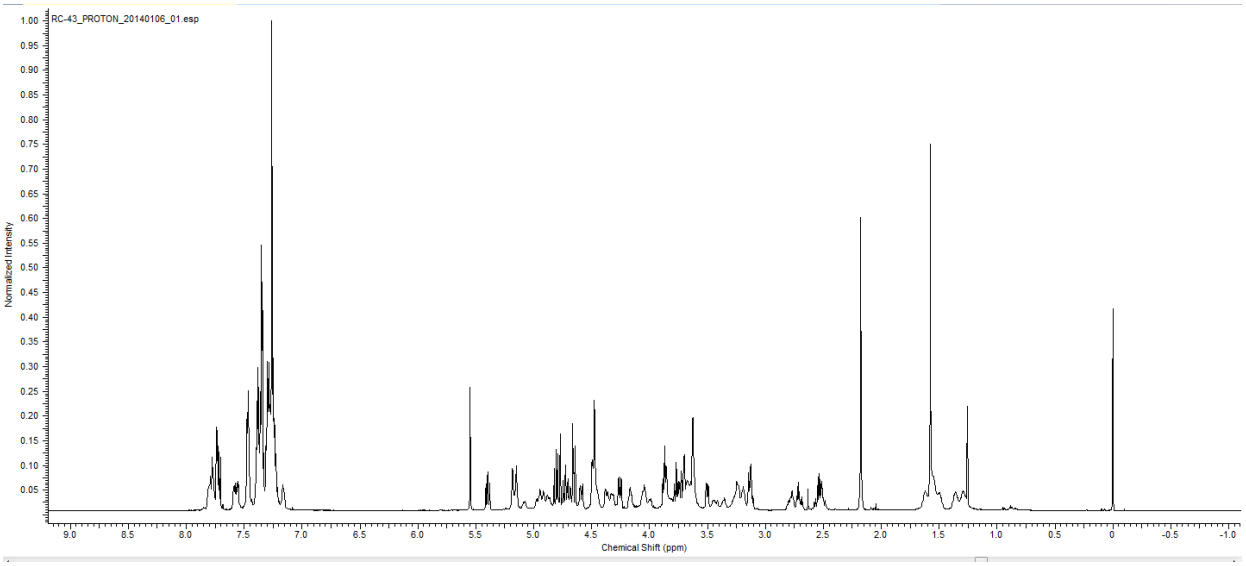
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



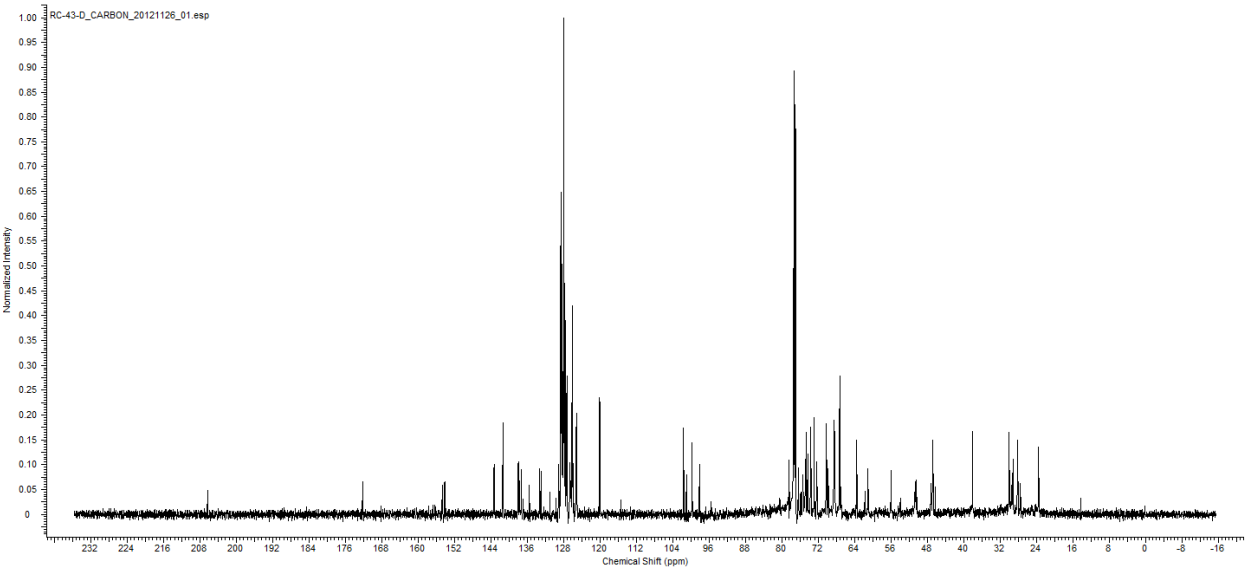


16

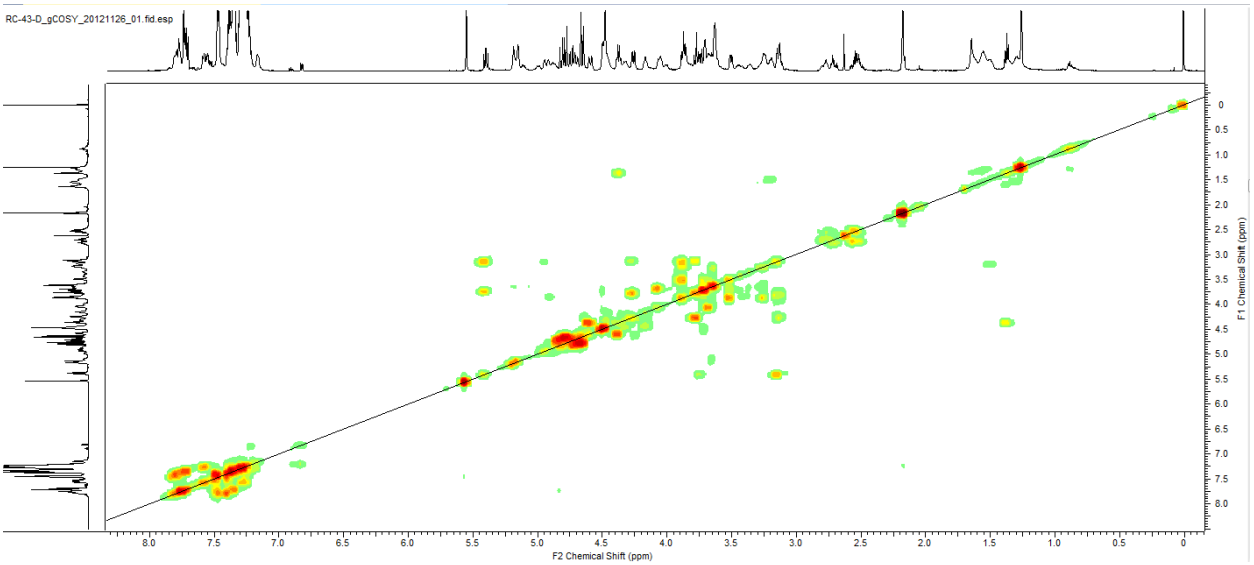
^{1}H NMR (600 MHz, $CDCl_3$)



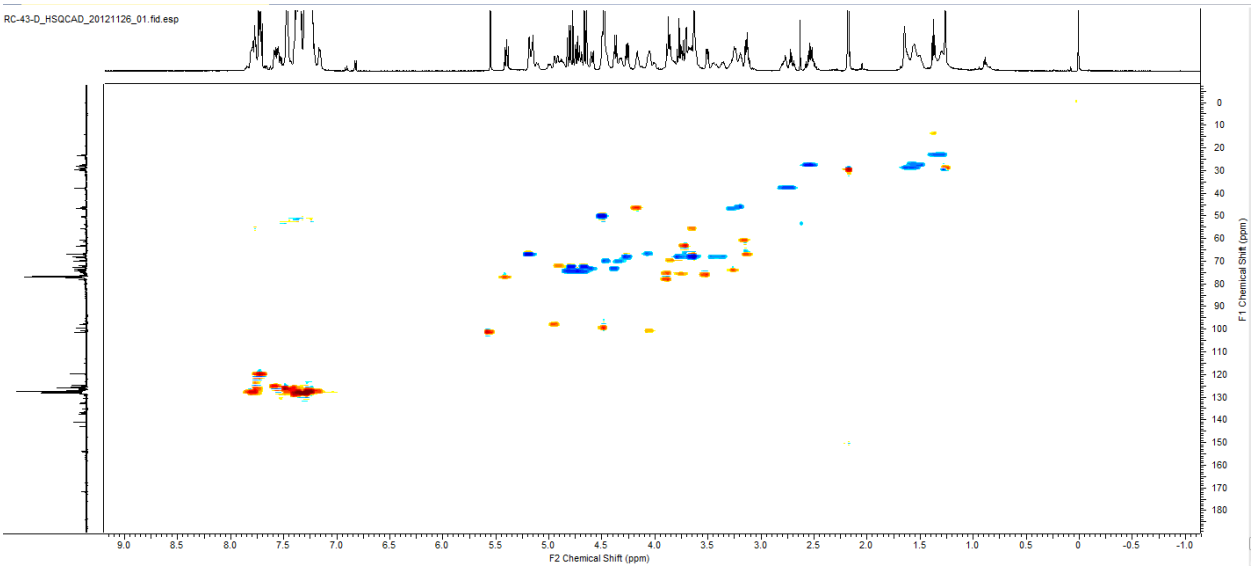
^{13}C NMR (150 MHz, $CDCl_3$)

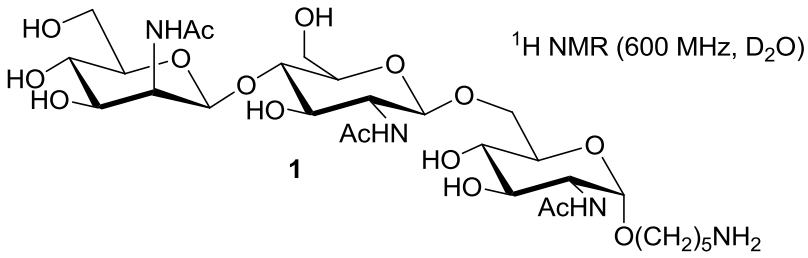


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



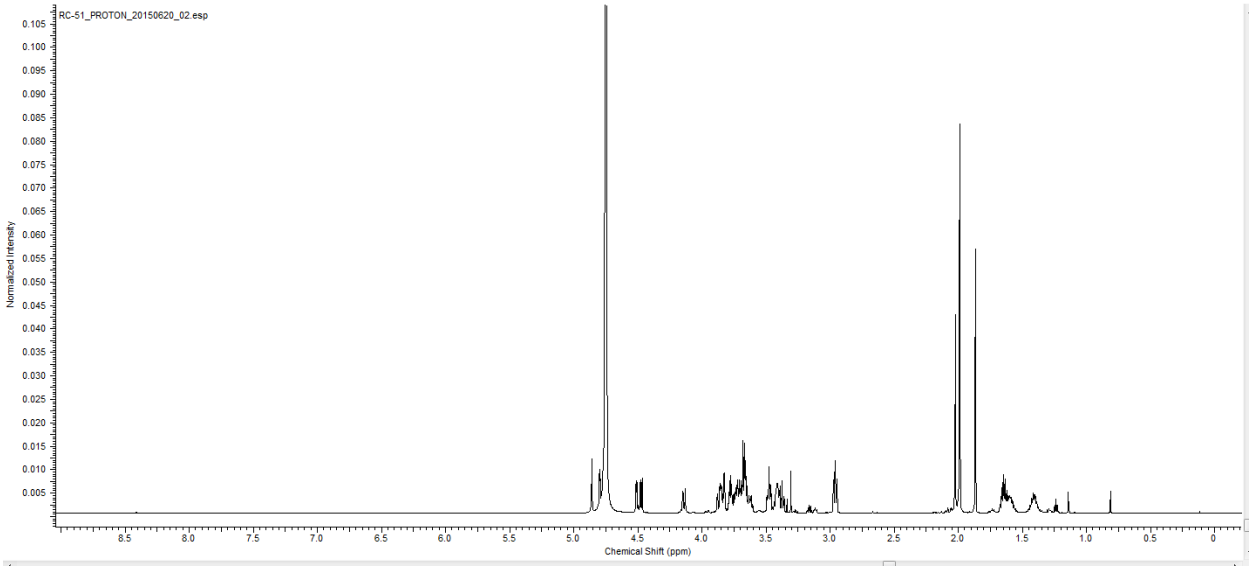
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



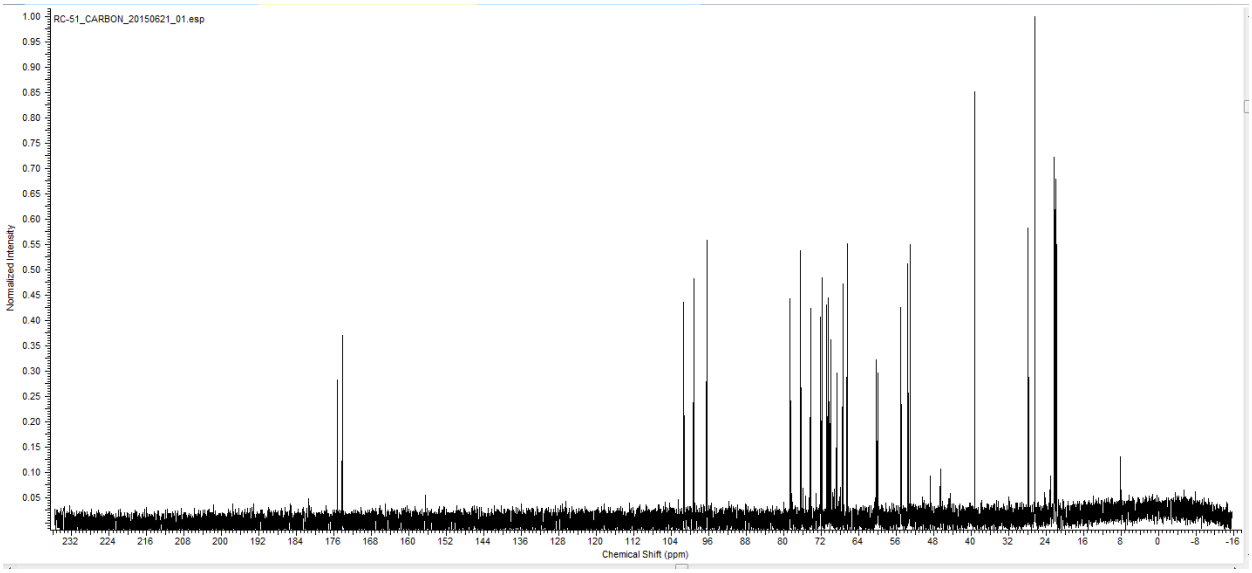


1

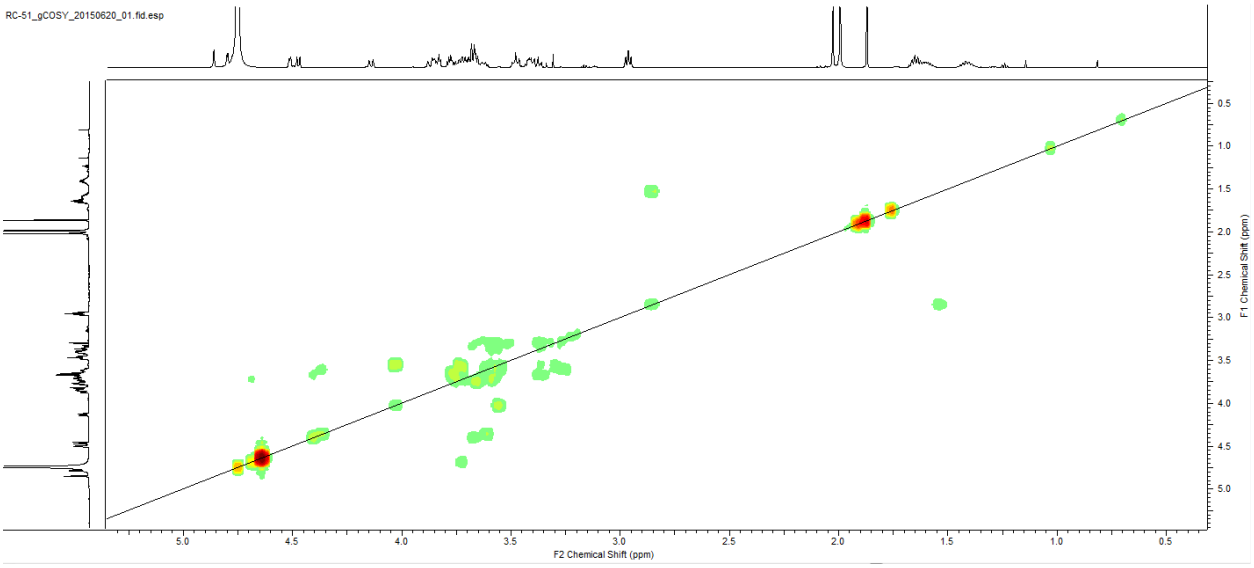
^{1}H NMR (600 MHz, D_2O)



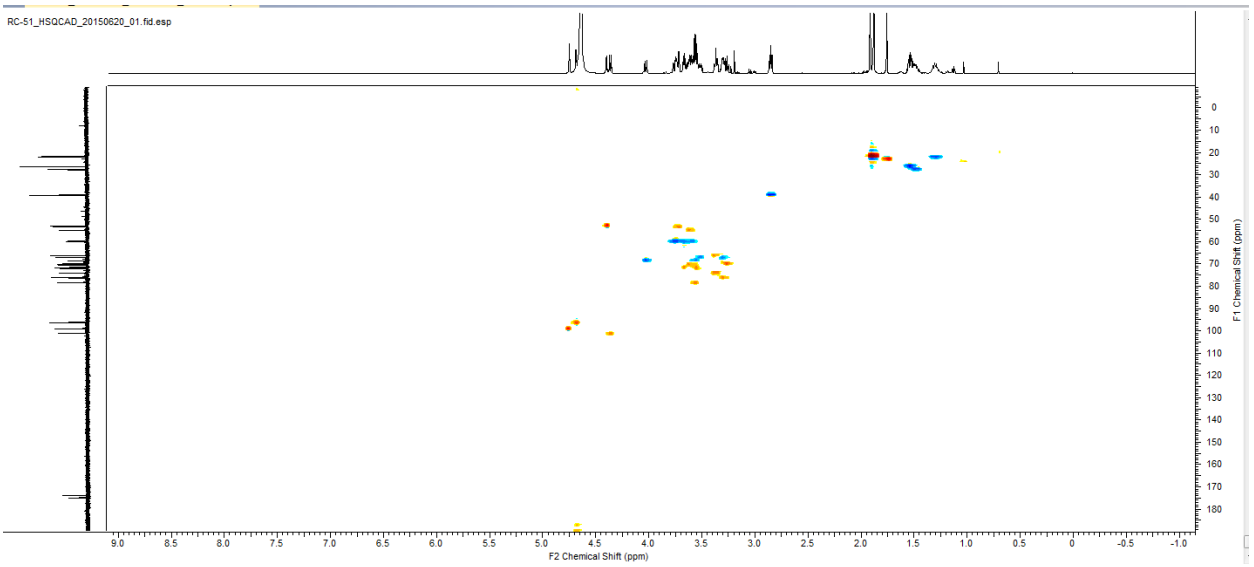
^{13}C NMR (150 MHz, D_2O)

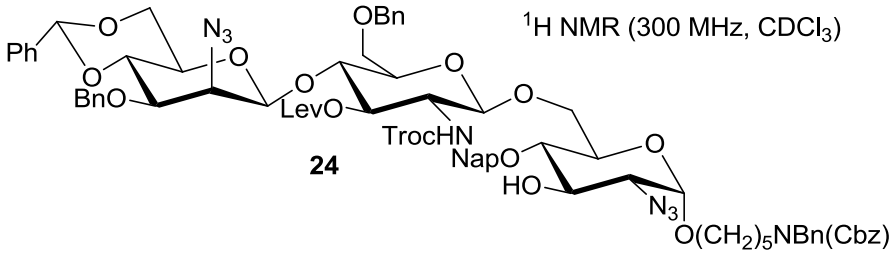


^{1}H-^{1}H COSY (600 MHz, D_2O)



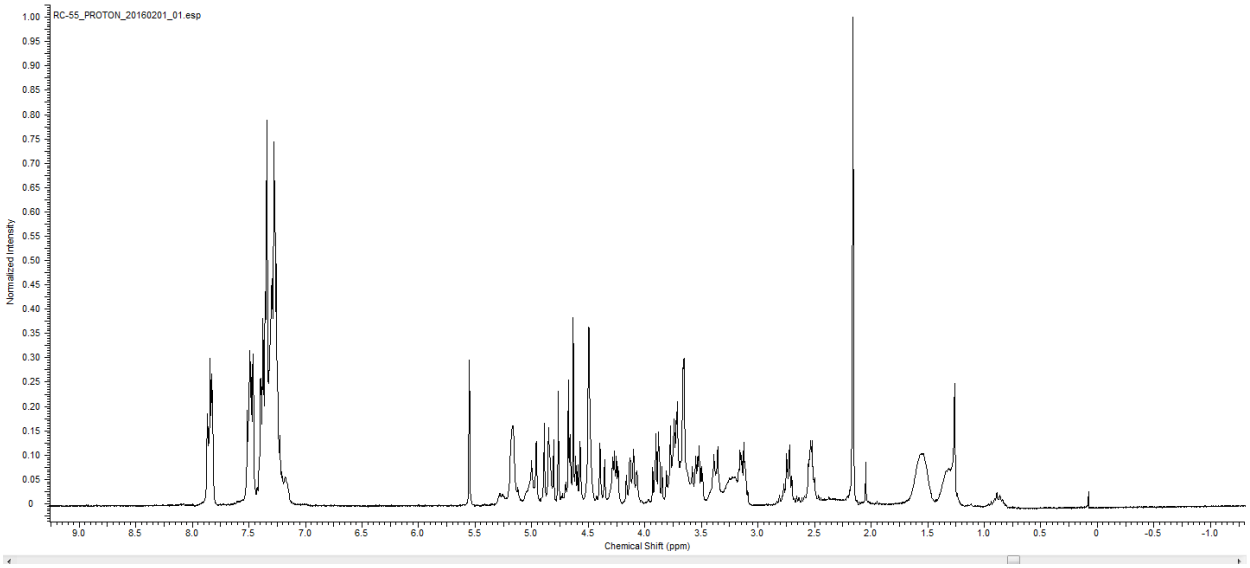
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



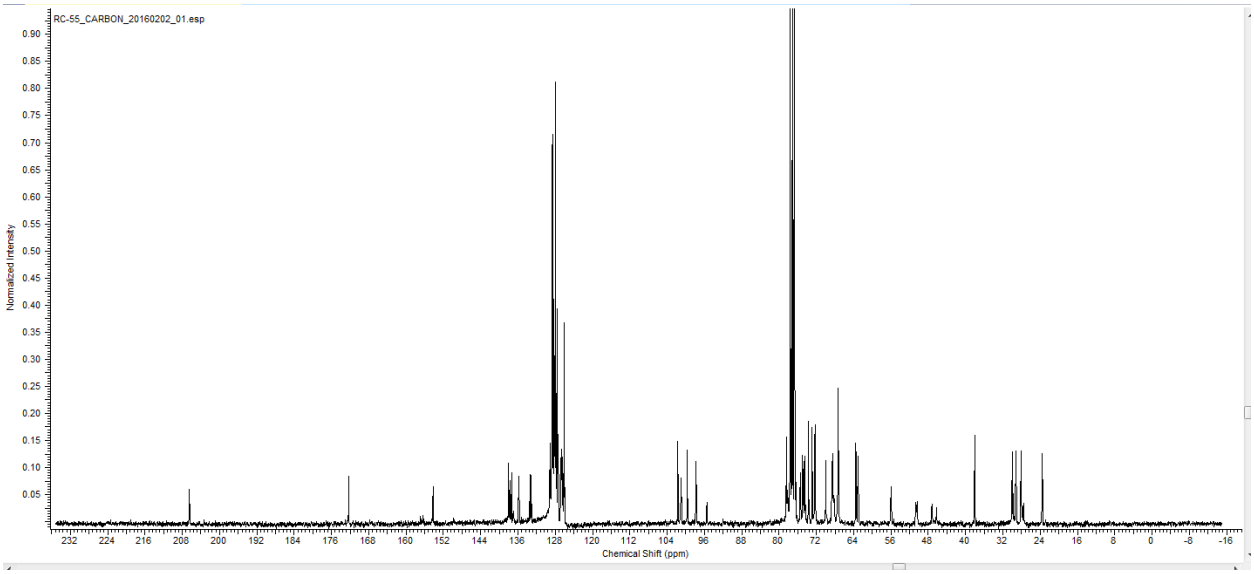


24

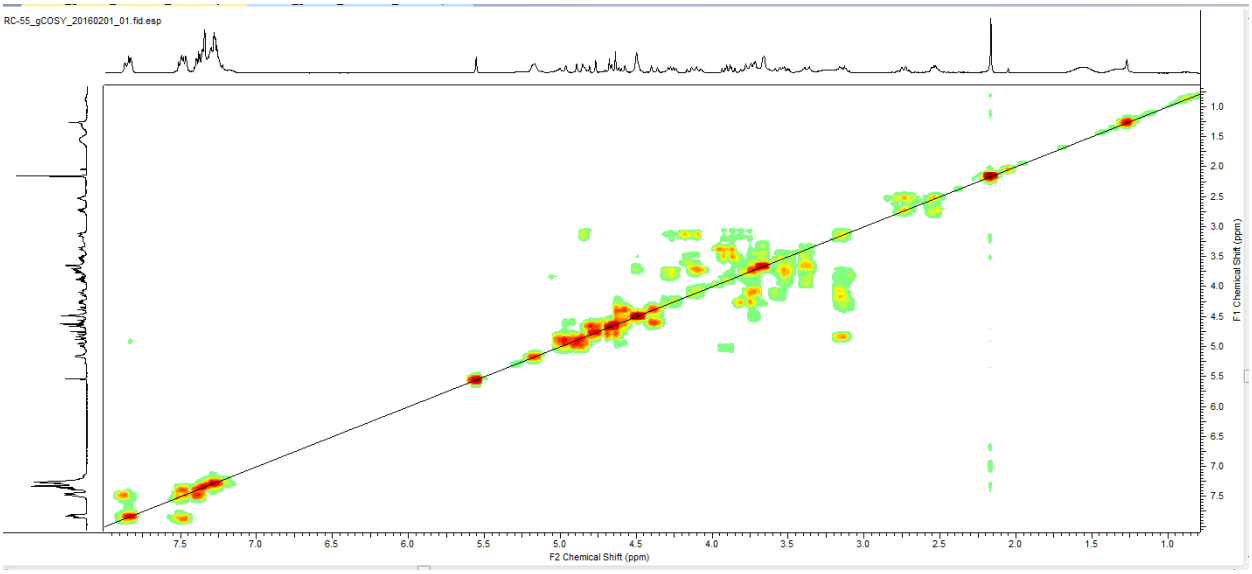
^{1}H NMR (300 MHz, $CDCl_3$)



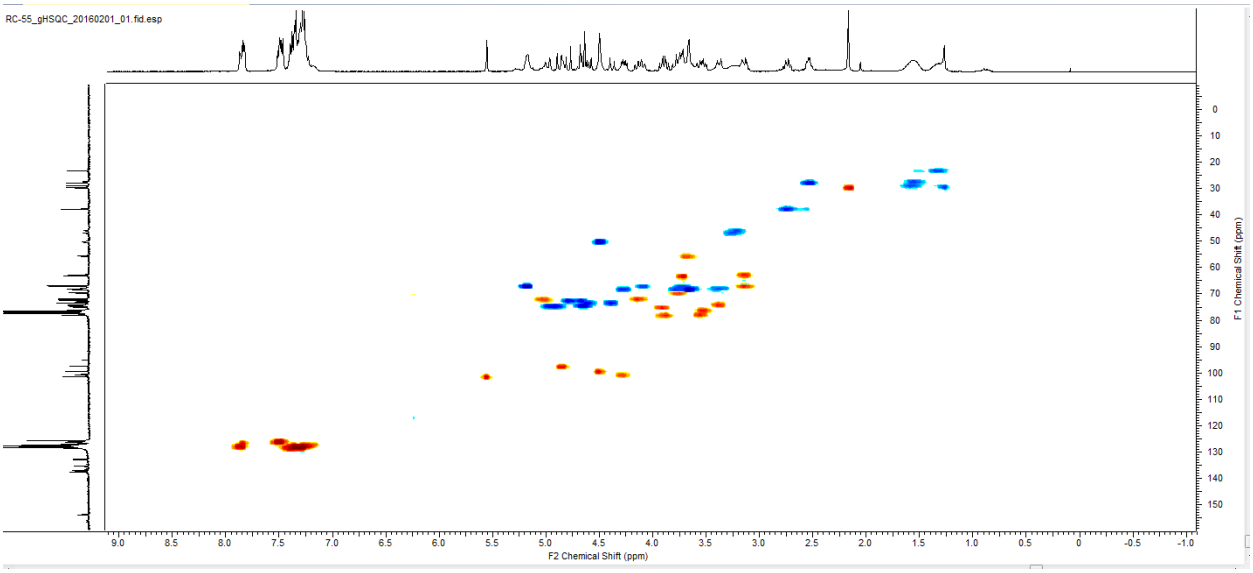
^{13}C NMR (75 MHz, $CDCl_3$)

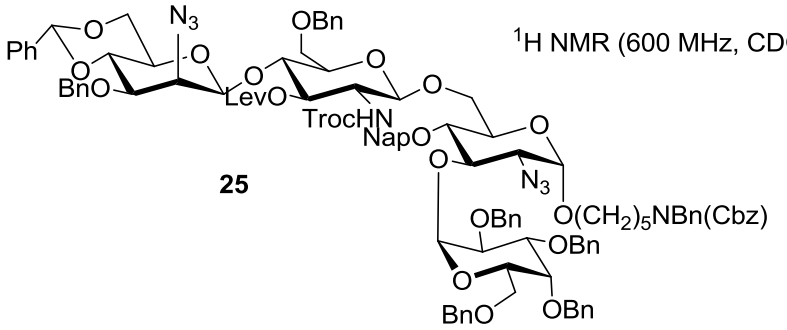


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



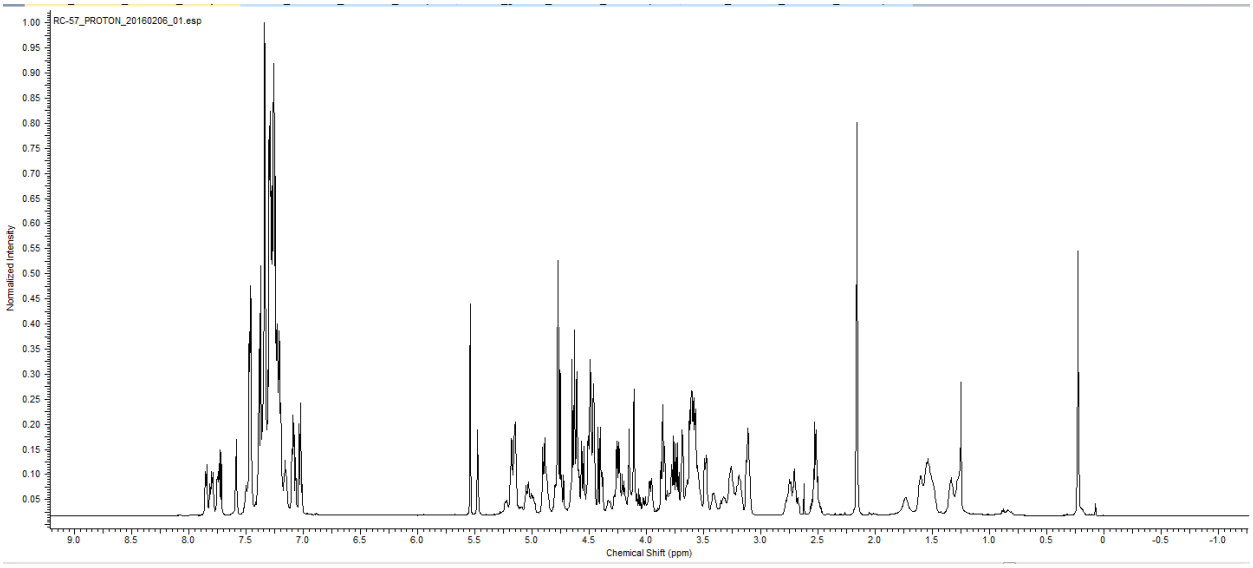
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



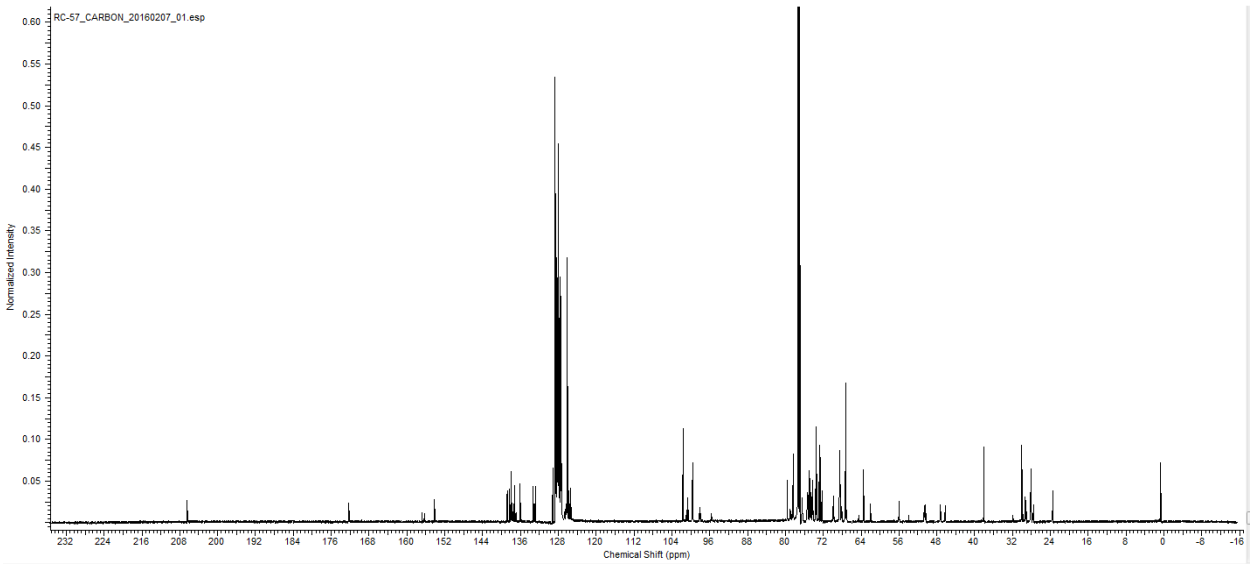


25

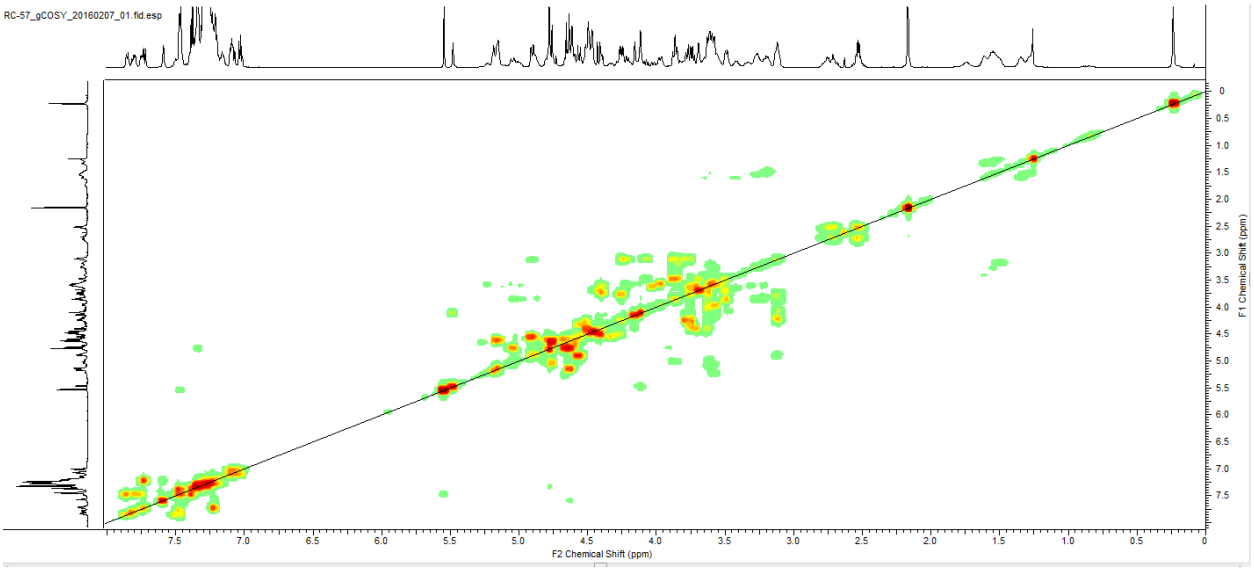
^{1}H NMR (600 MHz, $CDCl_3$)



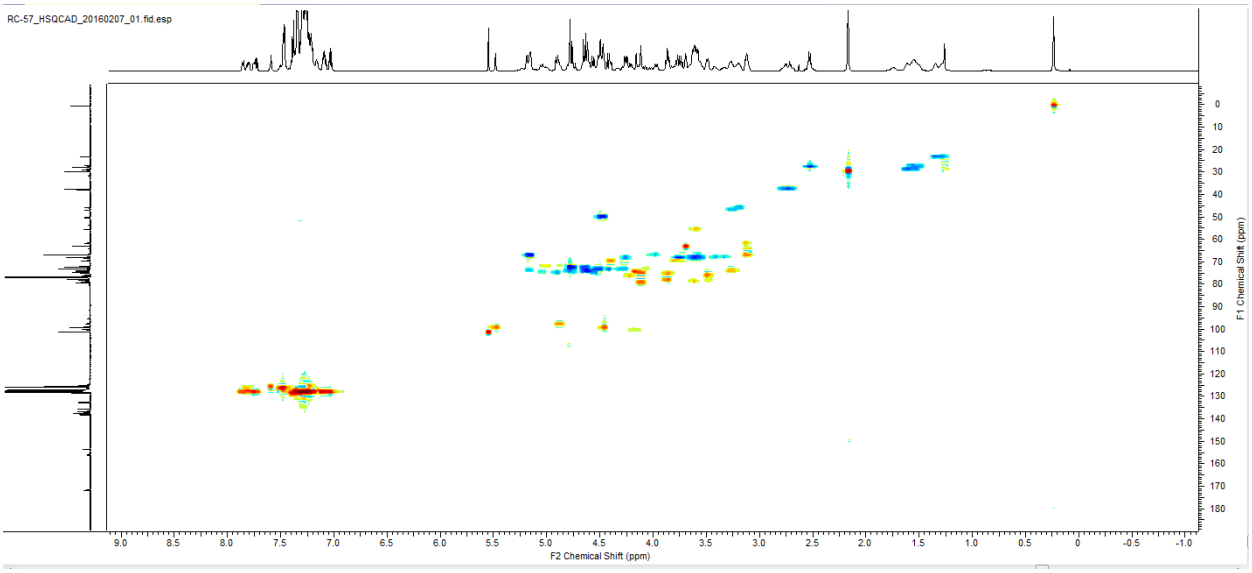
^{13}C NMR (150 MHz, $CDCl_3$)

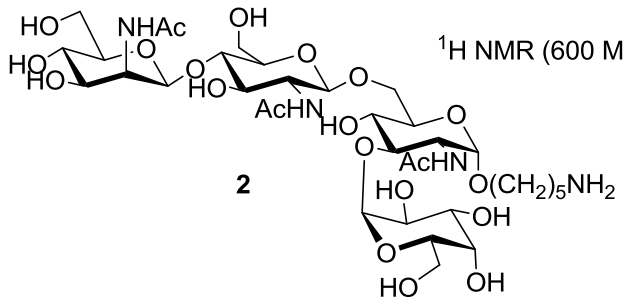


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



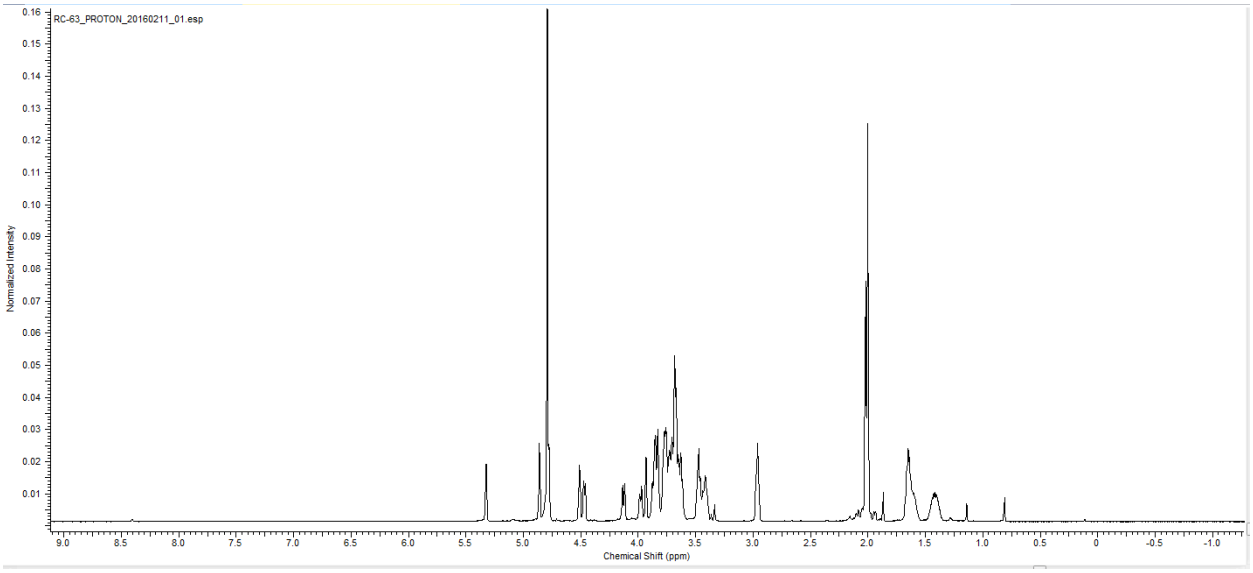
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



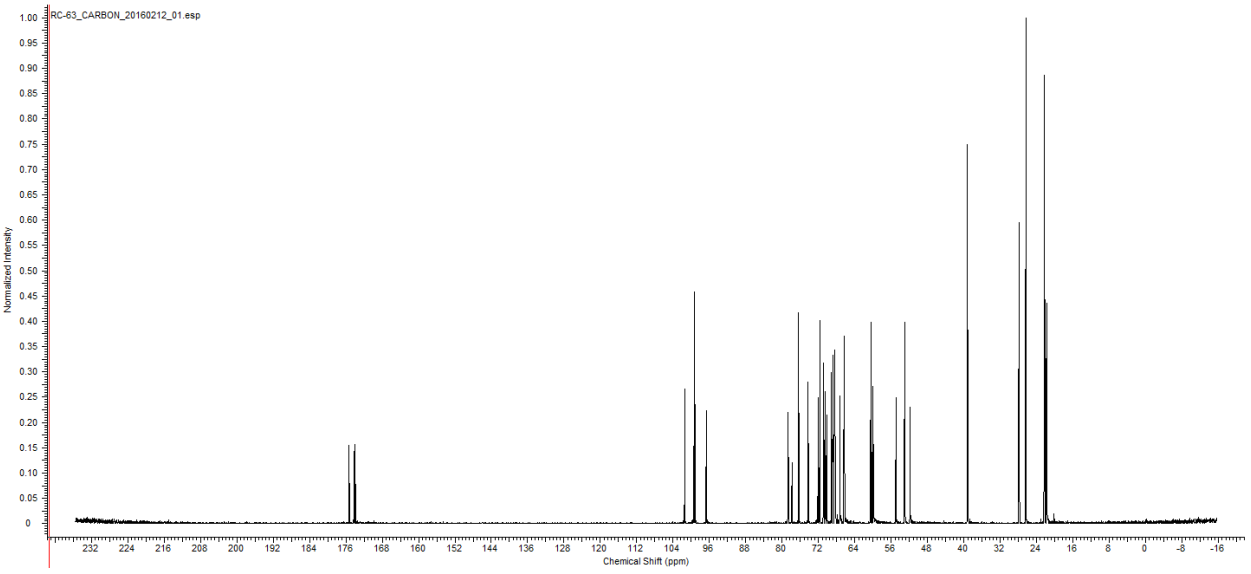


2

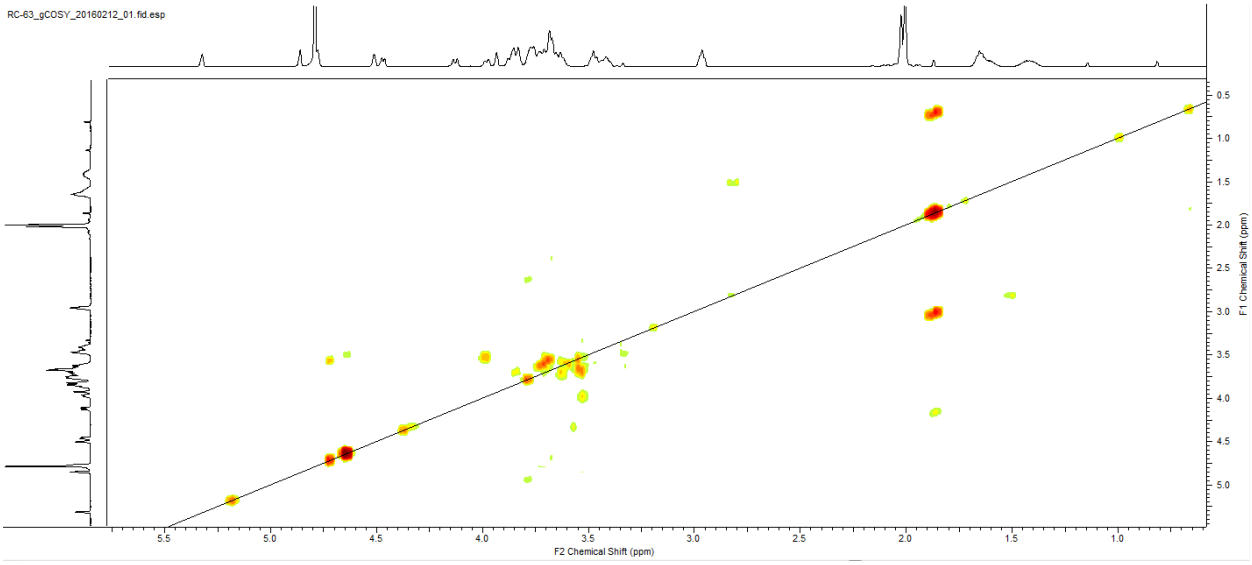
^{1}H NMR (600 MHz, D_2O)



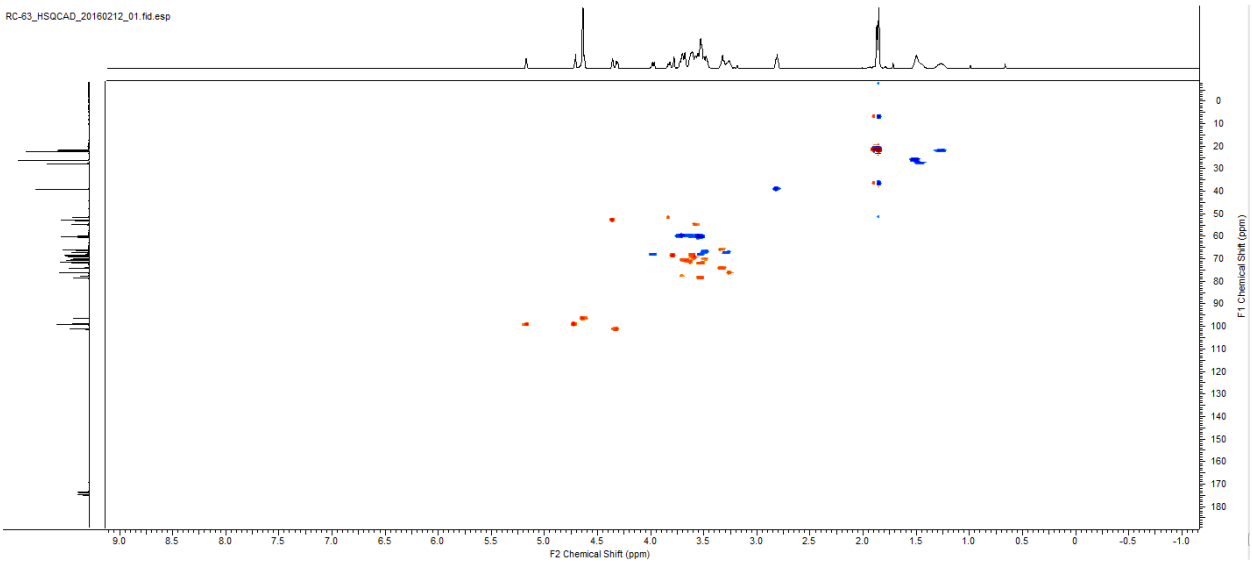
^{13}C NMR (150 MHz, D_2O)

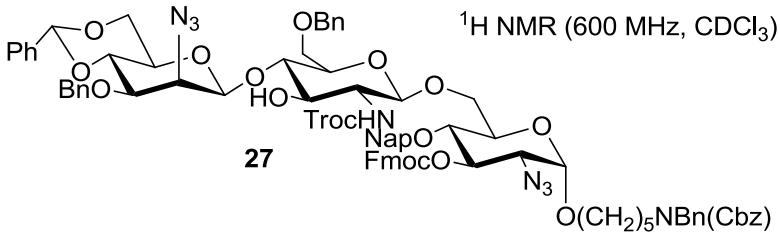


^{1}H-^{1}H COSY (600 MHz, D_2O)



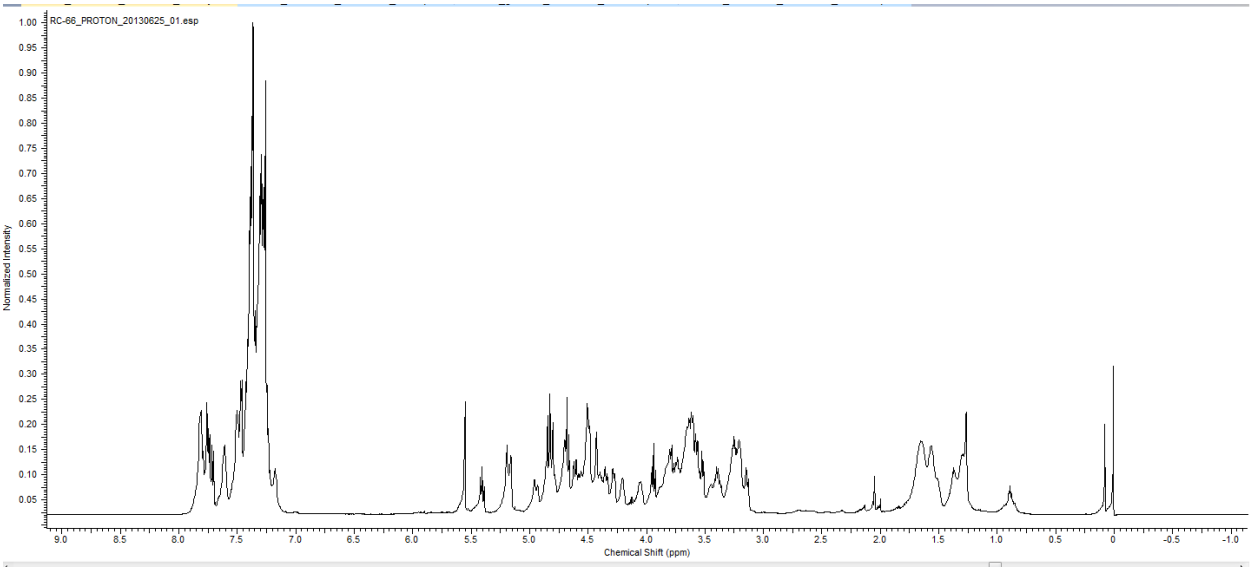
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



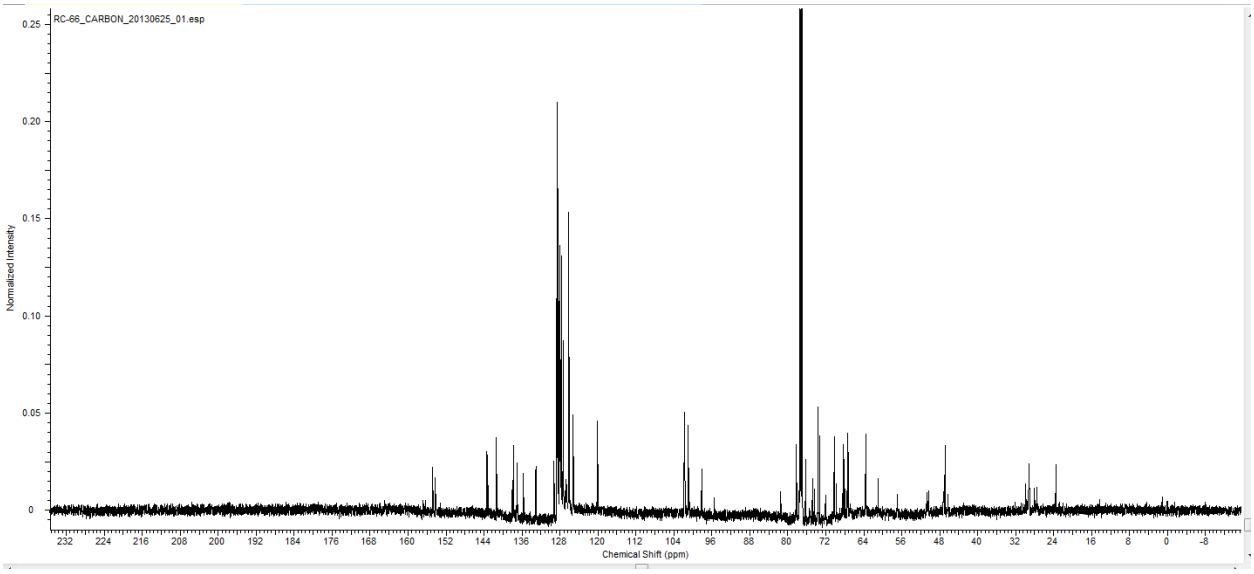


27

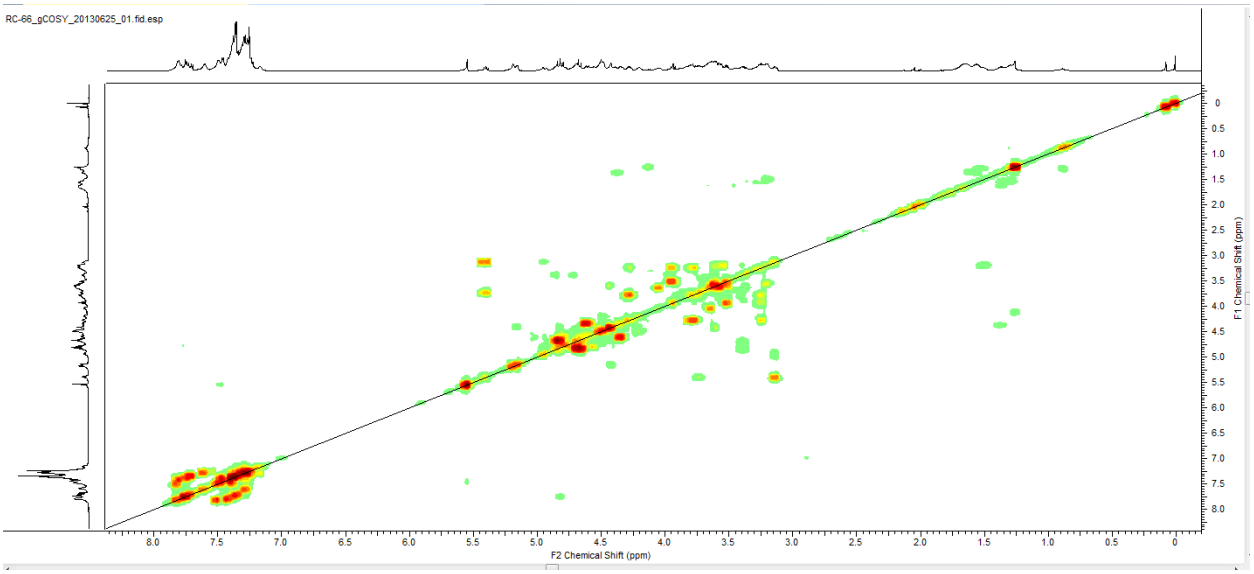
^{1}H NMR (600 MHz, $CDCl_3$)



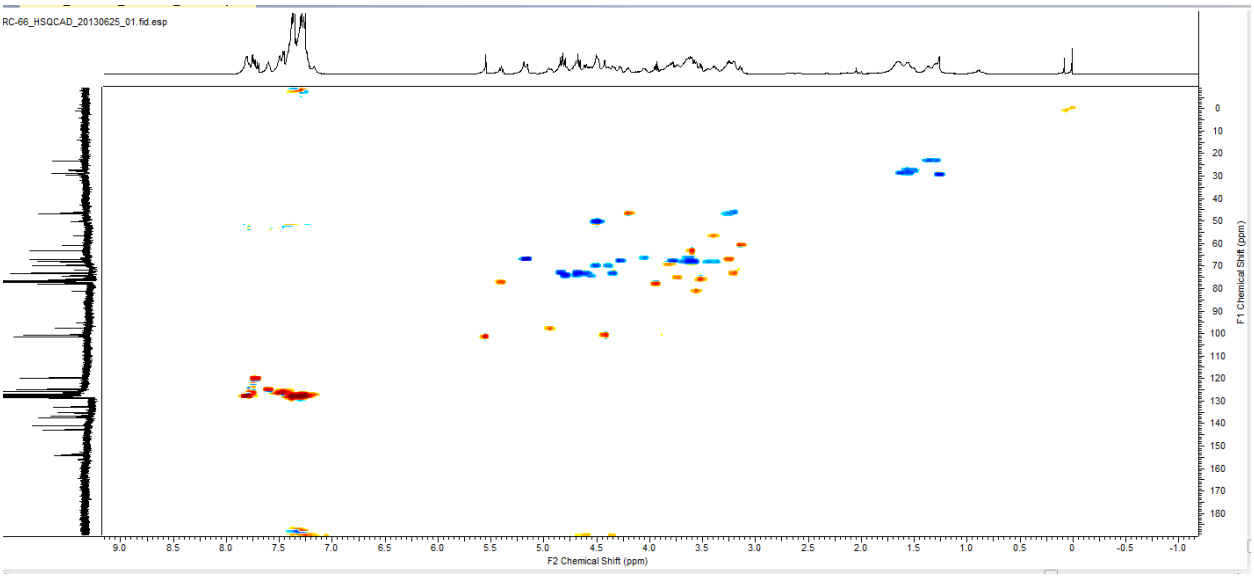
^{13}C NMR (150 MHz, $CDCl_3$)

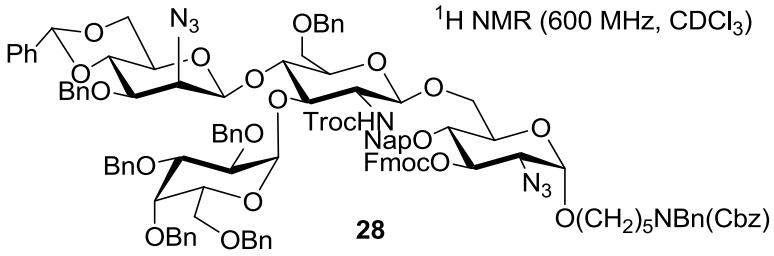


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



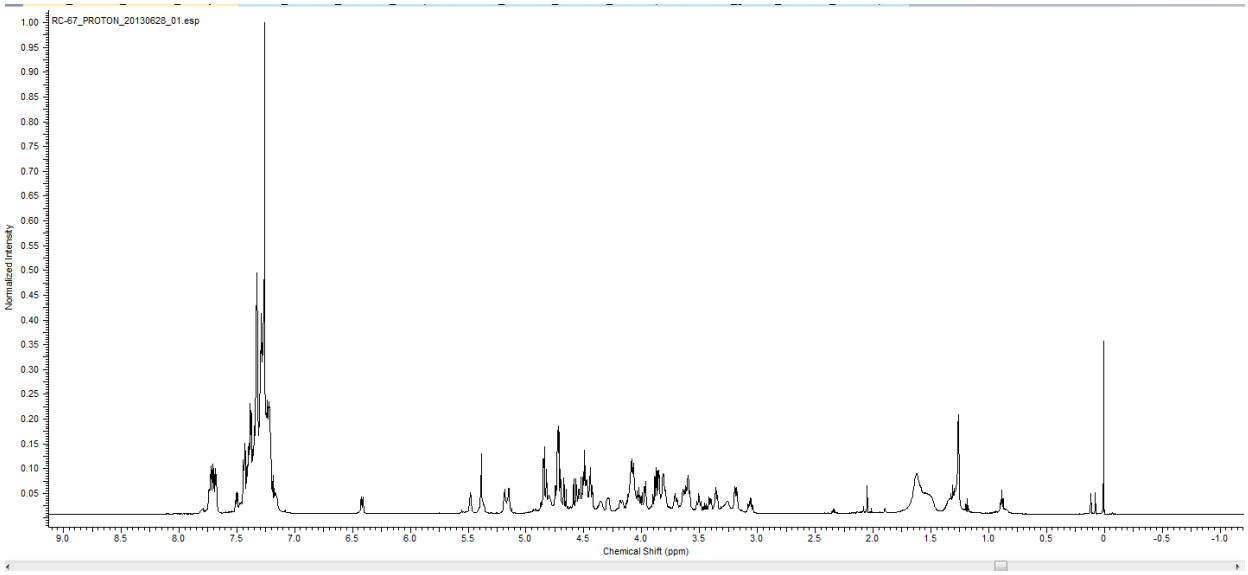
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



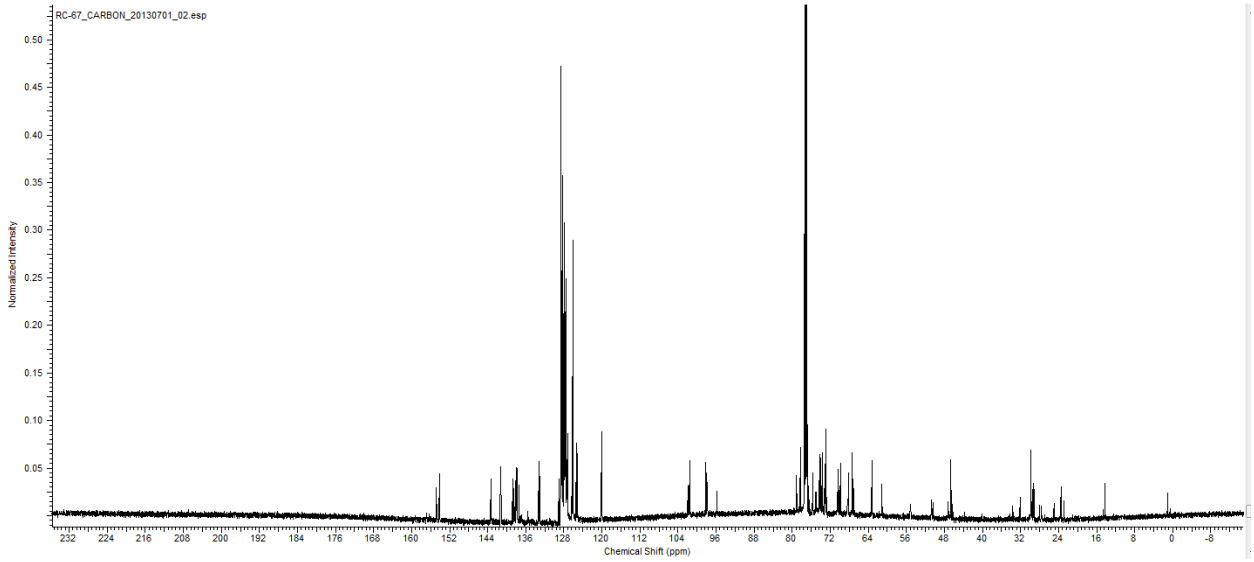


28

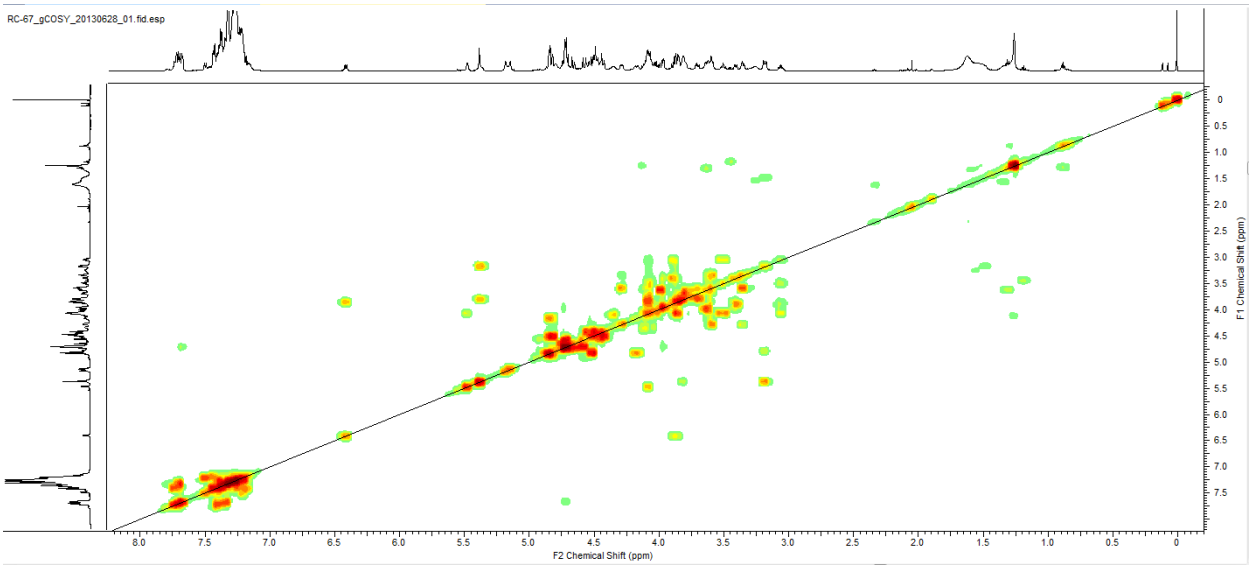
^{1}H NMR (600 MHz, $CDCl_3$)



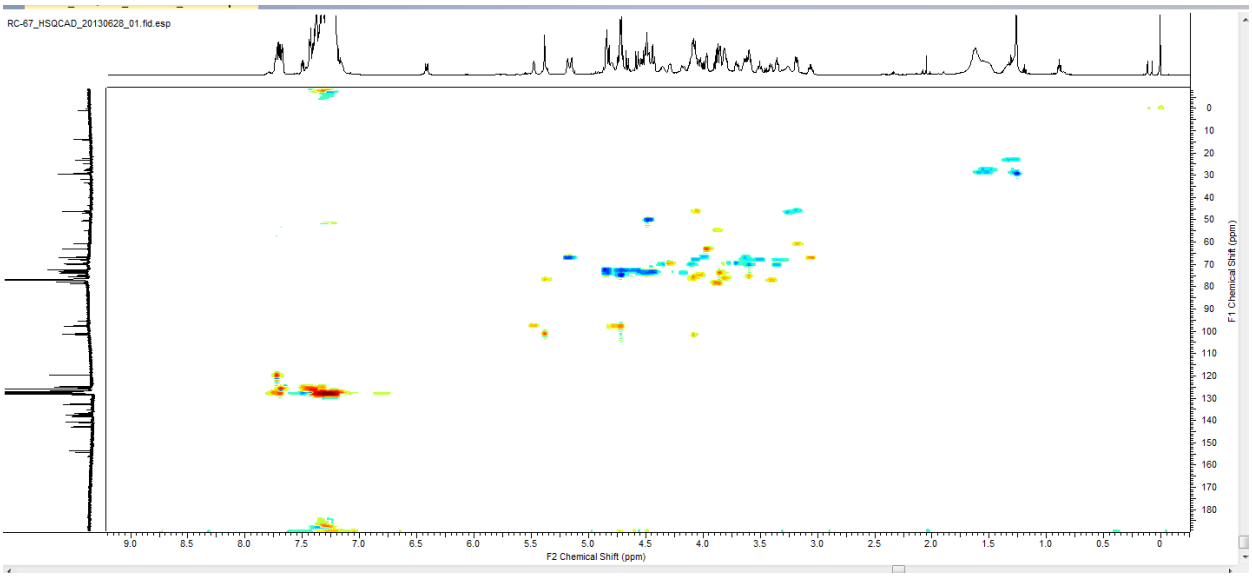
^{13}C NMR (150 MHz, $CDCl_3$)

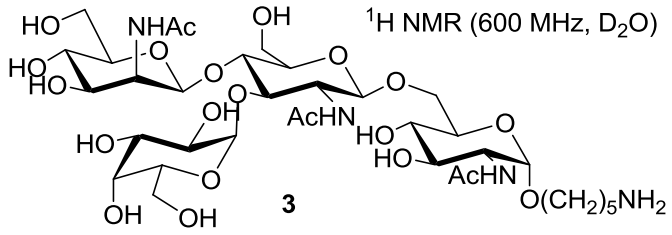


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



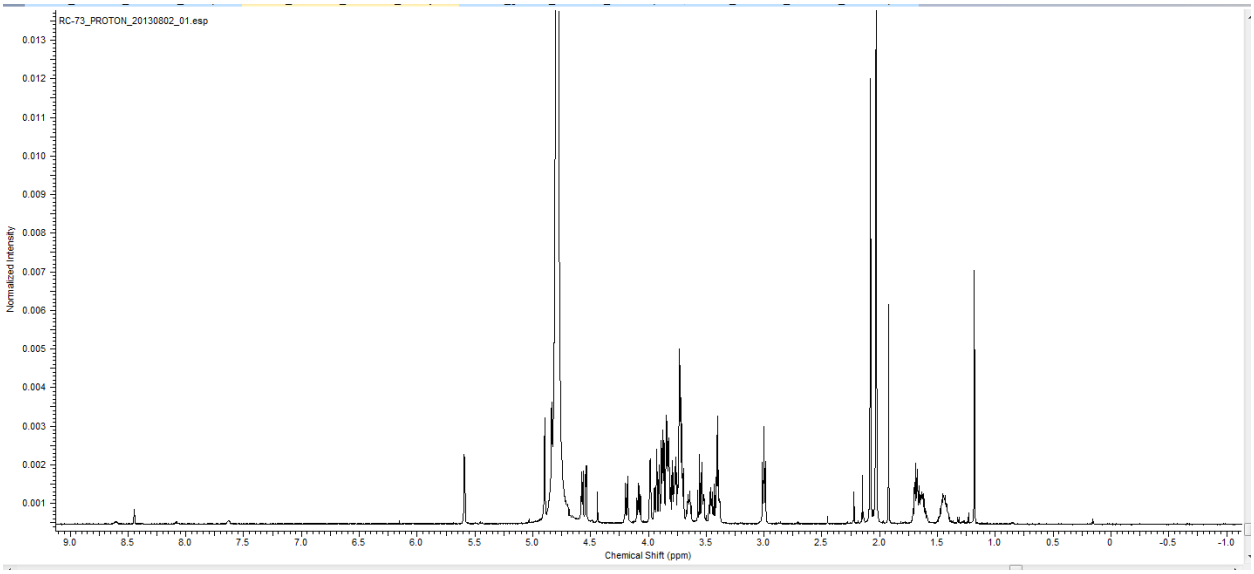
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



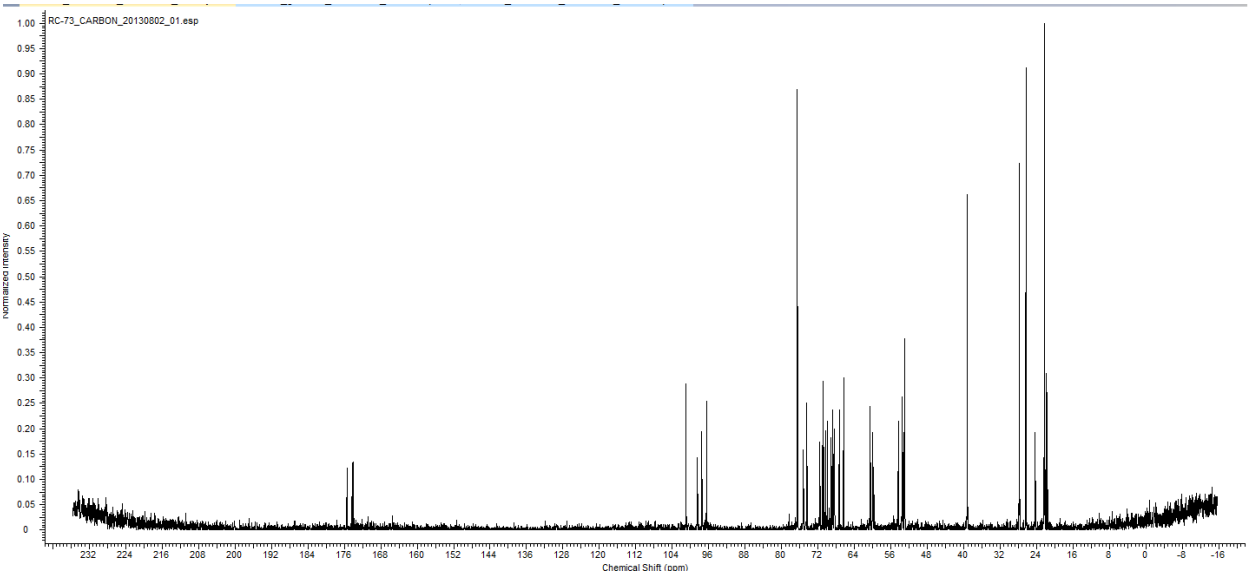


3

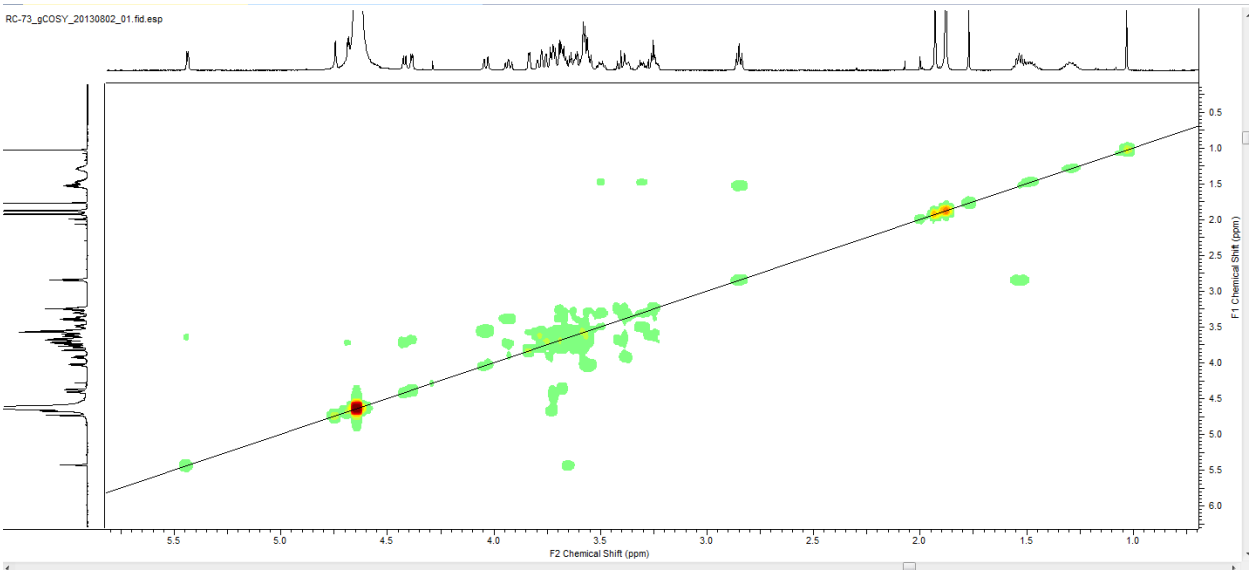
^{1}H NMR (600 MHz, D_2O)



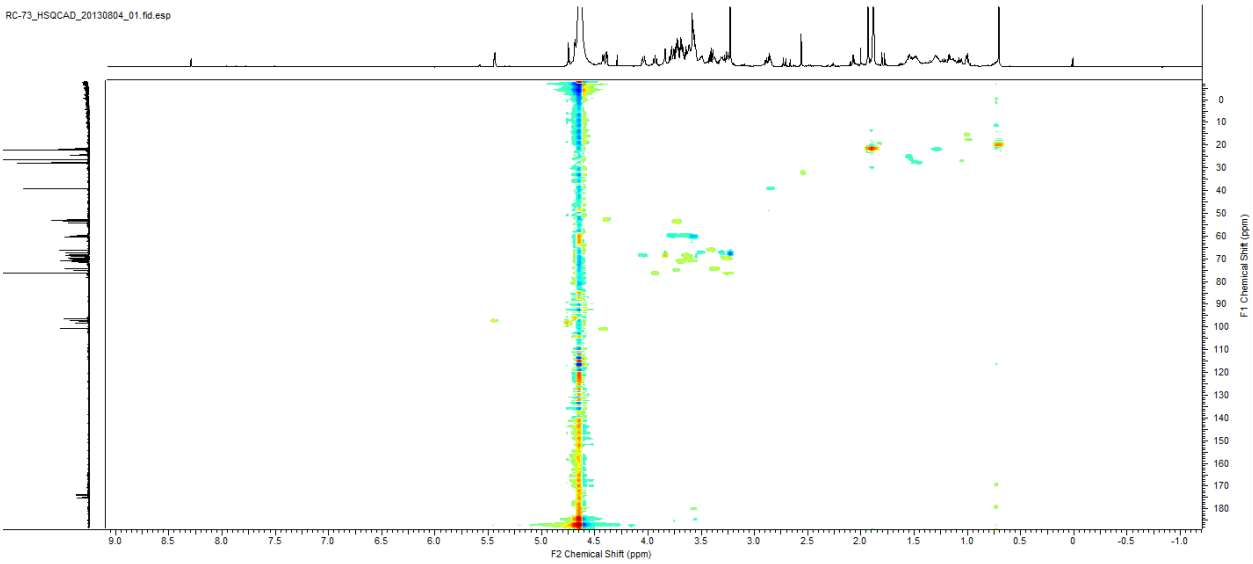
^{13}C NMR (150 MHz, D_2O)

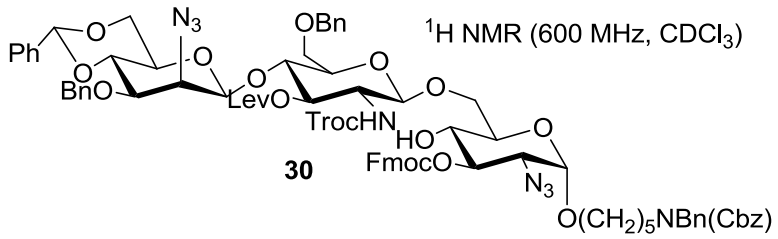


^{1}H-^{1}H COSY (600 MHz, D_2O)



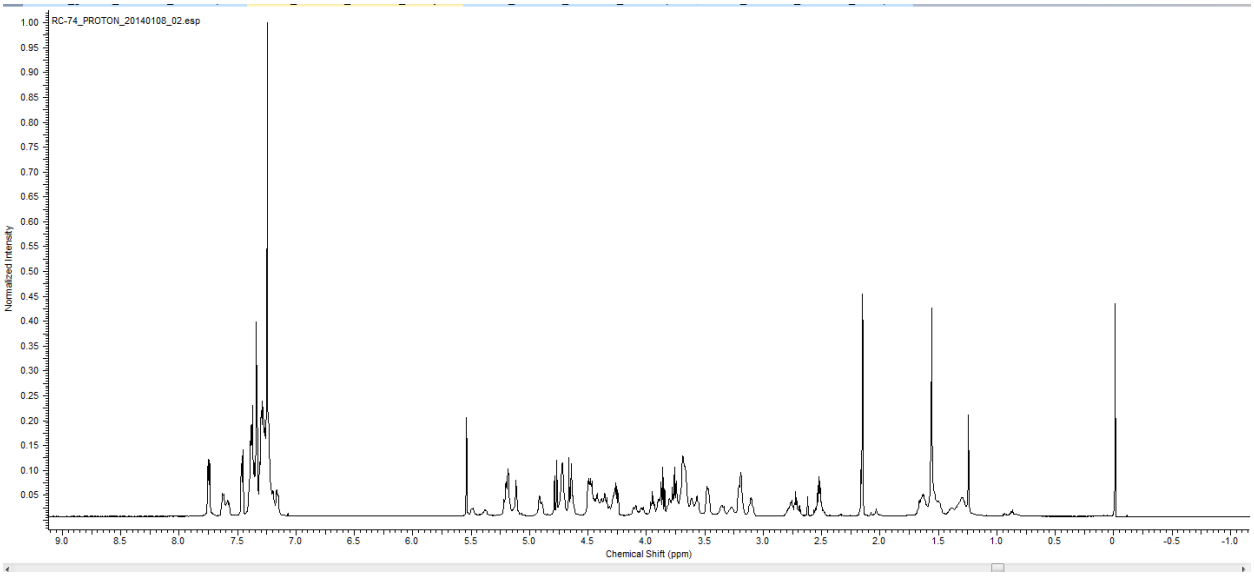
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



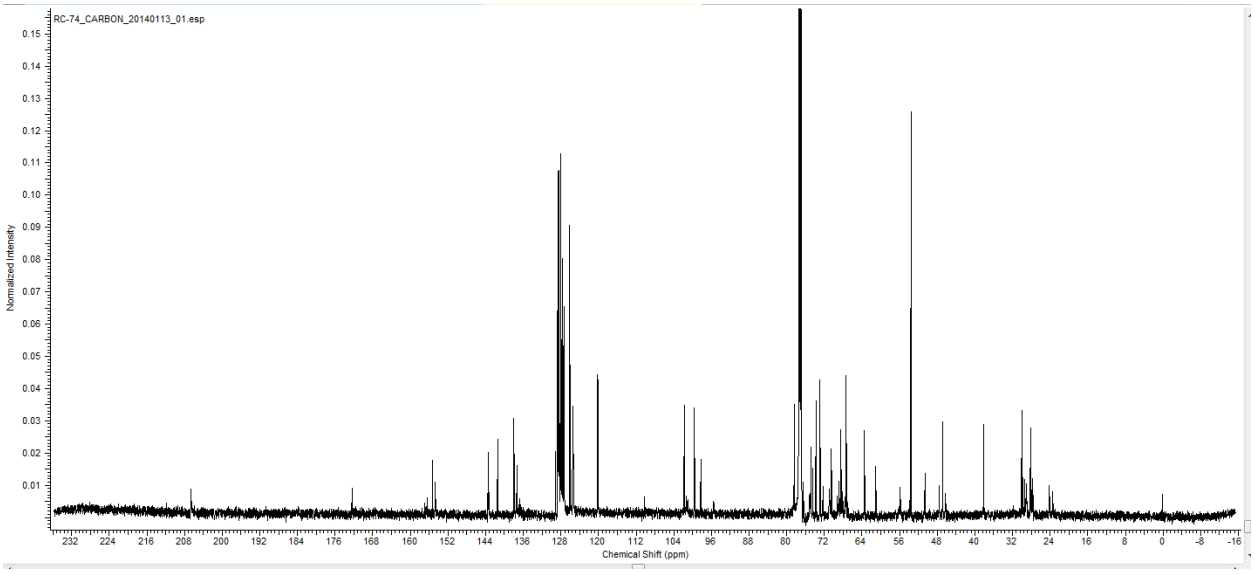


30

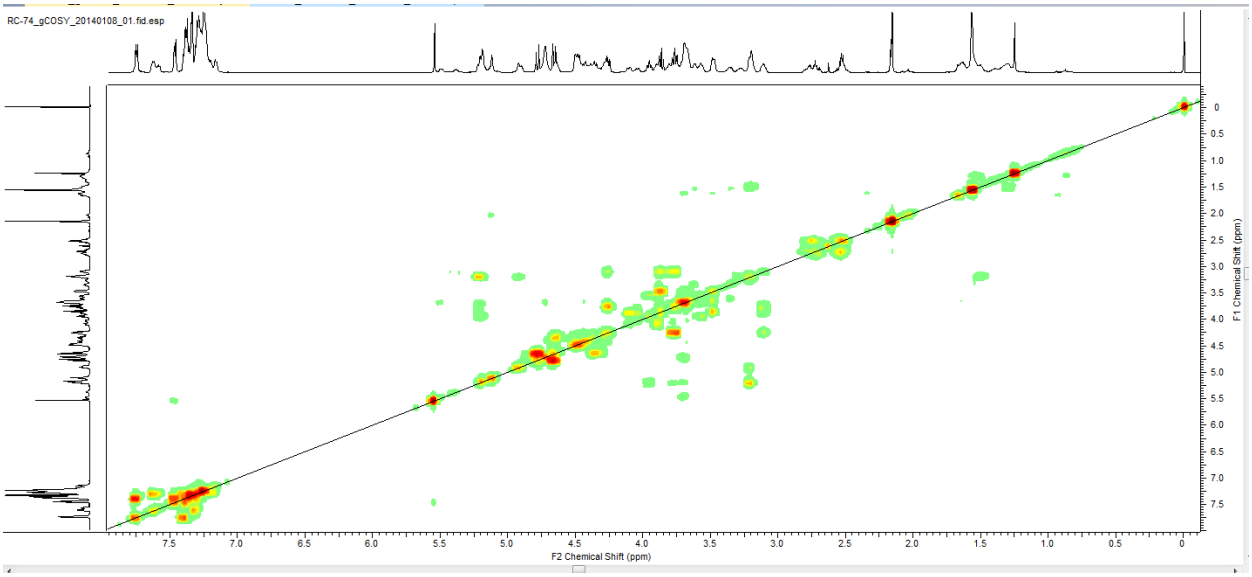
^{1}H NMR (600 MHz, $CDCl_3$)



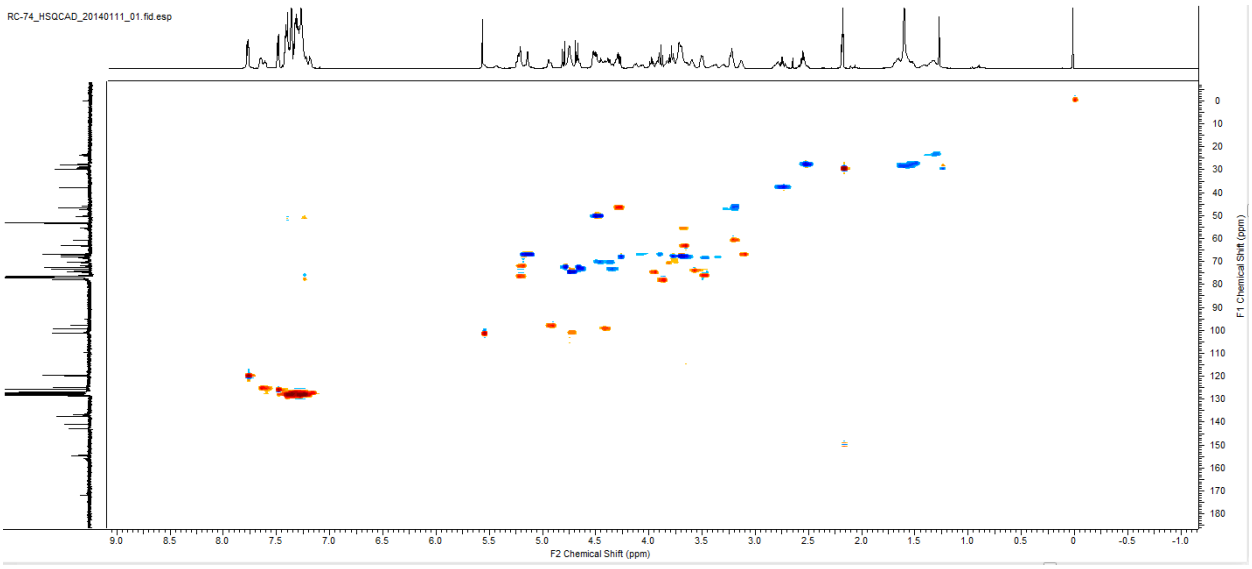
^{13}C NMR (150 MHz, $CDCl_3$)

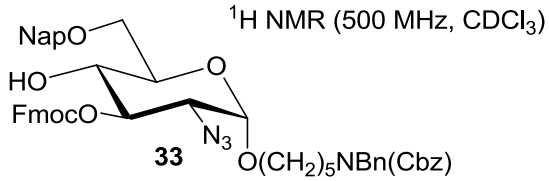


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



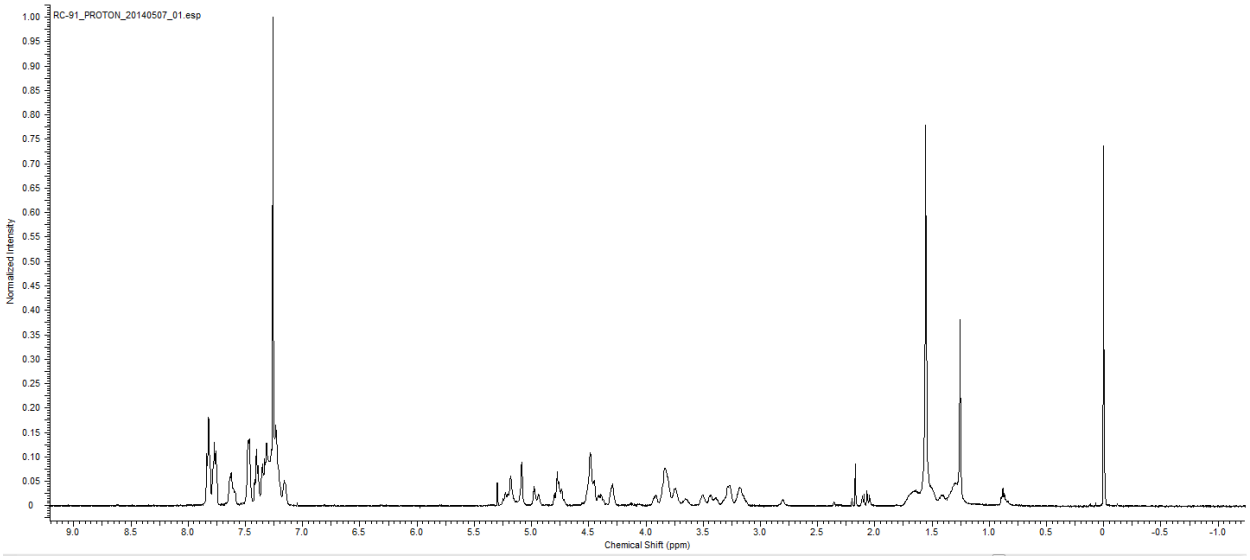
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



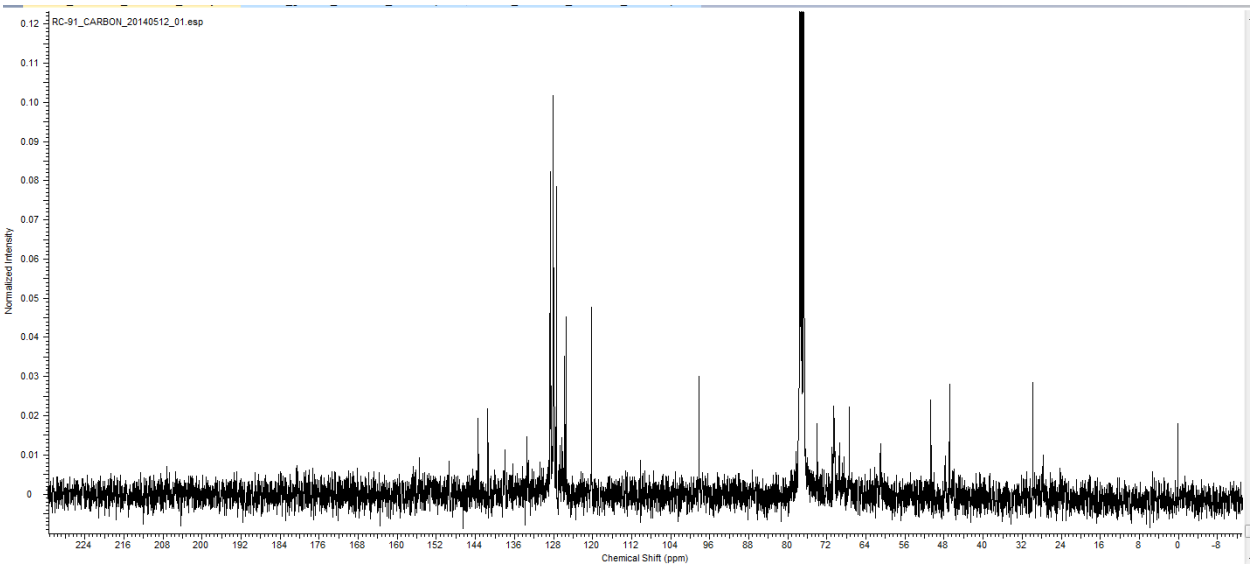


33

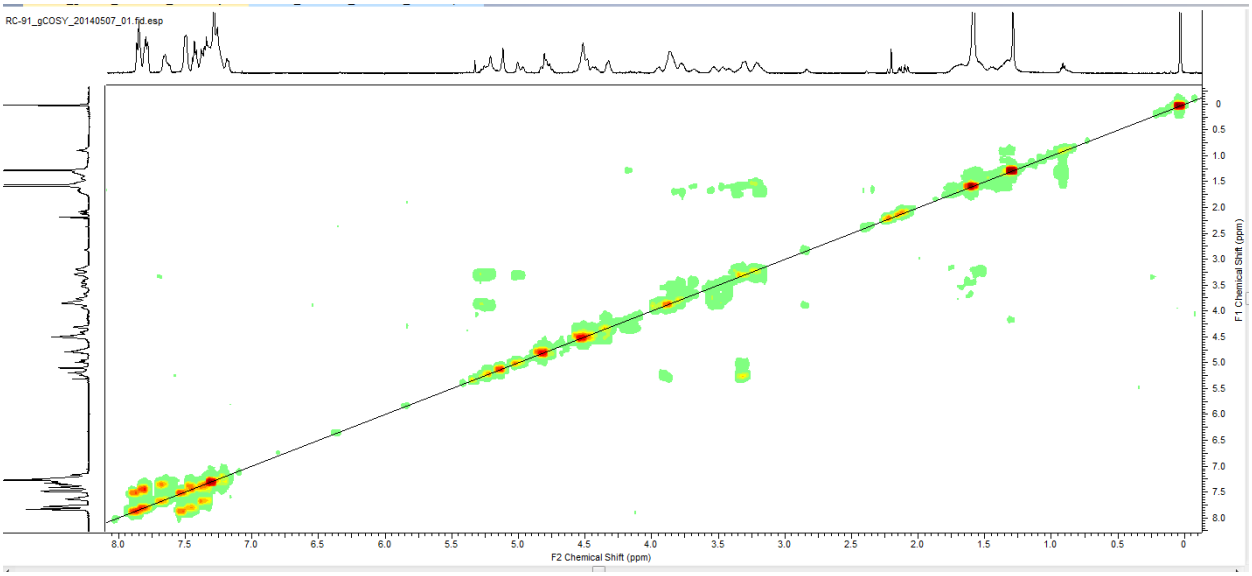
^{1}H NMR (500 MHz, $CDCl_3$)



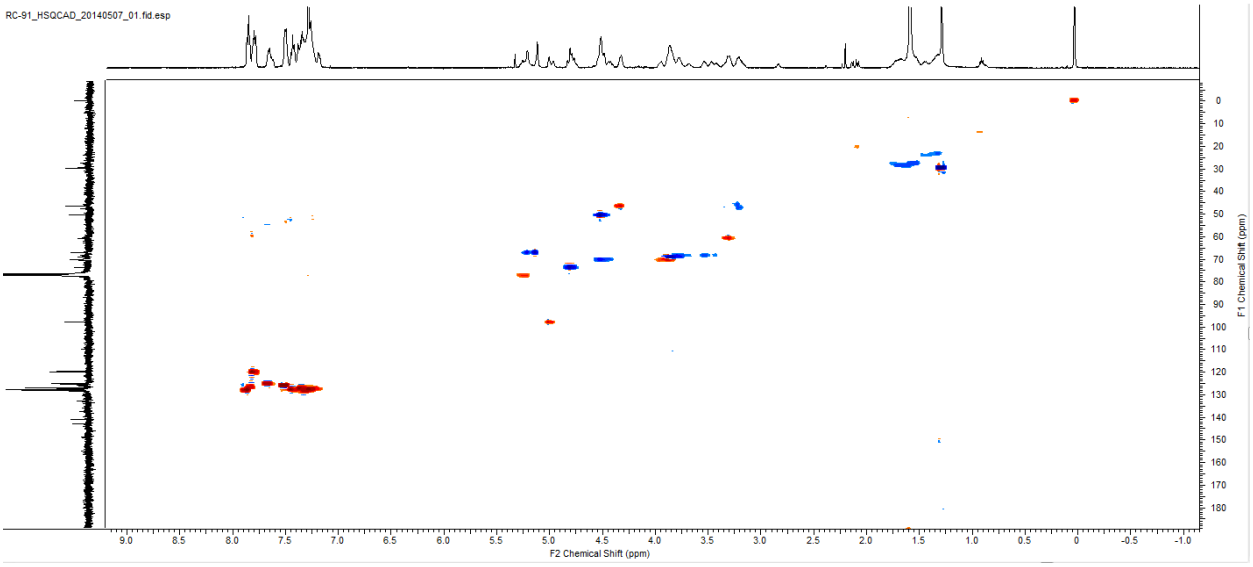
^{13}C NMR (75 MHz, $CDCl_3$)

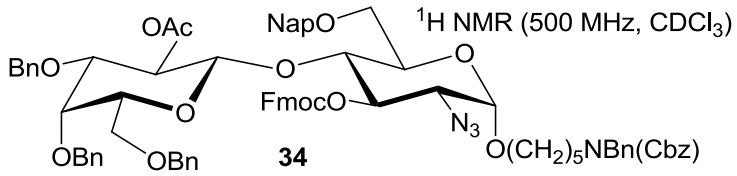


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



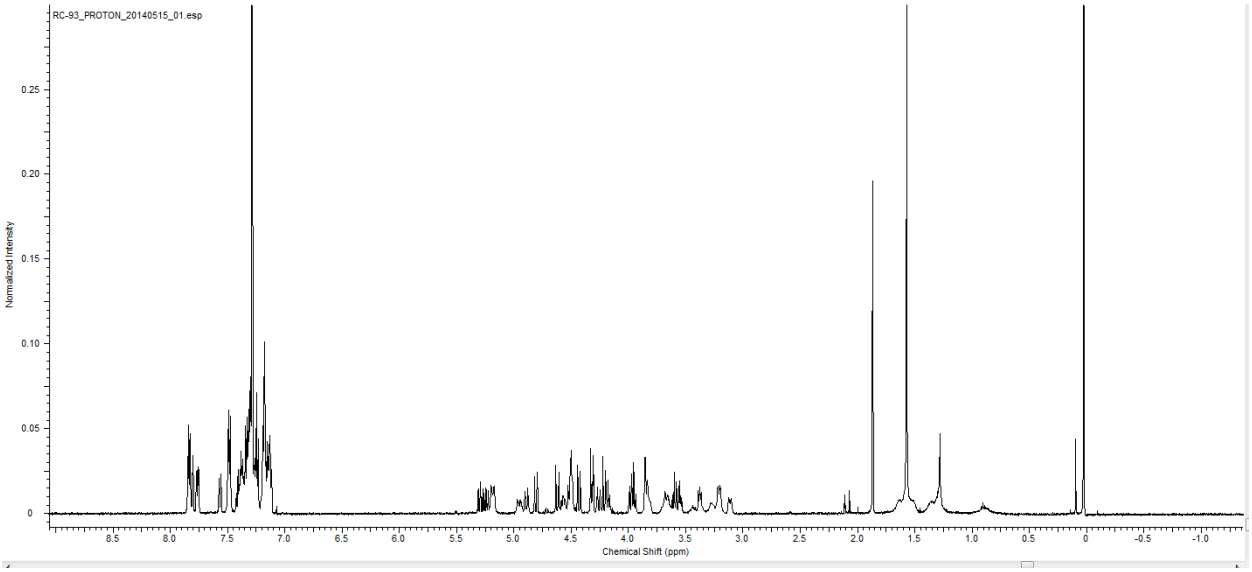
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)



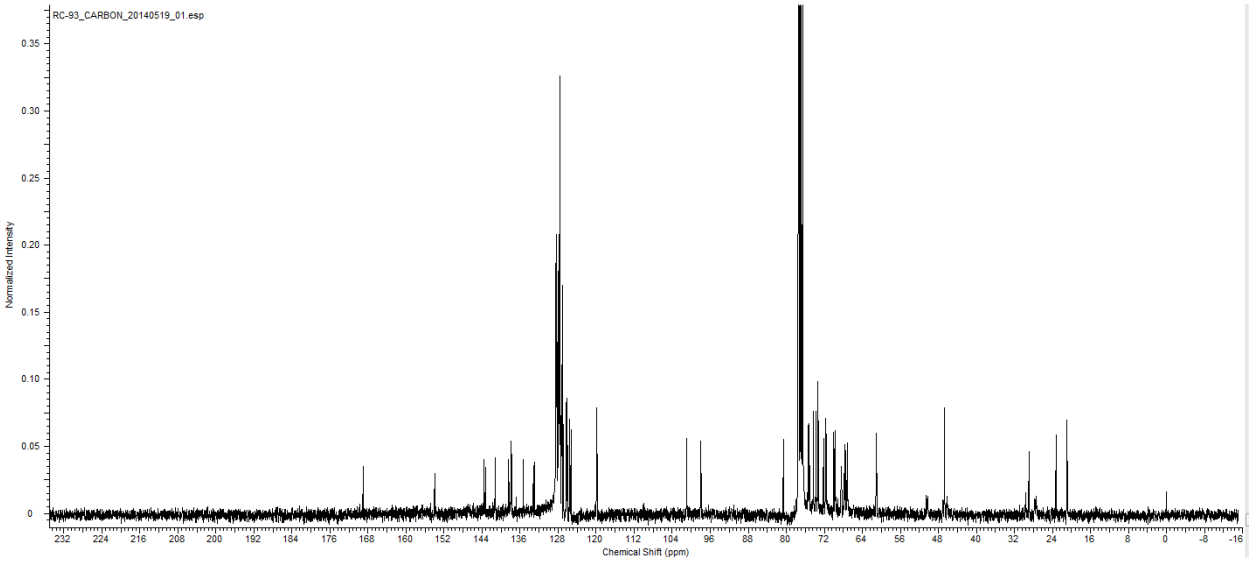


34

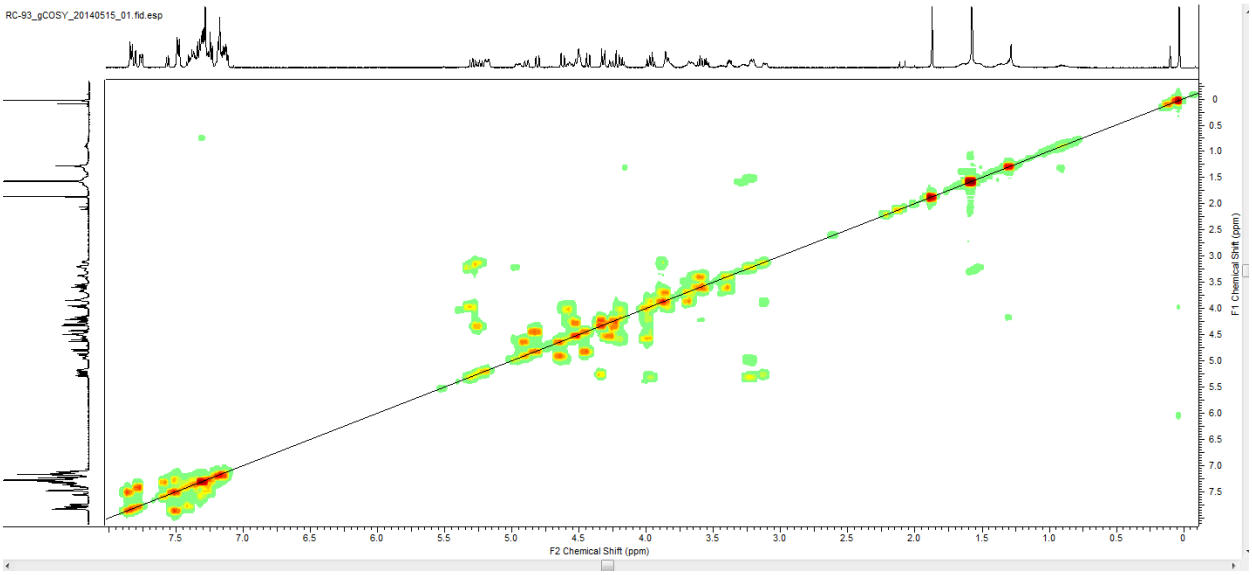
^{1}H NMR (500 MHz, $CDCl_3$)



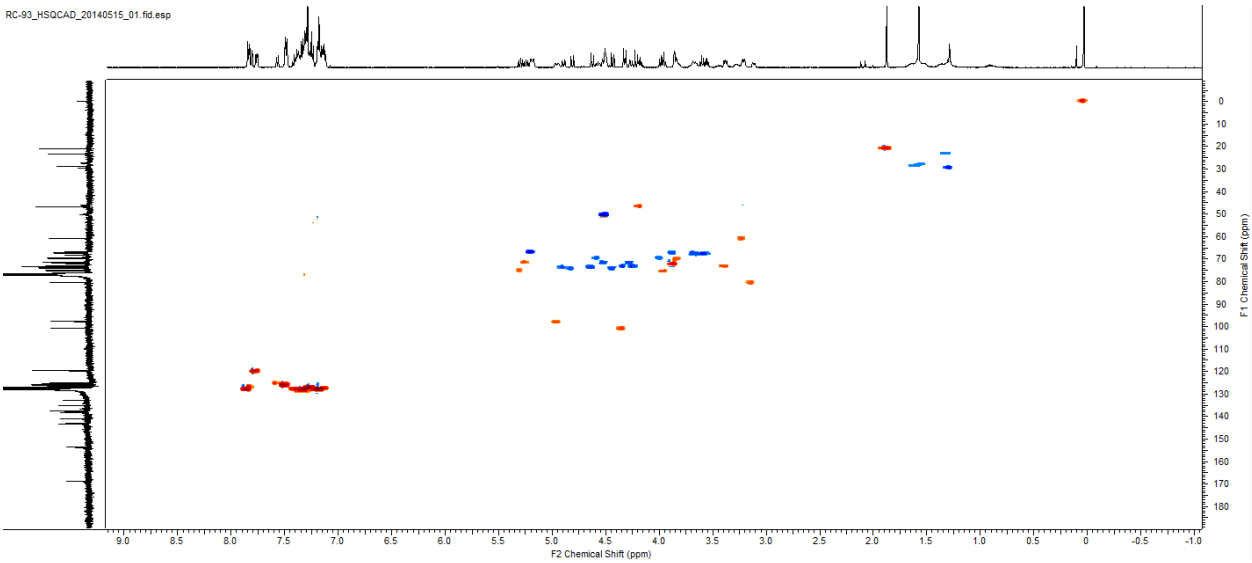
^{13}C NMR (75 MHz, $CDCl_3$)

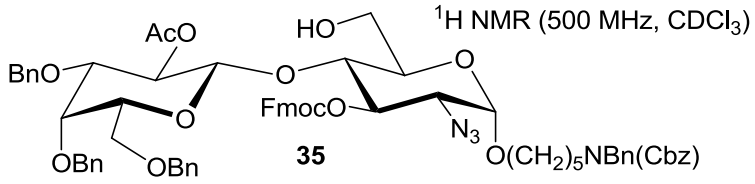


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



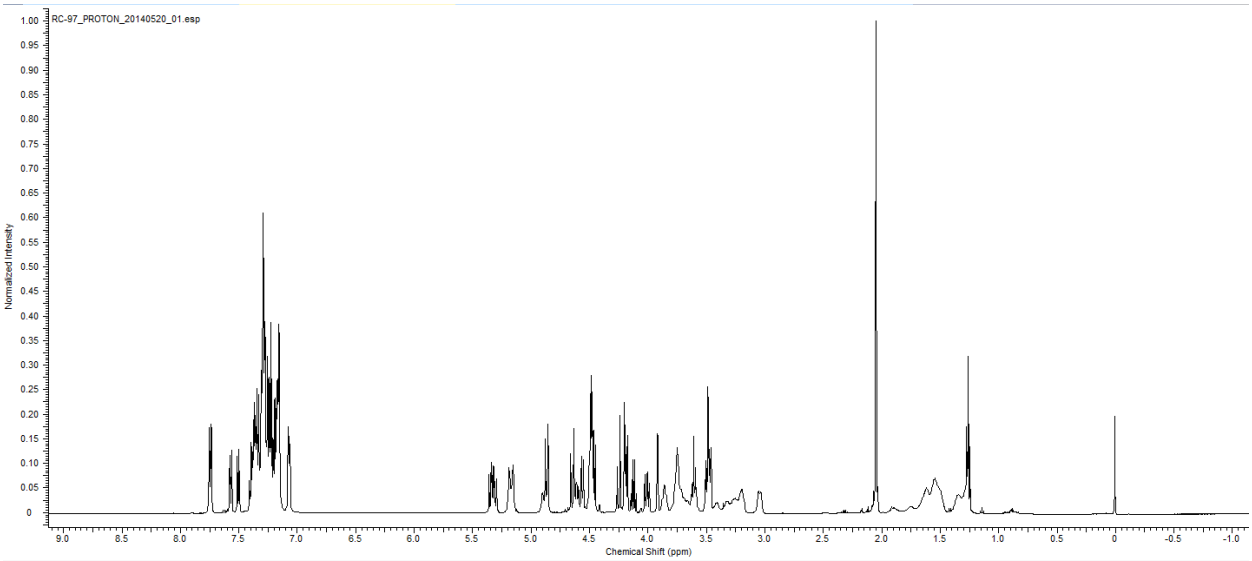
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)



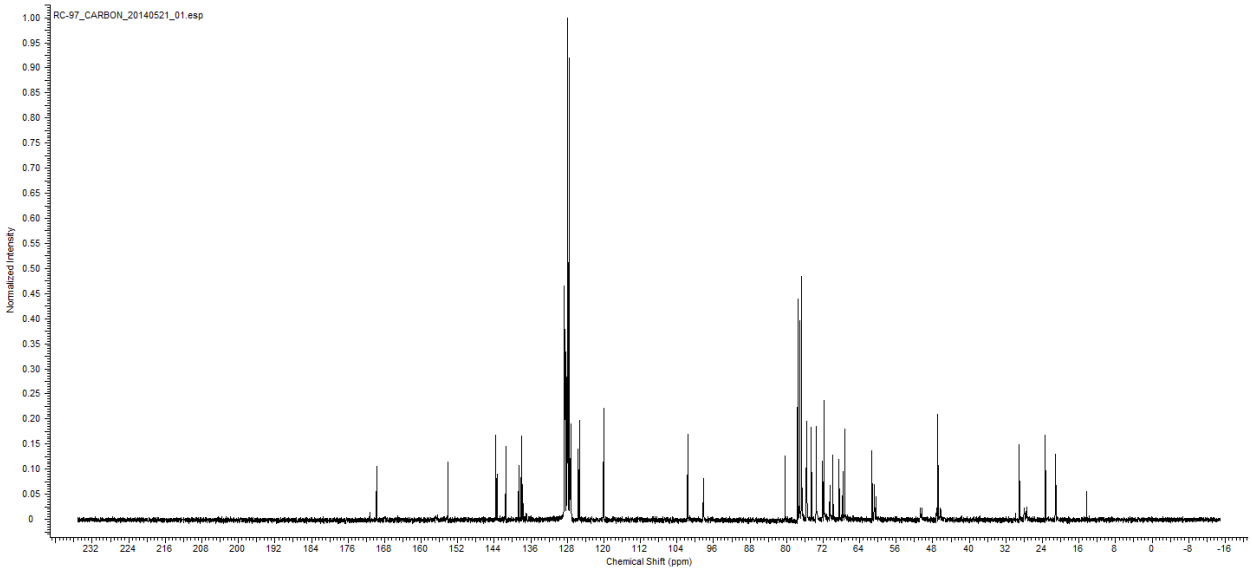


35

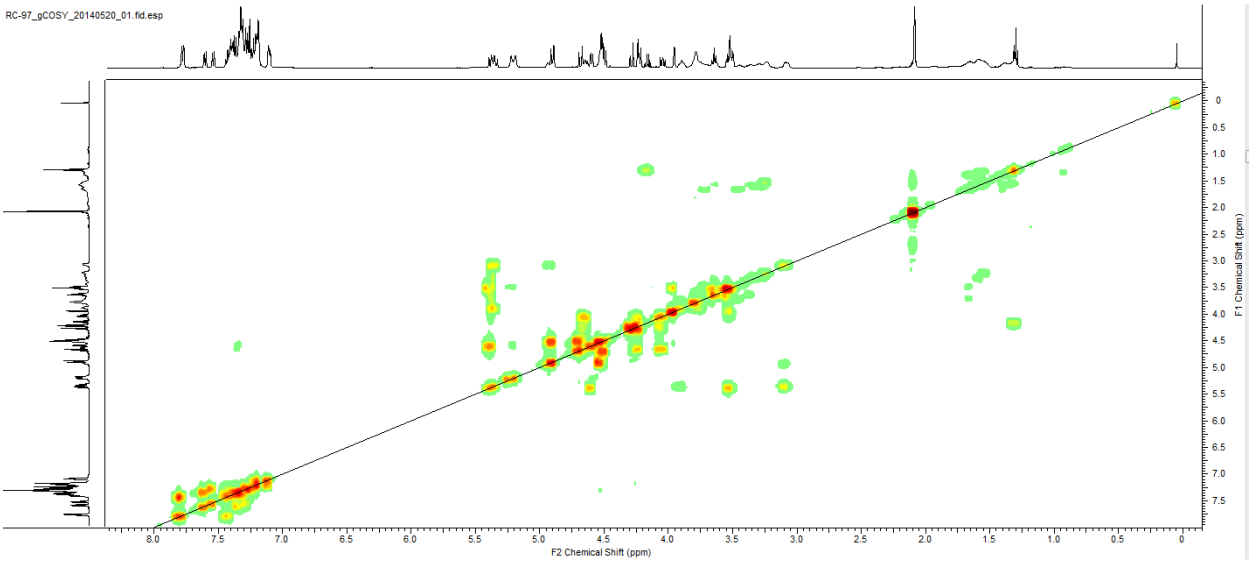
^{1}H NMR (500 MHz, $CDCl_3$)



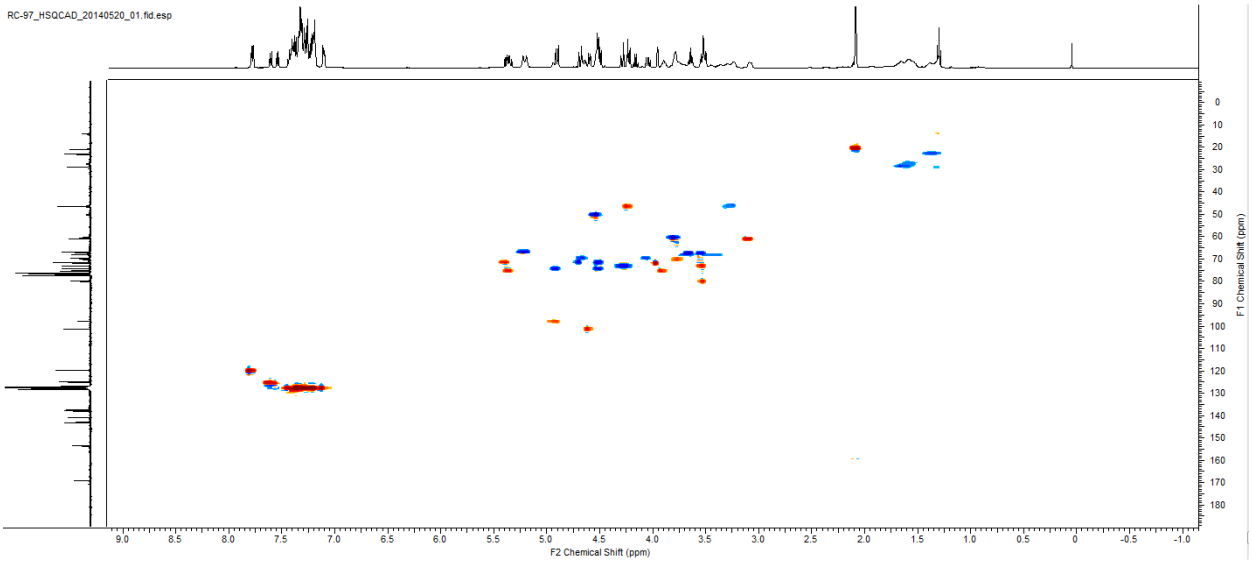
^{13}C NMR (75 MHz, $CDCl_3$)

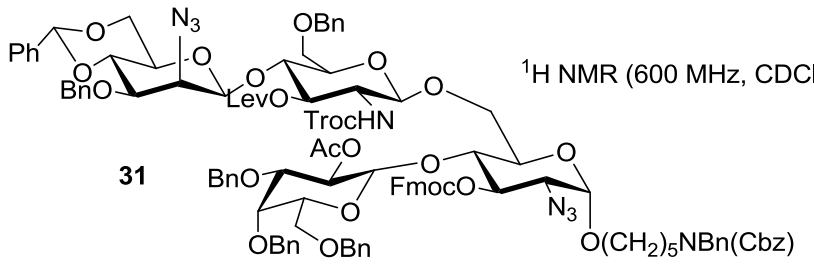


^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)

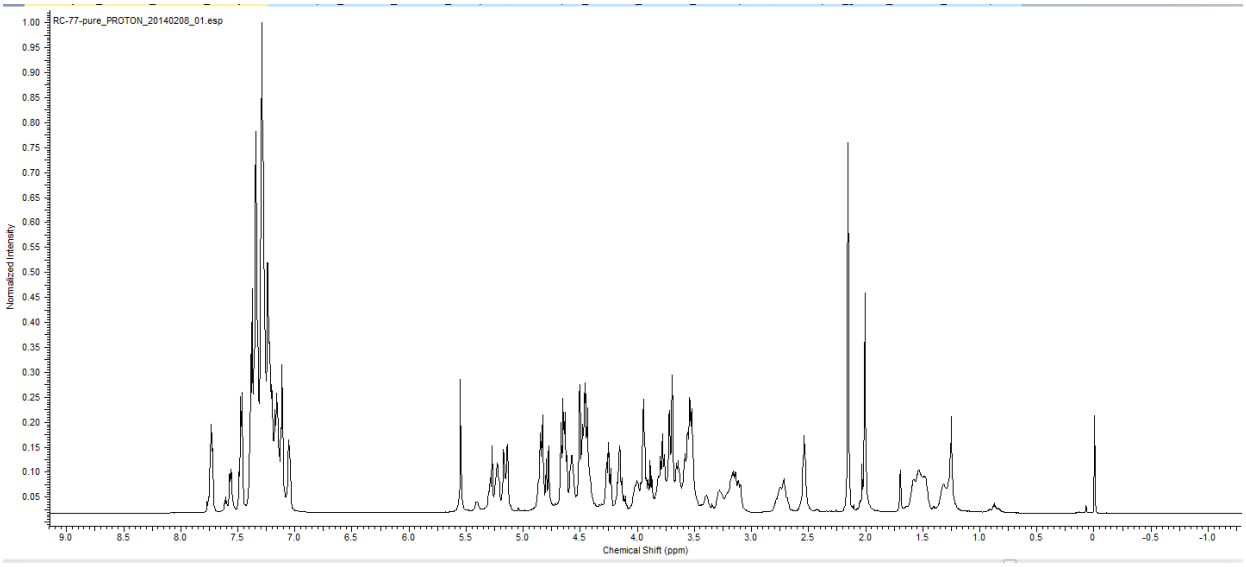


^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)

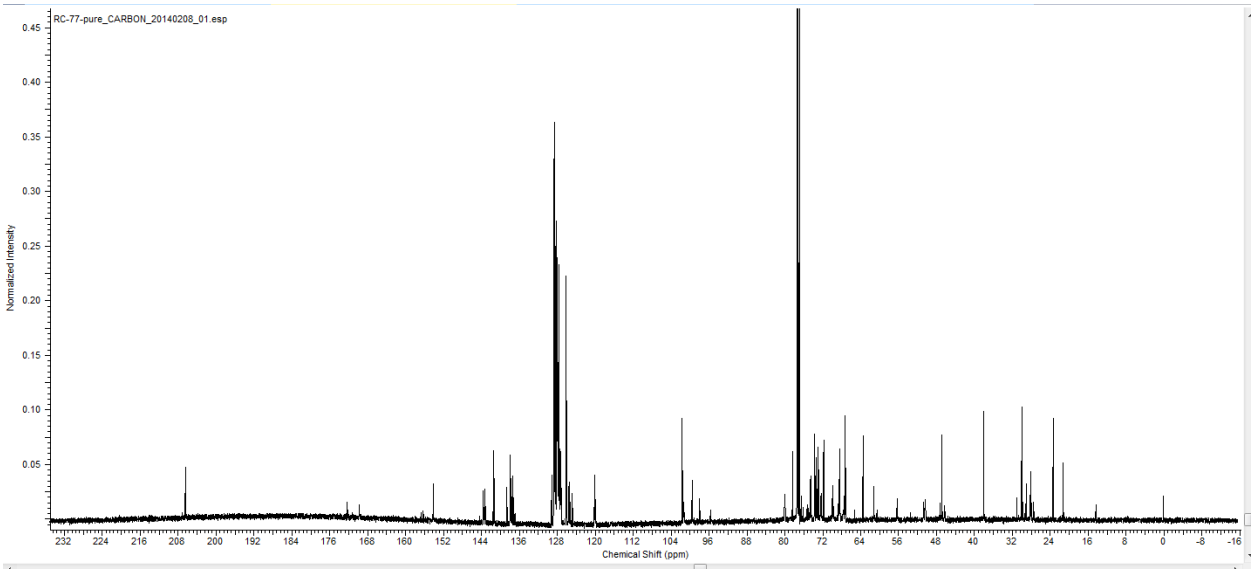




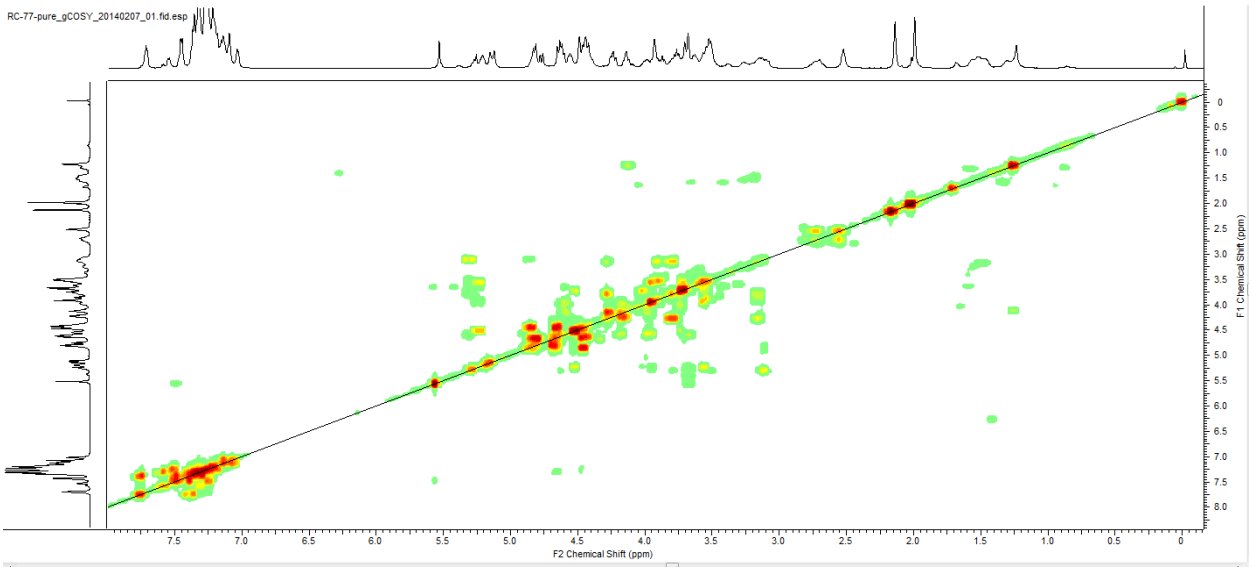
^{1}H NMR (600 MHz, $CDCl_3$)



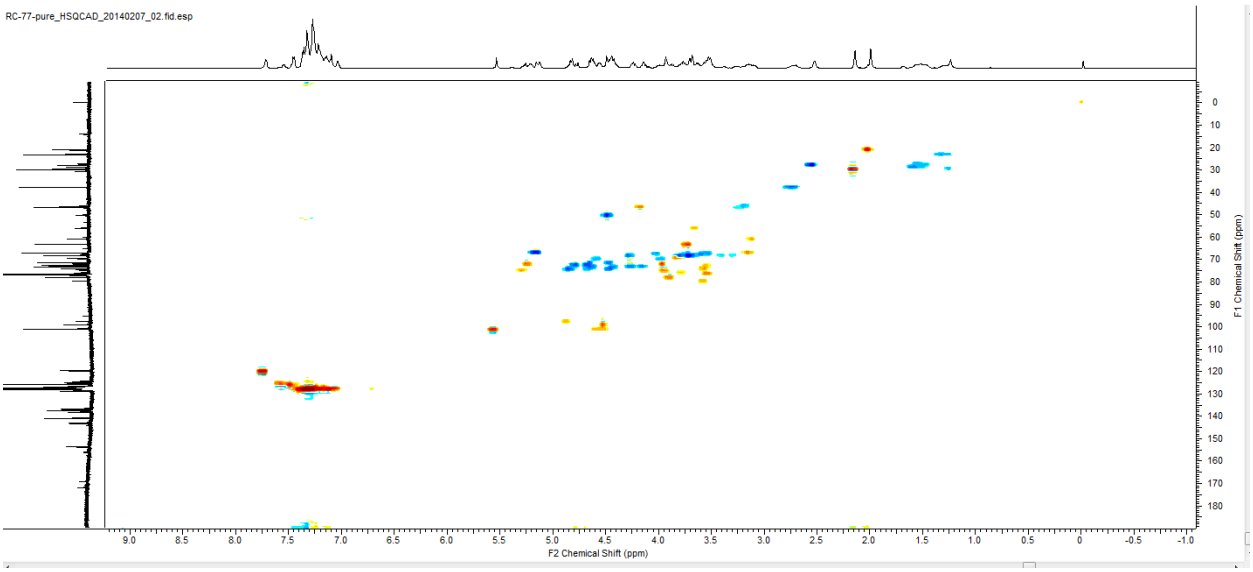
^{13}C NMR (150 MHz, $CDCl_3$)

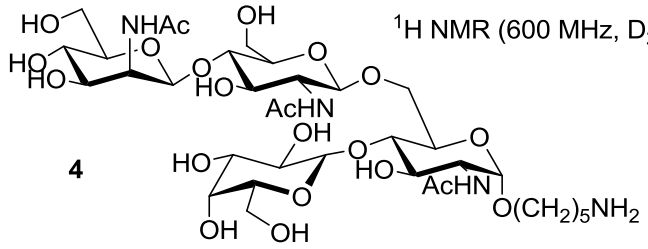


^{1}H-^{1}H COSY (600 MHz, $CDCl_3$)



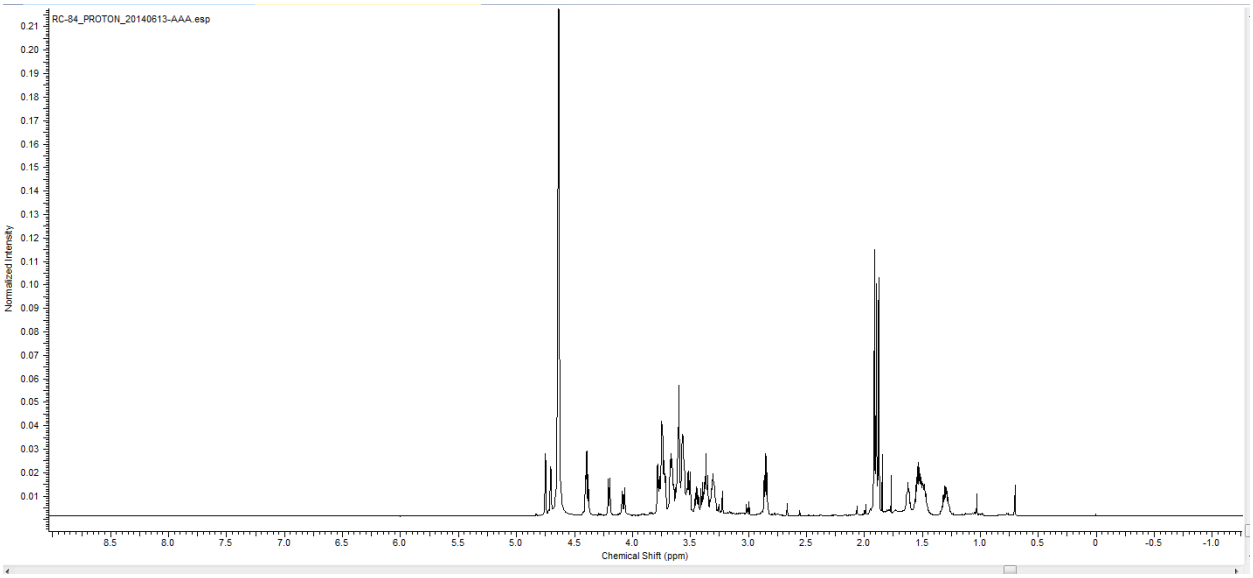
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



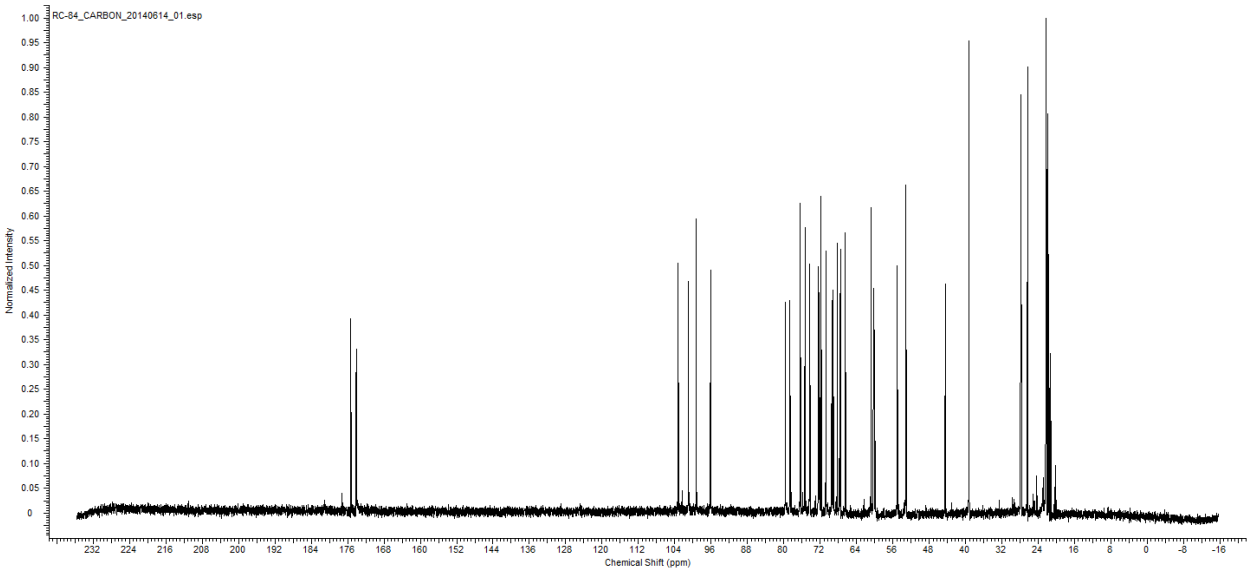


4

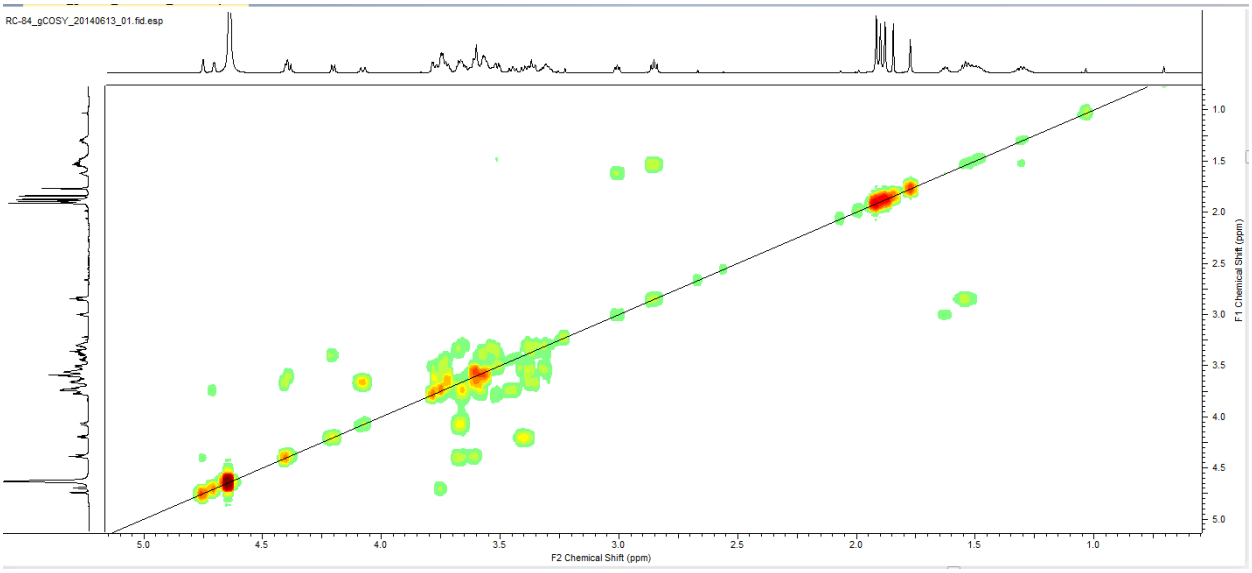
^{1}H NMR (600 MHz, D_2O)



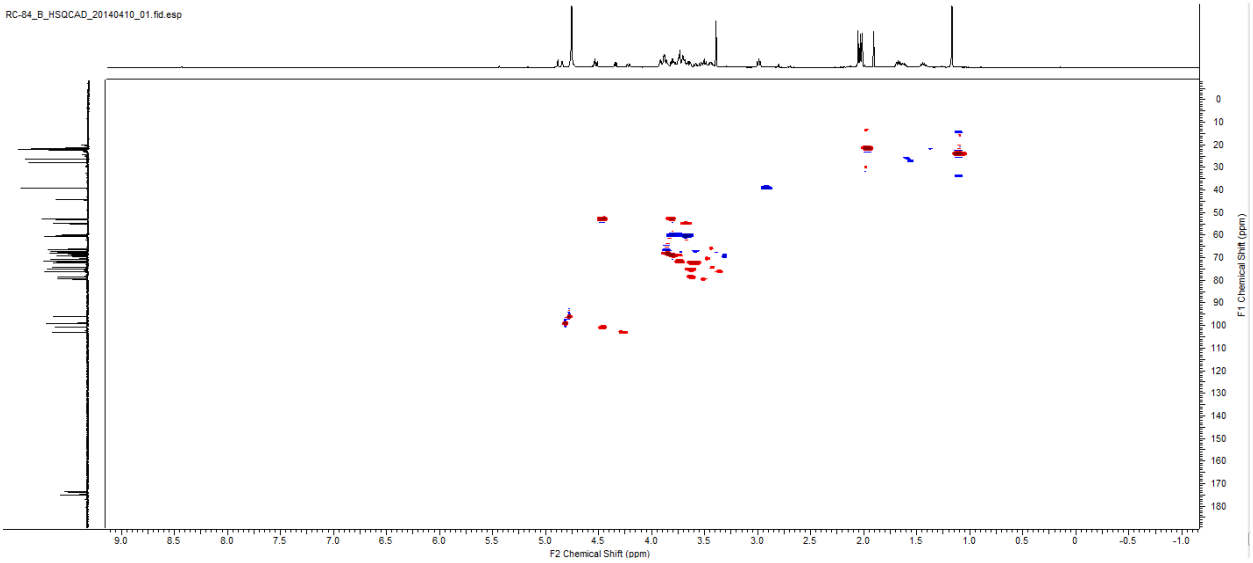
^{13}C NMR (150 MHz, D_2O)

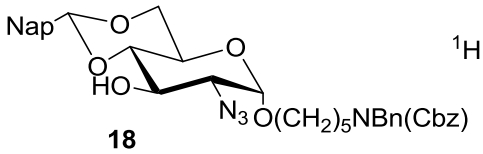


^{1}H-^{1}H COSY (600 MHz, D_2O)



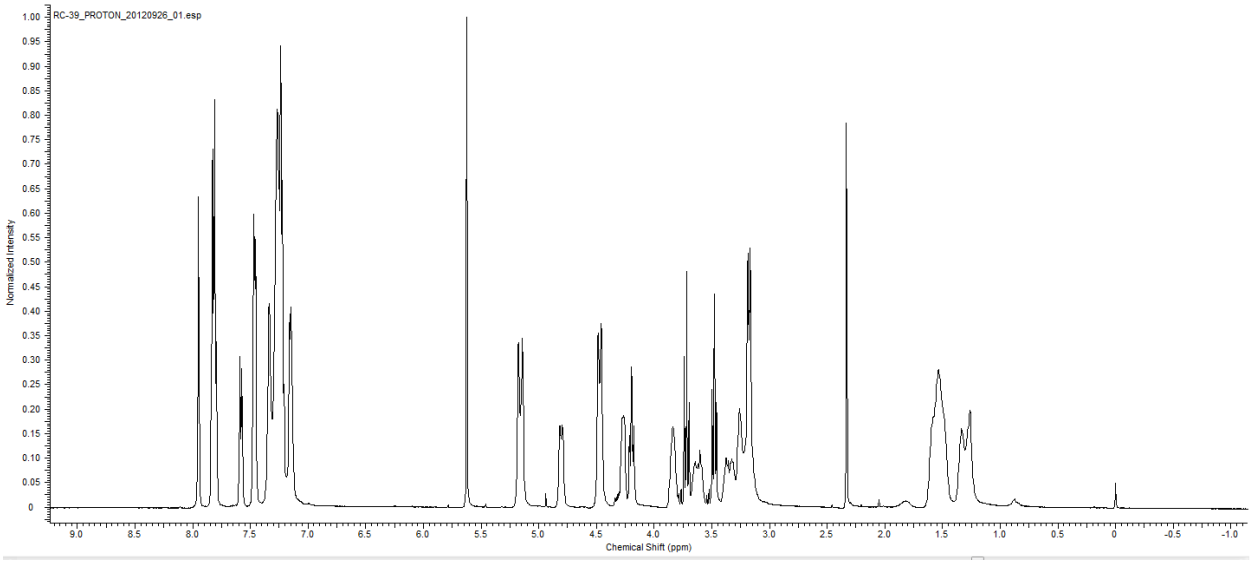
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, D_2O)



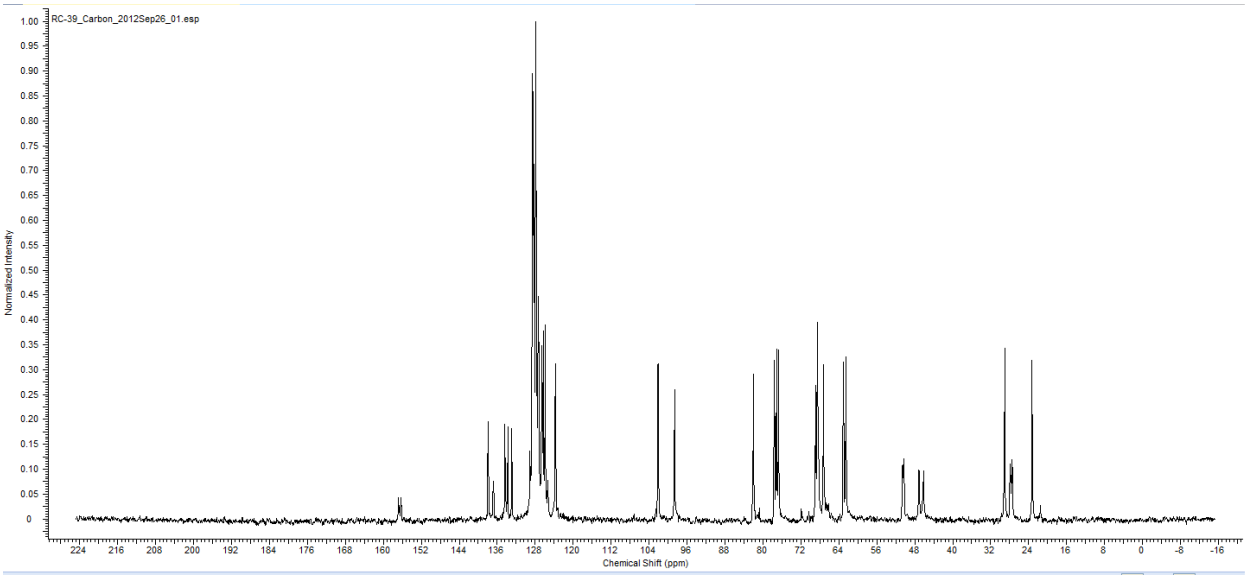


18

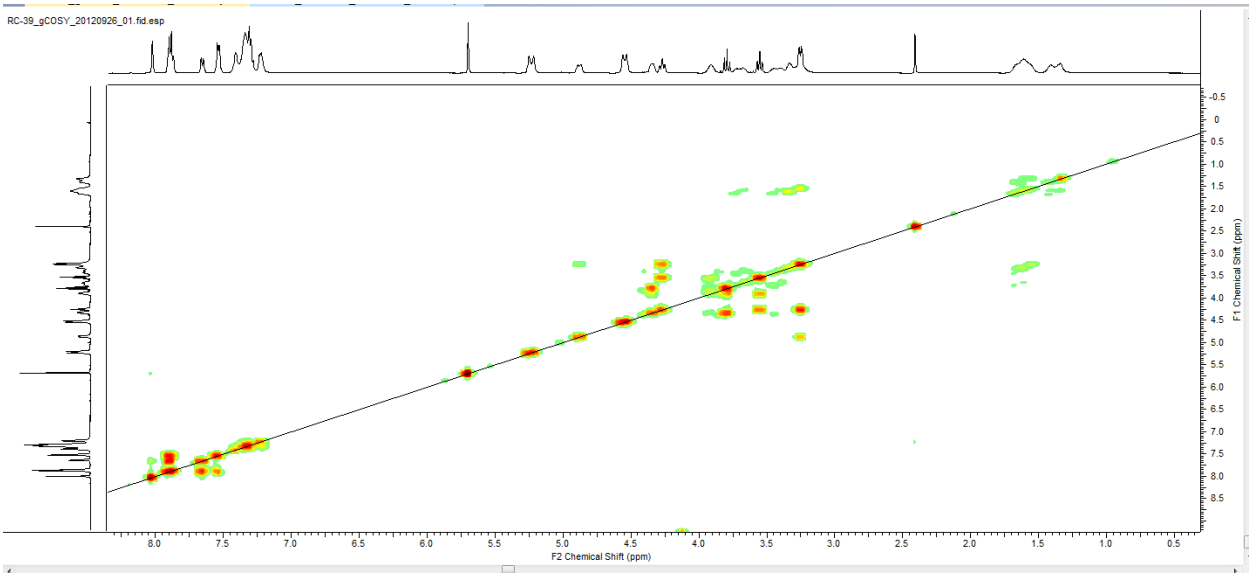
^{1}H NMR (300 MHz, $CDCl_3$)



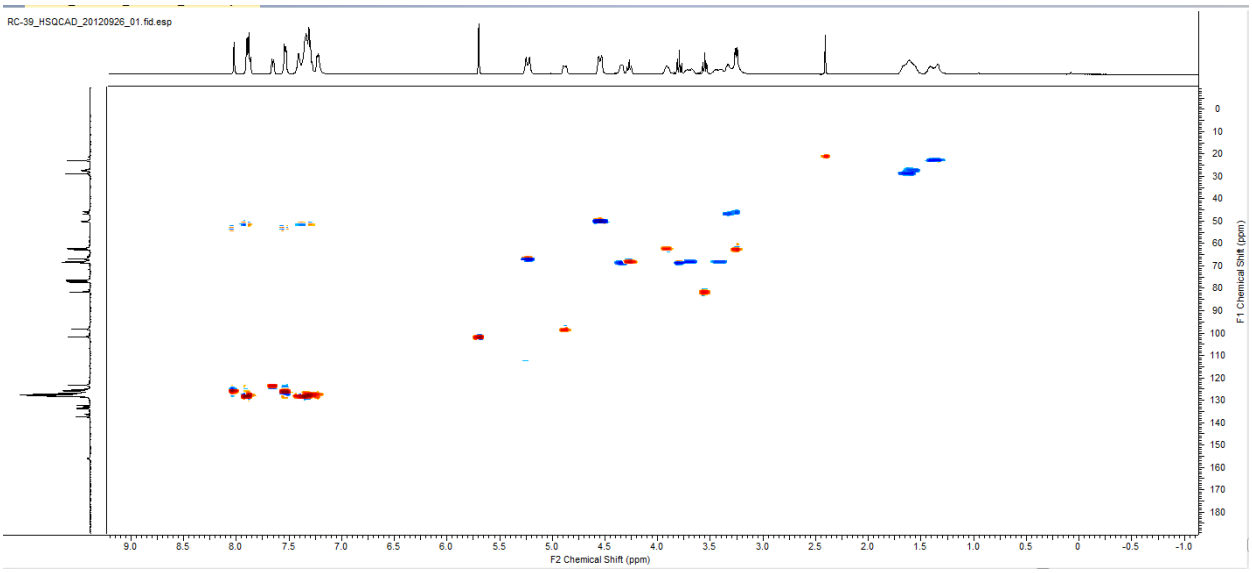
^{13}C NMR (75 MHz, $CDCl_3$)

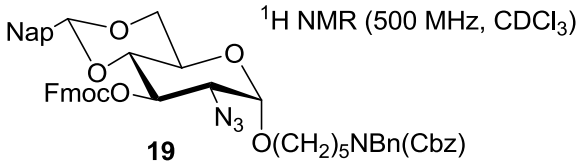


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



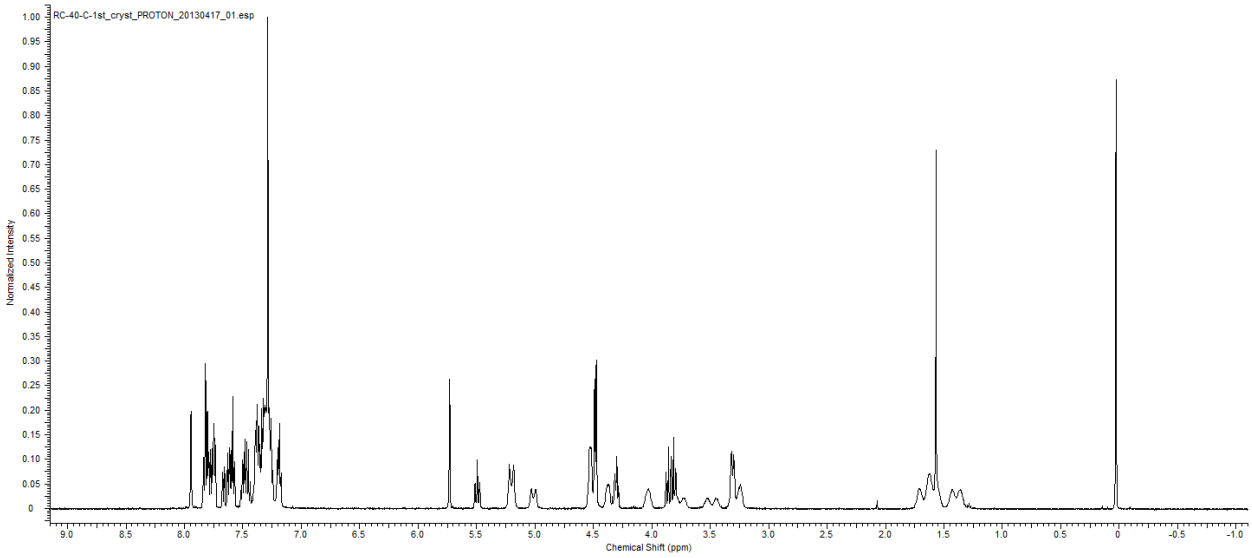
^{1}H-^{13}C HSQC (300 MHz for ^{1}H and 75 MHz for ^{13}C, $CDCl_3$)



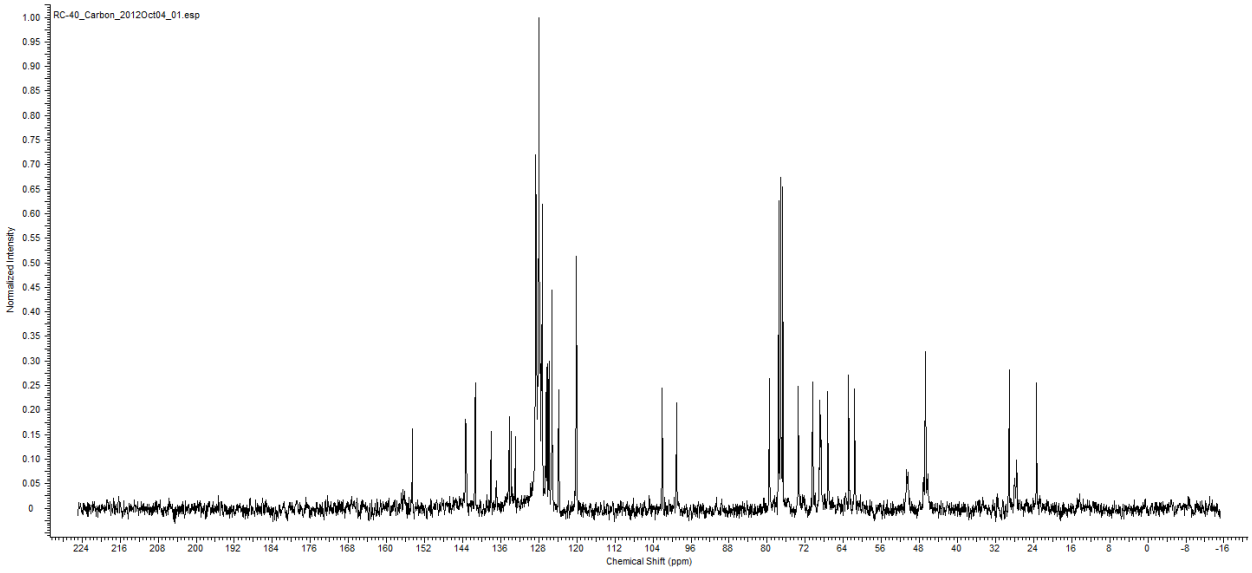


19

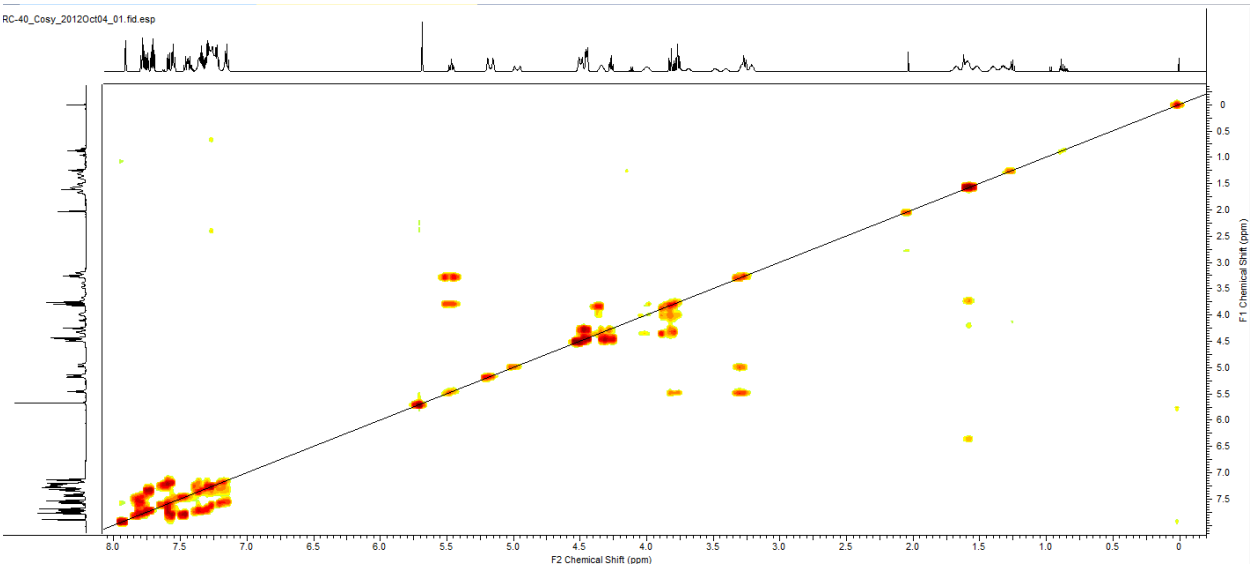
^{1}H NMR (500 MHz, $CDCl_3$)



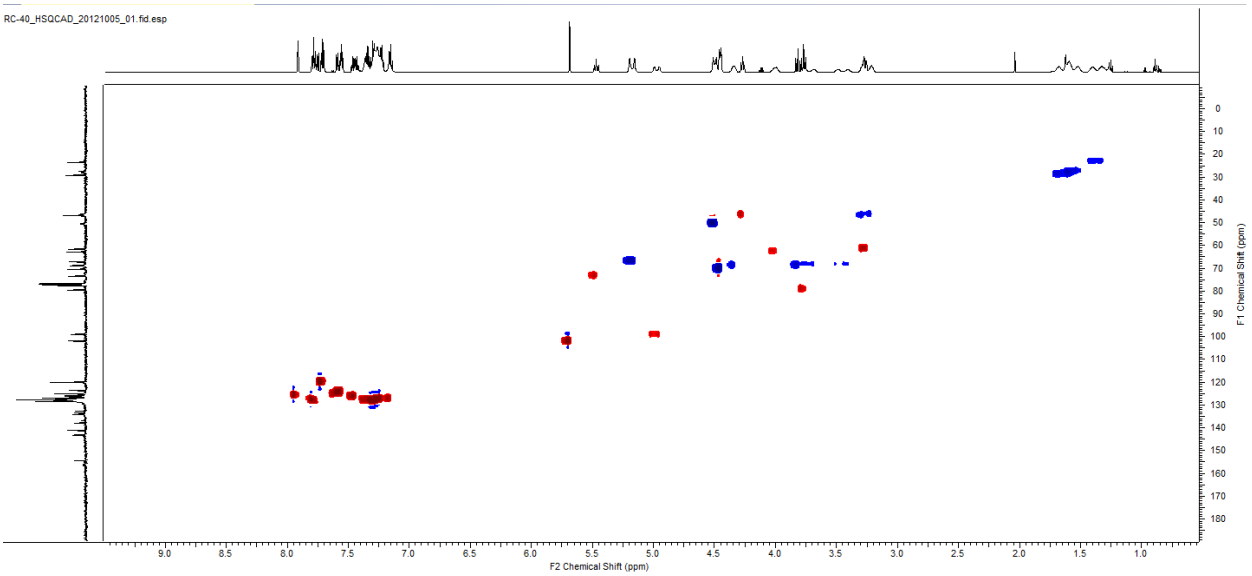
^{13}C NMR (75 MHz, $CDCl_3$)

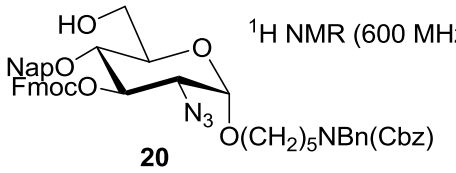


^{1}H-^{1}H COSY (300 MHz, $CDCl_3$)



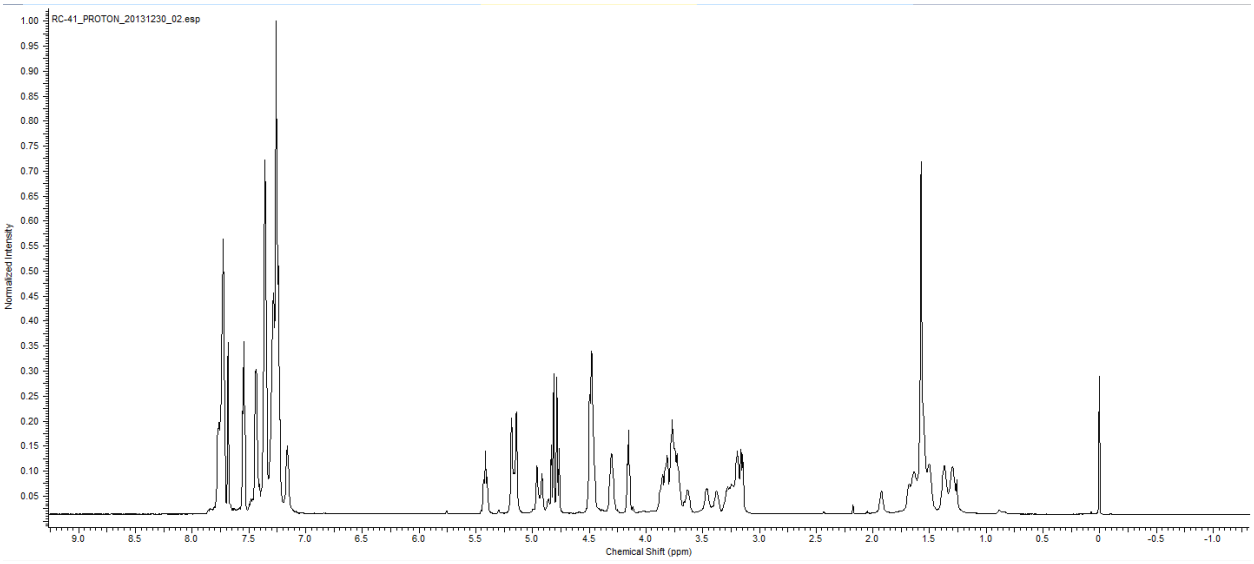
^{1}H-^{13}C HSQC (600 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$)



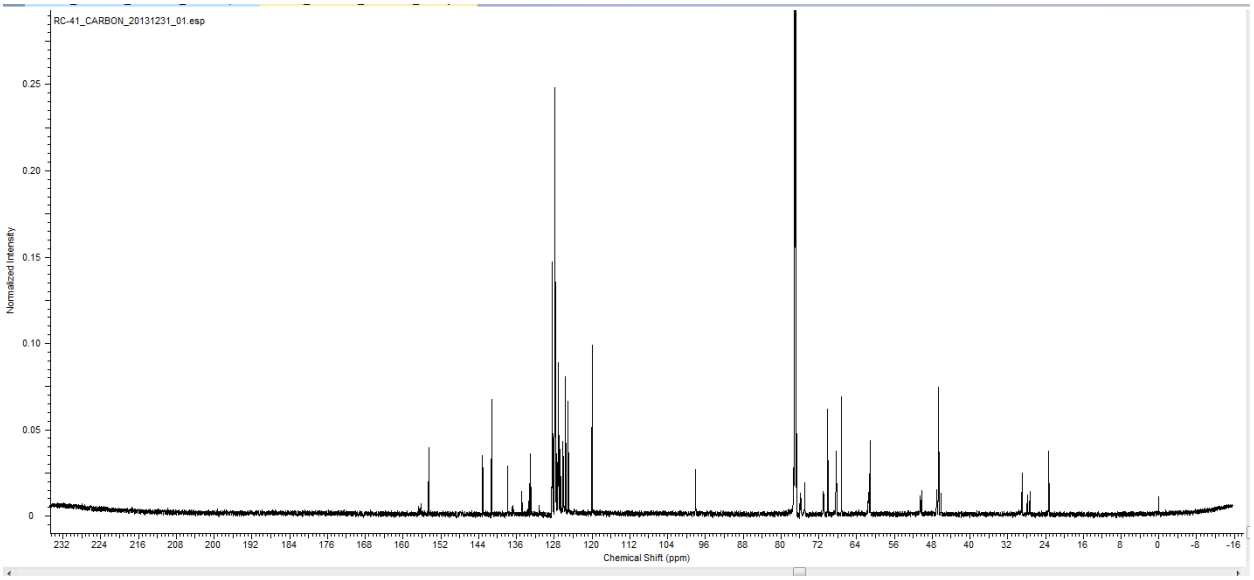


20

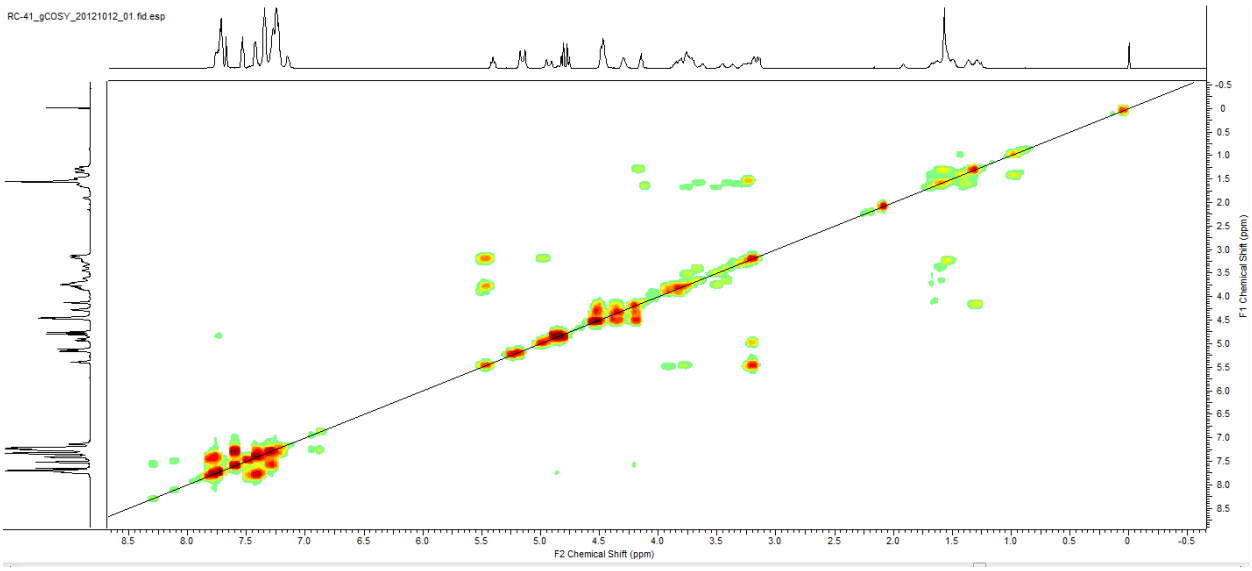
^{1}H NMR (600 MHz, $CDCl_3$)



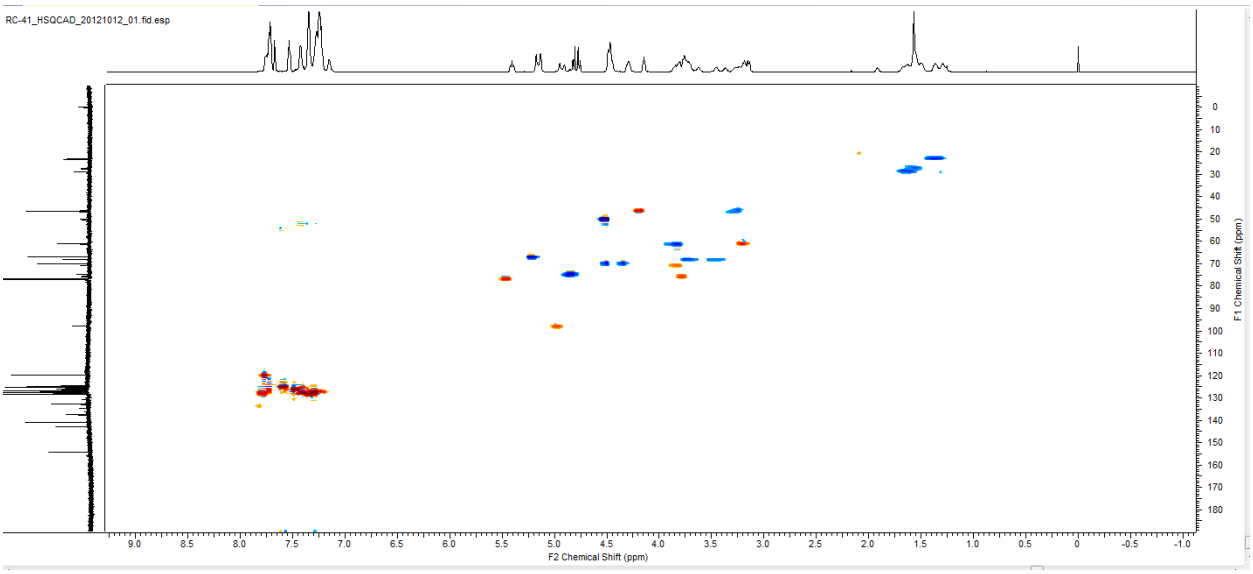
^{13}C NMR (150 MHz, $CDCl_3$)

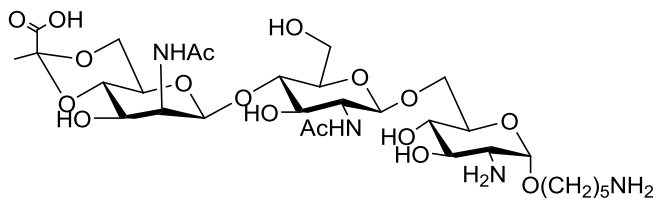


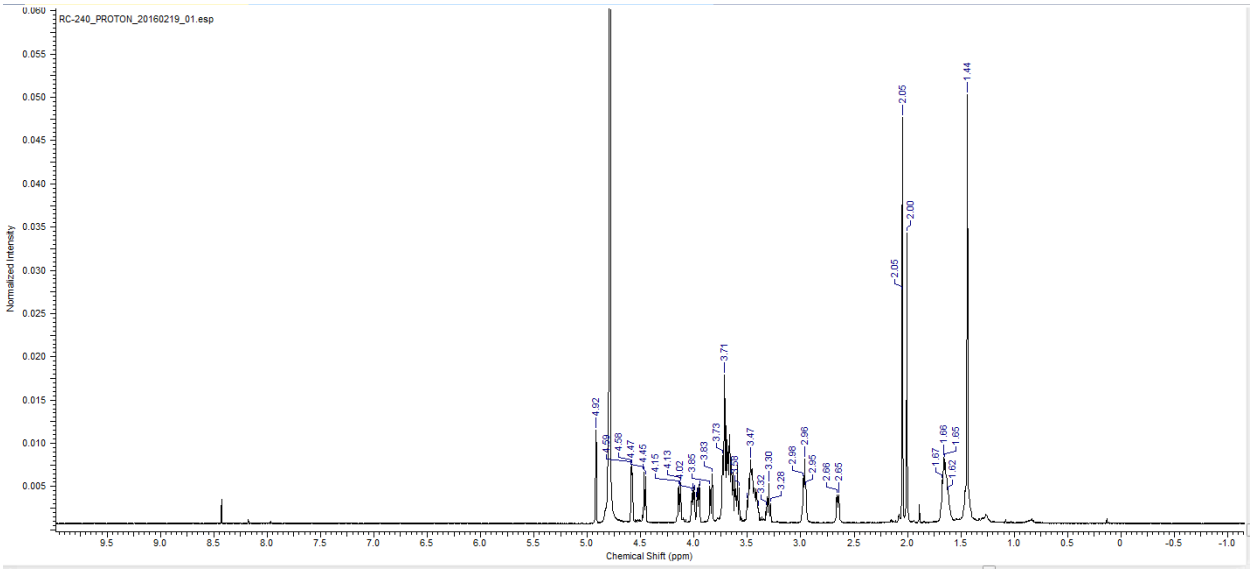
^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)

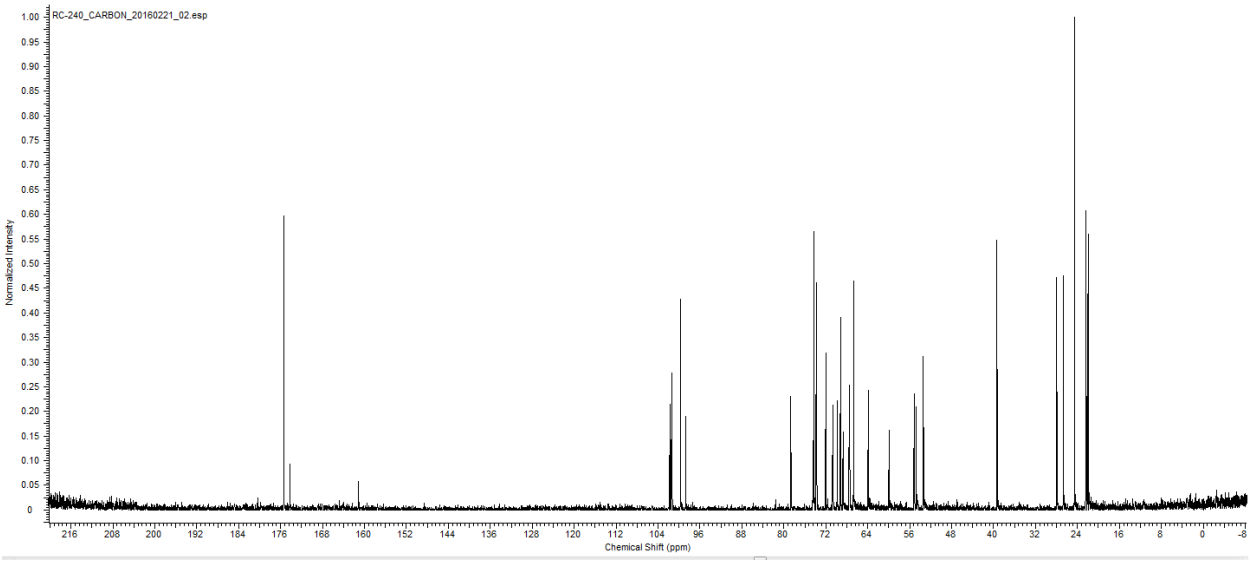


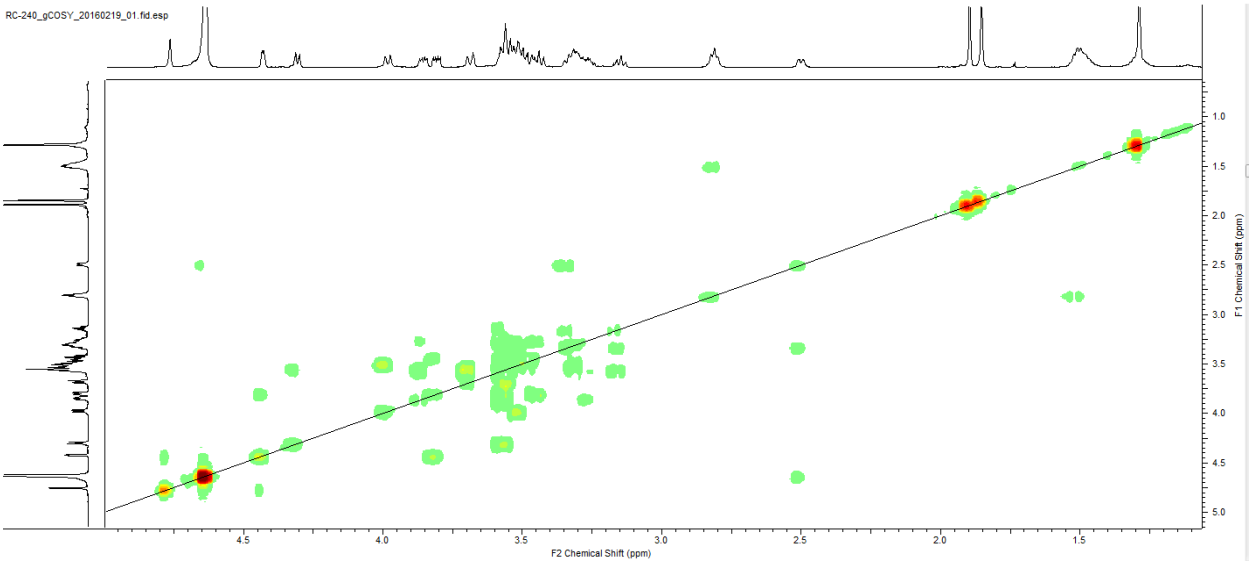
^{1}H-^{13}C HSQC (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)

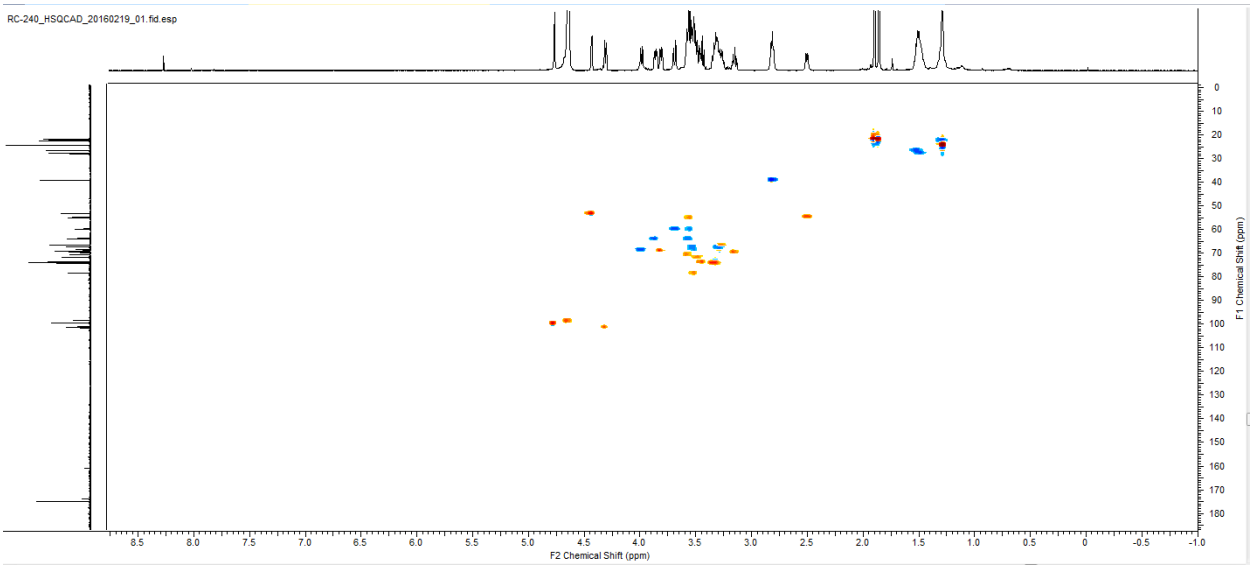



O
OH
NHAc
HO
O
HO
AcHN
HO
HO
H_2N
$O(CH_2)_5NH_2$


RC-240_PROTON_20160219_01.esp
Normalized Intensity
Chemical Shift (ppm)
4.92
4.59
4.58
4.47
4.45
4.15
4.13
4.05
3.95
3.83
3.73
3.71
3.47
3.30
3.28
2.96
2.96
2.95
2.69
2.65
2.05
2.00
2.05
1.67
1.66
1.62
1.65
1.44


RC-240_CARBON_20160221_02.esp
Normalized Intensity
Chemical Shift (ppm)


RC-240_gCOSY_20160219_01.fid.esp
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


RC-240_HSQCAD_20160219_01.fid.esp
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)