NOVEL HIGHLY CONVERGENT SYNTHESES OF BIOLOGICALLY IMPORTANT OLIGOSACCHARIDES AND GLYCOPEPTIDES

by

YUSUFBHAI U. VOHRA

(Under the Direction of GEERT-JAN BOONS)

ABSTRACT

The past couple of decades have seen a great improvement in chemical and enzymatic synthesis of structurally well-defined oligosaccharides. Mutant glycosidase enzymes have been developed which have made possible to synthesize oligosaccharides previously inaccessible due to the lack of corresponding glycosyltransferases. Chemoselective and orthogonal glycosylation techniques have allowed researchers an access to a number of fully synthetic oligosaccharides such as heparan sulfate, the tumor associated STn-antigen, blood group antigens (Le^x, Le^y), and Globo-H.

In order to further streamline the process of oligosaccharide synthesis, we developed a new class of one-pot reactions that addresses some of the shortcomings of the above approaches. We have combined triflic acid promoted glycosylations of trichloroacetimidates with reductive opening of benzylidene acetals using triflic acid and triethylsilane as one-pot procedures to provide access to a wide range of disaccharides and branched trisaccharides. The attraction of the approach is that it makes it possible to assemble branched oligosaccharides by a one-pot procedure, a task that cannot readily be accomplished by chemoselective, orthogonal, and iterative glycosylations.

In chapters III and IV of this dissertation we describe our efforts towards the synthesis of the glycopeptide recognition domain of PSGL-1 and analogs thereof. PSGL-1 has clearly been demonstrated to mediate the adhesion of leukocytes to selectins, resulting in transmigration of leukocytes to the site of injury. Uncontrolled migration of leukocytes can lead to chronic inflammation. Inhibition of leukocyte adhesion to selectins by blocking PSGL-1 represents a promising therapeutic approach against diseases in which inflammation has a destructive role (e.g.; ischemia, venous thrombosis, hemorrhage, atherosclerosis, asthma, skin inflammation and autoimmune diseases). We have developed a highly convergent synthesis of the PSGL-1 oligosaccharide linked to threonine based on the use of trichoroacetimidate donors and thioglycosyl acceptors that give products that can immediately be employed in a subsequent glycosylation step without the need for protecting group manipulations. We also demonstrate here for the first time a novel approach toward the synthesis of glycosulfopeptide mimics of PSGL-1 by exploiting the Huisgen [3 + 2] cycloaddition reaction to conjugate the oligosaccharide with the peptide.

INDEX WORDS: One-pot oligosaccharide synthesis, carbohydrates, glycopeptides, glycosulfopeptides, P-selectin glycoprotein ligand 1, PSGL-1, organic synthesis, glycopeptide mimetics, Solid-phase peptide synthesis, Microwave-assisted synthesis.

NOVEL HIGHLY CONVERGENT SYNTHESES OF BIOLOGICALLY IMPORTANT OLIGOSACCHARIDES AND GLYCOPEPTIDES

by

YUSUFBHAI U. VOHRA

B.S., Sardar Patel University, Vallabh Vidyanagar, India 1999

M.S., Sardar Patel University, Vallabh Vidyanagar, India 2001

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

2010

NOVEL HIGHLY CONVERGENT SYNTHESES OF BIOLOGICALLY IMPORTANT OLIGOSACCHARIDES AND GLYCOPEPTIDES

by

YUSUFBHAI U. VOHRA

Major Professor: Geert-Jan Boons

Committee: Robert S. Phillips
Vladimir V. Popik

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2010

DEDICATION

“He preferred the hard truth to his dearest illusions, that is the heart of science.”

--Carl Sagan on Johannes Kepler

To

My Parents

For their unconditional support and encouragement in all my endeavors.

ACKNOWLEDGEMENTS

I would like to thank Prof. Geert-Jan Boons for providing me with an opportunity to be a part of his research team. His support, encouragement, and guidance throughout my graduate degree have been phenomenal, for which I am truly grateful. I would also like to thank my committee members Dr. Popik and Dr. Phillips for their useful suggestions, support and for being available for all my Ph.D. requirements.

I would like to express my gratitude to Dr. Andre Venot and Dr. Therese Buskas for their constant advice and discussions during my stay at CCRC. Their guidance was a key to my success as carbohydrate and glycopeptide chemist. I not only learned critical aspects of chemical reactions with carbohydrates, but also the importance of keeping the lab clean and organized and following safety precautions. Without their help, my Ph.D. projects would have been incomplete.

My special thanks go to Pam Thompson for training me on the HPLC and peptide synthesizer, for being a wonderful friend, and for her unwavering support and patience proof reading all my written work. I would also like to thank Jessica Cardot, without whom work would have been less fun. I would like to express my appreciation toward all my lab mates and friends who have been very kind, helpful, and supportive.

I would like to thank Dr. John Glushka and Dr. Christian Heiss for helping me with NMR instrumentation and analysis. I would also like to thank Saeid for his technical help with computer and other software related issues, and the CCRC support staff who have been very friendly and helpful.

Finally, I would like to thank my parents and my family without whom much of this endeavor would have been meaningless.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

ABBREVIATIONS

Å	Angstrom
Ac	Acetyl
AcOH	Acetic acid
AgOTf	Silver triflate
Ar	Aromatic
BF_3•Et_2O	Borontrifluoride diethyletherate
Bn	Benzyl
Bz	Benzoyl
ACN	Acetonitrile
Cbz	Benzyloxycarbonyl
D	Doublet
Da	Dalton
DBU	1,8-Diazabiccycloundec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dicyano-5,6-dichloro quinone
dfBz	2,5-Difluorobenzoyl
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-(dimethylamino)pyridine

DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethylsulfonium triflate
DTBMP	Di-t-butyl-4-methylpyridine
EDC	3-(3-dimethylaminopropyl)-1-ethylcarbodiimide
Et_3SiH	Triethyl silane
EtOAc	Ethyl acetate
EtOH	Ethyl alcohol
H	Hour
HATU	O-(7-Azabenzotriazol)- 1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HBTU	O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate
$HfCp_2Cl_2$	Bis(cyclopentadienyl)hafnium dichloride
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	Hydroxybenzotriazole
HPLC	High pressure liquid chromatography
HR-MALDI	High resolution-matrix assisted laser desorption/ionization
Hz	Hertz
IDCP	Iodonium dicollidine perchlorate
Le^x	$Lewis^x$
Le^y	$Lewis^y$
M	Multiplet
m/z	Mass to charge ratio

MBz	p-Methylbenzoate
MeOH	Methanol
MeOTf	Methyl trifluromethanesulfonate
mM	Millimolar
mmol	Millimole
MP	p-Methoxyphenyl
MS	Molecular sieves
$NaCNBH_3$	Sodium cyanoborohydride
NaH	Sodium hydride
NAM	Naphthyl methyl ether
NAP	Naphthyl
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
PAPS	Adenosine 3'-phosphate 5'-phosphosulfate
Pent	Pentenyl
PSGL-1	P-Selectin glycoprotein ligand 1
p-TolSCl	p-Toluenesulfenyl chloride
q	Quartet
Rf	Retention factor
RRV	Relative reactivity values
rt	Room temperature
s	Singlet

Sbox	Thiobenzoxazolyl
SDMP	2,6-Dimethylthiophenyl
SEt	Thioethyl
sLe^x	Sialyl $Lewis^x$
SPPS	Solid-phase peptide synthesis
Staz	S-thiazolyl
Stol	Thiotolyl
t	Triplet
TBAF	Tetrabutyl ammoniumfluoride
TBAI	Tetrabutyl ammoniumiodide
t-BuOH	tert-Butyl alcohol
TDS	Thexyl dimethyl silyl
TEA	Triethylamine
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TIPS	Triisopropyl silane
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluromethanesulfonate
TPST-1	Tyrosylprotein sulfotransferase
Troc	Trichloroethoxy carbonyl

Chapter I

Introduction and Literature Review

1.1 General Introduction

Conventionally, carbohydrates have been believed to be hydrates of carbon designated by a general formula $C_m(H_2O)_n$. Over time the term "carbohydrates" has evolved to encompass a large family of compounds including monosaccharides, disaccharides, oligosaccharides and polysaccharides. Monosaccharides are the smallest units of this class, often coexisting in their open-chain forms and closed ring forms. These monosaccharides, when linked together through glycosidic linkages, form oligo- or polysaccharides. This structurally diverse class of compounds, when covalently linked to lipids and proteins, form highly complex biopolymers known as glycolipids and glycoproteins, respectively.

As our understanding of this class of compounds has increased, so has our knowledge of the number of functions associated with them. Today, the functions of carbohydrates have been shown to range from being primary sources of energy and structural components in living organisms to being involved in complex biological processes such as cell recognition, proliferation and differentiation, fertilization, embryogenesis, and neuronal development. Carbohydrate interactions are also important in health science and are involved in the invasion and attachment of pathogens, inflammation, cancer metastasis, blood group immunology, and xenotransplantation (Figure **1.1**)[1].

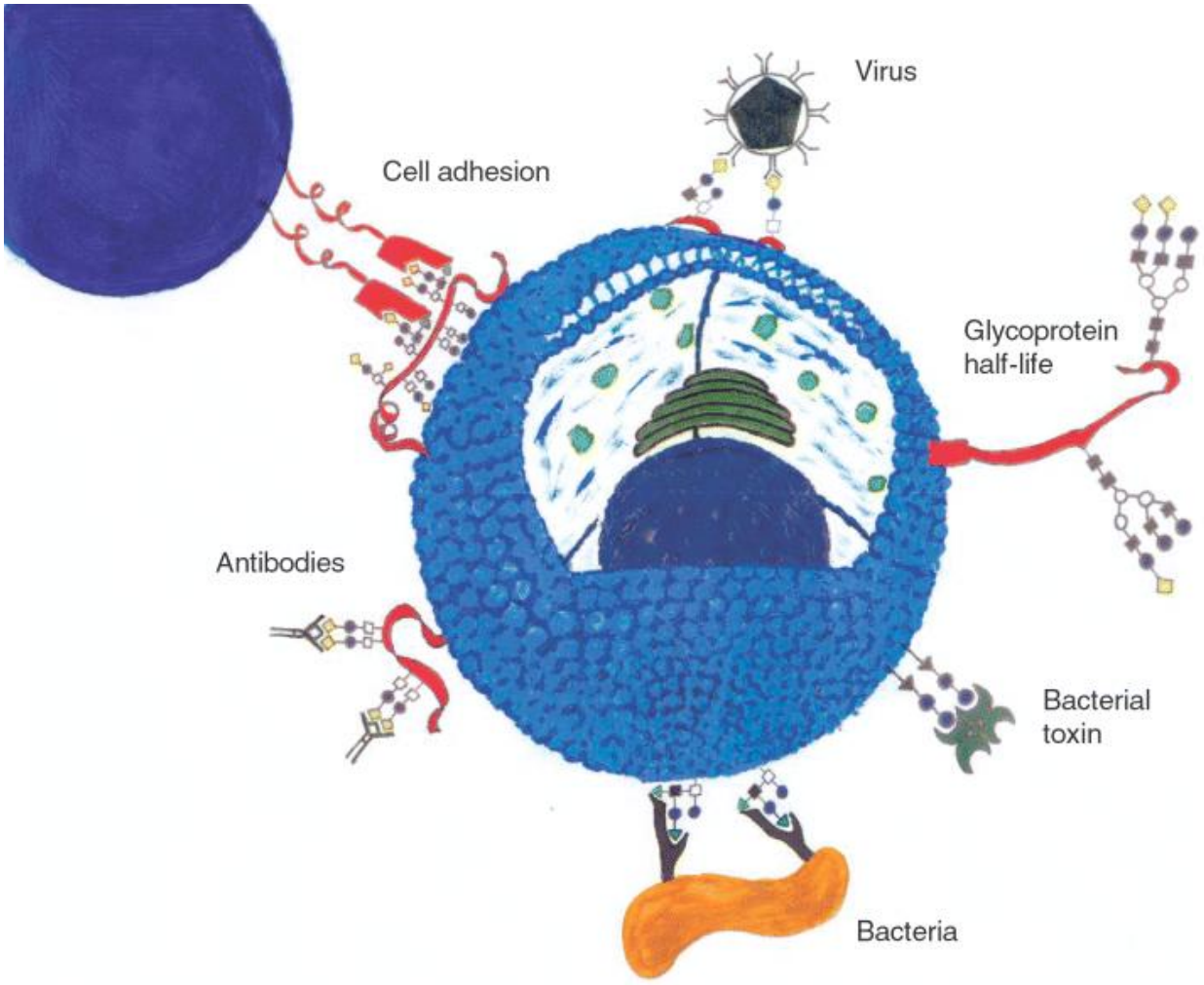


Figure 1.1 Protein-carbohydrate interactions at the cell surface

Many microbes, including viruses, bacteria and bacterial toxins, have evolved to bind with cell-surface carbohydrates of the hosts. These interactions are prerequisites for infection to occur.[2] Figure **1.1** illustrates the protein-carbohydrate interactions at the cell surface mediating cell-cell binding, cell-microbe (bacterial, viral and bacterial toxin) adhesion and cell-antibody binding.

1.2 Cell Adhesion

The surface of living cells is densely covered with a wide variety of glycoproteins, glycolipids, glycosphingolipids and proteoglycans. Amongst their numerous functions,[3] intracellular recognition events account for the majority of research in glycobiology. Processes such as blood clotting, inflammation, immune responses, bacterial adhesion, homeostasis, and wound repair have been attributed to protein-carbohydrate interactions.[4]

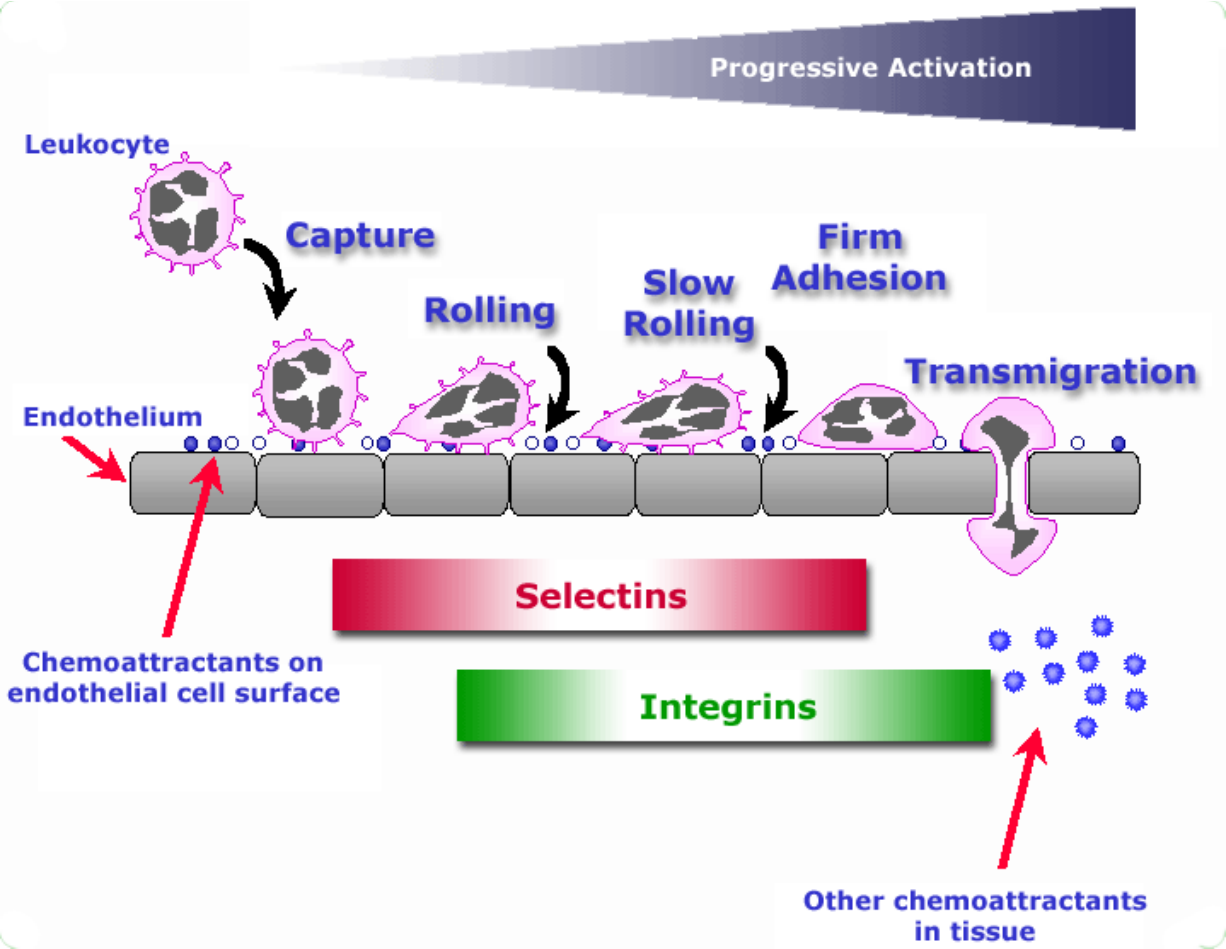
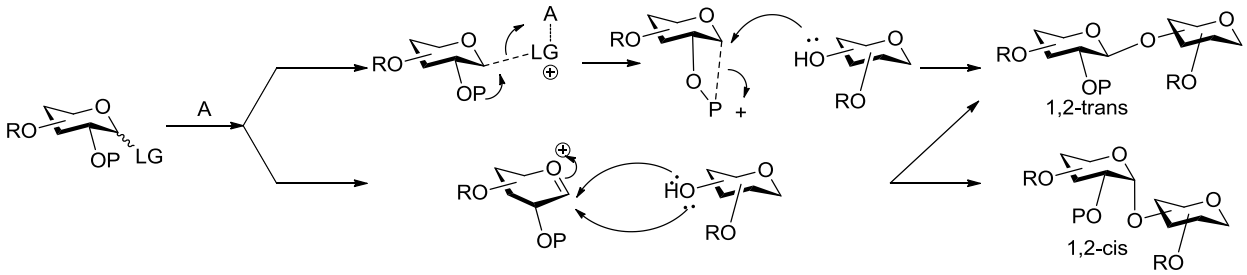


Figure 1.2 Leukocyte adhesion cascade.

Among these processes, inflammation is a nonspecific immune response to harmful stimuli, such as pathogens, tissue damage or irritants.[5] The inflammatory response begins with increased blood flow by which white blood cells or leukocytes are transported to the inflamed area. First, glycoproteins such as P-, E- and, L-selectins are activated by chemoattractants and are expressed on the endothelial cell surfaces in the affected area. These selectins act as adhesion molecules to the passing leukocytes. This adhesion is at the origin of the leukocyte recruitment process, which occurs in a cascade like fashion from capture to rolling to a systematic decrease of rolling velocity to firm adhesion and transmigration. This process, known as the leukocyte adhesion cascade (Figure **1.2**),[6] results into transmigration of leukocytes at the site of injury to start the healing process. Continuation of the transmigration process for a prolonged duration can however lead to chronic inflammation, characterized by simultaneous destruction and healing of tissue.[7-9]

1.3 Oligosaccharide Synthesis

Carbohydrates in their various forms are ubiquitous in nature. These compounds are often found in low concentrations and in micro-heterogeneous forms, greatly complicating their isolation and characterization. This lack of pure and structurally well-defined carbohydrates and glycoconjugates represents a major obstacle to advances in glycobiology. The solution to this predicament is the procurement of well-defined oligosaccharides by organic synthesis. The chemical synthesis of oligosaccharides is much more complex compared to the synthesis of other biopolymers such as peptides and oligonucleotides, but the past few decades have seen a huge amount of progress in the construction of oligosaccharides based on a variety of synthetic strategies. The use of highly convergent strategies has enabled us to synthesize oligosaccharides consisting of as many as 20 monosaccharide units. In such strategies, most of the effort is directed towards the synthesis of advanced monosaccharide building blocks. Using combinatorial and highly regioselective methods to protect the individual hydroxyl groups of the monosaccharide units can furnish these advanced building blocks rather quickly and alleviate the amount of labor involved. These building blocks can then be linked together utilizing a minimum number of highly efficient synthetic steps with excellent stereoselectivities.



LG - leaving group
P - Participating / Non-participating group
R - Protecting group
A - Activator

Scheme 1.1 General approach for chemical glycosylations

A glycosidic linkage is generally formed by coupling a glycosyl donor having a leaving group at its anomeric center while all the other hydroxyl groups are protected and a glycosyl acceptor which has one hydroxyl free to act as a nucleophile. The most common leaving groups employed as glycosyl donors are halides, trichloroacetimidates, thioglycosides, and *n*-pentenyl glycosides (Scheme **1.1**). After the initial activation of the glycosyl donor (typically using a Lewis acid), the bond between the anomeric carbon atom and the leaving group is either partially broken or completely broken to form an oxo-carbenium ion. This intermediate can then undergo a nucleophilic attack by the hydroxyl group of the acceptor to form the glycosidic bond. Often, the product is a mixture of two possible diastereoisomers, the separation of which is quite cumbersome resulting in poor yields. Various strategies have been developed to achieve complete control over the stereochemical outcome of these reactions based on neighboring group participation, exploiting the anomeric effect, a C-2 auxiliary,[10-11] temperature of the reaction, and the type of solvent, leaving group and promoter used.[12]

1.4 Enzymatic and Chemoenzymatic Synthesis

The most challenging aspect in oligosaccharide synthesis has been the stereoselective installation of the glycosidic linkage. The chemoenzymatic approach combines the flexibility of chemical synthesis with the high regio- and stereoselectivities of enzyme-catalyzed reactions, making it a powerful tool to obtain complex oligosaccharides. Glycosyl transferases, when employed in catalytic amounts, transfer a sugar residue from a sugar-nucleotide mono- and di-phosphate to a growing oligosaccharide chain.

In case of monosaccharides such as sialic acids, the neighboring group participation cannot be exploited to achieve stereochemical control. The presence of an electron-withdrawing carboxyl group at the anomeric position, a sterically hindered quaternary anomeric carbon and a deoxy carbon next to the anomeric center makes its glycosylation a formidable challenge. The abundance of sialic acid residues as terminal units in many glycans and glycoconjugates in nature makes them important targets for oligosaccharide synthesis. In 2007, Chen and co-workers reported the synthesis of sialyl disaccharides containing a mannose or ManNAc at the reducing end. They exploited the high expression level of bacterial sialoside biosynthetic enzymes in *E. coli* expression systems.[13] These enzymes showed high activity and substrate promiscuity which was demonstrated by the synthesis of sialic acid containing natural and non-natural functionalities *via* one-pot three-enzyme chemoenzymatic approach.[14-15]

Chemoenzymatic approaches have been employed to construct a range of glycopeptides derived from *N*-terminus of P-selectin glycoprotein ligand-1.[16] These methods are discussed in detail in the latter part of this chapter (Section **1.7.3**). Glycosidases, which are responsible for the cleavage of glycosidic bonds in nature, have also been employed in synthesis of oligosaccharides by driving the reaction in a thermodynamically unfavorable direction. Withers and co-workers converted glycosidases into glycosynthases using site-directed mutagenesis, rendering them hydrolytically incompetent.[17] These enzymes have been shown to perform transglycosylation reactions without hydrolyzing the product, thus increasing the yield of target oligosaccharides.

1.5 Polymer-supported Oligosaccharide Synthesis

The synthesis of biopolymers such as oligonucleotides and peptides has been greatly improved by exploiting solid-phase chemistry. Automated synthesizers have been routinely used to accomplish this task. The major advantages of solid-phase synthesis are that the excess reagents used to drive the reactions to completion can be removed by simply washing the resin and a single purification step at the end of the synthesis minimizes the number of chromatographic purifications at each step. Based on the immense impact that automated solid-phase peptide and oligonucleotide synthesis has had in acquiring these biopolymers, it is prudent to apply this technology to oligosaccharide synthesis. The first attempts at solid-phase oligosaccharide synthesis were reported in the 1970s;[18] however, due to a dearth of efficient glycosylation conditions these efforts were futile. The past decade has seen a renewed interest in solid-phase oligosaccharide synthesis.[19-20] In 2001, the first automated synthesizer for solid-phase oligosaccharide synthesis was introduced by Seeberger and co-workers.[21] They attached a mannose trichloroacetimidate donor to the solid support *via* a cleavable linker (octenediol) by a TMSOTf-promoted glycosylation, followed by removal of a temporary protecting group to reveal a free alcohol. The oligosaccharide chain was prepared by repeating the glycosylation and de-protection steps to obtain a heptamannoside. A cross-metathesis reaction with ethene using Grubb's catalyst released the oligosaccharide from the solid support. The heptasaccharide was obtained in 20 h with an overall yield of 42%. This strategy was later applied to the synthesis of Le^x, Le^y and the Le^y-Le^x nonasaccharide.[22]

Although solid-phase oligosaccharide synthesis has seen a number of successes, there are inherent limitations to this method. A large excess of reagents, including glycosyl donors, are required to drive the reactions to completion. Other important limitations of this technique

include a lack of anomeric control for the installation of 1,2-cis glycosidic linkages, unpredictability of glycosylations, and the installation, stability, and cleavability of the linkers.

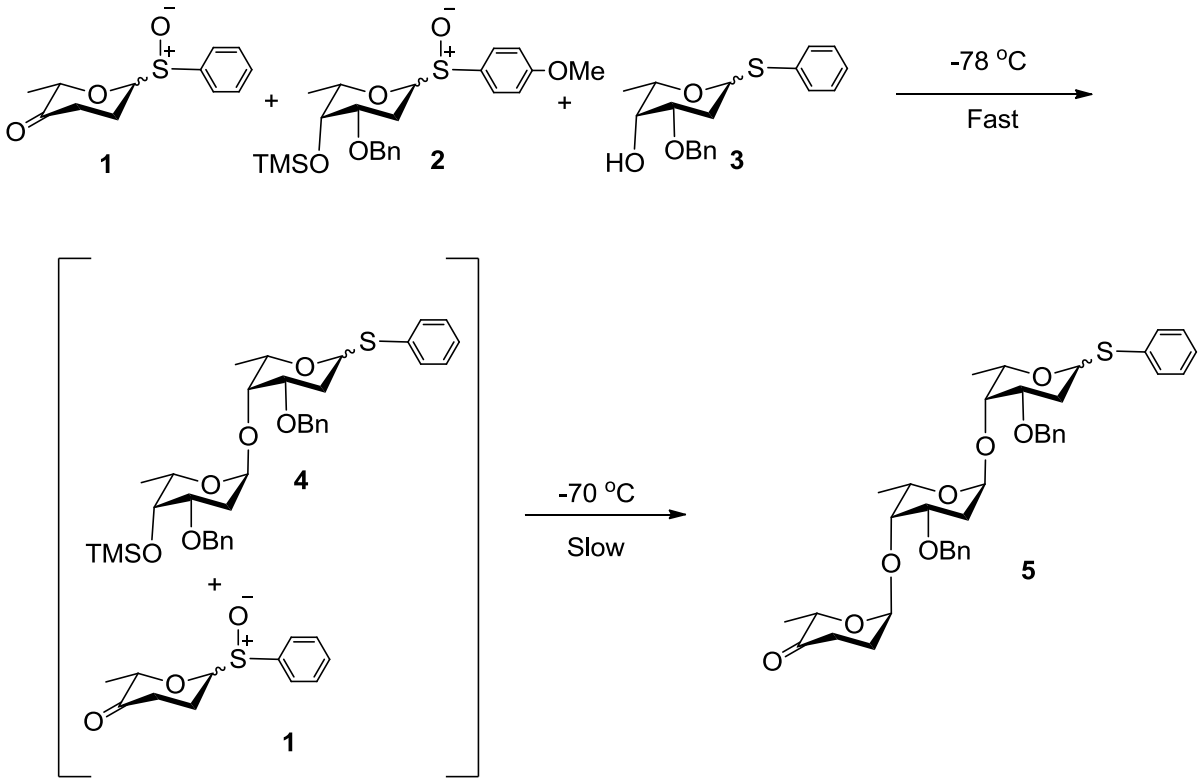
1.6 One-Pot Synthesis of Oligosaccharides

Although the methods for oligosaccharide synthesis have improved considerably during the past decade, the construction of complex carbohydrates remains a significant challenge due to the combined demands of elaborate procedures for glycosyl donor and acceptor preparation and the requirements of regio- and stereoselectivity in glycoside bond formation. To streamline the preparation of complex oligosaccharides, one-pot multistep approaches for selective monosaccharide protection and oligosaccharide assembly are being pursued, which do not require intermediate workup and purification steps and hence speed up the process of chemical synthesis considerably.

Overview

In a one-pot glycosylation procedure, several glycosyl donors are allowed to react with an acceptor in the same reaction flask resulting in a single main oligosaccharide product. Thus several glycosylation steps are integrated into one synthetic operation to furnish the target oligosaccharide without the need of protecting group manipulations or isolation and purification of intermediates. The one-pot synthesis of an oligosaccharide was first demonstrated by Kahne and co-workers in 1993 for the synthesis of Ciclamycin trisaccharide.[23] They utilized the reactivity difference between a *p*-methoxyphenyl sulfoxide donor and an unsubstituted

phenylsulfoxide donor as well as higher nucleophilicity of free alcohol versus a silyl-protected alcohol (Scheme **1.2**).



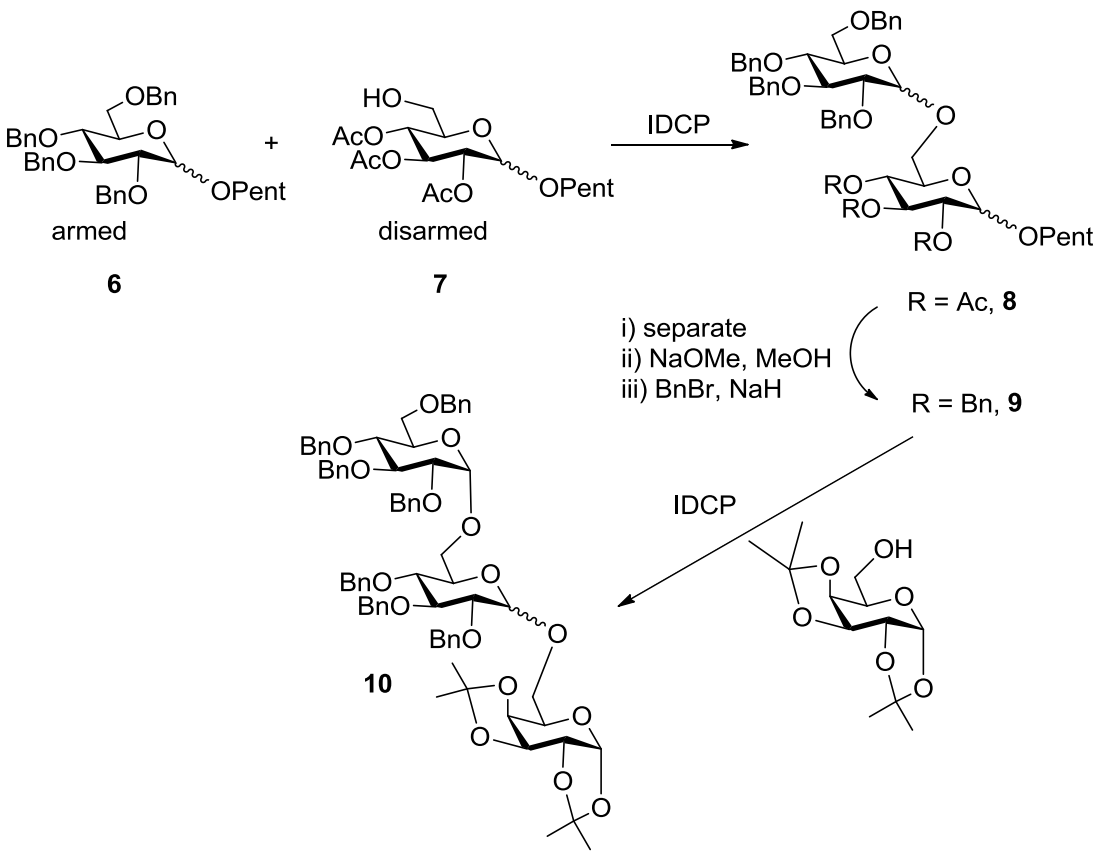
Scheme 1.2 First reported one-pot glycosylation

Thus, in presence of catalytic amount of triflic acid (TfOH), donor **2** is first activated and reacts with the readily available C-4 hydroxyl of compound **3** to form the disaccharide **4**. It is imperative to note that the less reactive silyl ether on compound **2** does not interfere in the reaction. The silyl ether on compound **4** then reacts with the less reactive donor **1** over a period of 45 min to give trisaccharide **5** as the major product in a yield of 25%. Less than 5% of the disaccharide coupling product of compound **1** and **3** was observed. Despite the relatively low yield of the coupling reactions, this methodology provided an efficient route to the synthesis of the Ciclamycin trisaccharide.

Since then, a variety of chemical strategies have been reported for one-pot oligosaccharide synthesis. These strategies can be broadly classified based on three major concepts: (i) Chemoselective activation, (ii) Orthogonal activation, and (iii) Pre-activation. Also of significance is the multi-enzyme one-pot glycosylation technique which will not be discussed in this chapter.

Chemoselective Activation

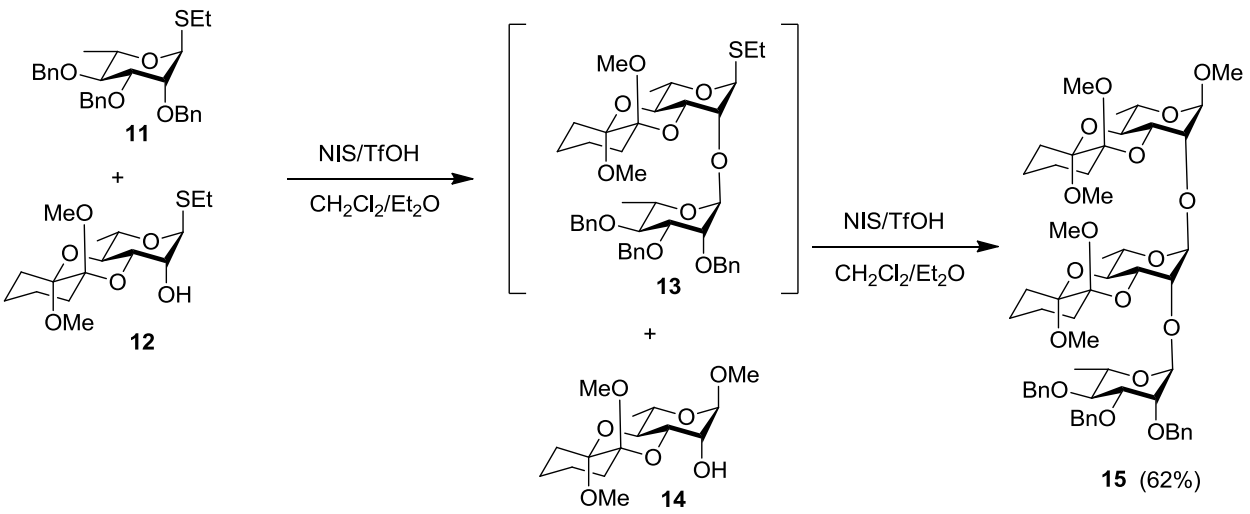
The chemoselective activation strategy exploits the differences in the reactivities of glycosyl donors and acceptors. It is well known that electron-donating groups on the hydroxyls of the donor saccharide increase the propensity of the leaving group to depart by stabilizing the positive charge generated in the transition state, thus arming the donor. On the contrary, electron-withdrawing groups deactivate the leaving group resulting in disarming of the donor.[24] In 1988, Fraser-Reid and co-workers demonstrated tuning the reactivity of *n*-pentenyl glycosides by using benzyl ethers and acetyl esters to obtain armed and disarmed donors, respectively (Scheme **1.3**). Thus, the coupling of compound **6** with **7** mediated by the mild activator iodonium dicollidine perchlorate (IDCP) afforded the disaccharide **8** in a yield of 62%.The disaccharide formed from the self-coupling of **7** was not observed. Thus, the *O*-acetyl groups of **7** effectively disarmed the pentenyl glycoside thereby ensuring that only **6** served as a glycosyl donor.



Scheme 1.3 Chemoselective glycosylations with *n*-pentenyl glycosides

The replacement of the acetyl esters with benzyl ethers armed the disaccharide, which was then employed as a donor using IDCP to obtain trisaccharide **10** in a yield of 60%. Later, this replacement of protecting groups was avoided by using a strong activator such as *N*-iodosuccinimide (NIS).[25]

The armed-disarmed approach was extended to other types of glycosyl donors, such as thioglycosides[26] and glycals.[27] A wide range of thioglycosyl donors and acceptors have been developed with differential reactivities to accomplish chemoselective activation.[28-30] Ley and co-workers prepared a trisaccharide unit which is derived from the common polysaccharide antigen of a group **B** *Streptococci* by a facile one-pot two-step synthesis (Scheme **1.4**).[31]

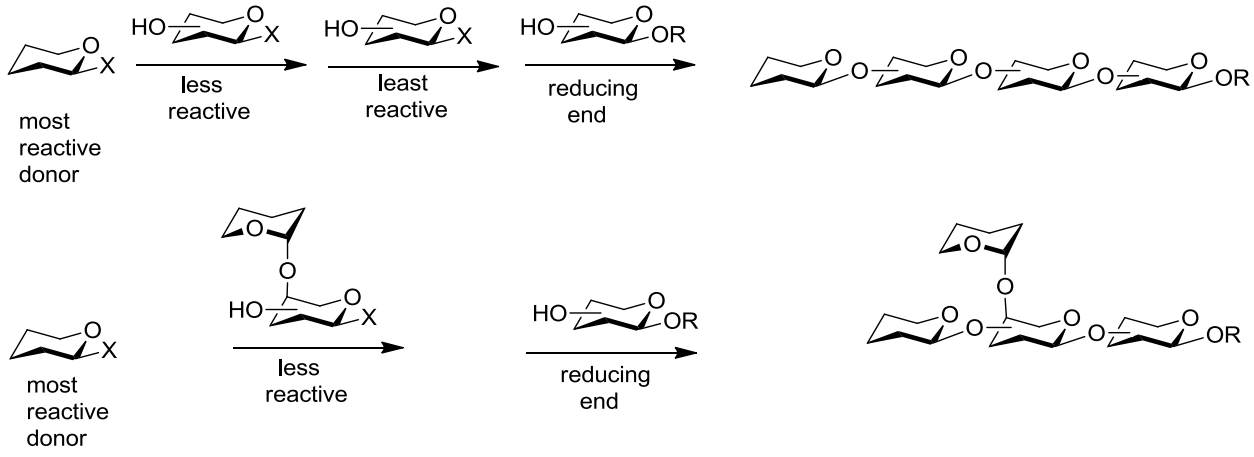


Scheme 1.4 Ley's one-pot synthesis of trisaccharide **15**.

The Ley group utilized a cyclohexane-1,2-diacetal protecting group to achieve a torsional disarming effect. The cyclic protecting group on **12** rendered the anomeric thioglycoside less reactive by hampering the formation of a planar oxo-carbenium ion. Thus, the benzylated activated thioglycosyl donor **11** was chemoselectively coupled with **12** to give disaccharide **13**. After addition of the acceptor **14**, the less reactive thioglycoside of **13** was activated to give trisaccharide **15** in a overall yield of 62%.

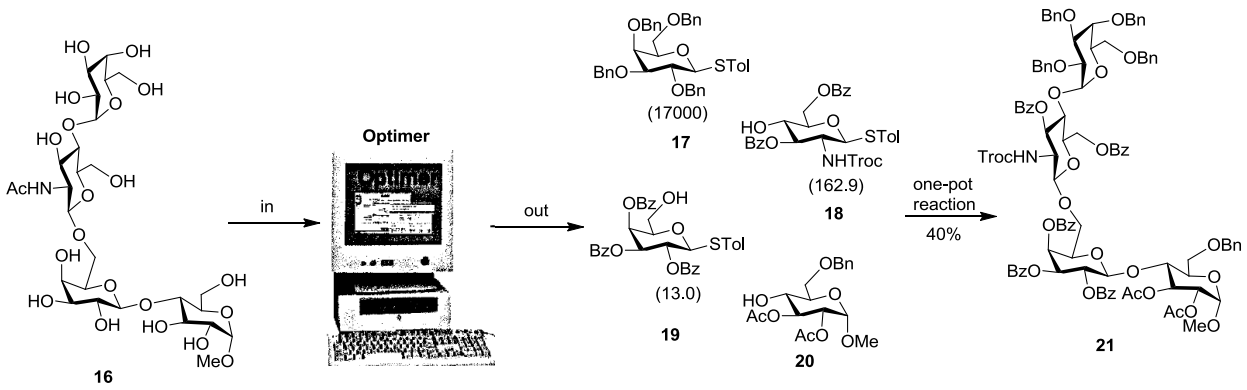
There has been a tremendous increase in the number of one-pot syntheses of complex oligosaccharides by exploiting large disparities in the reactivities of glycosyl donors. This created a need to quantify the reactivities of glycosyl donors. The Wong group has made a tremendous contribution to this effort. They have synthesized a number of thioglycoside building blocks and reported a general procedure for the quantitative measurement of relative reactivities of various glycosyl donors.[32] The relative reactivity values (RRVs) were established by performing competition experiments and monitoring them by HPLC. With the RRV database in hand, they generated a computer program called OptiMer, which can search the database to

identify the optimal combinations of glycosyl building blocks for a given oligosaccharide synthesis.



Scheme 1.5 Strategy for programmable one-pot synthesis.

In this approach, once the target oligosaccharide is entered, the program searches the database and presents the best combination of the available characterized building blocks. These building blocks can then be used sequentially starting from the most reactive donor, building towards the reducing end of the target oligosaccharide (Scheme **1.5**). This technique, known as programmable one-pot synthesis, has been successfully utilized for the synthesis of a number of oligosaccharides. For example, as outlined in scheme **1.6**,[32] once tetrasaccharide sequence **16** was entered, the program established the best available combination of building blocks (**17**, **18**, **19** and **20)**. These four building blocks were subjected to sequential one-pot glycosylations to give the protected oligosaccharide **21**. Programmable one-pot oligosaccharide synthesis has been applied with success for the synthesis of oligosaccharides including Globo-H hexasaccharide,[33] colon cancer antigen Le^y,[34] sLe^x,[35] fucosyl GM_1 oligosaccharide,[36] tumor associated antigen N3 minor octasaccharide,[37] and other biologically significant oligosaccharides.[38-40]

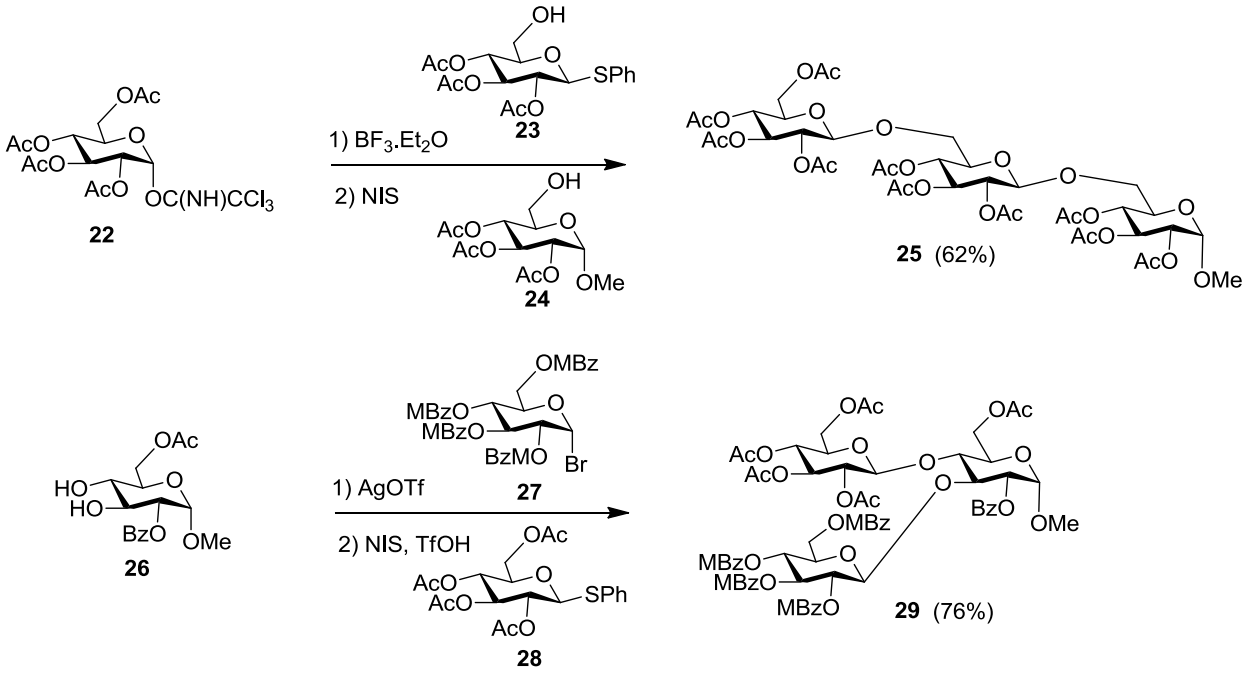


Scheme 1.6 Optimer-programmed one-pot synthesis.

Although numerous oligosaccharides have been synthesized employing the chemoselective one-pot synthesis, it should be noted that this type of reaction requires exceptionally high yields for individual steps and high chemoselectivity and diastereoselectivity.

Orthogonal Activation

The orthogonal one-pot glycosylation strategy is based on the selective activation of one leaving group over another. In this approach, different anomeric leaving groups are employed which can also act as protecting groups until they are subjected to the right activating conditions (i.e., halides, sulfides and trichloroacetimidates). One clear advantage of orthogonal over chemoselective activation is that it allows the condensation of the building blocks irrespective of the relative reactivities of the leaving groups.

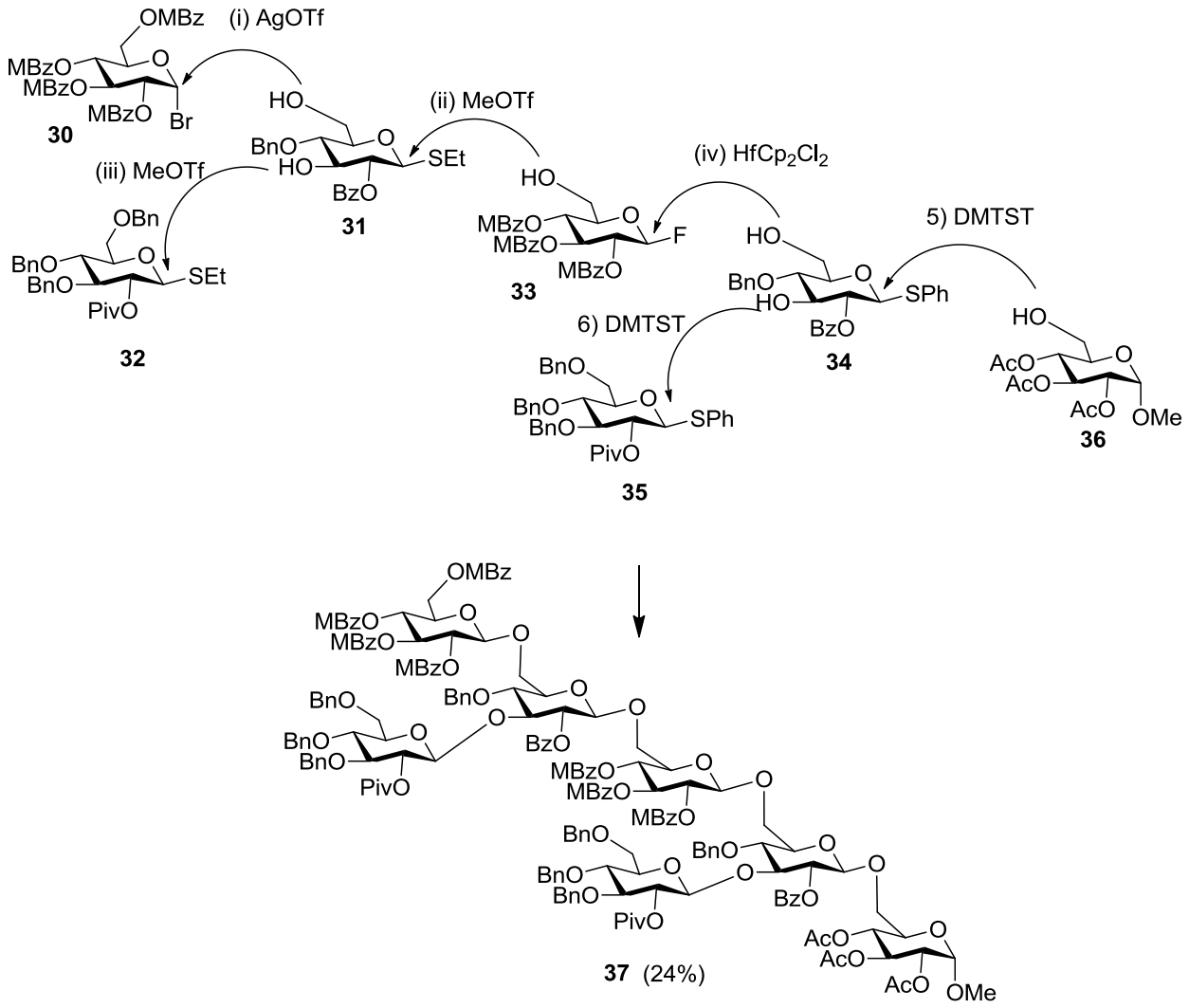


Scheme 1.7 Takahashi's one-pot synthesis of trisaccharides **25** and **29**.

Takahashi and co-workers were the first to report the orthogonal activation strategy for the one-pot synthesis of linear and branched trisaccharides **25**[41] and **29**,[42] respectively. Thus, trichloroacetimidate donor **22** was selectively activated in presence of thioglycoside **23** using BF_3-etherate as the promoter. After the first coupling, acceptor **24** was added followed by

activation of the newly formed disaccharide using NIS to give the single trisaccharide product **25** in an overall yield of 62%. Similarly, branched trisaccharide **29** was obtained by selective activation of the anomeric bromide **27** in presence of diol acceptor **26**. The coupling occurred at the more reactive C-3 position followed by addition of thioglycoside donor **28** and NIS to form a glycosidic linkage at the C-4 position.

Takahashi and co-workers extended the use of halides and thioglycosides in a tandem synthesis of a phytoalexin elicitor heptasaccharide (Scheme **1.8**).[43] The synthesis was carried out using seven different building blocks possessing four different leaving groups in six sequential glycosylation steps with high chemo- and regioselectivities. The assembly was performed using a parallel manual synthesizer (Quest 210). The reaction sequence entailed three steps: i) a regioselective glycosylation of glycosyl bromide **30** at the primary alcohol of disarmed thioglycoside **31** in presence of a secondary alcohol at C-3; ii) glycosylation of the resulting disaccharide containing a thioethyl leaving group with the primary alcohol of glycosyl fluoride **33**; iii) glycosylation of the secondary alcohol at C-3 of the newly formed trisaccharide with the thioglycoside **32** to give the tetrasaccharide intermediate. Successive glycosylations of the tetrasaccharide with building blocks **34**, **36** and **35**, respectively, gave the heptasaccharide **37** in a 24% overall yield with an average yield of 80% for each step. It is to be noted that all the glycosylations were stereoselectively giving β-glycosides due to neighboring group participation by C-2 functionalities.



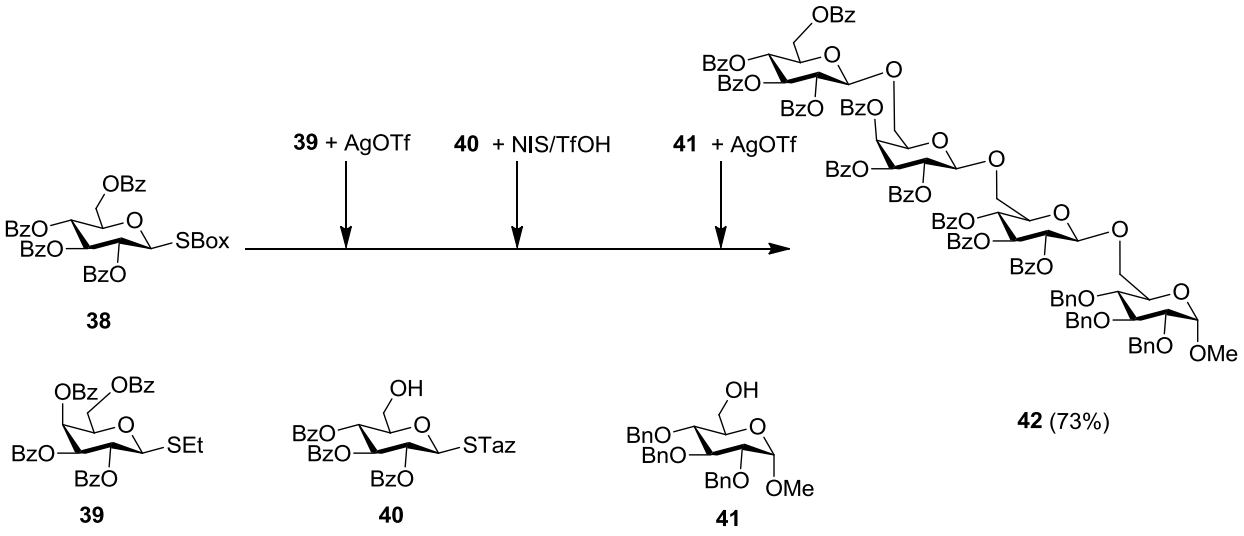
Scheme 1.8 One-pot synthesis of phytoalexin elicitor by Takahashi and co-workers.

Later, the Takahashi group extended the one-pot multi-step strategy to synthesis of sialic acid-containing branched and linear glycosyl amino acids.[44] They also reported a library of 54 linear and 18 branched trisaccharides synthesized by solution-phase one-pot multi-step glycosylations using a manual Quest 210 synthesizer.[45]

Ley and co-workers exploited orthogonal activation of fluoro-, seleno- and thioglycosides and the disarming effect of 1,2-diacetal protecting groups to achieve a one-pot synthesis of a pentamannoside.[46] The Fraser-Reid group reported a synthetic approach in which the tuning of

reactivity of *n*-pentenylglycosides, thioglycosides and trichloroacetimidate donors was achieved using metal triflates as activators to synthesize branched mannosyl trisaccharide.[47]

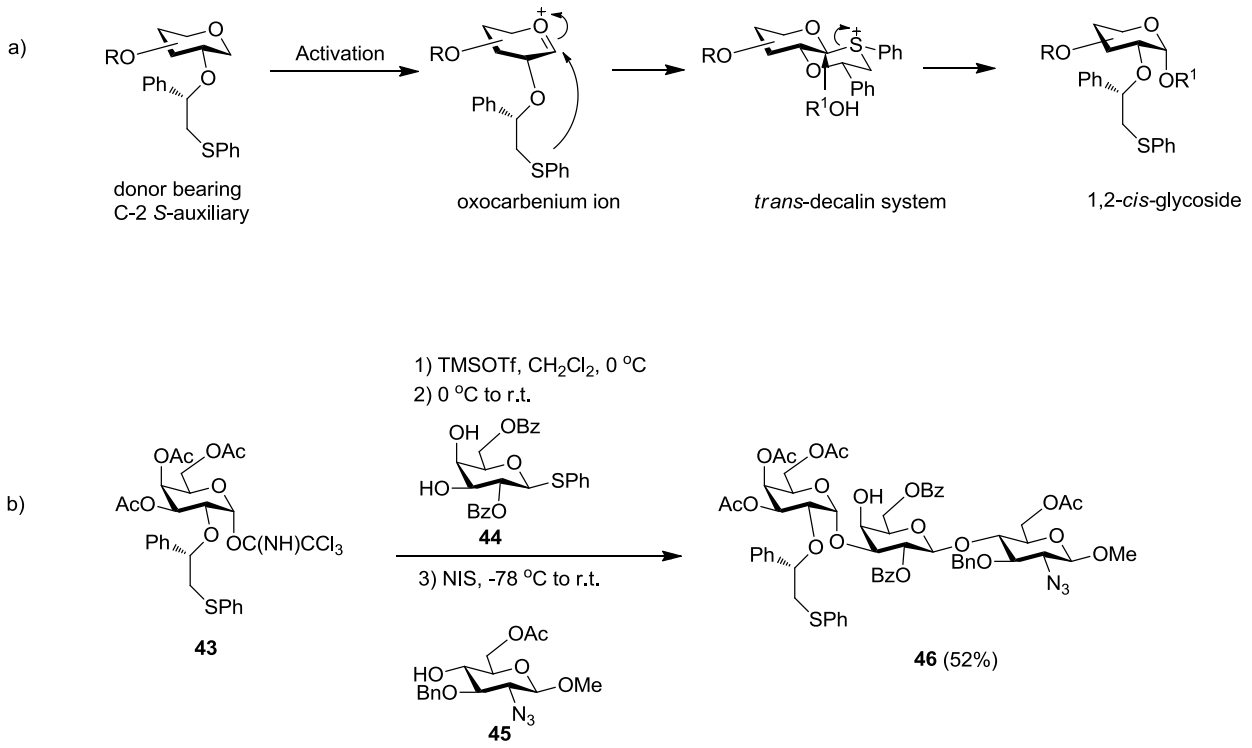
Recently, Demchenko and co-workers reported the one-pot synthesis of tetrasaccharide **42** by exploiting orthogonal activation of thioethyl (SEt), S-benzoxazolyl (SBox)[48] and S-thiazolyl (STaz)[49] glycosides (Scheme **1.9**).[50] While SBox and STaz could be activated using silver triflate, the SEt moiety was found to be stable under these conditions. Also, STaz was found to be stable under NIS/TfOH conditions used to activate the SEt leaving group. Thus, using the activation sequence of SBox + SEt + STaz, the one-pot synthesis of tetrasaccharide was achieved with a 73% overall yield.



Scheme 1.9 One-pot synthesis of tetrasaccharide by Demchenko and co-workers.

In most of the examples described above, 1,2-*trans* linkages were formed efficiently by exploiting neighboring group participation by a C-2 functionality. However, in cases where a 1,2-*cis* linkage is required, the application of one-pot glycosylation methodology is not efficient. In many cases, the optimal conditions required to install these linkages are not compatible with

the standard one-pot glycosylation conditions. The Boons group recently provided a solution to this problem.[10-11] They developed a (1*S*)-phenyl-2-(phenylsulfanyl)ethyl auxiliary which can be installed at the C-2 position of the sugar. The auxiliary forms a six membered 1,2-*trans* linked intermediate after activation of the anomeric leaving group, which blocks the *trans*-face of the sugar leaving only the *cis*-face open for the nucleophilic attack by the alcohol (Scheme **1.10a**). This novel strategy in combination with the traditional participating functionalities allowed the synthesis of trisaccharide **46** with both 1,2-*cis* and 1,2-*trans* linkages in excellent stereoselectivities and good overall yields (Scheme **1.10b**).



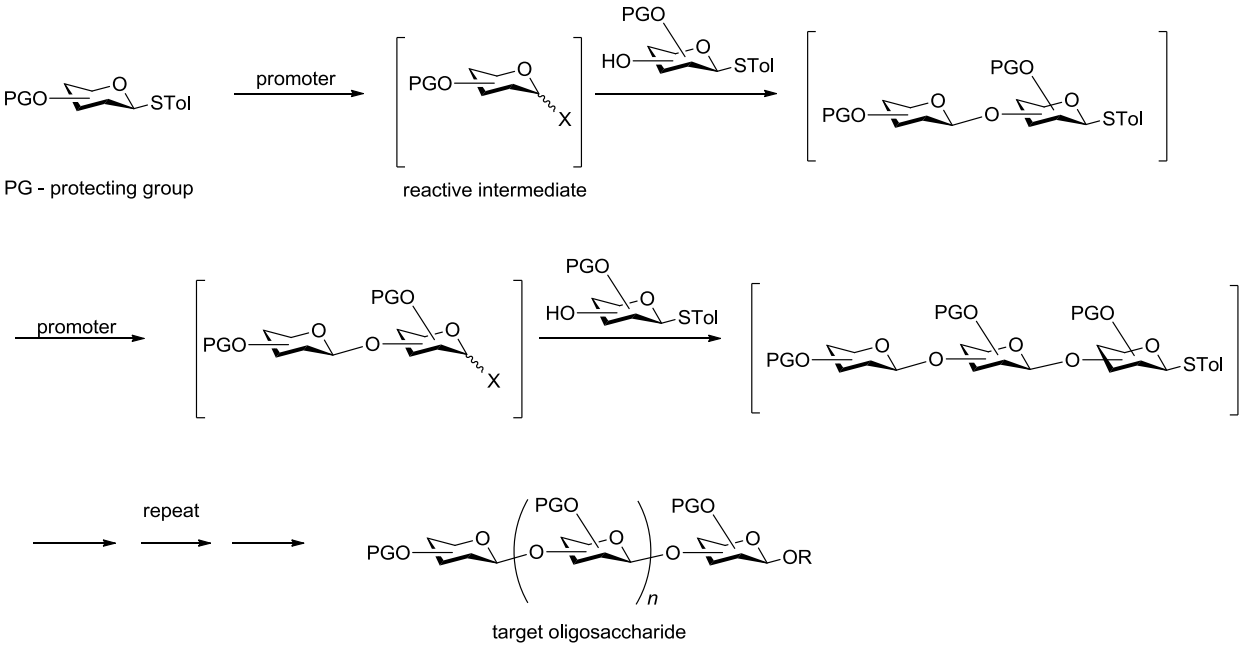
Scheme 1.10 One-pot synthesis of trisaccharide with 1,2-cis linkage by Boons and co-workers.

Although chemoselective and orthogonal glycosylation strategies have greatly influenced a wide variety of one-pot oligosaccharide syntheses, it remains a challenging enterprise. The design of these reactions requires extensive planning and careful selection of protecting groups.

Tuning the reactivities of the anomeric leaving groups by careful selection of the protecting groups requires excessive synthetic operations and thus affects the overall efficiency of the synthetic scheme.

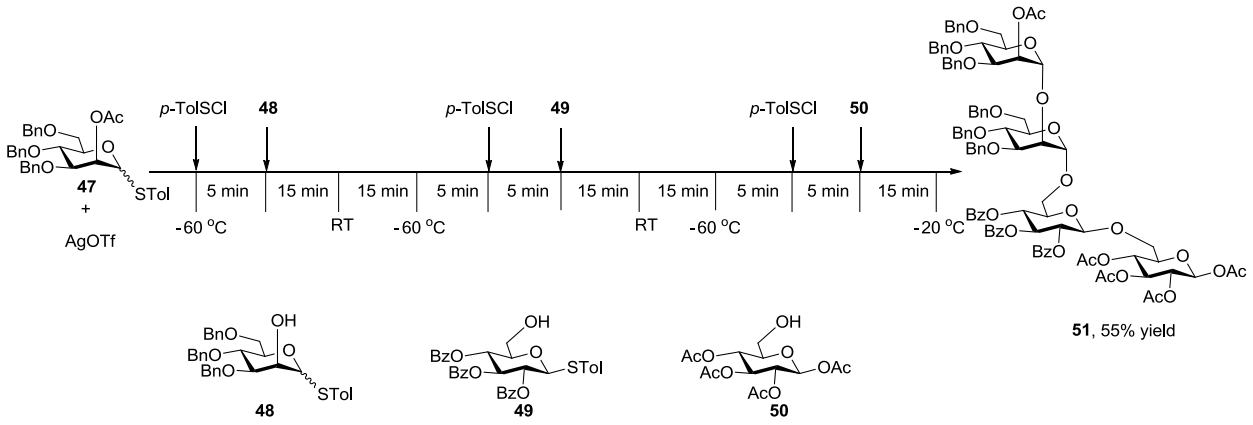
Pre-activation Strategy

Unlike chemoselective activation, where the reactivity of the donor is tuned by the arming or disarming effects, or orthogonal activation, where the activation is achieved by the inherent properties of the leaving groups, a pre-activation strategy, in principle, does not require the tuning of the building block reactivities or the selection of orthogonal leaving groups. Thus, pre-activation offers a good alternative to the above mentioned strategies.



Scheme 1.11 Iterative one-pot synthesis of oligosaccharides based on pre-activation strategy.

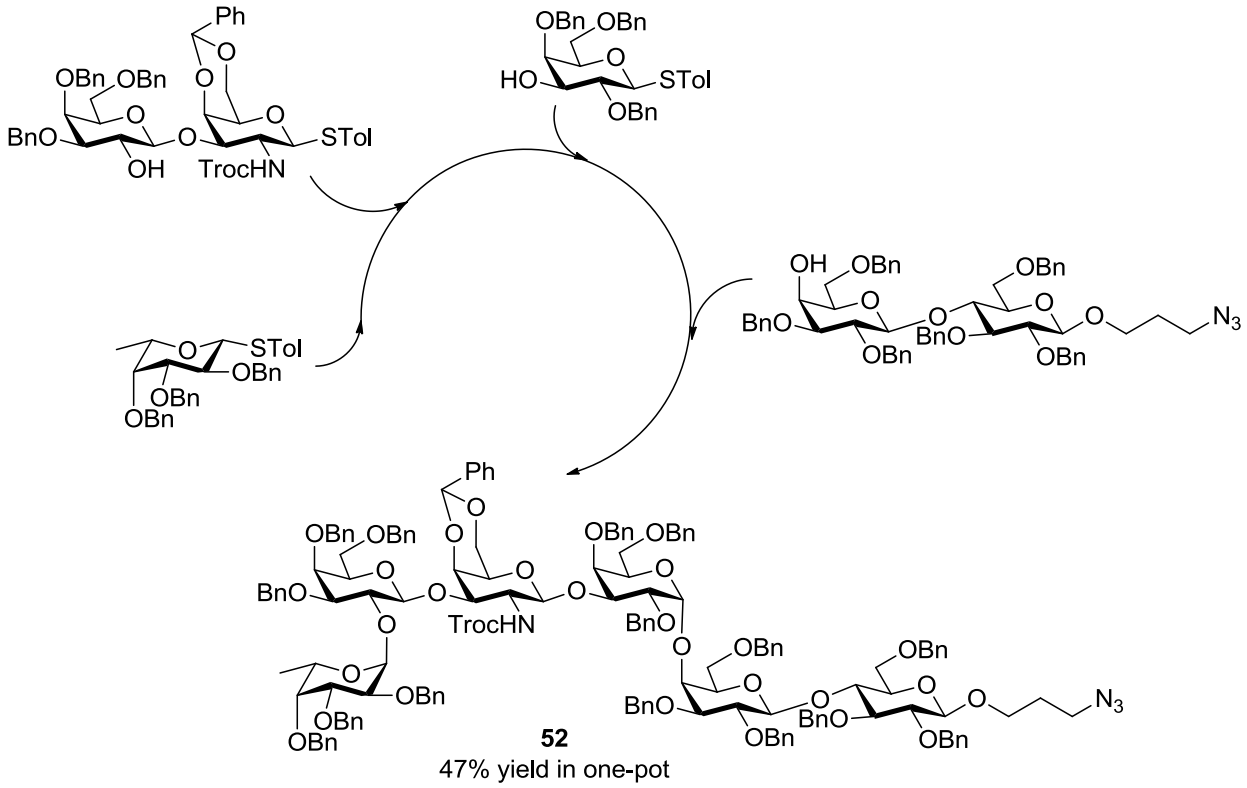
In 2004, Huang and co-workers developed a general one-pot method independent of the differential glycosyl donor reactivities.[51] This was achieved by pre-activating the donor (in absence of the acceptor), which generates a reactive intermediate (Scheme **1.11**). Upon addition of the second building block to the pre-activated donor, a disaccharide was obtained having the same leaving group at the anomeric center. Addition of fresh activator to the reaction flask activates the disaccharide for the next coupling. This process can be repeated multiple times in the same reaction vessel to achieve the target oligosaccharide. In order to accomplish a successful one-pot synthesis, the following requirements need to be met: 1) activation of the donor must be achieved by using stoichiometric amount of the activator; 2) the activator must be completely consumed without the formation of reactive byproducts to prevent activation of the second building block or interference in the glycosylation step; and 3) the intermediate generated after pre-activation of the donor must be stable until the addition of the acceptor, but should promptly react, furnishing high yields once the acceptor is added.



Scheme 1.12 Iterative one-pot synthesis of tetrasaccharide **51** by Huang and co-workers.

To prove their principle, Huang and co-workers synthesized tetrasaccharide **51** (Scheme **1.12**). They utilized *p*-toluenesulfenyl triflate as the activator and thiotolyl as the leaving group.

p-Toluenesulfenyltriflate was generated *in situ* from *p*-toluenesulfenylchloride and silver triflate. As shown in scheme **1.12**, preactivation of thiotolyl donor **47** was achieved by stoichiometric addition of *p*-toluenesulfenyltriflate, followed by the addition of glycosyl acceptor **48**. The reaction sequence was repeated with coupling of building blocks **49** with **50** to give tetrasaccharide **51** in a 55% overall yield.



Scheme 1.13 Iterative one-pot synthesis of Globo-H hexasaccharide.

As part of a program to establish the scope of their methodology, Huang and co-workers reported the synthesis of Globo-H hexasaccharide **52** containing both α- and β-anomeric linkages.[52] They performed a one-pot three-step glycosylation to obtain the target oligosaccharide in a 47% overall yield within 7 h. The use of only one type of glycosyl donor (i.e., *p*-tolyl thioglycosides), greatly simplified the synthetic design. Also, the pre-activation

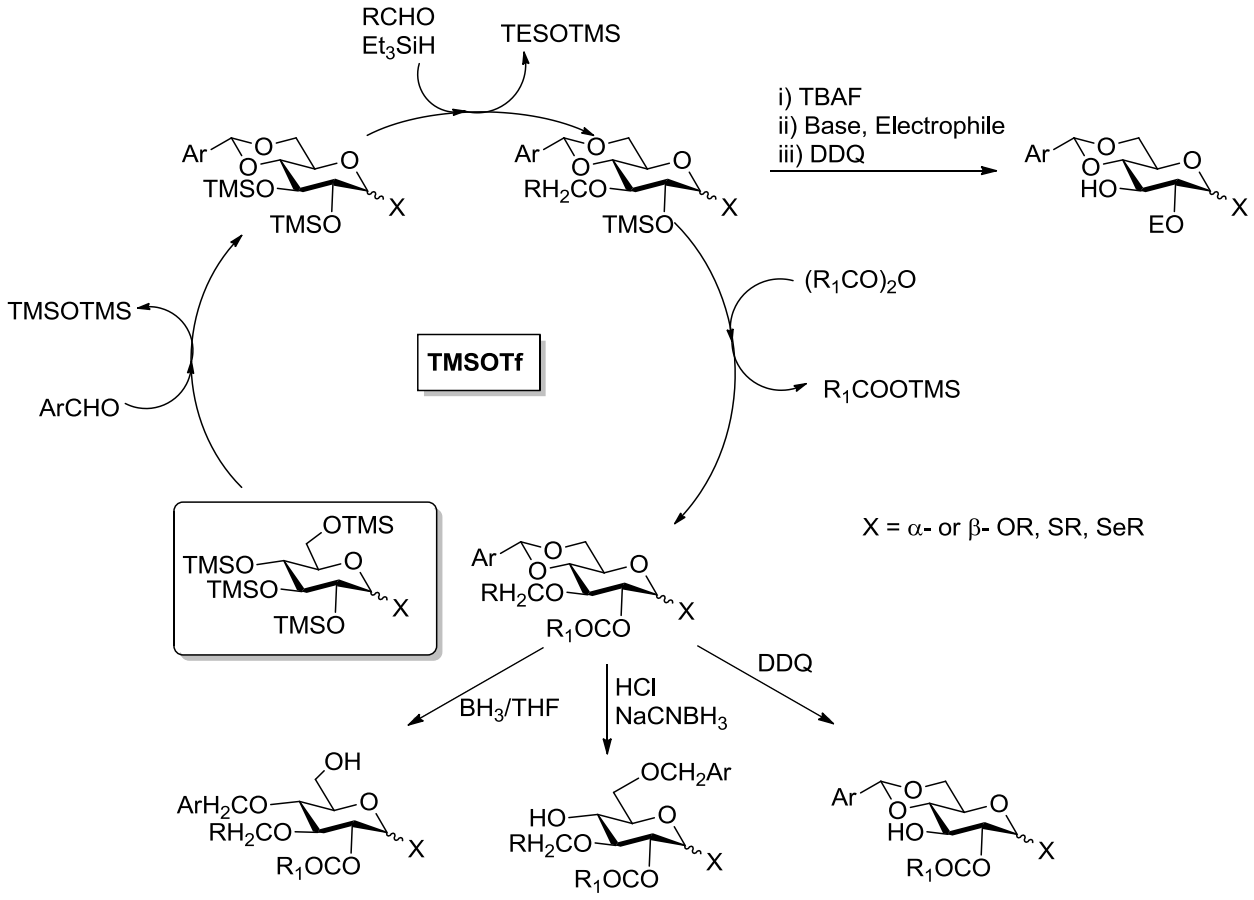
approach allowed them to perform glycosylations without the need to follow decreasing anomeric reactivities of donors, thus granting the freedom to choose the protecting groups that matched the donors and acceptors. This approach is a significant improvement over the reactivity-based one-pot strategy, which was demonstrated by assembly of chitotetroses[53] and hyaluronic acid oligosaccharides[54] in acceptable yields. However, it should be noted that this approach does suffer from limitations. In some cases, the pre-activated donor does not react with the acceptors, thus demanding improvements in the glycosylation conditions by the development of new and more powerful activators. Also the aglycon transfer phenomenon, which occurs occasionally due to the use of thioglycosides with the same anomeric reactivities, results in the regeneration of the donor and destruction of the acceptor. Efforts have been made to minimize this aglycon transfer of thioglycosides; however, more investigations into the nature of this side reaction and its application to pre-activation based one-pot reactions are required.

One-Pot Protection of Monosaccharides

To truly streamline the process of oligosaccharide assembly, it is imperative to develop procedures to obtain monosaccharide building blocks relatively quickly. Thus, protection of monosaccharide building blocks with a strategically positioned hydroxyl group or an appropriate leaving group at the anomeric center is a formidable challenge. In 2007, Hung and co-workers reported a parallel combinatorial and highly regioselective method to protect the individual hydroxyl groups of glucose *via* a one-pot strategy (Scheme **1.14**).[55-56] They selected glucose derivatives bearing anomeric groups such as α- or β-OR, -SR and –SeR and TMS-protected C-2, C-3, C-4 and C-6 hydroxyl groups. They exploited the reactivity differences between substituted

and unsubstituted benzyl ethers. Using a single catalyst system (TMSOTf), the desired building blocks with differential protecting group pattern were obtained. The per-*O*-silylation provided the required TMS-protected sugar, while enhancing the solubility of the monosaccharide in organic solvents. The general protocol involved four steps: 1) protection of the C-4 and C-6 alcohols as an arylidene acetal, followed by regioselective reductive arylmethyl ether formation at the C-3 position to give C-2 alcohols; 2) the reactions in step 1 were followed by either acetylation or etherification at the C-2 position to give either a participating or non-participating functionality; 3) the reactions in step 1 and step 2 were performed to obtain a *p*-methoxybenzyl or 2-naphthylmethyl ether at C-3 followed by removal of the C-3 protection by DDQ; and 4) the reactions in step 1 and step 2 were followed by regioselective ring opening of arylidene acetal to form either C-4 or C-6 alcohols.

More than 70 different monosaccharide building blocks for glycosylations were prepared using this one-pot methodology. In the same year, Francais *et al*.[57] also reported the one-pot regioselective protection of glucosides using $Cu(OTf)_2$ as the catalyst.



Scheme 1.14 TMSOTf-catalysed one-pot protection of carbohydrates.

Thus, considerable progress has been made for the streamlining of the synthesis of oligosaccharides. One-pot glycosylation and monosaccharide preparation has contributed considerably to this effect. However, there are still limitations that need to be conquered to achieve wider applicability of these technologies. In case of one-pot synthesis of branched oligosaccharides, glycosylation is performed using an acceptor with a diol having differential reactivities. There is always a risk of glycosylation at the less reactive alcohol resulting in the undesired product. In chapter II of this thesis we have demonstrated a new class of one-pot oligosaccharide synthesis that tries to address some of these issues.

1.7 P-Selectin Glycoprotein Ligand-1 (PSGL-1)

Structure and Function of PSGL-1

Although there are many ligands for selectins, P-selectin glycoprotein ligand-1 (PSGL-1), located on leukocytes, has been shown to be one of the most important ligands for selectins.[58-60] PSGL-1 is a dimeric, mucin-type 120kDa glycoprotein ligand (Figure **1.3**).[61] It is expressed on the surfaces of monocytes, lymphocytes, granulocytes and in some $CD34^+$ stem cells. It is a 402 residue type **I** membrane protein, containing a 70 residue cytoplasmic domain, a single 24 residue hydrophobic transmembrane domain and a 308 residue Ser/Thr/Pro-rich extracellular domain.

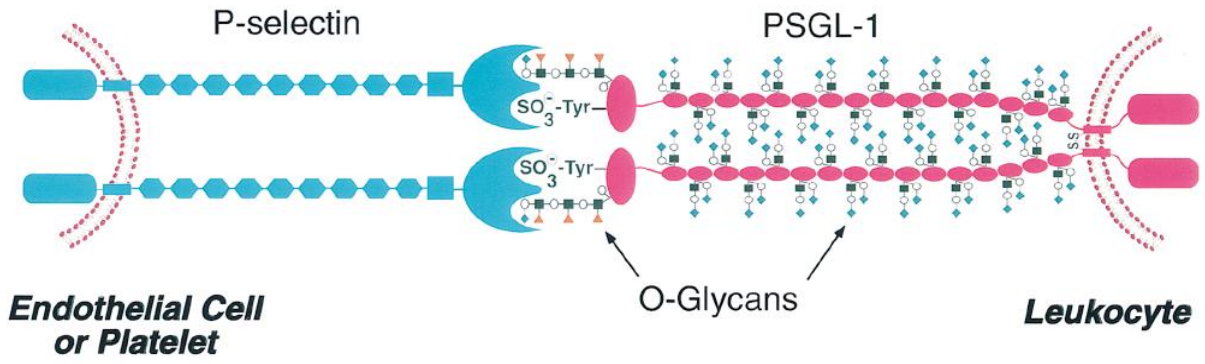


Figure 1.3 Model for interactions between P-selectin and PSGL-1.

Its extracellular domain contains one or more *N*-linked glycans, and many sialylated *O*-linked oligosaccharides. It also contains an 18 amino acid signal sequence spanning residues 42 to 59 at its extreme *N*-terminus. This signal sequence has an *O*-linked oligosaccharide and three tyrosine sulfate residues. The *N*-terminal region of PSGL-1 has been at the focal point of research on cell adhesion molecules, as it contains the binding regions for the selectins. P-selectin binds to the *N*-terminus of PSGL-1 by interacting with the stereospecifically clustered

three tyrosine sulfate residues and a nearby core-2-*O*-glycan with the sialyl Lewis^x (SLe^x) epitope.[62]

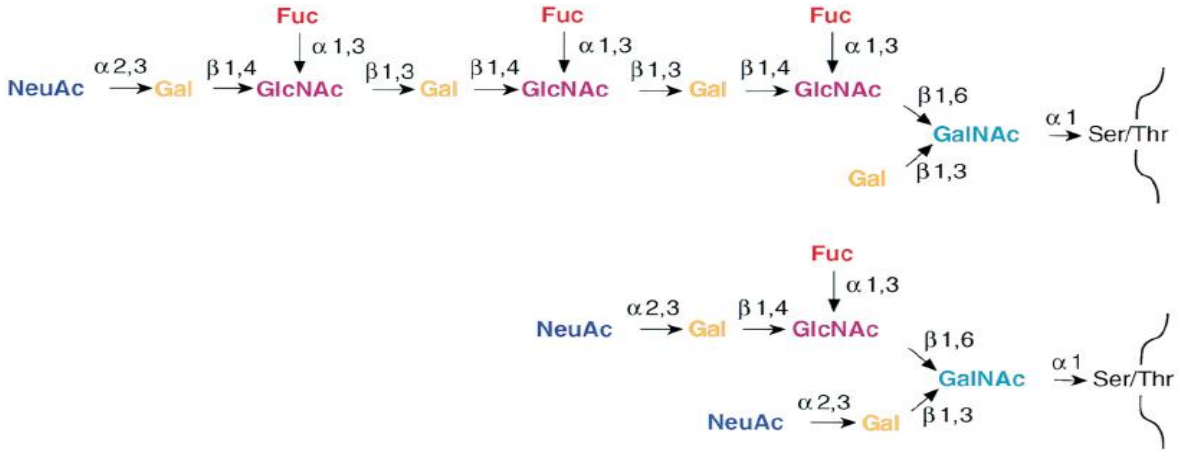


Figure 1.4 Structure of the two fucosylated, core-2 *O*-glycans on PSGL-1 derived from HL-60 cells.

The α2,3-linked sialic acid and α1,3-linked fucoside of SLe^x are important modifications required for binding with P-selectin. While L-selectin binding occurs in a similar fashion, E-selectin binding is sulfate-independent, requiring SLe^x and glycosylations of PSGL-1 by α-(1,3)-fucosyl transferases.

Therapeutic Potential

PSGL-1 binding with the selectins has been implicated in a wide variety of diseases. The differences in glycosylation patterns, affinity binding and qualitative and quantitative expressions of PSGL-1 between various leukocyte subtypes suggest that blocking PSGL-1 should not have a broad negative impact on the physiology and host defense. Thus, inhibition of leukocyte migration by blocking PSGL-1 represents a promising therapeutic approach towards diseases in which inflammation has a destructive role (e.g.; ischemia, venous thrombosis,

hemorrhage, atherosclerosis, asthma, skin inflammation and autoimmune diseases).[63] In this respect, a recombinant truncated form of a PSGL-1 immunoglobulin fusion protein has already demonstrated effectiveness as such an inhibitor.[64-67] Thus, a consistent amount of data suggests that blocking selectin dependent leukocyte adhesion may offer novel therapeutic strategies for modulating inflammatory responses in a broad spectrum of diseases.

Synthesis

Since the realization of the therapeutic potential of PSGL-1 in inflammatory processes, several groups have attempted to synthesize PSGL-1 glycopeptides and analogous structures. As discussed above, the binding of P-selectin to PSGL-1 requires the *N*-terminus of PSGL-1 with an *O*-linked sialyl Lewis^x glycan at Thr57 and one or more *O*-sulfated tyrosine residues at Tyr46, Tyr48, and Tyr51.[61] In 1999, Cummings and co-workers became the first to report an enzymatic procedure for the synthesis of truncated forms of PSGL-1 glycopeptides (Figure **1.5**).[16]

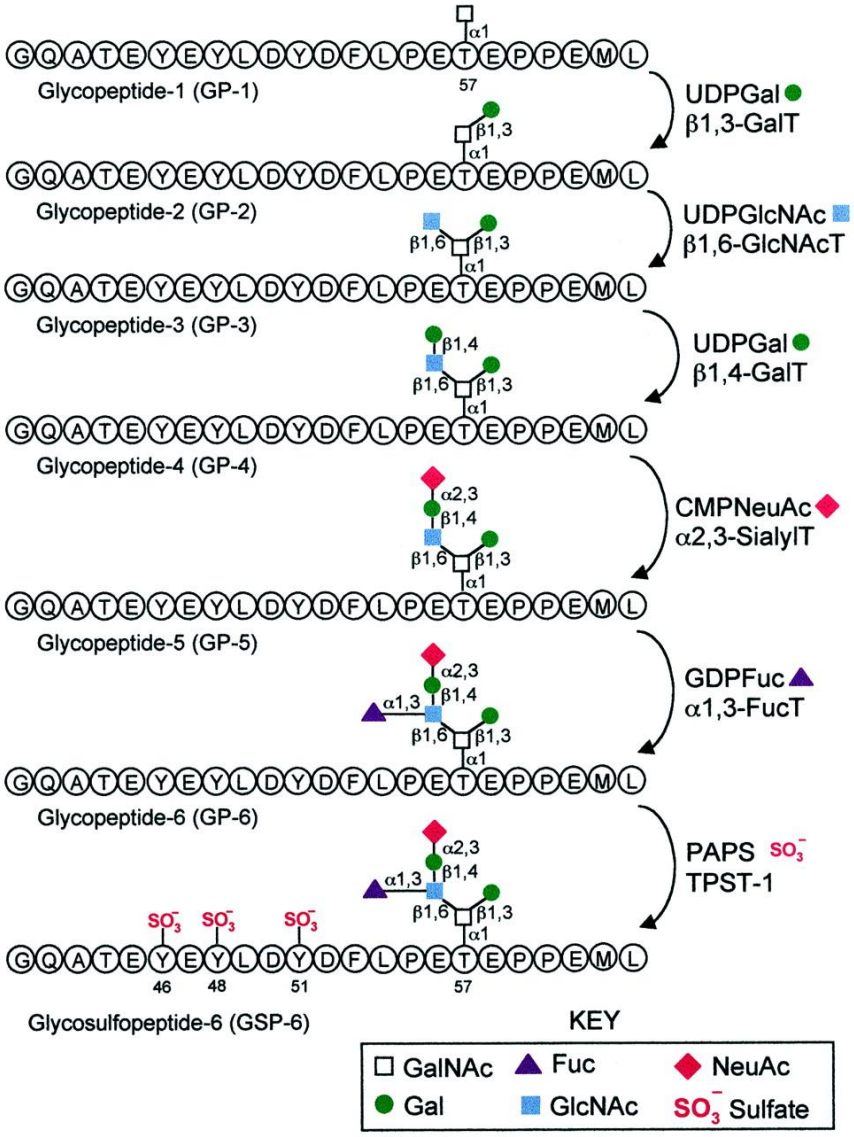
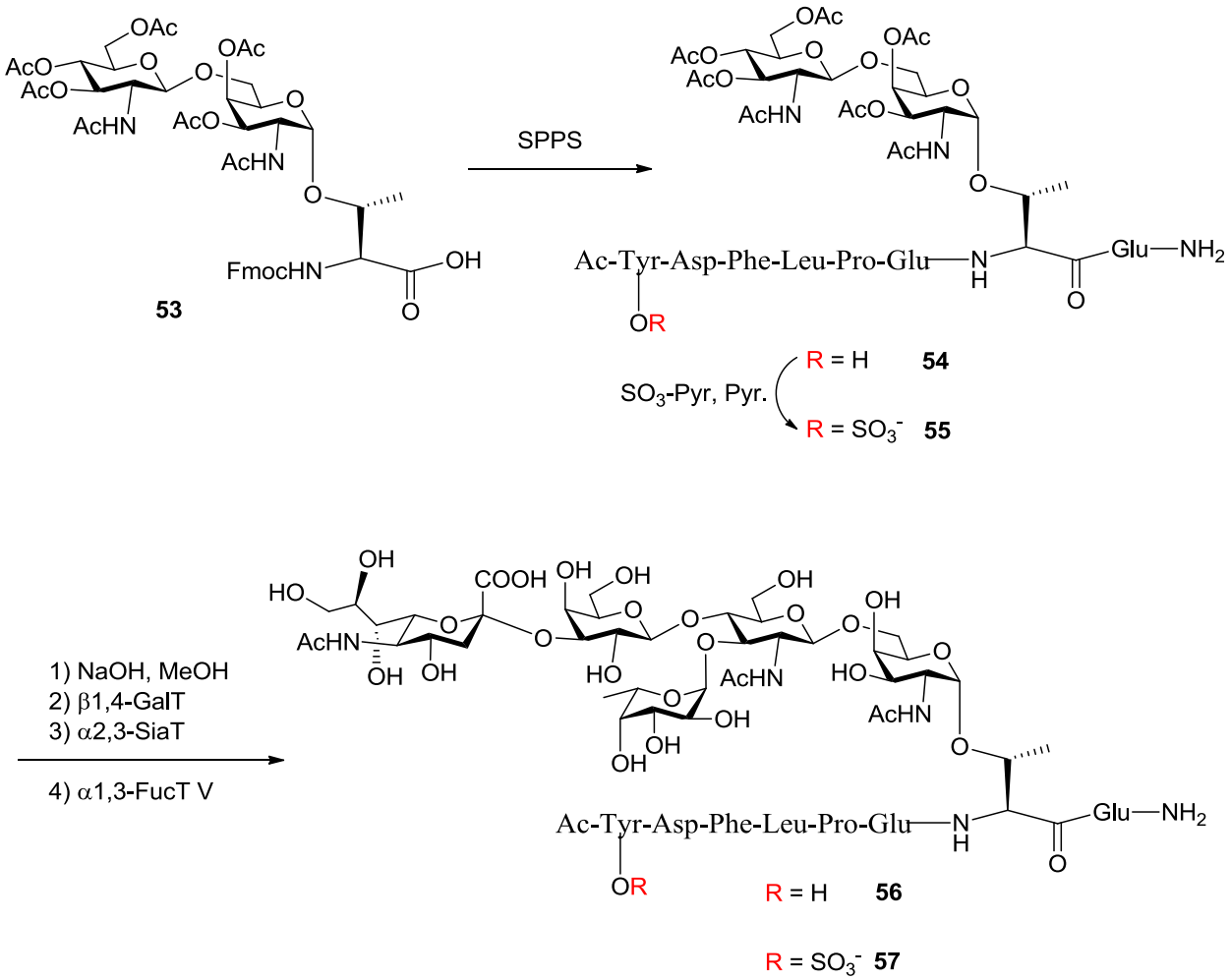


Figure 1.5 Enzymatic synthesis of PSGL-1 derived glycosulfopeptide.

They synthesized a PSGL-1 glycopeptide with a 23-amino acid sequence using Fmoc-based solid phase peptide synthesis. The tri-*O*-acetyl-GalNAc-α-Fmoc-Thr residue was incorporated into the peptide during the solid phase synthesis. Using appropriate glycosyl transferases, a sialyl Lewis^{x} hexasaccharide was generated on the GalNAc residue and finally enzymatic sulfation was achieved using TPST-1 (tyrosylprotein sulfotransferase) and PAPS

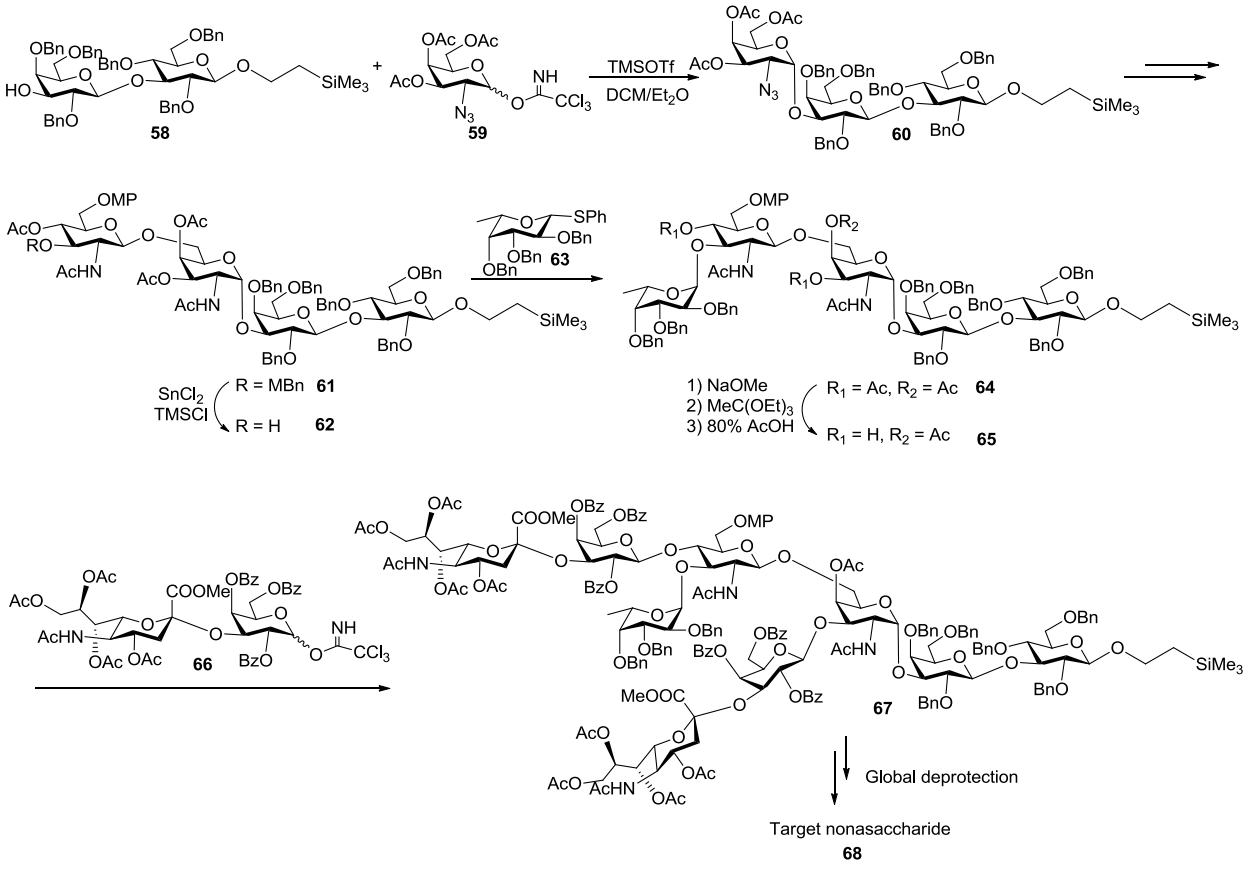
(adenosine 3'-phosphate 5'-phosphosulfate). The glycopeptide exhibited tight binding to the immobilized P-selectin with a K_d value of 350nm.



Scheme 1.15 Chemoenzymatic synthesis of PSGL-1 fragments by Wong and co-workers.

Later in 2000, Wong and co-workers reported a chemoenzymatic strategy to achieve the synthesis of a smaller fragment of PSGL-1 glycopeptide (Scheme **1.15**).[68-69] First, the disaccharide-threonine conjugate **53** was incorporated into the Fmoc-based solid-phase peptide synthesis of the unsulfated and sulfated glycopeptides **54** and **55**, respectively. After deacetylation of the glycopeptide, they studied glycosyltransferase-catalysed extensions of the

O-glycan. They used recombinant bovine β-1,4-galactosyltransferase, recombinant human α-1,3-fucosultransferase and rat liver α-2,3-sialyltransferase to extend the glycan. Their results indicated that while the unsulfated glycopeptide was accepted as a good substrate, sulfation of tyrosine had a marked impact on the activity of glycosyltransferases especially the α-2,3-sialyltransferase.[70] The application of a sialyltransferase over-expressed from *Escherichia coli* proved to be better with sulfated glycopeptide. Later the procedure was further streamlined *via* a multi-enzyme one-pot strategy to obtain the glycopeptides **56** and **57**.



Scheme 1.16 Chemical synthesis of spacer-armed *O*-glycan on PSGL-1 by Kiso and co-workers.

Although, chemoenzymatic approaches are efficient for the preparation of PSGL-1 glycopeptides, they are ineffective for obtaining substantial amounts of pure material for enhanced biological studies. The problems of this approach include difficulties of preparing sufficient quantities of glycosyltransferases, which often require a eukaryotic cell expression system and the need of expensive sugar nucleotides or the use of a complicated *in-situ* recycling system. Furthermore, the high selectivity of glycosyltransferases also complicates the preparation of analogs that may exhibit more desirable pharmacological properties.

Kiso and co-workers demonstrated a purely chemical method to obtain the *O*-glycan part of the PSGL-1 glycopeptide in 2000 (Scheme **1.16**).[71] They reported the synthesis of a novel nonasaccharide **68** containing the sialyl Lewis^x determinant in the spacer-armed core-2 structure. The formation of the α-linkage between the GalNAc residue and the spacer arm **58** was achieved by using tricholroacetimidate donor **59** in a 1:1 mixture of DCM and diethyl ether at 0 ^oC. The use of *p*-methoxybenzyl group as a temporary protecting group made it possible to incorporate the α-1,3-fucoside *via* an NIS promoted glycosylation. Finally, deacetylation of the pentasaccharide followed by 3,4-orthoester formation at the GalNac residue and subsequent opening of the orthoester, revealed the two alcohols on compound **65**. The alcohols were then glycosylated using sialyl disaccharide **66** to give the nonasaccharide **67**. Finally, global deprotection of the oligosaccharide gave the target deprotected nonasaccharide **68**.

With the current progress in chemical oligosaccharides synthesis, several laboratories are striving to achieve a more practical approach to obtain the PSGL-1 oligosaccharide and its analogues. Recently, Kunz and co-workers reported the chemical synthesis of properly protected glycopeptide recognition domain of PSGL-1.[72] Although the problems such as anomeric selectivity and acid-base sensitivity were addressed, this approach suffered from poor

regioselectivities. Also interconversion of protecting groups at late stage in the synthesis affected the overall yield of the product. In the chapters II and III of this dissertation, new methods are described to solve some of these issues. Chapter II describes a highly convergent synthesis of the *O*-glycan of PSGL-1 using orthogonal, chemoselective one-pot glycosylation reactions as key steps. Chapter III describes a new approach toward the synthesis of glycopeptide mimics of PSGL-1 to obtain a variety of analogues with little time and effort.

1.8 References

1. Holgersson, J.; Gustafsson, A.; Breimer, M. E., Characteristics of protein-carbohydrate interactions as a basis for developing novel carbohydrate-based antirejection therapies. *Immunol Cell Biol* **2005,** *83* (6), 694-708.
2. Karlsson, K. A., Pathogen-host protein-carbohydrate interactions as the basis of important infections. *Adv Exp Med Biol* **2001,** *491*, 431-443.
3. Varki, A., Biological Roles of Oligosaccharides - All of the Theories Are Correct. *Glycobiology* **1993,** *3* (2), 97-130.
4. Hrachovinova, I.; Cambien, B.; Hafezi-Moghadam, A.; Kappelmayer, J.; Camphausen, R. T.; Widom, A.; Xia, L. J.; Kazazian, H. H.; Schaub, R. G.; McEver, R. P.; Wagner, D. D., Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med* **2003,** *9* (8), 1020-1025.
5. Ley, K., The role of selectins in inflammation and disease. *Trends Mol Med* **2003,** *9* (6), 263-8.
6. http://bme.virginia.edu/ley/.
7. Geng, J.-G.; Chen, M.; Chou, K.-C., P-selectin cell adhesion molecule in inflammation, thrombosis, cancer growth and metastasis. *Curr. Med. Chem.* **2004,** *11* (16), 2153-2160.
8. McNicol, A.; Israels, S. J., Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovascular & Hematological Disorders: Drug Targets* **2008,** *8* (2), 99-117.
9. Platt, O. S., Sickle cell anemia as an inflammatory disease. *Journal of Clinical Investigation* **2000,** *106* (3), 337-338.
10. Kim, J. H.; Yang, H.; Boons, G. J., Stereoselective glycosylation reactions with chiral auxiliaries. *Angew Chem Int Edit* **2005,** *44* (6), 947-949.

11. Kim, J. H.; Yang, H.; Park, J.; Boons, G. J., A general strategy for stereoselective glycosylations. *J Am Chem Soc* **2005,** *127* (34), 12090-12097.

12. Boons, G.-J.; Hale, K., *Organic synthesis with carbohydrates*. Sheffield Academic Press ; Blackwell Science: Sheffield, England
Malden, MA U.S.A., 2000; p xi, 336 p.

13. Huang, S. S.; Yu, H.; Chen, X., Disaccharides as sialic acid aldolase substrates: Synthesis of disaccharides containing a sialic acid at the reducing end. *Angew Chem Int Edit* **2007,** *46* (13), 2249-2253.

14. Yu, H.; Chokhawala, H. A.; Huang, S. S.; Chen, X., One-pot three-enzyme chemoenzymatic approach to the synthesis of sialosides containing natural and non-natural functionalities. *Nat Protoc* **2006,** *1* (5), 2485-2492.

15. Muthana, S.; Yu, H.; Huang, S.; Chen, X., Chemoenzymatic synthesis of size-defined polysaccharides by sialyltransferase-catalyzed block transfer of oligosaccharides. *J Am Chem Soc* **2007,** *129* (39), 11918-+.

16. Leppanen, A.; Mehta, P.; Ouyang, Y. B.; Ju, T. Z.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D., A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. *J Biol Chem* **1999,** *274* (35), 24838-24848.

17. Shaikh, F. A.; Withers, S. G., Teaching old enzymes new tricks: engineering and evolution of glycosidases and glycosyl transferases for improved glycoside synthesis. *Biochem Cell Biol* **2008,** *86* (2), 169-177.

18. Frechet, J. M.; Schuerch, C., Solid-Phase Synthesis of Oligosaccharides .1. Preparation of Solid Support - Poly[-Para (-Propen-3-Ol-L-Yl)Styrene]. *J Am Chem Soc* **1971,** *93* (2), 492-&.

19. Seeberger, P. H., Automated oligosaccharide synthesis. *Chem Soc Rev* **2008,** *37* (1), 19-28.

20. Seeberger, P. H.; Haase, W. C., Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chem Rev* **2000,** *100* (12), 4349-+.

21. Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Automated solid-phase synthesis of oligosaccharides. *Science* **2001,** *291* (5508), 1523-1527.

22. Love, K. R.; Seeberger, P. H., Automated solid-phase synthesis of protected tumor-associated antigen and blood group determinant oligosaccharides. *Angew Chem Int Edit* **2004,** *43* (5), 602-605.

23. Raghavan, S.; Kahne, D., A One-Step Synthesis of the Ciclamycin Trisaccharide. *J Am Chem Soc* **1993,** *115* (4), 1580-1581.

24. Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraserreid, B., Armed and Disarmed N-Pentenyl Glycosides in Saccharide Couplings Leading to Oligosaccharides. *J Am Chem Soc* **1988,** *110* (16), 5583-5584.

25. Fraserreid, B.; Udodong, U. E.; Wu, Z. F.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R., N-Pentenyl Glycosides in Organic-Chemistry - a Contemporary Example of Serendipity. *Synlett* **1992,** (12), 927-942.

26. Veeneman, G. H.; Vanboom, J. H., An Efficient Thioglycoside-Mediated Formation of Alpha-Glycosidic Linkages Promoted by Iodonium Dicollidine Perchlorate. *Tetrahedron Lett* **1990,** *31* (2), 275-278.

27. Friesen, R. W.; Danishefsky, S. J., On the Use of the Haloetherification Method to Synthesize Fully Functionalized Disaccharides. *Tetrahedron* **1990,** *46* (1), 103-112.

28. Boons, G. J.; Grice, P.; Leslie, R.; Ley, S. V.; Yeung, L. L., Dispiroketals in Synthesis .5. A New Opportunity for Oligosaccharide Synthesis Using Differentially Activated Glycosyl Donors and Acceptors. *Tetrahedron Lett* **1993,** *34* (52), 8523-8526.

29. Geurtsen, R.; Holmes, D. S.; Boons, G. J., Chemoselective glycosylations .2. Differences in size of anomeric leaving groups can be exploited in chemoselective glycosylations. *J Org Chem* **1997,** *62* (23), 8145-8154.

30. Boons, G. J.; Geurtsen, R.; Holmes, D., Chemoselective Glycosylations .1. Differences in Size of Anomeric Leaving Groups Can Be Exploited in Chemoselective Glycosylations. *Tetrahedron Lett* **1995,** *36* (35), 6325-6328.

31. Ley, S. V.; Priepke, H. W. M., Cyclohexane-1,2-Diacetals in Synthesis .2. A Facile One-Pot Synthesis of a Trisaccharide Unit from the Common Polysaccharide Antigen of Group-B Streptococci Using Cyclohexane-1,2-Diacetal (Cda) Protected Rhamnosides. *Angewandte Chemie-International Edition in English* **1994,** *33* (22), 2292-2294.

32. Zhang, Z. Y.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C. H., Programmable one-pot oligosaccharide synthesis. *J Am Chem Soc* **1999,** *121* (4), 734-753.

33. Burkhart, F.; Zhang, Z. Y.; Wacowich-Sgarbi, S.; Wong, C. H., Synthesis of the Globo H hexasaccharide using the programmable reactivity-based one-pot strategy. *Angew Chem Int Edit* **2001,** *40* (7), 1274-+.

34. Mong, K. K. T.; Wong, C. H., Reactivity-based one-pot synthesis of a Lewis Y carbohydrate hapten: A colon-rectal cancer antigen determinant. *Angew Chem Int Edit* **2002,** *41* (21), 4087-4090.

35. Zhang, Z. Y.; Niikura, K.; Huang, X. F.; Wong, C. H., A strategy for the one-pot synthesis of sialylated oligosaccharides. *Can J Chem* **2002,** *80* (8), 1051-1054.

36. Mong, T. K. K.; Lee, H. K.; Duron, S. G.; Wong, C. H., Reactivity-based one-pot total synthesis of fucose GM(1) oligosaccharide: A sialylated antigenic epitope of small-cell lung cancer. *P Natl Acad Sci USA* **2003,** *100* (3), 797-802.

37. Lee, J. C.; Wit, C. Y.; Apon, J. V.; Siuzdak, G.; Wong, C. H., Reactivity-based one-pot synthesis of the tumor-associated antigen N3 minor octasaccharide for the development of a photocleavable DIOS-MS sugar array. *Angew Chem Int Edit* **2006,** *45* (17), 2753-2757.

38. Mong, T. K. K.; Huang, C. Y.; Wong, C. H., A new reactivity-based one-pot synthesis of N-acetyllactosamine oligomers. *J Org Chem* **2003,** *68* (6), 2135-2142.

39. Ritter, T. K.; Mong, K. K. T.; Liu, H. T.; Nakatani, T.; Wong, C. H., A programmable one-pot oligosaccharide synthesis for diversifying the sugar domains of natural products: A case study of vancomycin. *Angew Chem Int Edit* **2003,** *42* (38), 4657-4660.

40. Lee, H. K.; Scanlan, C. N.; Huang, C. Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C. H., Reactivity-based one-pot synthesis of oligomannoses: Defining antigens recognized by 2G12, a broadly neutralizing anti-HIV-1 antibody. *Angew Chem Int Edit* **2004,** *43* (8), 1000-1003.

41. Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T., One-Pot Sequential Glycosylation - a New Method for the Synthesis of Oligosaccharides. *Tetrahedron Lett* **1994,** *35* (23), 3979-3982.

42. Yamada, H.; Kato, T.; Takahashi, T., One-pot sequential glycosylation: A new method for the synthesis of branched oligosaccharides. *Tetrahedron Lett* **1999,** *40* (24), 4581-4584.

43. Tanaka, H.; Adachi, M.; Tsukamoto, H.; Ikeda, T.; Yamada, H.; Takahashi, T., Synthesis of di-branched heptasaccharide by one-pot glycosylation using seven independent building blocks. *Org Lett* **2002,** *4* (24), 4213-4216.

44. Tanaka, H.; Adachi, M.; Takahashi, T., One-pot synthesis of sialo-containing glycosyl amino acids by use of an N-trichloroethoxycarbonyl-beta-thiophenyl sialoside. *Chem-Eur J* **2005,** *11* (3), 849-862.

45. Takahashi, T.; Adachi, M.; Matsuda, A.; Doi, T., Combinatorial synthesis of trisaccharides via solution-phase one-pot glycosylation. *Tetrahedron Lett* **2000,** *41* (15), 2599-2603.

46. Baeschlin, D. K.; Green, L. G.; Hahn, M. G.; Hinzen, B.; Ince, S. J.; Ley, S. V., Rapid assembly of oligosaccharides: 1,2-diacetal-mediated reactivity tuning in the coupling of glycosyl fluorides. *Tetrahedron-Asymmetr* **2000,** *11* (1), 173-197.

47. Jayaprakash, K. N.; Fraser-Reid, B., One-pot chemo-, regio-, and stereoselective double-differential glycosidation mediated by lanthanide triflates. *Org Lett* **2004,** *6* (23), 4211-4214.

48. Demchenko, A. V.; Kamat, M. N.; De Meo, C., S-benzoxazolyl (SBox) glycosides in oligosaccharide synthesis: Novel glycosylation approach to the synthesis of beta-D-glucosides, beta-D-galactosides, and alpha-D-mannosides. *Synlett* **2003,** (9), 1287-1290.

49. Demchenko, A. V.; Pornsuriyasak, P.; De Meo, C.; Malysheva, N. N., Potent, versatile, and stable: Thiazolyl thioglycosides as glycosyl donors. *Angew Chem Int Edit* **2004,** *43* (23), 3069-3072.

50. Pornsuriyasak, P.; Demchenko, A. V., Glycosyl thioimidates in a highly convergent one-pot strategy for oligosaccharide synthesis. *Tetrahedron-Asymmetr* **2005,** *16* (2), 433-439.

51. Huang, X. F.; Huang, L. J.; Wang, H. S.; Ye, X. S., Iterative one-pot synthesis of oligosaccharides. *Angew Chem Int Edit* **2004,** *43* (39), 5221-5224.

52. Wang, Z.; Zhou, L. Y.; El-Boubbou, K.; Ye, X. S.; Huang, X. F., Multi-component one-pot synthesis of the tumor-associated carbohydrate antigen Globo-H based on preactivation of thioglycosyl donors. *J Org Chem* **2007,** *72* (17), 6409-6420.

53. Huang, L. J.; Wang, Z.; Li, X. N.; Ye, X. S.; Huang, X. F., Iterative one-pot syntheses of chitotetroses. *Carbohyd Res* **2006,** *341* (10), 1669-1679.

54. Huang, L. J.; Huang, X. F., Highly efficient syntheses of hyaluronic acid oligosaccharides. *Chem-Eur J* **2007,** *13* (2), 529-540.

55. Wang, C. C.; Kulkarni, S. S.; Lee, J. C.; Luo, S. Y.; Hung, S. C., Regioselective one-pot protection of glucose. *Nat Protoc* **2008,** *3* (1), 97-113.

56. 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* (7138), 896-899.

57. Francais, A.; Urban, D.; Beau, J. M., Tandem catalysis for a one-pot regioselective protection of carbohydrates: The example of glucose. *Angew Chem Int Edit* **2007,** *46* (45), 8662-8665.

58. Carlow, D. A.; Gossens, K.; Naus, S.; Veerman, K. M.; Seo, W.; Ziltener, H. J., PSGL-1 function in immunity and steady state homeostasis. *Immunol Rev* **2009,** *230*, 75-96.

59. Cummings, R. D., Structure and function of the selectin ligand PSGL-1. *Braz J Med Biol Res* **1999,** *32* (5), 519-28.

60. Moore, K. L.; Stults, N. L.; Diaz, S.; Smith, D. F.; Cummings, R. D.; Varki, A.; Mcever, R. P., Identification of a Specific Glycoprotein Ligand for P-Selectin (Cd62) on Myeloid Cells. *Journal of Cell Biology* **1992,** *118* (2), 445-456.

61. McEver, R. P.; Cummings, R. D., Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* **1997,** *100* (3), 485-91.

62. Moore, K. L.; Eaton, S. F.; Lyons, D. E.; Lichenstein, H. S.; Cummings, R. D.; Mcever, R. P., The P-Selectin Glycoprotein Ligand from Human Neutrophils Displays Sialylated, Fucosylated, O-Linked Poly-N-Acetyllactosamine. *J Biol Chem* **1994,** *269* (37), 23318-23327.

63. Constantin, G., PSGL-1 as a novel therapeutic target. *Drug News & Perspectives* **2004,** *17* (9), 579-586.

64. Hicks, A. E. R.; Nolan, S. L.; Ridger, V. C.; Hellewell, P. G.; Norman, K. E., Recombinant P-selectin glycoprotein ligand-1 directly inhibits leukocyte rolling by all 3 selectins in vivo: complete inhibition of rolling is not required for anti-inflammatory effect. *Blood* **2003,** *101* (8), 3249-3256.

65. Kyriakides, C.; Favuzza, J.; Wang, Y.; Austen, W. G.; Moore, F. D.; Hechtman, H. B., Recombinant soluble P-selectin glycoprotein ligand 1 moderates local and remote injuries following experimental lower-torso ischaemia. *British Journal of Surgery* **2001,** *88* (6), 825-830.

66. Hayward, R.; Campbell, B.; Shin, Y. K.; Scalia, R.; Lefer, A. M., Recombinant soluble P-selectin glycoprotein ligand-1 protects against myocardial ischemic reperfusion injury in cats. *Cardiovascular Research* **1999,** *41* (1), 65-76.

67. Wang, K.; Zhou, X. R.; Zhou, Z. M.; Tarakji, K.; Qin, J. X.; Sitges, M.; Shiota, T.; Forudi, F.; Schaub, R. G.; Kumar, A.; Penn, M. S.; Topol, E. J.; Lincoff, A. M., Recombinant soluble P-selectin glycoprotein ligand-Ig (rPSGL-Ig) attenuates infarct size and myeloperoxidase activity in a canine model of ischemia-reperfusion. *Thrombosis and Haemostasis* **2002,** *88* (1), 149-154.

68. Koeller, K. M.; Smith, M. E. B.; Wong, C. H., Chemoenzymatic synthesis of PSGL-1 glycopeptides: Sulfation on tyrosine affects glycosyltransferase-catalyzed synthesis of the O-glycan. *Bioorgan Med Chem* **2000,** *8* (5), 1017-1025.

69. Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C. H., Chemoenzymatic synthesis of a PSGL-1 N-terminal glycopeptide containing tyrosine sulfate and alpha-O-linked sialyl Lewis X. *J Am Chem Soc* **2000,** *122* (17), 4241-4242.

70. Koeller, K. M.; Smith, M. E. B.; Wong, C. H., Tyrosine sulfation on a PSGL-1 glycopeptide influences the reactivity of glycosyltransferases responsible for synthesis of the attached O-glycan. *J Am Chem Soc* **2000,** *122* (4), 742-743.

71. Otsubo, N.; Ishida, H.; Kiso, M., The first, highly efficient synthesis of spacer-armed O-glycans on GlyCAM-1 and PSGL-1: the counter-receptors for L- and P-selectin. *Tetrahedron Lett* **2000,** *41* (20), 3879-3882.

72. Baumann, K.; Kowalczyk, D.; Kunz, H., Total synthesis of the glycopeptide recognition domain of the P-selectin glycoprotein ligand 1. *Angew Chem Int Edit* **2008,** *47* (18), 3445-3449.

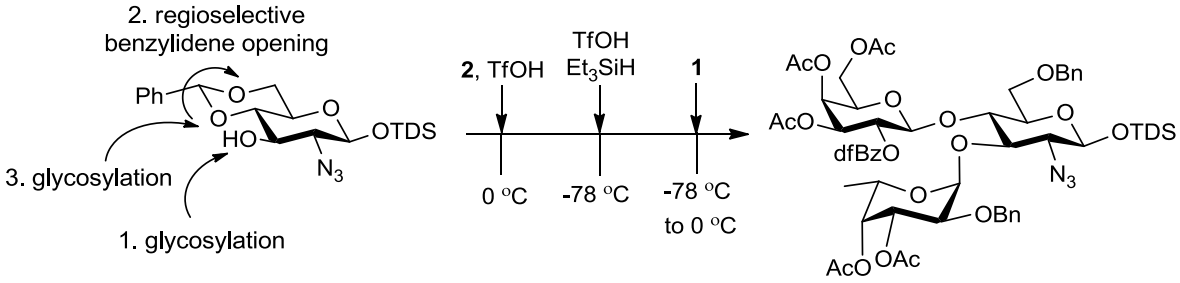
Chapter II

One-pot synthesis of Oligosaccharides by Combining Protecting Group Manipulations and Glycosylations*

* **Yusuf Vohra**, Mahalakshmi Vasan, Andre Venot, and Geert-Jan Boons; *Org. lett.* **2008,** *10*, 3247-3250.
Reprinted here with permission of the publisher.

2.1 Abstract

To streamline the process of oligosaccharide synthesis, several groups are pursuing one-pot multi-step approaches. These approaches have been broadly classified based on chemoselective, orthogonal, and pre-activation strategies for glycosylations and regioselective one-pot protection of monosaccharides. We report here a new class of one-pot reactions that addresses some of the shortcomings of the above approaches. By combining triflic acid promoted glycosylations of trichloroacetimidates with reductive opening of benzylidene acetals using triflic acid and triethylsilane in a one-pot procedure, an easy access to a wide range of di- and branched trisaccharides is made possible. The attraction of this approach is that it allows us to assemble branched oligosaccharides by a one-pot procedure, a task that cannot readily be accomplished by chemoselective, orthogonal, and iterative glycosylations.



2.2 Introduction

Protein- and lipid-bound saccharides are ubiquitous in biological systems involved in many important molecular processes such as fertilization, embryogenesis, neuronal development, hormone activities, and the proliferation of cells and their organization into specific tissues.[1-2] These interactions are also important in health science and are involved in the invasion and attachment of pathogens, inflammation, metastasis, blood group immunology, and xenotransplantation.[3-5] A major obstacle to advances in glycobiology is the lack of pure and structurally well-defined carbohydrates and glycoconjugates. These compounds are often found in low concentrations and in micro-heterogeneous forms, greatly complicating their isolation and characterization. In many cases, well-defined oligosaccharides can only be obtained by organic synthesis.[6-9]

2.3 Research Objectives

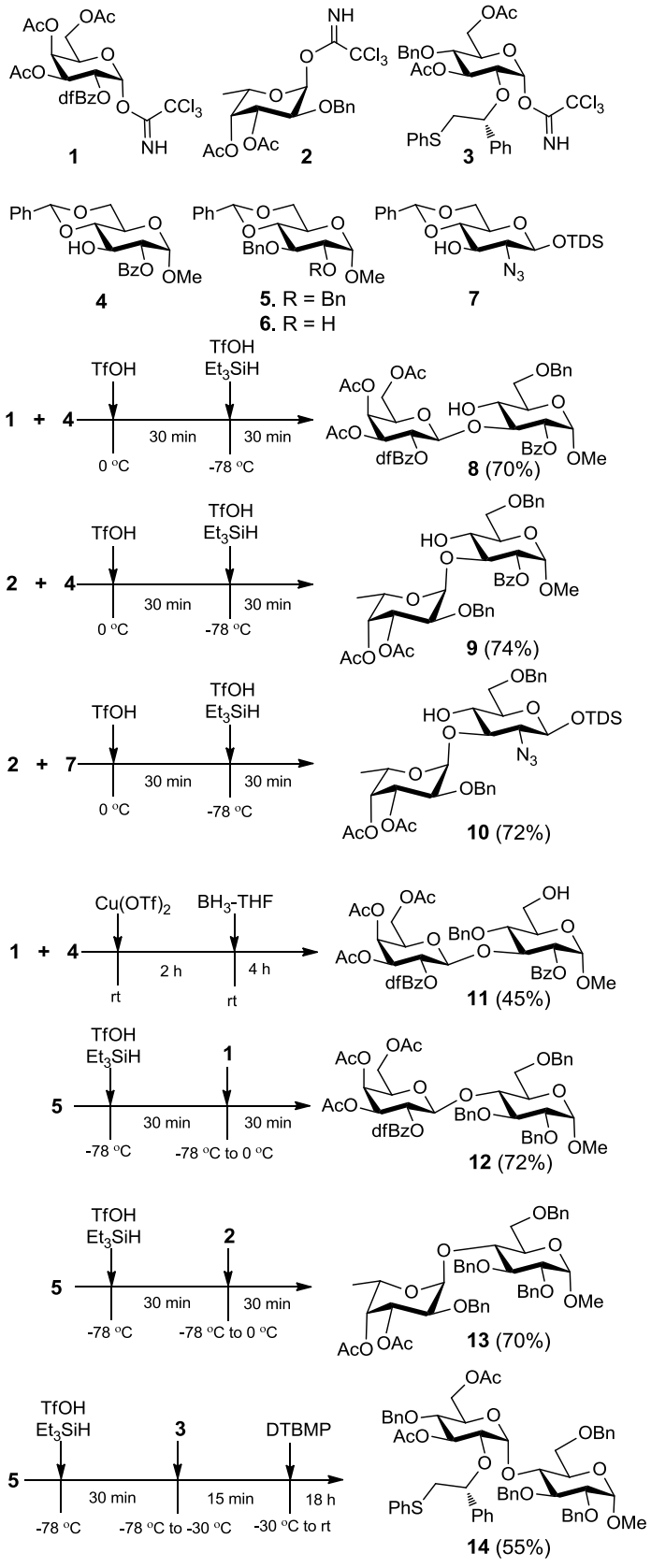
Although the methods for oligosaccharide synthesis have improved considerably during the past decade, the construction of complex carbohydrates remains a significant challenge due to the combined demands of elaborate procedures for glycosyl donor and acceptor preparation and the requirements of regio- and stereoselectivity in glycoside bond formation. To streamline the preparation of complex oligosaccharides, one-pot multi-step approaches for selective monosaccharide protection and oligosaccharide assembly are being pursued, which do not require intermediate work-up and purification step and hence speed-up the process of chemical synthesis considerably.[10-11] For example, the observation that acetal formation, regioselective reductive opening of arylidene acetals, reductive etherification and acetylation can be catalyzed by triflic acid (TfOH) or trimethylsilyl triflate (TMSOTf) made it possible to program these

reactions in tandem by sequential addition of reagents to give easy access to a wide variety of selective protected monosaccharide building blocks. Furthermore, many laboratories have demonstrated that chemoselective, orthogonal and iterative glycosylation strategies, which exploit differential reactivities of anomeric leaving groups, allow several selected glycosyl donors to react in a specific order resulting in a single oligosaccharide product.[12-18] Several reports have also shown that removal of silyl and trityl ethers can be combined with glycosylations.[19-22]

To further streamline the process of oligosaccharide assembly, we report here a strategy whereby a regioselective opening of a benzylidene acetal and glycosylations are combined in a one-pot multi-step synthetic procedure. The attraction of the approach is that it makes it possible to assemble branched oligosaccharides by a one-pot procedure; a task that cannot readily be accomplished by chemoselective, orthogonal and iterative glycosylations. In this respect, differential reactivities of hydroxyls have been exploited for the synthesis of branched oligosaccharides by one-pot procedures[23-30] but the scope of this approach is limited because of the need of exceptional high regioselectivities.

2.4 Results and Discussion

Trichloroacetimidates were selected as glycosyl donors because their activation requires only catalytic TfOH or TMSOTf.[31] Furthermore, TfOH combined with triethylsilane (Et_3SiH) was employed for the opening of a 4,6-*O*-benzylidene acetals[32-33] because these conditions provide in general high regioselectivies and excellent yields, and furthermore it was anticipated that these reaction conditions would be compatible with TfOH mediated glycosylations of trichloroacetimidates.



Scheme 2.1 One-pot synthesis of disaccharides by glycosylation and benzylidene acetal opening.

Thus, a mixture of trichloroacetimidate **1**[34] and benzylidene acetal protected glycosyl acceptor **4**[35] in dichloromethane at 0 °C was treated with a catalytic amount of TfOH. After a reaction time of 30 min., TLC and MALDI-MS analysis indicated consumption of the starting material and the formation of the expected disaccharide. The reaction mixture was cooled to -78 °C followed by addition of TfOH (1.8 eq.) and Et_3SiH (2.0 eq.). After stirring for 30 minutes at -78 °C, the reaction was quenched by the addition of triethylamine and methanol, and purification by silica gel column chromatography gave disaccharide **8** in a yield of 70%. As expected, only a β-galactoside was formed due to the presence of a 2,5-difluorobenzoyl group[36] at C-2 of the galactosyl donor **1**.[34] This protecting group is an excellent neighboring group participant and unlike benzoyl and pivaloyl esters can be cleaved under mild conditions using a catalytic amount of sodium methoxide in methanol.

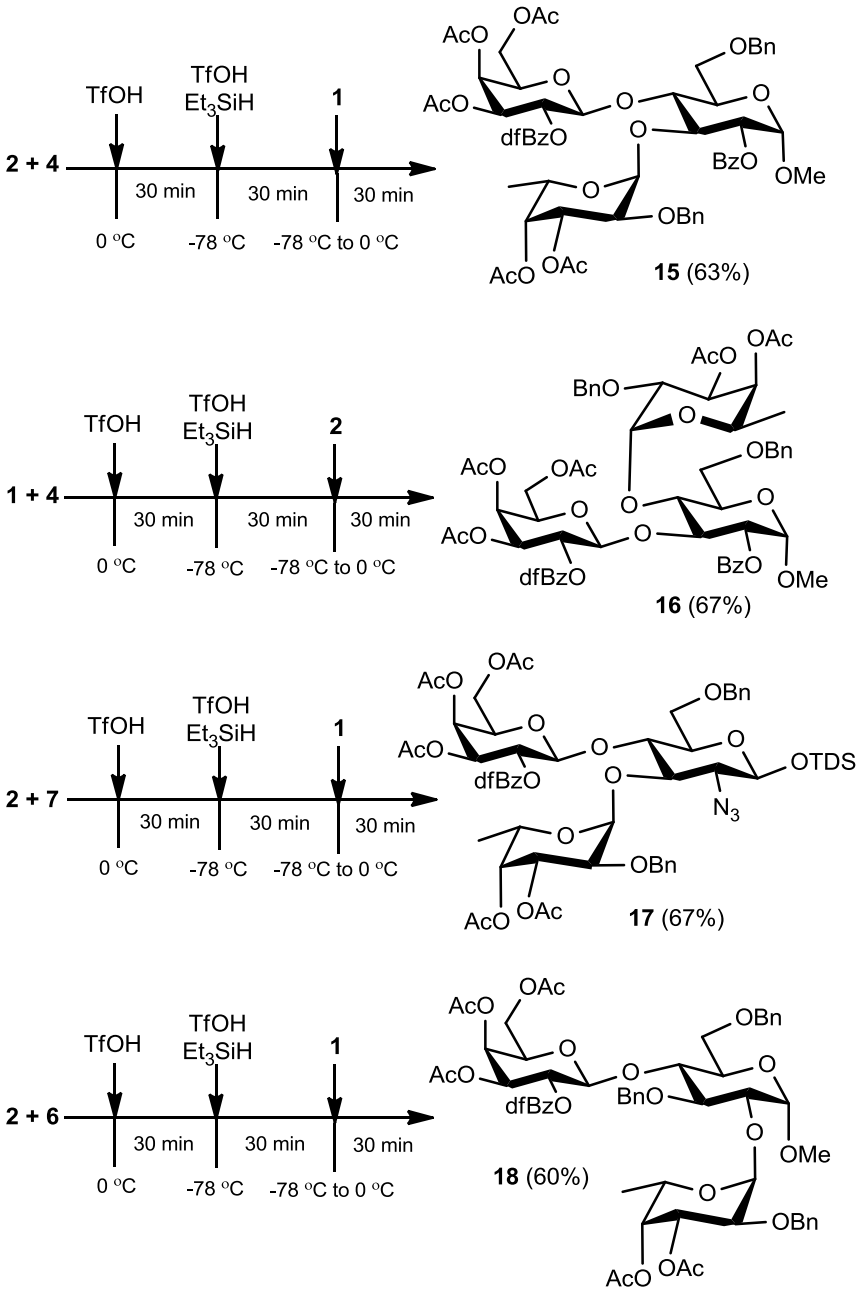
In the next set of experiments, the sequence of reactions was repeated, however, in this case the fucosyl donor **2** [37] was employed instead of **1**. Fortunately, disaccharide **9** was isolated in an overall yield of 74% as a single regio- and stereoisomer demonstrating that the methodology is compatible with acid sensitive fucosides. It was also found that fucosylation of 2-azido-containing glycosyl acceptor **7**[38] followed by a regioselective opening of the benzylidene acetal using standard conditions of the resulting disaccharide led to the facile formation of disaccharide **10**.

It has been established that the regioselective ring opening of 4,6-*O*-benzylidene acetals can be reversed to provide saccharides with a free C-6 hydroxyl by employing $Cu(OTf)_2$ in combination with borane[39] instead of TfOH and Et_3SiH. Furthermore, it was anticipated that trichloroacetimidates can be activated by $Cu(OTf)_2$ and thus it may be possible to glycosylate the C-3 hydroxyl of **4** followed by a regioselective opening of the benzylidene acetal of the resulting

product to give a disaccharide having a C-6 hydroxyl. Indeed, a $Cu(OTf)_2$ (0.15 eq.) mediated glycosylation of glucosyl donor **1** with acceptor **4** gave, after a reaction time of 2h at room temperature, a disaccharide, which was treated with borane-THF complex to provide after stirring for an additional 4 h at ambient temperature, disaccharide **11** in a yield of 45%. In our efforts to improve the reaction yield, it was found that prolonged reaction times led to decomposition and formation of by-products.

Next, attention was focused on a reaction sequence whereby a regioselective benzylidene acetal opening is followed by glycosylation. Thus, Et_3SiH and TfOH were added to a cooled (-78 $^{\circ}$C) solution of compound **5** in dichloromethane and after a reaction time of 30 min, glycosyl donor **1** or **2** was added followed gradually raising the temperature to 0 $^{\circ}$C over a period of 30 min. Standard work-up and purification by silica gel column chromatography gave regio- and stereo-isomerically pure disaccharides **12** and **13**, respectively, in yields of 72 and 70%.

Recently, we demonstrated that glycosylations with glycosyl donors modified at C-2 with a (*S*)-(phenylthiomethyl)benzyl moiety give exclusively α-anomeric selectivity due to neighboring group participation resulting in an intermediate *trans*-fused 1,2-sulfonium ion.[40-41] To explore the possibility of a one-pot procedure involving benzylidine acetal opening and glycosylation with such a donor, compound **5**[42] was treated with TfOH and Et_3SiH followed by subsequent addition of **3**[40] and DTBMP to give **14** as only the α-anomer.



Scheme 2.1 One-pot synthesis of trisaccharides by glycosylation and benzylidene acetal opening.

Next, we explored the possibility of synthesizing branched trisaccharides by a reaction sequence involving glycosylation, reductive benzylidene acetal opening followed by another glycosylation (Scheme 2). Thus, a mixture of trichloroacetimidate **2** and benzylidene protected glycosyl acceptor **4** in dichloromethane at 0 °C was treated with a catalytic amount of TfOH.

After a reaction time of 30 min., the reaction mixture was cooled to -78 °C followed by addition of TfOH (1.8 eq.) and Et_3SiH (2.0 eq.) and after a further reaction time of 30 min, galactosyl donor **1** was added and the reaction mixture was allowed to warm to 0 °C over a period of 30 min, to give after standard work-up and purification by silica gel column chromatography, trisaccharide **15** in a yield of 63%. A similar procedure gave trisaccharide **16** by a glycosylation of **1** with **4**, followed by reductive opening of the benzylidene acetal and glycosylation of the resulting acceptor with fucosyl donor **2**. Furthermore, a TfOH promoted glycosylation of 2-azido-2-deoxy-glucoside **7** with **2** followed by benzylidene acetal opening with TfOH/Et_3SiH gave a disaccharide acceptor, which was glycosylated with galactosyl donor **1** to give protected Lewis^x trisaccharide **17** in an excellent yield of 67%. Removal of anomeric TDS protecting group of **17** followed by conversion of the resulting lactol into leaving group provides an opportunity to prepare more complex oligosaccharides. Finally, it was demonstrated that the methodology can also be employed for the preparation of 2,4-branched trisaccharides by glycosylation of acceptor **6** with fucosyl donor **2** to give a 2,4-linked disaccharide, which was subjected to Et_3SiH/TfOH to regioselectively open the benzylidene to afford a glycosyl acceptor having a C-4 hydroxyl. Addition of galactosyl donor **1** to the latter compound followed by standard work-up and purification by silica gel column chromatography gave trisaccharide **18** in 60% yield.

2.5 Conclusion

In conclusion, it has been demonstrated that one pot-procedures involving reductive opening of benyzlidene acetals and glycosylations can give easy access to a wide range of di- and trisaccharides. It is to be expected that the utility of the methodology can be further extended by combining acid catalyzed reductive etherifications and acetylations with glycosylations. Also the use of a thioglycoside as the initial acceptor may provide an opportunity to prepare more complex structures by employing the resulting thioglycoside product as a glycosyl donor.

2.6 Experimental Section

General remarks:

Column chromatography was performed on silica gel G60 (SiliCycle, 60-200μm 60 Å), reactions were monitored by TLC on Silicagel 60 F_{254} (EMD Chemicals Inc.). The compounds were detected by examination under the UV light, by charring with 10% sulfuric acid in methanol and Cerium ammonium molybdate in 20% sulfuric acid in water. Solvents were removed under reduced pressure at < 35 °C. CH_2Cl_2 were distilled from CaH_2 prior to use in reactions. All the starting materials were kept *in vacuo* with P_2O_5 prior to use. ^{1}H-NMR spectra were recorded in $CDCl_3$ at 500 MHz (Varian), ^{13}C-NMR spectra were recorded in $CDCl_3$ at 75 MHz (Varian). High-resolution mass spectra were obtained by using MALDI-ToF (Applied Biosystems 4700 Proteomics Analyzer) with 2,5-dihydroxybenzoic acid as an internal standard matrix.

Methyl 4,6-*O*-benzylidene-3-*O*-benzyl-α-D-glucopyranoside (6):

To a solution of methyl 4,6-benzylidene-α-D-glucopyranoside (50 mg, 0.18 mmol) in DCM (1.0 mL) was added benzaldehyde (22 μL, 0.21mmol) and triethylsilane (34 μL, 0.21 mmol), and the mixture was stirred under an atmosphere of argon for 30 min. The mixture was cooled (-78 °C) and TfOH (0.3 eq) was added. The reaction mixture was quenched after 30 min. with pyridine (50 μL), diluted with DCM (5 mL), and washed with sat. aq. $NaHCO_3$ (3 mL) and brine (3 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and purified by silical gel column chromatography (hexane/ethylacetate, 4/1, v/v) to give **6** as a white solid in 50% yield.

General procedure for the synthesis of 8, 9 and 10:

A mixture of glycosyl acceptor (0.13 mmol) and trichloroacetimidate donor (0.2 mmol) in CH_2Cl_2 (1 ml) was placed under an atmosphere of Ar and cooled to 0 °C. TfOH (0.013 mmol, 0.13M solution in DCM) was added and stirring at 0 °C was continued for 30 min. The reaction mixture was then cooled to -78 °C followed by addition of TfOH (0.23 mmol) and triethylsilane (0.26 mmol). The reaction mixture was then stirred at -78 °C for 30 min to 1 hr. The progress of each reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of triethylamine (25 μL) and methanol (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

Methyl 3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (8)

Compound **8** was obtained from **1** and **4**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→35%) to afford compound **8** in 70% yield. Analytical data for **8**: R_f = 0.30 (ethyl acetate – hexanes, 1/2, v/v); ^{1}H-n.m.r: δ, 7.63-6.59 (m, 13H, aromatic), 5.39-5.35 (m, 2H, H-2', H-4'), 5.10 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 10.4 Hz, H-3'), 4.94-4.91 (m, 2H, H-1, H-2), 4.72 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.56 (s, 2H, CH_2, Bn), 4.16-4.08 (m, 2H, H-6'a, H-6'b), 4.04-3.98 (m, 2H, H-5', H-3), 3.77-3.66 (m, 4H, H-6a, H-5, H-4, H-6b), 3.57 (s, 1H, O*H*), 3.23 (s, 3H, OCH_3), 2.10 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3), 1.81 (s, 3H, COCH_3) ppm; ^{13}C-n.m.r.: δ, 169.45, 169.09, 168.93, 164.12, 160.88, 158.17, 158.04, 154.96, 154.64, 137.25, 132.07, 128.35, 127.32, 127.18, 126.53, 100.75 (C-1'), 95.65 (C-1),

81.70, 72.56, 71.35, 70.15, 69.57, 69.48, 68.76, 68.17, 67.68, 65.88, 60.30, 54.20, 19.59, 19.58, 19.30 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{40}H_{42}F_2O_{16}$ $[M+Na]^+$ calcd 839.2339; found 839.2341.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-2-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**9**)

Compound **9** was obtained from **2** and **4**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 5→25%) to afford compound **9** in 74% yield. Analytical data for **9**: R_f = 0.35 (ethyl acetate – hexanes, 1/2, v/v); ^{1}H-n.m.r: δ, 7.98-6.92 (m, 15H, aromatic), 5.22-5.20 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.5 Hz, H-3), 5.16 (bd, 1H, H-4'), 5.11 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1'), 5.09-5.07 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 4.99 (d, 1H, $J_{1,2}$ = 3.8 Hz H-1), 4.61-4.53 (q, 2H, CH_2, Bn), 4.41-4.38 (q, 1H, H-5'), 4.24-4.21(d, 1H, C*H*H, Bn), 4.14-4.10 (m, 2H, C*H*H, Bn, H-3), 3.78-3.69(m, 4H, H-4, H-5, H-6a, H-6b), 3.68-3.65 (dd, 1H, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 3.52 (d, 1H, O*H*), 3.31 (s, 3H, OCH_3), 1.99 (s, 3H, COCH_3), 1.82 (s, 3H, COCH_3), 0.89 (d, 3H, C$H_{3\text{fucose}}$) ppm. ^{13}C-n.m.r.: δ, 169.38, 168.91, 164.55, 136.94, 136.64, 132.09, 128.80, 127.43, 127.34, 127.22, 126.72, 126.68, 126.64, 126.46, 96.04 (C-1, C-1'), 78.16, 72.69, 71.57, 71.55, 71.38, 70.49, 69.06, 68.89, 68.80, 68.71, 64.26, 54.28, 19.72, 19.57, 14.67 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{38}H_{44}O_{13}$ $[M+Na]^+$ calcd 731.2680; found 731.2683.

Thexyldimethylsilyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (10)

Compound **10** was obtained from **2** and **7**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 5→40%) to afford compound **10** in 72% yield. Analytical data for **10**: R_f = 0.35 (ethyl acetate – hexanes, 1/2, v/v); ^{1}H-n.m.r: δ, = 7.17-7.06 (m, 10H, aromatic), 5.14-5.11 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3'4'}$ = 3.0 Hz, H-3'), 5.10-4.98 (bd, 1H, H-4'), 4.57-4.25 (m, 6H, 2 x CH_2, Bn, H-1, H-5'), 3.70-3.67 (dd, 1H, $J_{1'2'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 3.56-3.49 (m, 2H, H-6a,b), 3.40-3.36 (m, 1H, H-4), 3.22-3.09 (m, 3H, H-5, H-3, H-2), 1.92 (s, 3H, COCH_3), 1.79 (s, 3H, COCH_3), 1.50-1.40 (m, 1H, SiC(CH$_3$)$_2$CH(CH$_3$)$_2$), 0.90 (d, 3H, OC$H_{3\text{fucose}}$), 0.70-0.69 (m, 12H, SiC(CH_3)$_2$CH(CH_3)$_2$), 0.00 (s, 6H, Si(CH_3)$_2$) ppm. ^{13}C-n.m.r.: δ, 173.71, 173.31, 141.25, 141.07, 131.67, 131.32, 131.12, 131.00, 130.84, 100.73 (C-1'), 100.49 (C-1), 86.40, 77.71, 77.57, 76.91, 76.16, 76.10, 74.72, 74.29, 73.95, 73.32, 73.25, 73.05, 72.54, 70.59, 69.80, 68.73, 37.16, 28.04, 24.09, 23.90, 23.23, 23.09, 21.76, 21.63, 19.16, 1.18, 0.00 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{38}H_{55}N_3O_{11}Si$ $[M+Na]^+$ calcd 780.3504; found 780.3501.

Methyl 3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-glucopyranoside (11):

Premixed galactosyl donor **1** (115 mg, 0.20 mmol) and glucosyl acceptor **4** (50 mg, 0.13 mmol) were dissolved in CH_2Cl_2 (2 ml) and placed under an atmosphere of Ar at room temperature. Copper triflate (7.0 mg, 0.02 mmol) was added to the mixture and stirring was continued for 2 hr. Borane in tetrahydrofuran (0.26 ml, 0.26 mmol) was added and the resulting mixture was stirred at room temperature for 4 hr. The progress of the reaction was monitored by

TLC and MALDI-ToF MS. The reaction was quenched by the addition of triethylamine (25 μL) and methanol (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→45%) to afford compound **11** (48 mg, 45%). Analytical data for **11**: R_f = 0.50 (ethyl acetate – hexanes, 1/1, v/v); ^{1}H-n.m.r.: δ, 7.92-6.85 (m, 13H, aromatic), 5.42-5.38 (m, 2H, H-2', H-4'), 5.12 (bd, 1H, CH*H*, Bn), 5.00-4.98 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 4.96 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.90-4.88 (m, 2H, H-1, H-2), 4.64 (d, 1H, CH*H*, Bn), 4.50-4.46 (m, 1H, H-3), 4.11-4.09 (m, 2H, H-6'a, H-6'b), 3.98-3.95 (m, 1H, H-5'), 3.83-3.74 (m, 2H, H-6a, H-6b), 3.71-3.63 (m, 2H, H-5, H-4), 3.28 (s, 3H, OC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$), 1.85 (s, 3H, COC*H*$_3$) ppm. ^{13}C-n.m.r.: δ, 170.75, 170.59, 170.54, 170.42, 170.39, 170.26, 170.23, 170.18, 169.63, 165.55, 162.18, 162.13, 162.10, 159.60, 159.56, 159.50, 156.36, 156.32, 156.14, 156.10, 138.50, 133.74, 129.71, 129.11, 128.75, 128.54, 128.51, 128.11, 121.57, 121.45, 121.25, 121.13, 118.52, 118.41, 118.18, 118.08, 117.97, 117.63, 101.37 (C-1'), 96.98 (C-1), 78.12, 75.65, 75.33, 74.42, 70.99, 70.87, 70.80, 70.64, 67.07, 62.05, 60.91, 55.38, 20.91, 20.81, 20.61 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{40}H_{42}F_2O_{16}$ $[M+Na]^+$ calcd 839.2339; found 839.2335.

General procedure for the synthesis of 12 and 13:

Compound **5** (0.11 mmol) was dissolved in CH_2Cl_2 (1 ml), placed under an atmosphere of Ar and cooled to -78 °C. TfOH (0.20 mmol) and triethylsilane (0.22 mmol) were added to the mixture followed by stirring at -78 °C for 30 min. The trichloroacetimidate donor (0.20 mmol) in CH_2Cl_2 (1 ml) was added and the temperature was increased to 0 °C and stirring was continued for 30 min. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of triethylamine (25 μL) and methanol (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo.* The residue was purified using silica gel column chromatography.

Methyl 3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-*O*-benzyl-α-D-glucopyranoside (**12**)

Compound **12** was obtained from **1** and **5**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→55%) to afford compound **12** in 72% yield. Analytical data for **12**: R_f = 0.55 (ethyl acetate – Hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 7.53-7.11 (m, 18H, aromatic), 5.38-5.35 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 5.30 (d, 1H, $J_{3',4'}$ = 3.1 Hz, H-4'), 5.00 (d, 1H, C*H*H, Bn), 4.90-4.87 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.4 Hz, H-3'), 4.87 (d, 1H, C*H*H, Bn), 4.81 (d, 1H, C*H*H, Bn), 4.69 (d, 1H, C*H*H, Bn), 4.65 (d, 1H, C*H*H, Bn), 4.58-4.56 (m, 2H, H-1, H-1'), 4.33 (d, 1H, C*H*H, Bn), 4.05-4.01(dd, 1H, $J_{5',6'}$ = 8.2 Hz, $J_{6'b,6'a}$ = 11.1 Hz, H-6'a), 3.97-3.86 (m, 3H, H-4, H-6'b, H-3), 3.68 (dd, 1H, $J_{5,6}$ = 2.9 Hz, $J_{6b,6a}$ = 10.7 Hz, H-6a), 3.59-3.49 (m, 4H, H-5, H-5', H-2, H-6b), 3.34 (s, 3H, OCH_3), 2.13 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3), 1.93 (s, 3H, COCH_3) ppm. ^{13}C-n.m.r.: δ, 170.44, 170.40,

170.21, 162.08, 162.05, 162.03, 161.99, 159.92, 159.88, 159.78, 159.74, 156.67, 156.64, 156.37, 156.33, 139.50, 138.52, 137.98, 128.86, 128.60, 128.38, 128.36, 128.34, 128.29, 128.03, 127.73, 127.53, 122.18, 122.06, 121.86, 121.74, 119.33, 119.23, 119.17, 119.07, 119.03, 118.92, 118.69, 118.58, 118.52, 118.18, 99.97 (C-1'), 98.64 (C-1), 79.96, 79.15, 76.39, 75.38, 73.73, 73.65, 71.24, 71.05, 70.62, 69.76, 67.84, 67.10, 60.85, 55.59, 20.88, 20.83, 20.73 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{47}H_{50}F_2O_{15}$ $[M+Na]^+$ calcd 915.3015; found 915.3010.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (13)

Compound **13** was obtained from **2** and **5**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 5→45%) to afford compound **13** in 70% yield. Analytical data for **13**: R_f = 0.50 (ethyl acetate – Hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 7.31-7.14 (m, 20H, aromatic), 5.23-5.20 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.06-5.03 (d, 1H, C*H*H, Bn), 5.01 (d, 1H, H-1'), 4.94-4.93 (bd, 1H, H-4'), 4.68-4.54 (m, 3H, 3 x C*H*H, Bn), 4.52 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.44-4.35 (m, 3H, 3 x C*H*H, Bn), 4.29-2.26 (d, 1H, C*H*H, Bn), 4.21-4.17 (m, 1H, H-5'), 3.85 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.74 (t, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.70-3.64 (m, 3H, H-5, H-2', H-6a), 3.54-3.50 (m, 2H, H-6b, H-2), 3.27 (s, 3H, OCH_3), 2.00 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3), 0.53 (d, 3H, C$H_{3fucose}$) ppm. ^{13}C-n.m.r.: δ, 170.73, 170.13, 138.96, 138.31, 138.22, 138.17, 128.71, 128.62, 128.60, 128.52, 128.45, 128.27, 128.19, 128.16, 128.14, 128.07, 128.00, 127.92, 127.76, 98.02 (C-1), 97.65 (C-1'), 80.92, 80.33, 80.28, 75.97, 74.73, 74.27, 74.05, 73.57, 73.47, 72.04, 70.54, 70.44, 68.61, 64.95, 55.35, 21.09, 20.91, 15.60 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{45}H_{52}O_{12}$ $[M+Na]^+$ calcd 807.3356; found 807.3358.

Methyl 3,6-di-*O*-acetyl-4-*O*-benzyl-2-*O*-{(1*S*)-phenyl-2-(phenylsulfanyl)ethyl}-α-D-glucopyranoside -(1→4)-2,3,6-tri-*O*-benzyl- α-D-glucopyranoside (14):

Compound **5** (50 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (1 ml), placed under an atmosphere of Ar and cooled to -78 °C. TfOH (17.2 μL, 0.20 mmol) and triethylsilane (2.0 eq., 0.22 mM) were added to the mixture followed by stirring at -78 °C for 30 min. The trichloroacetimidate donor **3** (137 mg, 0.20 mmol) dissolved in CH_2Cl_2 (1 ml) was added and the temperature was increased to -30 °C over a period of 15 min. followed by the addition of 2,6-di-*t*-butyl-4-methyl pyridine (66 mg, 0.33 mmol). The reaction mixture was allowed to warm to room temperature and stirring was continued for 18 hrs. The reaction was quenched by the addition of triethylamine (25 μL) and methanol (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate – hexanes gradient elution 10→55%) to afford compound **14** (61 mg, 55%). Analytical data for **14**: R_f = 0.40 (ethyl acetate – hexanes, 1/1, v/v); ^{1}H-n.m.r.: δ, 7.32-6.91 (m, 30H, aromatic), 5.57 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1'), 5.38 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 9.3 Hz, H-3'), 5.01-4.89 (q, 2H, C*H*$_2$, Bn), 4.66 (d, 1H, CH*H*, Bn), 4.55 (d, 1H, CH*H*, Bn), 4.54 (m, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 4.45 (bd, 2H, C*H*$_2$, Bn), 4.41-4.30 (q, 2H, C*H*$_2$, Bn), 4.27 (t, 1H, $J_{7',8a'} = J_{7',8b'}$ = 6.6 Hz, H-7'), 4.08 (t, 1H, $J_{3,2} = J_{3,4}$ = 9.2 Hz, H-3), 4.01-3.95 (m, 2H, H-6'a, H-4), 3.92-3.86 (m, 2H, H-6'b, H-5'), 3.84-3.82 (dd, 1H, $J_{5,6}$ = 3.4 Hz, $J_{6b,6a}$ = 11.0 Hz, H-6a), 3.64-3.62 (m, 1H, H-5), 3.56-3.54 (m, 1H, H-6b), 3.53-3.50 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.27 (s, 3H, OC*H*$_3$), 3.27-3.22 (m, 2H, H-8'a, H-4'), 3.16-3.14 (dd, 1H, $J_{1',2'}$ = 3.4 Hz, $J_{2',3'}$ = 9.9 Hz, H-2'), 2.82-2.78 (dd, 1H, $J_{7',8'b}$ = 6.6 Hz, $J_{8'b,8'a}$ = 13.6 Hz, H-8'b), 1.89 (s, 3H, COC*H*$_3$), 1.74 (s, 3H, COC*H*$_3$) ppm. ^{13}C-n.m.r.: δ, 170.73, 169.89, 140.29,

139.66, 138.34, 138.24, 137.78, 136.92, 129.04, 128.68, 128.65, 128.54, 128.47, 128.43, 128.38, 128.13, 127.63, 127.36, 127.23, 127.16, 125.78, 98.13 (C-1), 95.17 (C-1'), 81.76, 81.12, 80.22, 77.44, 76.32, 74.57, 74.45, 73.49, 73.08, 69.87, 68.96, 63.04, 55.50, 40.75, 21.26, 21.08 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{59}H_{64}O_{13}S$ $[M+Na]^+$ calcd 1035.3965; found 1035.3961.

General procedure for the synthesis of trisaccharides 15, 16, 17 and 18:

A mixture of glycosyl acceptor (0.13 mmol) and trichloroacetimidate donor (0.19 mmol) in CH_2Cl_2 (1 ml) was placed under an atmosphere of Ar and cooled to 0 °C. TfOH (0.013 mmol, 0.13M solution in DCM) was added and stirring at 0 °C was continued for 30 min. The reaction mixture was then cooled to -78 °C followed by addition of TfOH (0.23 mmol) and triethylsilane (0.26 mmol). The reaction mixture was then stirred at -78 °C for 30 min to 1 hr. The second trichloroacetimidate donor (0.23 mmol) dissolved in CH_2Cl_2 (1 ml) was added and the mixture was allowed to warm to 0 °C. The progress of the reactions was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of triethylamine (25 μL) and methanol (0.2 ml), diluted with CH_2Cl_2 (30 ml), and washed with sat. aq. $NaHCO_3$ solution (20 ml), water (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→4)]-2-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (15)

Compound **15** was obtained from **1**, **2** and **4**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→55%) to afford compound **15** in 63% yield. Analytical data for **15**: R_f = 0.30 (ethyl acetate – hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 8.02-6.81 (m, 18H, aromatic), 5.52 (d, 1H, H-1', $J_{1',2'}$ = 3.9 Hz), 5.39-5.38 (bd, 1H, H-4'), 5.34-5.33 (bd, 1H-H-4''), 5.27-5.23 (dd, 1H, $J_{1'',2''} = J_{2'',3''}$ = 8.3 Hz, H-2''), 5.20-5.17 (m, 2H, H-2, H-3'), 5.10(d, 1H, H-1, $J_{1,2}$ = 3.9 Hz), 5.08-5.07 (m, 1H, H-5'), 4.89 (d, 1H, C*H*H, Bn), 4.82-4.79 (dd, $J_{2'',3''} = J_{3'',4''}$ = 10.3 Hz, 1H, H-3''), (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1''), 4.49-4.47 (dd, 1H, $J_{5'',6''a}$ =

6.4 Hz, $J_{6''b,6''a}$ = 11.5 Hz, H-6''a), 4.42-4.32 (m, 3H, C*H*H, Bn, H-3, H-6''b), 4.24 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 4.20-4.02 (dd, 2H, CH_2,Bn), 3.86-3.84 (dd, 1H, $J_{5,6}$ = 9.0 Hz, $J_{6b,6a}$ = 10.8 Hz, H-6a), 3.65-3.59 (m, 2H, H-5, H-6b), 3.54 (t, 1H, $J_{4'',5''}$ = 7.0 Hz, $J_{5'',6''a}$ = 6.8 Hz, H-5''), 3.29 (s, 3H, OCH_3), 2.20 (s, 3H, COCH_3), 2.08 (s, 6H, 2 x COCH_3), 1.93 (s, 3H, COCH_3), 1.89 (s, 3H, COCH_3), 1.31 (d, 3H, C$H_{3fucose}$) ppm. ^{13}C-n.m.r.: δ, 170.92, 170.77, 170.68, 170.51, 170.08, 169.88, 165.42, 161.88, 161.86, 161.83, 161.80, 159.98, 159.94, 159.82, 159.80, 156.74, 156.70, 156.42, 156.39, 137.90, 137.63, 133.61, 129.99, 129.84, 129.26, 129.15, 128.91, 128.81, 128.75, 128.65, 128.60, 128.54, 128.44, 128.39, 128.16, 127.50, 127.42, 125.52, 122.32, 122.19, 121.99, 121.88, 119.12, 119.02, 118.95, 118.86, 118.79, 118.68, 118.62, 118.29, 99.55 (C-1''), 97.29 (C-1), 96.97 (C-1'), 75.68, 73.99, 73.05, 72.84, 72.42, 72.25, 71.88, 71.22, 71.06, 70.57, 70.31, 70.10, 67.46, 67.15, 64.29, 61.13, 55.70, 29.91, 21.04, 20.99, 20.88, 20.85, 20.73, 16.18 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{57}H_{62}F_2O_{22}$ $[M+Na]^+$ calcd 1159.3598; found 1159.3593.

Methyl 3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→3)-[3,4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→4)]2-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**16**)

Compound **16** was obtained from **1**, **2** and **4**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→55%) to afford compound **16** in 67% yield. Analytical data for **16**: R_f = 0.30 (ethyl acetate – hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 7.84-6.89 (m, 18H, aromatic), 5.46 (bd, 1H, H-4'), 5.42 (bd, 1H, H-4''), 5.33-5.29 (m, 2H, H-2', H-3''), 5.20-5.17 (m, 2H, H-1, H-5''), 5.06 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 5.03-5.00 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.97 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1), 4.95-4.93 (m, 1H, H-3'), 4.62- 4.43 (m, H-6'a, 2 x CH_2, Bn, H-3), 4.39-4.35 (dd, 1H, $J_{5',6'}$ = 8.5 Hz, $J_{6'b,6'a}$ = 11.0 Hz, H-6'b), 4.09 (t, 1H, H-4), 4.03-3.99 (m, 1H, H-5'), 3.95-3.92 (m, 2H, H-6a, H-2''), 3.84-3.82 (m, 1H, H-5), 3.61-

3.59 (d, 1H, H-5), 3.30 (s, 3H, OCH_3), 2.38 (s, 3H, COCH_3), 2.21 (s, 3H, COCH_3), 2.19 (s, 3H, COCH_3), 2.12 (s, 3H, COCH_3), 1.97 (s, 3H, COCH_3), 1.88 (s, 3H, COCH_3), 1.35 (d, 3H, OC$H_{3fucose}$) ppm. ^{13}C-n.m.r.: δ, 170.97, 170.91, 170.58, 170.15, 169.91, 165.41, 161.92, 159.58, 159.46, 156.34, 156.03, 138.18, 138.09, 133.74, 129.74, 129.26, 129.12, 128.77, 128.66, 128.48, 128.45, 128.20, 128.01, 127.92, 125.52, 121.45, 121.33, 121.12, 121.01, 119.43, 119.34, 119.26, 119.17, 118.46, 118.35, 118.13, 118.02, 117.92, 117.93, 117.59, 101.53 (C-1'), 97.36 (C-1''), 96.84 (C-1), 75.52, 74.75, 74.41, 73.96, 73.70, 72.47, 72.16, 71.17, 71.00, 70.94, 70.87, 70.15, 67.70, 67.04, 64.61, 61.00, 55.22, 21.68, 21.11, 21.08, 20.96, 20.90, 20.59, 16.13 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{57}H_{62}F_2O_{22}$ $[M+Na]^+$ calcd 1159.3598; found 1159.3596.

Thexyldimethylsilyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→4)]-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**17**)

Compound **17** was obtained from **1**, **2** and **7**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→55%) to afford compound **17** in 67% yield. Analytical data for **17**: R_f = 0.35 (ethyl acetate – hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 7.35-6.95 (m, 13H, aromatic), 5.44 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.21 (d, 1H, H-4'), 5.19 (d, 1H, H-4), 5.10-5.04 (m, 2H, H-2, H-3'), 4.86-4.82 (m, 1H, H-5'), 4.78-4.76 (dd, 1H, H-3), 4.65-4.48 (m, 4H, 3 x C*H*H, Bn, H-1), 4.33 (d, 1H, $J_{1'',2''}$ = 7.6 Hz, H-1''), 4.29-4.26 (m, 2H, C*H*H, Bn, H-6a), 4.19-4.16 (dd, 1H, $J_{5,6b}$ = 8.0 Hz, $J_{6b,6a}$ = 11.5 Hz , H-6b), 3.94 (t, 1H, H-4''), 3.73-3.71 (dd, 1H, $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 3.57-3.48 (dd, 1H, $J_{5'',6''}$ = 9.0 Hz, $J_{6''a,\ 6''b}$ = 11.5 Hz, H-6''a), 3.48 (t, 1H, H-5), 3.40-3.36 (m, 2H, H-3'', H-6''b), 3.29-3.26 (dd, 1H, $J_{1'',2''}$ = 7.6 Hz, $J_{2'',3''}$ = 10.2 Hz, H-2''), 3.03-3.01 (m, 1H, H-5''), 2.02 (s, 3H, COCH_3), 1.94 (s, 3H, COCH_3),

1.90 (s, 3H, COCH_3), 1.88 (s, 3H, COCH_3), 1.82 (s, 3H, COCH_3), 1.79 (s, 3H, COCH_3), 1.74 (s, 3H, COCH_3), 1.52-1.48 (m, 1H, SiC$(CH_3)_2$C$H(CH_3)_2$), 1.09 (d, 3H, OC$H_{3fucose}$), 0.73-0.69 (m, 12H, SiC$(CH_3)_2$CH$(CH_3)_2$), 0.00 (s, 6H, Si$(CH_3)_2$) ppm. ^{13}C-n.m.r.: δ, 173.84, 173.58, 173.49, 172.97, 172.82, 164.67, 162.77, 162.60, 159.55, 159.23, 141.15, 140.74, 131.76, 131.35, 131.24, 130.97, 130.83, 130.74, 125.05, 124.85, 124.73, 121.96, 121.86, 121.62, 121.52, 121.41, 121.07, 121.08, 102.48 (C-1), 100.32 (C-1''), 99.90 (C-1'), 77.96, 77.59, 76.70, 75.94, 75.87, 75.46, 75.12, 74.07, 73.41, 73.39, 71.98, 70.47, 70.06, 67.17, 64.00, 36.91, 27.86, 23.91, 23.90, 23.73, 23.56, 23.04, 22.86, 21.57, 21.41, 19.00, 0.86, 0.00 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{57}H_{73}F_2N_3O_{20}Si$ $[M+Na]^+$ calcd 1208.4422; found 1208.4425.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→2)-[3,4,6-tri-*O*-acetyl-2-diflurobenzoyl-β-D-galactopyranosyl-(1→4)]-3,6-*O*-benzyl-α-D-glucopyranoside (18)

Compound **18** was obtained from **1**, **2** and **6**, and purified by silica gel column chromatography (ethyl acetate - hexanes gradient elution 10→50%) to afford compound **18** in 60% yield. Analytical data for **18**: R_f = 0.35 (ethyl acetate – Hexanes, 1/1, v/v); ^{1}H-n.m.r: δ, 7.44-6.96 (m, 18H, aromatic), 5.32-5.29 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3'4'}$ = 3.0 Hz, H-3'), 5.26-5.20 (m, 2H, H-3'', H-4'), 5.16-5.15 (bd, 1H, H-4''), 5.02-4.99 (d, 1H, C*H*H, Bn), 4.95 (d, 1H, $J_{1'2'}$ = 4.0 Hz, H-1'), 4.76-4.73 (m, 3H, C*H*H, Bn, H-3'', H-1), 4.58-4.54 (m, 2H, C*H*H, Bn, H-1''), 4.36-4.20 (m, 4H, 3 x C*H*H, Bn, H-5'), 3.29-3.72 (m, 4H, H-4', H-6''a,b, H-3), 3.68-3.66 (dd, 1H, $J_{1'2'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz , H-2'), (dd, 1H, $J_{5,6a}$ = 8.0 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.55-3.51 (m, 2H, H-5, H-2), 3.46-3.43 (m, 1H, H-6b), 3.36-3.33 (m, 1H, H-5), 3.24 (s, 3H, OCH_3), 2.01 (s, 3H, COCH_3), 1.97 (s, 3H, COCH_3), 1.93 (s, 3H, COCH_3), 1.88 (s, 3H, COCH_3), 1.82 (s, 3H, COCH_3), 1.00 (d, 3H, OC$H_{3fucose}$) ppm. ^{13}C-n.m.r.: δ, 170.73, 170.46, 170.35, 170.21,

139.81, 138.22, 138.04, 128.81, 128.37, 128.27, 128.22, 128.07, 127.70, 126.96, 126.88, 122.13, 122.01, 121.81, 121.69, 119.37, 119.28, 119.21, 119.11, 119.02, 118.89, 118.66, 118.55, 118.21, 100.43 (C-1'), 99.93 (C-1''), 99.21 (C-1), 81.72, 78.46, 76.35, 74.65, 73.64, 73.09, 72.63, 71.78, 71.24, 71.09, 70.59, 70.21, 69.50, 67.98, 67.01, 64.93, 60.82, 55.11, 29.91, 21.07, 20.89, 20.85, 20.78, 20.72, 16.27 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{57}H_{64}F_2O_{21}$ $[M+Na]^+$ calcd 1145.3806; found 1145.3799.

2.7 References

1. Kobata, A., Structures and Functions of the Sugar Chains of Glycoproteins. *European Journal of Biochemistry* **1992,** *209* (2), 483-501.
2. Varki, A., Nothing in glycobiology makes sense, except in the light of evolution. *Cell* **2006,** *126* (5), 841-845.
3. Dube, D. H.; Bertozzi, C. R., Glycans in cancer and inflammation. Potential for therapeutics and diagnostics. *Nat. Rev. Drug Discov.* **2005,** *4* (6), 477-488.
4. Walsh, G.; Jefferis, R., Post-translational modifications in the context of therapeutic proteins. *Nature Biotechnology* **2006,** *24* (10), 1241-1252.
5. Opdenakker, G.; Rudd, P. M.; Ponting, C. P.; Dwek, R. A., Concepts and Principles of Glycobiology. *Faseb Journal* **1993,** *7* (14), 1330-1337.
6. Davis, B. G., Recent developments in glycoconjugates. *Journal of the Chemical Society-Perkin Transactions 1* **1999,** (22), 3215-3237.
7. Demchenko, A. V., Stereoselective chemical 1,2-cis O-glycosylation: from 'sugar ray' to modern techniques of the 21st century. *Synlett* **2003,** (9), 1225-1240.
8. Boons, G.-J., Recent developments in chemical oligosaccharide synthesis. *Contemp. Org. Synth.* **1996,** *3* (3), 173-200.
9. Seeberger, P. H.; Werz, D. B., Synthesis and medical applications of oligosaccharides. *Nature* **2007,** *446* (7139), 1046-51.
10. Francais, A.; Urban, D.; Beau, J. M., Tandem catalysis for a one-pot regioselective protection of carbohydrates: The example of glucose. *Angewandte Chemie-International Edition* **2007,** *46* (45), 8662-8665.

11. 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* (7138), 896-899.

12. Yu, B.; Yang, Z.; Cao, H., One-pot glycosylation (OPG) for the chemical synthesis of oligosaccharides. *Current Organic Chemistry* **2005,** *9* (2), 179-194.

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

14. Codee, J. D. C.; Litjens, R.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A., Thioglycosides in sequential glycosylation strategies. *Chemical Society Reviews* **2005,** *34* (9), 769-782.

15. Wang, Y. H.; Ye, X. S.; Zhang, L. H., Oligosaccharide assembly by one-pot multi-step strategy. *Org Biomol Chem* **2007,** *5* (14), 2189-2200.

16. Wang, Y. H.; Zhang, L. H.; Ye, X. S., Oligosaccharide synthesis and library assembly by one-pot sequential glycosylation strategy. *Comb Chem High T Scr* **2006,** *9* (1), 63-75.

17. Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L., Tuning glycoside reactivity: new tool for efficient oligosaccharide synthesis. *J. Chem. Soc., Perkin Trans. 1* **1998,** (1), 51-65.

18. Koeller, K. M.; Wong, C. H., Synthesis of complex carbohydrates and glycoconjugates: enzyme-based and programmable one-pot strategies. *Chem. Rev.* **2000,** *100* (12), 4465-4493.

19. Demchenko, A.; Boons, G.-J., A highly convergent synthesis of a hexasaccharide derived from the oligosaccharide of group B type III Streptococcus. *Tetrahedron Letters* **1997,** *38* (9), 1629-1632.

20. Raghavan, S.; Kahne, D., A one step synthesis of the ciclamycin trisaccharide. *Journal of the American Chemical Society* **1993,** *115* (4), 1580-1.

21. Yu, B.; Xie, J.; Deng, S.; Hui, Y., First Synthesis of a Bidesmosidic Triterpene Saponin by a Highly Efficient Procedure. *Journal of the American Chemical Society* **1999,** *121* (51), 12196-12197.

22. Zhu, T.; Boons, G.-J., A two-directional approach for the solid-phase synthesis of trisaccharide libraries. *Angewandte Chemie, International Edition* **1998,** *37* (13/14), 1898-1900.

23. Boons, G. J.; Zhu, T., Novel regioselective glycosylations for the convergent and chemoselective assembly of oligosaccharides. *Synlett* **1997,** (7), 809-811.

24. Mong, K. K. T.; Wong, C. H., Reactivity-based one-pot synthesis of a Lewis Y carbohydrate hapten: a colon-rectal cancer antigen determinant. *Angew. Chem. Int. Ed.* **2002,** *41* (21), 4087-4090.

25. Takahashi, T.; Adachi, M.; Matsuda, A.; Doi, T., Combinatorial synthesis of trisaccharides via solution-phase one-pot glycosylation. *Tetrahedron Letters* **2000,** *41* (15), 2599-2603.

26. Tanaka, H.; Adachi, M.; Takahashi, T., One-pot synthesis of sialo-containing glycosyl amino acids by use of an N-trichloroethoxycarbonyl-beta-thiophenyl sialoside. *Chemistry-a European Journal* **2005,** *11* (3), 849-862.

27. Valverde, S.; Garcia, M.; Gomez, A. M.; Lopez, J. C., A combined intramolecular-intermolecular one-pot glycosylation approach for the synthesis of a branched trisaccharide. *Chem Commun* **2000,** (10), 813-814.

28. Yamada, H.; Kato, T.; Takahashi, T., One-pot sequential glycosylation: A new method for the synthesis of branched oligosaccharides. *Tetrahedron Letters* **1999,** *40* (24), 4581-4584.

29. Yamada, H.; Harada, T.; Takakhashi, T., Synthesis of an elicitor-active hexaglucoside analog by a one-pot, 2-step glycosidaton procedure. *J. Am. Chem. Soc.* **1994,** ***116***, 7919-7920.

30. Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T., One-Pot Sequential Glycosylation - a New Method for the Synthesis of Oligosaccharides. *Tetrahedron Letters* **1994,** *35* (23), 3979-3982.

31. Jung, K. H.; Muller, M.; Schmidt, R. R., Intramolecular O-glycoside bond formation. *Chem. Rev.* **2000,** *100* (12), 4423-+.

32. Wang, C. C.; Lee, J. C.; Luo, S. Y.; Fan, H. F.; Pai, C. L.; Yang, W. C.; Lu, L. D.; Hung, S. C., Synthesis of biologically potent alpha 1 -> 2-linked disaccharide derivatives via regioselective one-pot protection-glycosylation. *Angewandte Chemie-International Edition* **2002,** *41* (13), 2360-2362.

33. 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.

34. Cato, D.; Buskas, T.; Boons, G. J., Highly efficient stereospecific preparation of Tn and TF building blocks using thioglycosyl donors and the Ph_2SO/Tf_2O promotor system. *J. Carbohydr. Chem.* **2005,** *24* (4-6), 503-516.

35. Kim, S.; Chang, H.; Kim, W. J., Selective Benzoylation of Diols with 1-(Benzoyloxy)Benzotriazole. *Journal of Organic Chemistry* **1985,** *50* (10), 1751-1752.

36. Sjolin, P.; Kihlberg, J., Use of fluorobenzoyl protective groups in synthesis of glycopeptides: β-elimination of O-linked carbohydrates is suppressed. *Journal of Organic Chemistry* **2001,** *66*, 2957-2965.

37. Rencurosi, A.; Lay, L.; Russo, G.; Caneva, E.; Poletti, L., Glycosylation with trichloroacetimidates in ionic liquids: Influence of the reaction medium on the stereochemical outcome. *Journal of Organic Chemistry* **2005,** *70* (19), 7765-7768.

38. Muller, T.; Hummel, G.; Schmidt, R. R., Glycosyl Phosphites as Glycosyl Donors - a Comparative-Study. *Liebigs Ann Chem* **1994,** (4), 325-329.

39. Shie, C. R.; Tzeng, Z. H.; Kulkarni, S. S.; Uang, B. J.; Hsu, C. Y.; Hung, S. C., Cu(OTf)(2) as an efficient and dual-purpose catalyst in the regioselective reductive ring opening of benzylidene acetals. *Angewandte Chemie-International Edition* **2005,** *44* (11), 1665-1668.

40. Kim, J. H.; Yang, H.; Park, J.; Boons, G. J., A general strategy for stereoselective glycosylations. *J. Am. Chem. Soc.* **2005,** *127*, 12090-12097.

41. Kim, J. H.; Yang, H.; Boons, G. J., Stereoselective glycosylations using chiral auxiliaries. *Angew. Chem. Int. Ed.* **2005,** *44*, 947-949.

42. Ogawa, T.; Katano, K.; Matsui, M., Total Synthesis of Sorbistin-a-1 and a Positional Isomera,B. *Tetrahedron* **1980,** *36* (19), 2727-2733.

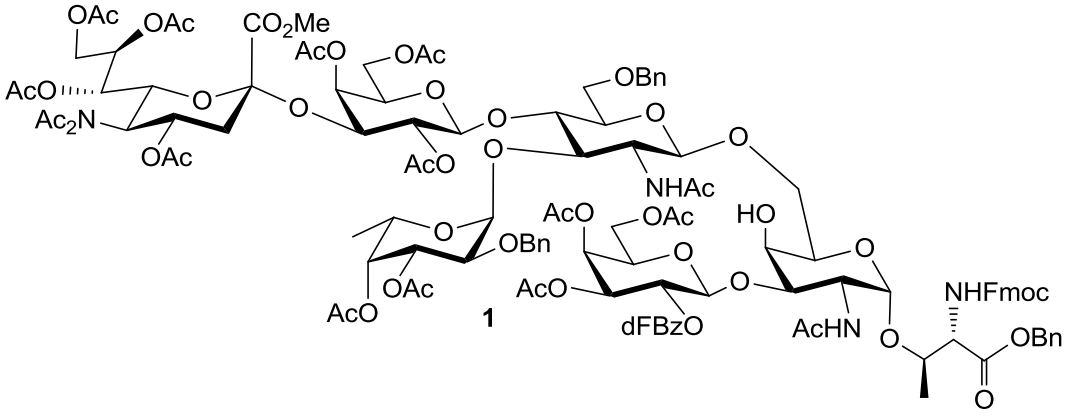
Chapter III

A Highly Convergent Strategy for the Assembly of a Glycosylated Amino Acid Derived from PSGL-1*

*** Yusuf Vohra**, Therese Buskas, and Geert-Jan Boons; *J. Org. Chem.* **2009,** *74*, 6064-6071. Reprinted here with permission of the publisher.

3.1 Abstract

P-selectin and P-selectin glycoprotein ligand 1 (PSGL-1) are vascular adhesion molecules that play an important role in the recruitment of leukocytes to inflamed tissue by establishing leukocyte–endothelial and leukocyte–platelet interaction. P-selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl Lewis^x (SLe^x) moiety linked to a properly positioned core-2 *O*-glycan and three tyrosine sulfate residues. We have developed a highly convergent synthesis of the PSGL-1 oligosaccharide linked to threonine based on the use of trichoroacetimidate donors and thioglycosyl acceptors that give products that can immediately be employed in a subsequent glycosylation step without the need for protecting group manipulations. Furthermore, by employing one-pot multi-step glycosylation sequences the number of purification steps could be minimized. The process of oligosaccharide assembly was further streamlined by combining protecting group manipulations and glycosylations as one-pot multi-step synthetic procedure. The resulting PSGL-1 oligosaccharide is properly protected for glycopeptide assembly. It is to be expected that the strategic principles employed for the synthesis of the target compound can be applied for the preparation of other complex oligosaccharides of biological and medical importance.



3.2 Introduction

The selectins are a family of three Ca^{2+} dependent membrane-bound glycoproteins that mediate the adhesion of leukocytes and platelets to vascular surfaces.[1-2] Several studies have demonstrated that they play important roles in inflammation, immune responses, hemeostasis and wound repair.[3] Selectins also contribute to a broad spectrum of diseases such as arteriosclerosis, thrombosis, organ-transplant rejection, arthritis, sickle cell anemia and tumor metastasis.[4-6]

Although there are many candidates for selectin ligands, only P-selectin glycoprotein ligand-1 (PSGL-1) has clearly been demonstrated to mediate the adhesion of leukocytes to selectins under flow. P-selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl Lewis^x (SLe^x) moiety linked to a properly positioned core-2 *O*-glycan and three tyrosine sulfate residues.[7-8]

Inhibitors of selectins may possess therapeutic properties for the treatment of a number of diseases.[9] In this respect, a recombinant truncated form of a PSGL-1 immunoglobulin fusion protein has already demonstrated effectiveness as such an inhibitor.[10-13] This glycoprotein can, however, only be produced in mammalian cells that are co-transfected with fucosyl- and core-2 GlcNAc transferases, making production of even small amounts of glycoprotein difficult. The Davis laboratory is beginning to address these problems by employing a PSGL-1 mimetic by incorporation of azidohomoalanine and cysteine in PSGL-1 using *E. coli B834* as a Met-auxotrophic expression system and selectively attach sialic acid containing oligosaccharides and a sulfated tyrosine mimic by employing the thiol and azide of the protein as chemical tags.[14] Also, it has been shown that conjugation of sialyl Lewis^x and sulfates tyrosine to a polyacrylamide gave a polymer with high affinity of L-selectin.[15]

The *N*-terminal glycosulfopeptide of PGSL-1 has also been obtained by chemo-enzymatic approaches.[14, 16-17] In these procedures, a glycosulfopeptide that contains an *N*-acetyl galactosamine linked to a threonine moiety was chemically assembled. Subsequently, glycosyl transferases were employed to assemble the complete oligosaccharide. The problems of this approach include difficulties of preparing sufficient quantities of glycosyltransferases, which often require a eukaryotic cell expression system and the need of expensive sugar nucleotides or the use of a complicated *in-situ* recycling system. Furthermore, the high selectivity of glycosyltransferases also complicates the preparation of analogs that may exhibit more desirable pharmacological properties.

Recent progress in chemical oligosaccharide synthesis is beginning to provide opportunities for the efficient and large-scale synthesis of complex oligosaccharides[18-23] and several laboratories have pursued the preparation of the oligosaccharide of PSGL-1.[24-30] In this respect, Kunz and coworkers have reported the chemical synthesis of a properly protected oligosaccharide of PSGL-1, which was attached to threonine for the preparation of a glycopeptide.[26] Although synthetic problems such as anomeric selectivity and the acid and base sensitivity of the PSGL-1 glycopeptide were addressed by employing properly protected saccharide building blocks, the synthetic approach suffered from poor regioselectivity in key glycosylations and a need for replacement of protecting groups at an advanced stage of synthesis. It is to be expected that a highly convergent approach for the synthesis of PSGL-1 will make it possible to prepare a wide range of glycopeptides structural analogs for structure activity relationships. Furthermore, it may offer an opportunity to make mimetics that have improved pharmacokinetic properties.

As part of a program to prepare the PGSL-1 analogs with improved properties, we report here a highly efficient and convergent synthesis of a properly protected oligosaccharide of PSGL-1 linked to threonine (**1**) that is appropriately protected for solid-phase glycopeptide synthesis. Key features of the approach include an orchestrated use of thioglycosides and trichoroacetimidates[31] for oligosaccharide assembly, which minimized protecting group manipulations and made it possible to employ one-pot multi-step glycosylations. The process of oligosaccharide assembly was further streamlined by combining protecting group manipulations and glycosylations as one-pot multi-step synthetic procedure.[22-23, 32-34] Furthermore, it is to be expected that the strategic principles employed for the synthesis of the target compound can be applied for the preparation of other complex oligosaccharides of biological and medical importance.

3.3 Results and Discussion

The synthesis of target compound **1** is complicated by the fact that *O*-glycosylated peptides are sensitive to acidic and basic conditions. In addition, sufficient quantities of such a complex glycosylated amino acid for glycosulfopeptide assembly can only be obtained by employing a highly convergent synthetic strategy, which uses properly protected monosaccharide building blocks that can be assembled into the target using a minimal number of synthetic steps. In this respect, strategies such as chemoselective, orthogonal, two-directional and one-pot multi-step glycosylations[22-23, 32-34] have engendered an increased efficiency of oligosaccharide synthesis by minimizing the number of protecting group manipulations on advanced intermediates. Furthermore, combining protecting group manipulations with glycosylations as a one-pot procedure can further expand the scope of these procedures.[35-39]

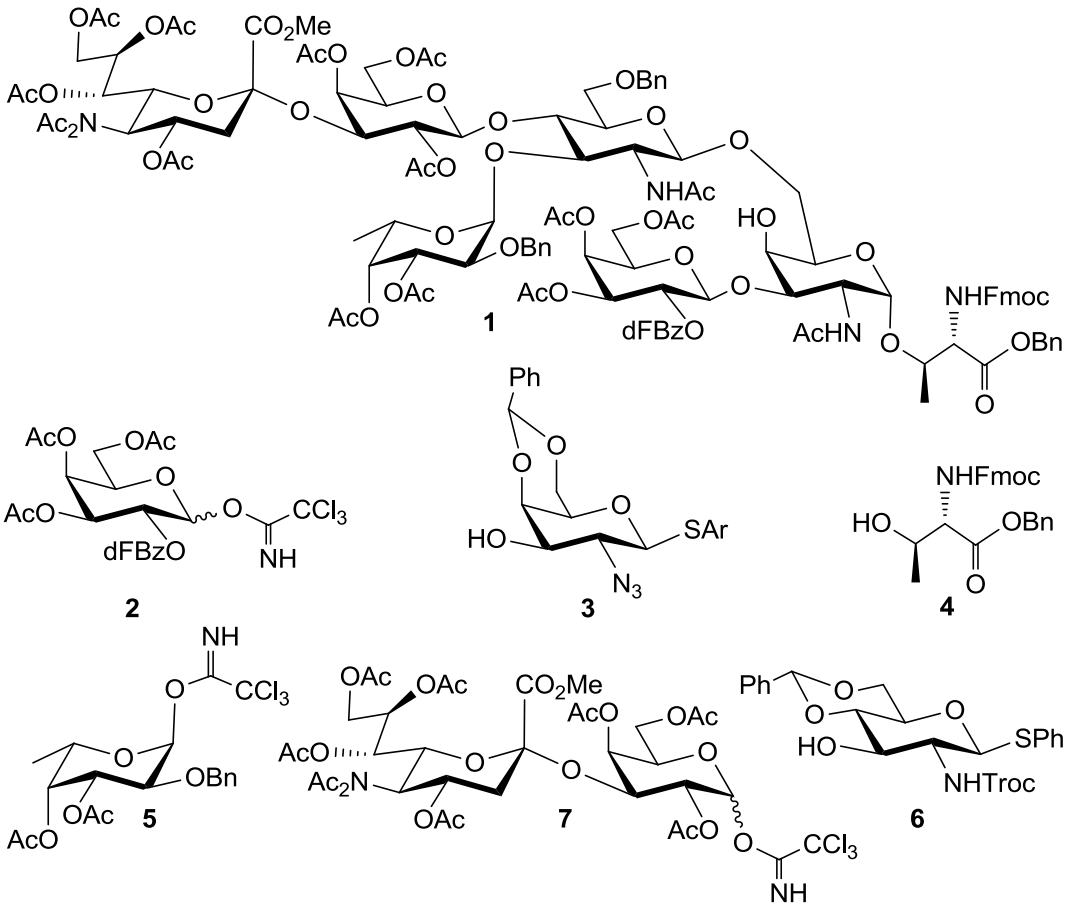
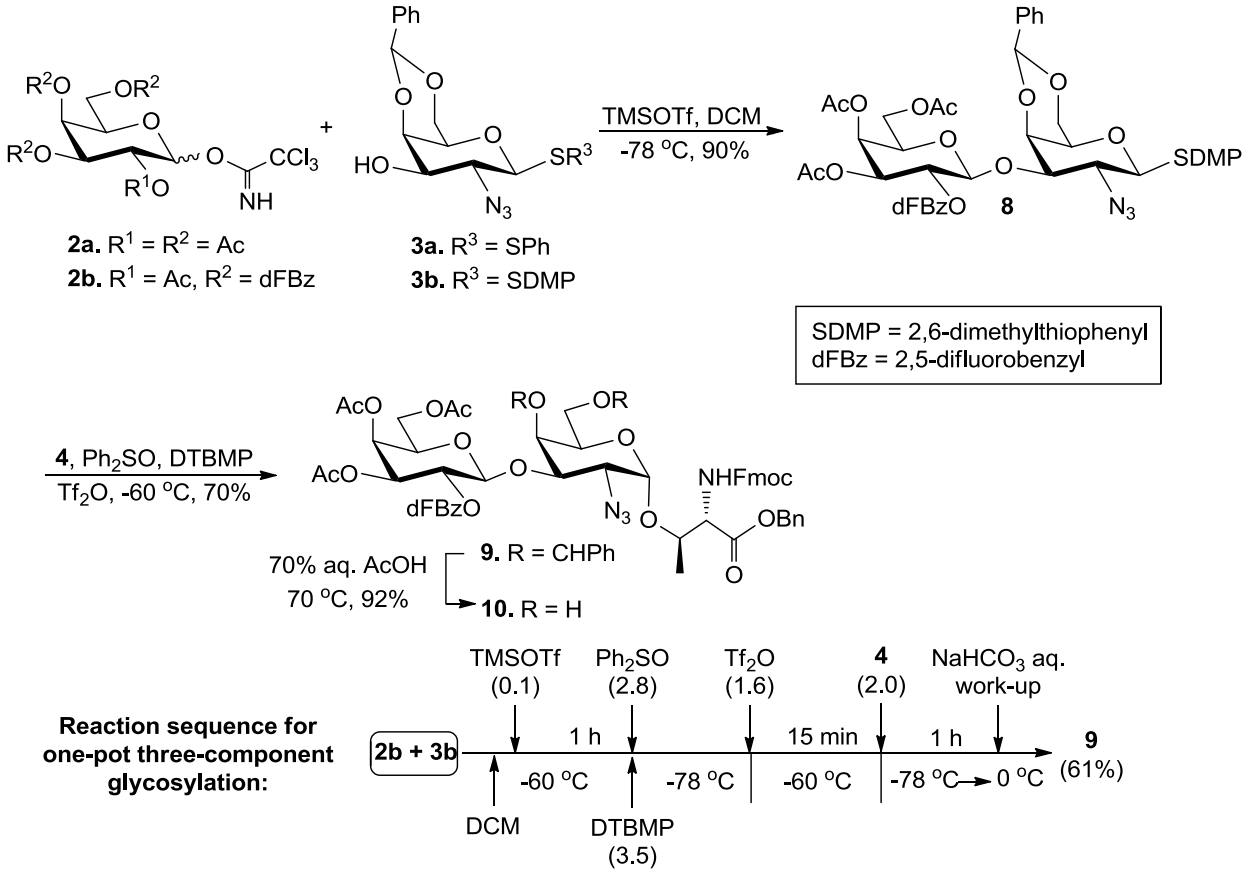


Figure 3.1 Target molecule and building blocks

It was envisaged that **1** could be prepared by a combination of chemo- and regioselective glycosylations and one-pot multi-step protocols that combine glycosylations and protecting group manipulations. Thus, the core-2 disaccharide linked to a properly protected threonine (Thr) was prepared by a chemoselective glycosylation of trichloroacetimidate **2** with thioglycoside **3** to give a disaccharide, which immediately can be activated with a thiophilic reagent for coupling with threonine acceptor **4**[40] (Figure 1). Removal of the benzylidene acetal of the resulting compound will give an acceptor for a regioselective coupling with a properly protected SLe^x derivative. It was envisaged the latter compound could be obtained by chemoselective glycosylations and one-pot reactions using compounds **5**,[41] **6**[42] and **7**.

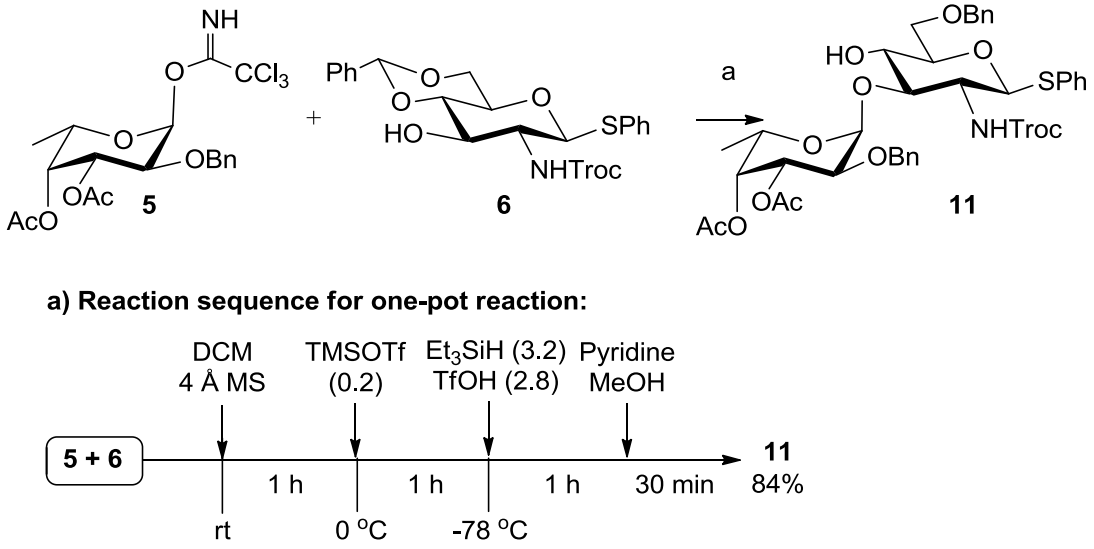
Unfortunately, coupling of galactosyl trichloroacetimidate **2a**[43] with thioglycosyl acceptor **3a**[44] in the presence of TMSOTf led to a complex mixture of products that included the required disaccharide, the corresponding ortho-ester,[45] and a thiophenyl galactoside derived from aglycon-transfer[46] (**Scheme 1**). It is well known that the use of C-2 benzoyl esters will suppress ortho-ester formation, however, this protecting group is not compatible with glycopeptide synthesis because the rather strong basic conditions required for its removal will result in β-elimination of the *O*-glycopeptide linkage.[47] Recently, it was shown that a 2,5-di-fluoro-benzoyl esters (dFBz) is an efficient neighboring group participant that suppresses ortho-ester formation. [48-49] This protecting group has, however, as an advantage that it can be removed under mild basic conditions without affecting threonine and serine glycosides. Thus, dFBz-protected glycosyl donor **2b**,[48-49] having a 2,5-di-fluoro-benzoyl (dFBz) ester at C-2 and acetyl esters at C-3, C-4 and C-6, was coupled with thioglycosyl acceptor **3a**[50] using TMSOTf as the catalyst.[51] Although orthoester formation was suppressed, the aglycon-transfer byproduct was still formed. Recently, it was reported that aglycon transfer of thioglycosyl acceptors can be avoided by employing a 2,6-dimethylthiophenyl glycoside.[52] The rationale of this observation is that the bulky 2,6-dimethylthiophenyl hinders reaction with an activated glycosyl donor, thereby reducing aglycon transfer. Indeed, trimethylsilyl triflate (TMSOTf) promoted glycosylation of **2b** with **3b**[52] gave the corresponding disaccharide **8** in an excellent yield of 90% as only the β-anomer. Next, the core-2 *O*-glycan **9** was obtained in high yield with exclusively α-selectivity by a diphenylsulfoxide/triflic anhydride mediated glycosylation[53] of thioglycoside **8** with threonine derivative **4.**



Scheme 3.1 One-pot three component reaction.

Having established efficient reaction conditions for the synthesis of **9**, attention was focused on its preparation by a one-pot procedure. Thus, coupling of galactosyl trichloroacetimidate **2b** with the galactosyl acceptor **3b** in presence of TMSOTf followed by activation of the resulting thio-disaccharide **8** by addition of diphenyl sulfoxide and triflic anhydride in presence of DTBMP[53] and coupling with threonine **4** gave oligosaccharide **9** in an overall yield of 61%. Finally, glycosyl acceptor **10** was obtained by the removal of the benzylidene acetal of **9** using aqueous acetic acid at 70 °C.

The next stage of the synthesis entailed the preparation of properly protected SLe^x glycosyl donor **15** for coupling with glycosyl acceptor **10** to give protected PSGL-1 **16**. Compound **15** was prepared from the readily available saccharide building blocks **5**, **6**, and **7** (Schemes 2 and 3). Thus, fucosyl trichloroacetimidate **5** was coupled with phenyl thioglycosyl acceptor **6** using TMSOTf as the promoter to give the corresponding disaccharide, exclusively as α-anomer, which was then treated with triethylsilane and trifluoromethanesulfonic acid (TfOH) for regioselective opening of the benzylidene acetal[39, 54] to provide glycosyl acceptor **11** in an overall yield of 84% with excellent stereo- and regio-selectivity. The regioselectivity of the latter reaction was confirmed by acetylation of compound **11** and the ^{1}H-NMR of the resulting derivative showed a significant down field shift for H-4 (4.94 ppm).



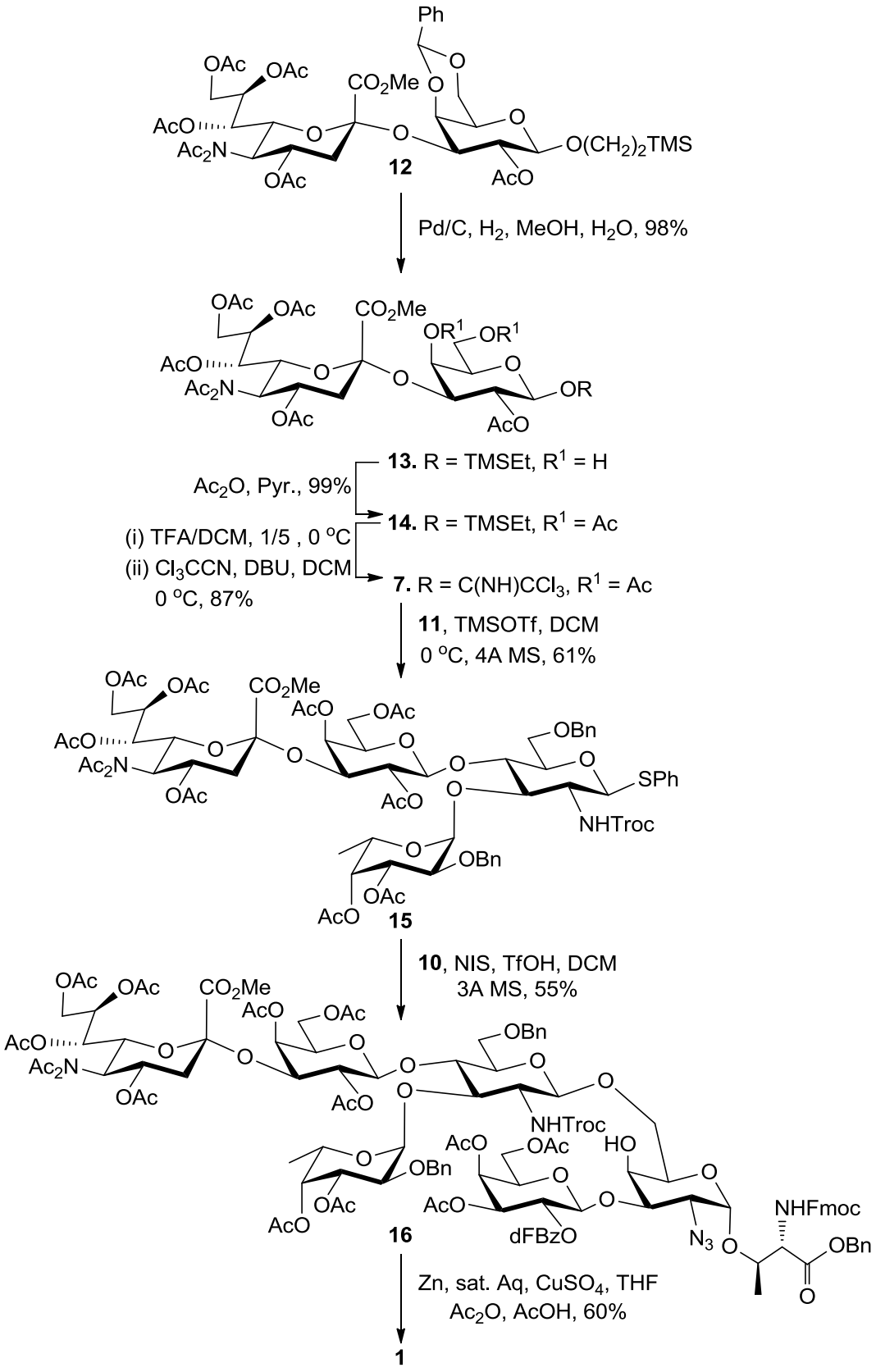
Scheme 3.2 One-pot glycosylation followed by reductive ring-opening of benzylidene acetal.

Glycosyl donor **7** could be obtained in facile manner from the known disaccharide **12**[55] by a four-step reaction sequence. Thus, hydrogenation of **12** over Pd/C to remove the benzylidene acetal was followed by acetylation of the hydroxyls of the resulting compound **13** to give **14** in a quantitative overall yield. Next, the anomeric trimethylsilylethyl moiety of **14** was

cleaved by treatment with trifluoroacetic acid in dichloromethane and the resulting lactol was converted into trichloroacetimidate **7** by reaction with trichloroacetonitrile and DBU in dichloromethane.

Next, a TMSOTf mediated coupling of trichloroactimidate **7** with **11** gave the properly protected SLe^x tetrasaccharide **15** in good yield. Up to this stage of the synthesis, the thiophenyl moiety of **15** has functioned as an effective anomeric-protecting group. However, in the next step it was activated with the thiophilic promoter NIS/TfOH for coupling with **10** to give the hexasaccharide **16** in a yield of 55%. As expected, no glycosylation of the less reactive C-4 hydroxyl of **11** was observed, which was confirmed by a range of two-dimensional NMR experiments. Thus, Heteronuclear Multiple Bond Correlation NMR Spectroscopy (HMBC) of **16** showed a cross peak between H-1 of β-GluNTroc (4.62 ppm) and C-6 of α-$GalN_3$ (69.3 ppm), confirming that the glycosylation had occurred at the C-6 hydroxyl of **10**. The latter was also supported by Nuclear Overhauser Enhancement Spectroscopy (NOESY), which revealed cross peaks between the H-1 of β-GluNTroc and H-6a and H-6b of the α-$GalN_3$ moiety. Furthermore, due to effective neighboring group participation of the *N*-Troc group of **15** only the β-glycoside was formed, which was confirmed by a large coupling constant between H-1 and H-2 (10.0 Hz).

Finally, the Troc and the azido moiety of **16** were converted into acetamido functions by reduction with $Zn/CuSO_4$ in a mixture of THF, acetic acid and acetic anhydride to give the target compound **1**.[56] It is envisaged that the benzyl ester of compound **1** can be removed by performing hydrogenolysis over palladium in a mixture of isopropanol and pyridine.[57] The benzyl ethers in the glycan can be removed after glycopeptide assembly by using the previously described "low TfOH" method.[58-59]



Scheme 3.3 Synthesis of the target compound.

3.4 Conclusion

In conclusion, a properly protected PSGL-1 oligosaccharide linked to threonine has been described that is appropriately protected for glycosulfopeptide assembly. A highly convergent strategy utilizing six strategically protected building blocks, combined with one-pot, chemoselective and regioselective glycosylations was employed to minimize the number of protecting group manipulations and purifications during oligosaccharide assembly. The longest linear sequence entailed only seven chemical steps and gave the target compounds in an excellent overall yield of 17%. Previous attempts to chemically synthesize the PSGL-1 oligosaccharide suffered from extensive replacement of protecting groups at advanced stages of the synthesis and poor regioselectivities in crucial glycosylation steps compromising the poor overall yield of target compound.[26] It is to be expected that the strategic principles employed for the synthesis of **1** will be relevant for the synthesis of many other complex oligosaccharides of biological and medical importance.

3.5 Experimental Section

General remarks:

All reactions were carried out under nitrogen with anhydrous solvents, unless otherwise stated. CH_2Cl_2 was distilled from CaH_2 prior to use in reactions. All the starting materials were kept *in vacuo* with P_2O_5 prior to use. Chemicals used were reagent grade as supplied except where noted. *N*-iodosuccinimide was used after recrystalization in dioxane/CCl_4. Column chromatography was performed on silica gel G60 (60-200 μm 60 Å), reactions were monitored by TLC on Silicagel 60 F_{254}. The compounds were detected by examination under the UV light and visualized by charring with 10% sulfuric acid in MeOH or cerium ammonium molybdate in 20% aq. sulfuric acid. Solvents were removed under reduced pressure at ≤ 30 °C. ^{1}H-NMR and HSQC spectra were recorded in $CDCl_3$ at 500 MHz on a Varian Inova spectrometer with tetramethylsilane as internal standard, unless otherwise stated. High-resolution mass spectra were obtained by using MALDI-ToF with 2,5-dihydroxybenzoic acid as matrix and the internal standards ultramark 1621 and PEG.

2,6-dimethylphenyl [2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)]-1-thio-α-D-galactopyranoside (8):

A mixture of galactosyl acceptor **3b** (93 mg, 0.22 mmol), galactosyl trichloroacetaimidate donor **2b** (200 mg, 0.34 mmol), and 4Å MS in CH_2Cl_2 (3 ml) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to -78 °C. TMSOTf (0.023 mmol, 0.23M solution in CH_2Cl_2) was added and the temperature was raised to -15 °C with stirring over a period of 2 h. The progress of the reaction was monitored by TLC and

MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (20 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford compound **8** (170 mg, 90%) as a white foam. Analytical data for **8**: R_f = 0.35 (Hexanes:EtOAc, 2:1, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.52-6.97 (m, 6H, aromatic), 5.49 (dd, 1H, $J_{1',2'}$ = 8.2 Hz, $J_{2',3'}$ = 10.2 Hz, H-2'), 5.43 (s, 1H, C*H*Ph), 5.38 (bd, 1H, J = 3.2 Hz, H-4'), 5.12 (dd, 1H, $J_{3',4'}$ = 3.1 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 4.96 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.18 (m, 2H, H-1, H-4), 4.15-4.05 (m, 3H, H-6a, H-6a', H-6b'), 3.91-3.85 (m, 2H, H-5', H-6b), 3.72 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.9 Hz, H-2), 3.46 (dd, 1H, $J_{3,4}$ = 3.1 Hz, $J_{2,3}$ = 9.9 Hz, H-3), 3.17 (bs, 1H, H-5), 2.51 (s, 6H, 2 x CH_3, SDMP), 2.11 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 102.3 (C-1'), 101.1 (*CH*Ph), 89.4 (C-1), 80.9 (C-3), 75.3 (C-4), 71.4 (C-5'), 71.2 (C-3'), 70.3 (C-2'), 70.0 (C-5), 69.6 (C-6), 67.3 (C-4'), 62.4 (C-2), 61.8 (C-6'), 22.9 (*CH*$_3$-SDMP), 21.0, 20.9, 20.8 (3 x OAc); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{40}H_{41}F_2N_3O_{13}S$ $[M+Na]^+$: 864.2226; found 864.2231.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine benzylester (9):**

Method A:

A mixture of disaccharide donor **8** (170 mg, 0.20 mmol), Ph_2SO (114 mg, 0.56 mmol), and 4Å MS in CH_2Cl_2 (5 ml) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to -60 °C after the addition of 2,6-di-*tert*-butyl-4-methylpyridine (124 mg, 0.60 mmol). Stirring was continued for 10 min. at the same temperature followed by the addition of Tf_2O (47 µL, 0.28 mmol). Stirring was continued for another 15 min at the same temperature followed by the addition of a solution of threonine acceptor **4** (173 mg, 0.40 mmol) in CH_2Cl_2 (2 ml). The temperature of the reaction mixture was raised to 0 °C over a period of 1 h. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (30 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (15 ml), water (15 ml), and brine (15 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford compound **9** (156 mg, 70%) as a white amorphous solid.

Method B:

A mixture of galactosyl acceptor **3b** (65 mg, 0.16 mmol), galactosyl trichloroacetaimidate donor **2b** (120 mg, 0.20 mmol), and 4Å MS in CH_2Cl_2 (2 ml) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to -60 °C. TMSOTf (0.016 mmol, 0.16M solution in CH_2Cl_2) was added and stirring was continued for 1 h at the same temperature. The reaction mixture was then cooled to -78 °C followed by addition of

Ph_2SO (88 mg, 0.44 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (112 mg, 0.55 mmol). After stirring for 10 min. at the same temperature, Tf_2O (37 μL, 0.22 mmol) was added followed by increasing the temperature to -60 °C over a period of 15 min. The reaction mixture was again cooled to -78 °C followed by addition of a solution of threonine acceptor **4** (100 mg, 0.23 mmol) in CH_2Cl_2 (1 ml). The temperature of the reaction mixture was raised to 0 °C over a period of 1 h. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (20 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford compound **9** (106 mg, 61%) as a white amorphous solid. Analytical data for **9**: R_f = 0.25 (Hexanes:EtOAc, 2:1, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.72-6.87 (m, 21H, aromatic), 5.66 (d, 1H, *J* = 9.4, N*H*Fmoc), 5.51-5.45 (m, 2H, C*H*Ph, H-2'), 5.40 (bd, 1H, H-4'), 5.15 (dd, 1H, $J_{3',4'}$ = 3.2 Hz, $J_{2',3'}$ = 10.2 Hz, H-3'), 5.10 (bt, 2H, CH_2, Bn), 4.88 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.83 (d, 1H, $J_{1,2}$ = 3.5, H-1), 4.46-4.25 (m, 5H, OC*H*CH_3 threonine, CH_2CH-Fmoc, C*H*COOBn threonine, H-4,), 4.21-4.08 (m, 4H, H-6a', CH_2C*H*-Fmoc, H-6b', H-6a), 3.96-3.92 (m, 3H, H-6b, H-3, H-5'), 3.69 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 3.56 (bs, 1H, H-5), 2.11 (s, 3H, OCH_3), 1.97 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3), 1.23 (d, 3H, OCHCH_3 threonine) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 102.3 (C-1'), 100.8 (*CH*Ph), 99.4 (C-1), 76.2 (O*CH*CH_3 threonine), 75.9 (C-4), 75.8 (C-3), 71.2 (C-5'), 71.1 (C-3'), 70.3 (C-2'), 69.3 (C-6), 68.0 (CH_2Ph), 67.6 (CH_2CH-Fmoc), 67.3 (C-4'), 63.7 (C-5), 61.5 (C-6'), 59.5 (C-2), 58.9 (*CH*COOBn threonine), 47.4 (CH_2*CH*-Fmoc), 21.0, 20.9, 20.7 (3 x OAc), 19.0 (OCH*CH*$_3$ threonine); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{58}H_{56}F_2N_4O_{18}$ $[M+Na]^+$: 1157.3455; found 1157.3460 $[M+Na]^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-azido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine benzyl ester (10):**

A solution of compound **9** (80 mg, 0.072 mmol) in 5 ml of 70% aq. acetic acid was heated at 70 °C for 3 h. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was cooled to rt and concentrated by co-evaporation with toluene *in vacuo.* The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 1:2, v:v) to afford compound **10** (59 mg, 92%) as a white amorphous solid. Analytical data for **10**: R_f = 0.25 (Hexanes:EtOAc, 1:2, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.72-6.94 (m, 16H, aromatic), 5.60 (d, 1H, *J* = 9.5 Hz, N*H*Fmoc), 5.50-5.46 (q, 1H, $J_{1',2'}$ = 8.6 Hz, $J_{3',2'}$ = 10.3 Hz, H-2'), 5.40 (bd, 1H, H-4'), 5.16 (q, 1H, $J_{3',4'}$ = 2.9 Hz, $J_{2',3'}$ = 10.3 Hz, H-3'), 5.12-5.01 (dd, 2H, *CH*$_2$Ph), 4.81 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.76 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.41-4.35 (m, 3H, OC*H*CH$_3$ threonine, C*H*COOBn threonine, C*H*H-Fmoc), 4.25-4.21 (m, 1H, C*H*H-Fmoc), 4.16-4.05 (m, 4H, H-6a', H-6b', H-4, CH$_2$C*H*-Fmoc), 3.96 (m, 1H, H-5'), 3.91-3.88 (dd, 1H, $J_{3,4}$ = 2.7 Hz, $J_{2,3}$ = 10.7 Hz, H-3), 3.83-3.70 (m, 3H, H-5, H-6a, H-6b), 3.46-3.44 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 2.75 (bs, 1H, C4-O*H*), 2.32 (bs, 1H, C6-O*H*), 2.13 (s, 3H, OC*H*$_3$), 2.00 (s, 3H, COC*H*$_3$), 1.90 (s, 3H, COC*H*$_3$), 1.24 (d, 3H, OCHC*H*$_3$) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 101.8 (C-1'), 99.2 (C-1), 78.1 (C-3), 76.2 (O*CH*CH$_3$ threonine), 71.7 (C-5'), 70.7 (C-3'), 69.8 (C-2'), 69.7 (C-5), 69.3 (C-4), 67.8 (*CH*$_2$Ph), 67.4 (*CH*$_2$CH-Fmoc), 67.2 (C-4'), 62.7 (C-6), 61.6 (C-6'), 59.0 (C-2), 58.7 (*CH*COOBn threonine), 47.4 (CH$_2$*CH*-Fmoc), 20.8, 20.7, 20.6 (3 x OAc), 18.6 (OCH*CH*$_3$ threonine); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{51}H_{52}F_2N_4O_{18}$ $[M+Na]^+$: 1069.3142; found 1069.3140.

Phenyl 3,4-di-*O*-acetyl-2-*O*-benzyl-6-deoxy-5-methyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)carbonyl amino-6-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (11):

A mixture of glycosyl acceptor **6** (50 mg, 0.09 mmol), trichloroacetimidate donor **5** (63 mg, 0.13 mmol), and 4Å MS in CH_2Cl_2 (1 ml) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to 0 °C. TMSOTf (0.018 mmol, 0.18M solution in CH_2Cl_2) was added and stirring was continued for 30 min at the same temperature. The reaction mixture was then cooled to -78 °C followed by addition of TfOH (23 μL, 0.26 mmol) and triethylsilane (48 μL, 0.30 mmol). The reaction mixture was then stirred at -78 °C for 30 min. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 μL) and MeOH (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford compound **11** (67 mg, 84%) as a white amorphous solid. Analytical data for **11**: R_f = 0.40 (Hexanes:EtOAc, 1:1, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.50-7.17 (m, 15H, aromatic), 5.46 (d, 1H, *J* = 6.6 Hz, N*H*Troc), 5.30-5.27 (m, 2H, H-3', H-4'), 5.09 (m, 2H, H-1, H-1'), 4.70-4.57 (m, 6H, 2 x C*H*$_2$, Bn, OC*H*$_2$CCl$_3$), 4.42 (m, 1H, H-5'), 3.89-3.83 (m, 3H, H-2', H-3, H-6a), 3.78-3.75 (dd, 1H, H-6b), 3.68 (bs, 1H, C4-OH), 3.58-3.55 (m, 2H, H-4, H-5), 3.31 (m, 1H, H-2), 2.13 (s, 3H, COC*H*$_3$), 1.98 (s, 3H, COC*H*$_3$), 1.12 (d, 3H, *J* = 6.6 Hz, CH_3 fucose) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 98.6 (C-1'), 85.9 (C-1), 83.8 (C-3), 78.7 (C-5), 74.1-73.6 (2 x *CH*$_2$Ph, *CH*$_2$Troc), 73.8 (C-2'), 71.2 (C-3'), 71.0 (C-4), 70.3 (C-4'), 70.2 (C-6), 66.0 (C-5'), 55.7 (C-3), 21.0, 20.8 (2 x OAc), 16.4 (C-6'); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{39}H_{44}Cl_3NO_{12}S$ $[M+Na]^+$: 878.1547; found 878.1543.

2-(Trimethylsilyl)ethyl [methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate]-(2→3)-*O*-β-D-galactopyranoside (13):

To a solution of **12** (250 mg, 0.283 mmol) in CH_2Cl_2:MeOH (30:1, v:v; 15 mL) under an argon atmosphere was added Pd, 10 wt. % on activated carbon, (150 mg) and the mixture stirred for 20 min. at room temperature. The argon was replaced with $H_{2(g)}$, and the reaction stirred for 8 h. The solution was diluted with CH_2Cl_2 (50 mL) and filtered through celite. The solvent was removed by evaporation under reduced pressure and the residue purified by silica gel column chromatography (Toluene:Acetone, 5:2, v:v) to afford compound **13** (220 mg, 98%) as a white amorphous solid. Analytical data for **13**: R_f = 0.48 (Toluene:Acetone, 1:1, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 5.52 (ddd, 1H, H-4'), 5.34 (dd, 1H, H-8'), 5.15 (dd, 1H, *J* = 8.2 Hz, H-7'), 4.94 (d, 1H, *J* = 10.3 Hz, H-6'), 4.42 (d, 1H, *J* = 7.6 Hz, H-1), 4.33 (dd, 1H, H-9a'), 4.21 (t, 1H, *J* = 10.2 Hz, H-5'), 4.12 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 4.08 (dd, 1H, H-9b'), 4.02 (m, 1H, OC*H*H), 3.91 (dd, 1H, H-6), 3.92-3.82 (m, 5H, H-6a, H-6b,COOCH_3), 3.70-3.60 (m, 3H, H-4, H-2, OC*H*H), 3.55 (t, 1H, H-5), 2.86 (dd, 1H, H-3'eq), 2.35, 2.28 (2 x s, 6H, N($COCH_3$)$_2$), 2.10, 2.09, 2.00, 1.97 (4 x s, 12H, 4 x $COCH_3$), 1.93 (dd, 1H, H-3'ax), 1.13-0.85 (m, 2H, CH_2SiMe$_3$), 0.01 (s, 9H, Si(CH_3)$_3$); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 102.7 (C-1), 77.2 (C-3), 73.8 (C-5), 70.4 (C-6'), 69.5 (C-2), 68.9 (C-4), 68.7 (C-8'), 67.3 (*C*H_2CH$_2$SiMe$_3$), 66.9 (C-7'), 66.8 (C-4'), 62.6 (C-6), 62.2 (C-9'), 56.9 (C-5'), 53.7 (COO*C*H_3), 38.6 (C-3'), 28.3, 26.3 (NAc$_2$), 21.4, 21.1, 21.0 (3 x OAc), 18.3 (*C*H_2SiMe$_3$); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{33}H_{53}NO_{19}Si$ $[M+Na]^+$: 818.2879; found 818.2880.

2-(Trimethylsilyl)ethyl [methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-glycero-α-D-galacto-2-non-ulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-galactopyranoside) (14):

Compound **13** (215 mg, 0.270 mmol) was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) and the reaction stirred for 14 h at room temperature. The solvent was removed by co-evaporation with toluene (3 x 50 mL). Silica gel column chromatography (Hexanes:EtOAc, 1:1, v:v) of the residue afforded compound **14** (246 mg, 99%) as a white solid. Analytical data for **14**: R_f = 0.55 (Hexanes:EtOAc, 1:3, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 5.53-5.47 (m, 2H, H-4', H-8'), 5.14 (dd, 1H, H-7', *J* = 9.3 Hz, 2.4 Hz), 4.98-4.94 (m, 2H, H-3, H-4), 4.58-4.53 (m, 3H, H-2, H-6', H-1), 4.28-4.25 (m, 2H, H-5', H-9a'), 4.08-3.98 (m, 3H, H-6a, H-6b, H-9b'), 3.97-3.92 (dt, 1H, OC*H*HCH$_2$Si(CH$_3$)$_3$), 3.85 (s, 3H, COOCH$_3$), 3.83 (t, 1H, H-5), 3.59-3.54 (dt, 1H, OCH*H*CH$_2$Si(CH$_3$)$_3$), 2.63 (dd, 1H, *J* = 5.4 Hz, 12.7 Hz, H-3'eq), 2.32, 2.25 (2 x s, 6H, N(COCH$_3$)$_2$), 2.17, 2.15, 2.04, 2.01, 2.00, 1.99, 1.91 (7 x s, 21H, 7 x COCH$_3$), 1.60 (t, 1H, *J* = 12.2 Hz, H-3'ax), 1.01-0.86 (m, 2H, C*H*$_2$Si(CH$_3$)$_3$), 0.00 (s, 9H, Si(CH$_3$)$_3$); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 100.8 (C-1), 71.8 (C-2), 70.6 (C-5), 70.4 (C-3), 69.6 (C-6'), 67.9 (C-4), 67.8 (C-4'), 67.6 (*CH*$_2$CH$_2$SiMe$_3$), 67.4 (C-7'), 67.3 (C-8'), 62.7 (C-6), 62.4 (C-9'), 56.5 (C-5'), 53.3 (COO*CH*$_3$), 38.7 (C-3'), 28.4, 27.0 (NAc$_2$), 22.0-20.6 (7 x OAc), 18.4 (*CH*$_2$SiMe$_3$), 1.3 (SiMe$_3$); HR HR-MALDI-ToF/MS: *m/z*: calc. for $C_{39}H_{59}NO_{22}Si$ $[M+Na]^+$: 944.3196; found 944.3194.

Methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl) trichloroacetimidate (7):

TFA (2 mL) was added to a solution of compound **14** (240 mg, 0.260 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the reaction stirred for 4 h at the same temperature. The solvent was removed by co-evaporation with toluene (5 x 20 mL). The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 2:5, v:v). Trichloroacetonitrile (130 µL, 1.26 mmol) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (14 µL, 94.8 µmol) were added to a solution of methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2- non-ulopyranosylonate-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranoside) (207 mg, 0.252 mmol), in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h and then concentrated in *vacuo*. Silica gel column chromatography (Hexanes:EtOAc, 1:2, v:v) of the syrup afforded compound **7** (220 mg, 87% over two steps, 3:2 α:β) as a white foam. Analytical data for **7**: R_f = 0.30 (Hexanes:EtOAc, 1:2, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 8.65 (s, 1H, NH), 8.61 (s, 1H, NH), 6.48 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1α), 5.93 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1β), 5.56-5.50 (m, 2H, H-4'α, H-8'α), 5.37-5.35 (m, 2H, H-4'β, H-8'β), 5.27-5.24 (m, 2H, H-2α, H-2β), 5.15-5.12 (m, 2H, H-7'α, H-7'β), 5.04 (bd, 1H, H-4β), 4.99 (dd, 1H, *J* = 3.4 Hz, 10.5 Hz, H-3α), 4.77 (dd, 1H, *J* = 3.4 Hz, 10.0 Hz, H-3β), 4.62 (m, 2H, H-6'α, H-6'β), 4.32-4.28 (m, 2H, H-5'α, H-6a), 4.23-4.06 (m, 4H, H-5'β, H-6b, H-9'aα, H-9'bα), 4.02-3.95 (m, 2H, H-9'aβ, H-9'bβ), 3.88 (s, 3H, COOCH_3α), 3.85 (s, 3H, COOCH_3β) 2.70 (dt, 1H, H-3'eq), 2.35, 2.33 (2 x s, 6H, NCOCH_3α), 2.28, 2.27 (2 x s, 6H, NCOCH_3β), 2.16-1.93 (7 x s, 21H, 7 x COCH_3), 1.68 (dd, 1H, H-3'ax); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 96.3 (C-1β), 94.2 C-1α), 72.0 (C-5), 71.2 (C-3β), 69.8 (C-6'), 68.4 (C-2), 68.3 (C-3α), 67.9 (C-4'α), 67.8 (C-8'α), 67.5 (C-4β), 67.3 (C-4'β), 67.2 (C-

8'β), 67.1 (C-7'), 62.5 (C-6), 62.3 (C-9'β), 61.8 (C-9'α), 56.6 (C-5'α), 56.1 (C-5'β), 53.3 (COO*CH*$_3$), 38.9 (C-3'), 28.4, 28.3 (NAc$_2$α), 26.9, 26.8 (NAc$_2$β), 23.0-21.8 (7 x OAc α/β);

Phenyl [*O*-methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-[6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (15):

A mixture of disaccharide acceptor **11** (30 mg, 0.035 mmol) and trichloroacetimidate donor **7** (50 mg, 0.052 mmol) in CH_2Cl_2 (4 ml) was placed under an atmosphere of argon and stirred at room temperature with 4Å MS for 1 h. The reaction mixture was then cooled to 0 °C. TMSOTf (3.0 μmol, 0.035M solution in CH_2Cl_2) was added and stirring was continued for 1 h at the same temperature. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 μL), diluted with CH_2Cl_2 (10 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography ($CHCl_3$:EtOAc, 1:1, v:v) to afford compound **15** (35 mg, 61%) as a white amorphous solid. Analytical data for **15**: R_f = 0.25 (Acetone:Toluene, 1:3, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.43-7.13 (m, 15H, aromatic), 5.56-5.51 (m, 2H, H-8''', H-4'''), 5.32 (d, 1H, *J* = 7.3 Hz, N*H*Troc), 5.27 (bs, 1H, H-4''), 5.21-5.14 (m, 3H, H-1, H-1'', H-3''), 4.99 (d, 1H, *J* = 1.0 Hz, H-4'), 4.91-4.86 (m, 2H, H-5'', H-2'), 4.83 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.79 (d, 1H, C*H*HPh), 4.68-4.90 (m, 7H, CH_2Ph, C*H*HPh, CH_2CCl$_3$, H-3', H-6''), 4.29 (t, $J_{4',5'} = J_{5',6'}$ = 10.1 Hz, H-5'), 4.23-4.14 (m, 4H, H-6a', H-6b', H-9a''', H-3), 4.03 (m, 1H, H-9b'''), 3.96 (t, 1H, $J_{3,4} = J_{5,4}$ = 9.0 Hz, H-4), 3.87-3.80 (m, 5H,

COOCH_3, H-6a, H-6b), 3.78 (m, 1H, H-5'), 3.52(bd, 1H, H-5), 3.10 (bm, 1H, H-2), 2.62 (m, 1H, H-3a'''), 2.33, 2.26 (2s, 6H, 2x NCOCH_3), 2.15-1.92 (9s, 27H, 9x COCH_3), 1.62 (m, 1H, H-3b'''), 1.17 (d, 3H, J = 6.6 Hz, CH_3 fucose) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 99.6 (C-1'), 97.7 (C-1''), 84.5 (C-1), 79.7 (C-5), 75.7 (C-3), 74.6 (*CH*$_2$Ph), 74.4 (C-2''), 73.9 (C-4), 73.2 (*CH*$_2$Ph), 72.3 (C-4''), 71.8 (C-3'), 71.2 (C-5'), 70.8 (C-5''), 70.6 (C-3''), 69.7 (C-6'''), 68.7 (C-6), 67.8 (C-4'), 67.5 (C-8'''), 67.4 (C-7'''), 67.3 (C-4'''), 64.8 (C-2'), 62.4 (C-9'''), 62.0 (C-6'), 57.9 (C-2), 56.2 (C-5'''), 53.4 (COO*CH*$_3$), 38.7 (C-3'''), 28.6, 27.2 (NAc_2), 21.6-20.5 (9 x OAc), 16.3 (C-6''); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{73}H_{89}Cl_3N_2O_{33}S$ $[M+Na]^+$: 1681.4032; found 1681.4029.

_N_-(9-Fluorenylmethyloxycarbonyl)-_O_-[2-azido-2-deoxy-3-_O_-(3,4,6-tri-_O_-acetyl-2-_O_-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-6-_O_-(_O_-methyl 5-(_N_-acetylacetamido)-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate)-(2→3)-_O_-(2,4,6-tri-_O_-acetyl-β-D-galactopyranosyl)-(1→4)-_O_-[(3,4-di-_O_-acetyl-2-_O_-benzyl-α-L-fucopyranosyl)-(1→3)]-_O_-(6-_O_-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α-D-galactopyranosyl]-L-threonine benzyl ester (16):

A mixture of disaccharide acceptor **10** (22 mg, 0.025 mmol) and tetrasaccharide donor **15** (32 mg, 0.019 mmol) in CH_2Cl_2 (2 ml) was placed under an atmosphere of argon and stirred at room temperature with 4Å MS for 1 h. The reaction mixture was then cooled to 0 °C. *N*-iodosuccinimide (22 mg, 0.096 mmol) and TfOH (0.019 mmol, 0.20M solution in CH_2Cl_2) were added sequentially and stirring was continued for 1 h at the same temperature. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction was diluted with CH_2Cl_2 (10 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10

ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexanes:EtOAc, 1:2, v:v) to afford compound **16** (29 mg, 55%) as a white amorphous solid. Analytical data for **16**: R_f = 0.30 (Hexanes:EtOAc, 1:2, v:v); ^{1}H-NMR (500 MHz, , $CDCl_3$) : δ = 7.77-6.42 (m, 26H, 3 x Bn, Fmoc, dFBz), 5.92-5.88 (m, 2H, H-4’’’’’, H-8’’’’’), 5.84 (t, 1H, H-2’), 5.74 (m, 2H, H-3’’’, H-4’’’), 5.61 (d, 1H, *J* = 3 Hz, H-1’’’), 5.47-5.41 (m, 4H, H-2’’’’, H-7’’’’’, H-4’, H-4’’’’), 5.34 (m, 1H, H-5’’’), 5.27 (m, 1H, H-3’), 5.19 (d, 1H, *J* = 8.1 Hz, H-1’’’’), 5.04-4.99 (m, 3H, H-6’’’’’, H-3’’’’, COOC*H*HPh), 4.95 (d, 1H, OC*H*HPh), 4.86-4.77 (m, 3H, COOCH*H*Ph, COOC*H*HCCl$_3$, OC*H*HPh), 4.73-4.49 (m, 9H, COOC*H*HCCl$_3$, H-6a’’’’, OC*H*HPh, H-1’’, H-1, H-5’’’’’, OC*H*CH$_3$ threonine, OC*H*HPh, H-6b’’’’), 4.44-4.18 (m, 9H, C*H*$_2$Fmoc, H-9a’’’’’, H-9b’’’’’, H-5’’’’, C*H*COOBn threonine, H-1’, H-3’’, H-4’’), 4.15-3.79 (m, 11H, H-6a, H-6b, H-2’’’, H-6a’, H-6b’, CH$_2$C*H*Fmoc, H-5, H-4, H-6a’, H-6b’, H-3), 3.77 (s, 3H, COOC*H*$_3$), 3.46 (m, 4H, H-2’’, H-2, H-5’, H-5’’), 2.85 (dd, 1H, H-3’’’’’), 2.26, 2.22 (2s, 6H, 2x NCOC*H*$_3$), 1.87 (m, 1H, H-3’’’’’), 1.88-1.65 (11s, 33H, 11x COC*H*$_3$), 1.60 (d, 1.623H, *J* = 6.5 Hz, C*H*$_3$ fucose), 1.57 (s, 3H, COC*H*$_3$), 1.34 (m, 3H, C*H*$_3$ threonine) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 101.6 (C-1’), 100.7 (C-1’’), 100.5 (C-1’’’’), 99.9 (C-1), 97.4 (C-1’’’), 78.4 (C-3), 76.5 (O*CH*CH$_3$ threonine), 75.6 (C-5’’), 74.9 (C-2’’’), 74.8 (C-3’’), 74.7 (C-4’’), 74.5 (*CH*$_2$Ph), 73.6(*CH*$_2$Ph), 73.1 (*CH*$_2$Troc), 72.6 (C-4’’’), 72.4 (C-3’’’’), 71.7 (C-5’), 71.5 (C-5’’’’), 71.1 (C-3’), 71.0 (C-2’’’’), 70.8 (C-7’’’’’), 70.6 (C-3’’’), 70.5 (C-6’’’’’), 70.3 (C-2’), 69.9 (C-5), 69.3 (C-6), 69.1 (C-6’’), 68.1 (C-4), 67.9 C-4’’’’), 67.7 (*CH*$_2$Fmoc), 67.6 (COO*CH*$_2$Ph threonine), 67.5 (C-4’), 67.4 (C-4’’’’’), 67.3 (C-8’’’’’), 65.2 (C-5’’’), 62.5 (C-9’’’’’), 62.1 (C-6’’’’), 61.6 (C-6’), 59.5 (O*CH*CH$_3$ threonine), 59.3 (C-2), 58.9 (C-2’’), 56.3 (C-5’’’’’), 52.9 (COO*CH*$_3$), 47.6 (CH$_2$*CH*Fmoc), 39.0 (C-3’’’’’), 21.0, 20.9 (NAc$_2$), 20.8-20.1 (12 x OAc), 18.9 (*CH*$_3$ threonine),

16.5 (C-6’’’); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{118}H_{135}Cl_3F_2N_6O_{51}$ $[M+Na]^+$: 2617.7086; found 2617.7091.

_N_-(9-Fluorenylmethyloxycarbonyl)-_O_-[2-(_N_-acetamido)-2-deoxy-3-_O_-(3,4,6-tri-_O_-acetyl-2-_O_-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-6-_O_-(_O_-methyl 5-(_N_-acetylacetamido)-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate)-(2→3)-_O_-(2,4,6-tri-_O_-acetyl-β-D-galactopyranosyl)-(1→4)-_O_-[(3,4-di-_O_-acetyl-2-_O_-benzyl-α-L-fucopyranosyl)-(1→3)]-_O_-(6-_O_-benzyl-2-deoxy-2-(N-acetamido)-β-D-glucopyranosyl)-α-D-galactopyranosyl]-L-threonine benzyl ester (1):

Zn dust (400 mg, 6.12 mmol) and saturated aq. $CuSO_4$ (25 µL) were added to a solution of **16** (20 mg, 7.70 µmol) in THF (3 mL), Ac_2O (2 mL), and AcOH (1 mL) and the reaction stirred at rt for 3 h. The reaction mixture was filtered and co-evaporated with toluene (3 x 5 mL). The residue was purified by silica gel column chromatography ($CHCl_3$:Acetone, 2/1, v/v) to afford compound **1** (11.5 mg, 60%) as a white amorphous solid. Analytical data for **1**: R_f = 0.30 ($CHCl_3$:Acetone, 2/1, v/v); ^{1}H-NMR (600 MHz, , acetone-D_6) : δ = 7.75-7.08 (m, 26H, 3 x Bn, Fmoc, dFBz), 6.99 (d, 1H, N*H*, GlcNAc), 6.51 (d, 1H, N*H*, GalNAc), 6.42 (d, 1H, N*H*Fmoc, threonine), 5.5 (m, 1H, H-8’’’’’), 5.45 (m, 1H, H-4’’’’’), 5.35 (d, 1H, *J* = 3.7 Hz, H-1’’’), 5.29 (d, 1H, *J* = 3.5 Hz, H-4’), 5.25 (dd, 1H, *J* = 8.1 Hz, *J* = 10.5 Hz, H-2’), 5.19 (bd, 1H, *J* = 2.6 Hz, H-4’’’), 5.12-5.08 (m, 3H, H-3’’’, H-7’’’’’, H-3’), 4.99 (bd, 1H, *J* = 3.7 Hz, H-4’’’’), 4.97-4.93 (dd, 2H, COOC*H*$_2$Ph, threonine), 4.88 (q, 1H, H-5’’’), 4.84-4.81 (m, 2H, H-2’’’’, H-1’’’’), 4.79 (d, 1H, *J* = 8.1 Hz, H-1’’), 4.67 (d, 2H, 2 x C*H*HPh), 4.60 (m, 1H, H-3’’’’), 4.57 (d, 1H, *J* = 2.8 Hz, H-1), 4.55 (dd, 1H, H-6’’’’’), 4.49 (d, 1H, *J* = 7.5 Hz, H-1’’), 4.45 (d, 1H, CH*H*Ph), 4.38 (d, 1H, CH*H*Ph), 4.35 (m, C*H*HFmoc), 4.27-4.22 (m, 2H, CH*H*Fmoc, H-2), 4.20 (m, 1H, H-5’’’’’),

4.15-4.05 (m, 8H, OC*H*CH_3 threonine, H-6a’’’’, C*H*COOBn threonine, C*H*Fmoc, H-6b’’’’, H-9a’’’’’, H-5’ H-6a’), 4.03-3.91(m, 5H, H-4’’, H-6b’, H-9b’’’’’, H-4, H-3’’), 3.87-3.81 (m, 3H, H-6a’’, H-2’’, H-5), 3.76-3.73 (m, 6H, H-5’’’’, COOCH_3, H-6b’’, H-3), 3.70 (dd, 1H, H-2’’’), 3.54 (m, 2H, H-6a, H-6b), 3.47 (m, 2H, C4-O*H*, H-5’’), 2.48 (dd, 1H, H-3’’’’’), 2.24, 2.20 (2 x s, 6H, NAc_2), 2.14-1.74 (14 x s, 42H, 12 x OAc, 2 x NHAc), 1.43 (t, 1H, H-3’’’’’), 1.16 (d, 3H, CH_3 threonine), 1.03 (d, 3H, *J* = 6.6 Hz, CH_3 fucose) ppm; ^{13}C from HSQC (150.9 MHz, $CDCl_3$) : δ = 104.3 (C-1’), 104.1 (C-1’’), 102.2 (C-1’’’’), 101.9 (C-1), 98.6 (C-1’’’), 81.0 C-3), 77.6 (C-5’’), 77.4 (O*CH*CH_3 threonine), 77.3 (C-3’’), 76.3 (C-2’’’), 76.1 (C-4’’’), 75.1 (*CH*$_2$Ph), 74.6 (C-4’’’), 74.2 (C-3’’’’), 73.8 (*CH*$_2$Ph), 73.6 (C-5’’’’), 73.5 (C-5’), 73.4 (C-3’), 73.1 (C-2’’’’), 72.8 (C-2’), 72.5 (C-3’’’), 72.3 (C-6’’’’’), 72.2 (C-6), 72.1 (C-5), 71.4 (C-4), 71.2 (C-6’’), 70.4 (C-8’’’’’), 70.2 (C-4’’’’), 70.0 (C-4’), 69.8 (C-7’’’’’), 69.5 (COO*CH*$_2$Ph), 69.2 (C-4’’’’’), 69.1 (*CH*$_2$CHFmoc), 66.4 (C-5’’’), 64.7 (C-9’’’’’), 64.1 (C-6’’’’), 63.9 (C-6’), 61.8 (O*CH*CH_3 threonine), 58.6 (C-2’’), 58.5 (C-5’’’’’), 55.2 (COO*CH*$_3$), 50.5 (C-2), 49.8 (CH_2*CH*Fmoc), 41.1 (C-3’’’’’), 29.8, 28.4 (NAc_2), 25.5-22.4 (12 x OAc, 2 x NHAc), 21.4 (*CH*$_3$ threonine), 18.3 (C-6’’’); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{119}H_{140}F_2N_4O_{51}$ $[M+Na]^+$: 2501.8350; found 2501.8353.

3.6 References

1. Ley, K.; Laudanna, C.; Cybulsky, M. I.; Nourshargh, S., Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nature Reviews Immunology* **2007,** *7* (9), 678-689.
2. Vestweber, D.; Blanks, J. E., Mechanisms that regulate the function of the selectins and their ligands. *Physiol. Rev.* **1999,** *79* (1), 181-213.
3. Hrachovinova, I.; Cambien, B.; Hafezi-Moghadam, A.; Kappelmayer, J.; Camphausen, R. T.; Widom, A.; Xia, L.; Kazazian, H. H.; Schaub, R. G.; McEver, R. P.; Wagner, D. D., Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat. Med. (N. Y., NY, U. S.)* **2003,** *9* (8), 1020-1025.
4. McNicol, A.; Israels, S. J., Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovascular & Hematological Disorders: Drug Targets* **2008,** *8* (2), 99-117.
5. Geng, J.-G.; Chen, M.; Chou, K.-C., P-selectin cell adhesion molecule in inflammation, thrombosis, cancer growth and metastasis. *Curr. Med. Chem.* **2004,** *11* (16), 2153-2160.
6. Platt, O. S., Sickle cell anemia as an inflammatory disease. *J. Clin. Invest.* **2000,** *106* (3), 337-338.
7. Cummings, R. D., Structure and function of the selectin ligand PSGL-1. *Braz. J. Med. Biol. Res.* **1999,** *32* (5), 519-28.
8. Moore, K. L.; Stults, N. L.; Diaz, S.; Smith, D. F.; Cummings, R. D.; Varki, A.; Mcever, R. P., Identification of a Specific Glycoprotein Ligand for P-Selectin (Cd62) on Myeloid Cells. *J. Cell Biol.* **1992,** *118* (2), 445-456.

9. Constantin, G., PSGL-1 as a novel therapeutic target. *Drug News & Perspectives* **2004,** *17* (9), 579-586.

10. Hicks, A. E. R.; Nolan, S. L.; Ridger, V. C.; Hellewell, P. G.; Norman, K. E., Recombinant P-selectin glycoprotein ligand-1 directly inhibits leukocyte rolling by all 3 selectins in vivo: complete inhibition of rolling is not required for anti-inflammatory effect. *Blood* **2003,** *101* (8), 3249-3256.

11. Kyriakides, C.; Favuzza, J.; Wang, Y.; Austen, W. G.; Moore, F. D.; Hechtman, H. B., Recombinant soluble P-selectin glycoprotein ligand 1 moderates local and remote injuries following experimental lower-torso ischaemia. *British J. Surg.* **2001,** *88* (6), 825-830.

12. Hayward, R.; Campbell, B.; Shin, Y. K.; Scalia, R.; Lefer, A. M., Recombinant soluble P-selectin glycoprotein ligand-1 protects against myocardial ischemic reperfusion injury in cats. *Cardiovasc. Res.* **1999,** *41* (1), 65-76.

13. Wang, K.; Zhou, X. R.; Zhou, Z. M.; Tarakji, K.; Qin, J. X.; Sitges, M.; Shiota, T.; Forudi, F.; Schaub, R. G.; Kumar, A.; Penn, M. S.; Topol, E. J.; Lincoff, A. M., Recombinant soluble P-selectin glycoprotein ligand-Ig (rPSGL-Ig) attenuates infarct size and myeloperoxidase activity in a canine model of ischemia-reperfusion. *Thromb. Haemostasis* **2002,** *88* (1), 149-154.

14. van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G., Expanding the diversity of chemical protein modification allows post-translational mimicry. *Nature* **2007,** *446* (7139), 1105-1109.

15. Enders, S.; Bernhard, G.; Zakrzewicz, A.; Tauber, R., Inhibition of L-selectin binding by polyacrylamide-based conjugates under defined flow conditions. *Bba-Gen Subjects* **2007,** *1770* (10), 1441-1449.

16. Zeng, S.; Gutierrez Gallego, R.; Dinter, A.; Malissard, M.; Kamerling, J. P.; Vliegenthart, J. F. G.; Berger, E. G., Complete enzymic synthesis of the mucin-type sialyl Lewis x epitope, involved in the interaction between PSGL-1 and P-selectin. *Glycoconj. J.* **1999,** *16* (9), 487-497.

17. Leppanen, A.; Mehta, P.; Ouyang, Y. B.; Ju, T. Z.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D., A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. *J. Biol. Chem.* **1999,** *274* (35), 24838-24848.

18. Davis, B. G., Recent developments in oligosaccharide synthesis. *J. Chem. Soc., Perkin Trans. 1* **2000,** (14), 2137-2160.

19. Sears, P.; Wong, C. H., Toward automated synthesis of oligosaccharides and glycoproteins. *Science* **2001,** *291* (5512), 2344-2350.

20. Boons, G. J., Recent developments in chemical oligosaccharide synthesis. *Contemp. Org. Synth.* **1996,** *3* (3), 173-200.

21. Seeberger, P. H.; Werz, D. B., Synthesis and medical applications of oligosaccharides. *Nature* **2007,** *446* (7139), 1046-1051.

22. Codee, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A., Thioglycosides in sequential glycosylation strategies. *Chem. Soc. Rev.* **2005,** *34* (9), 769-782.

23. Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L., Tuning glycoside reactivity: New tool for efficient oligosaccharide synthesis. *J. Chem. Soc., Perkin Trans. 1* **1998,** (1), 51-65.

24. Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C. H., Chemoenzymatic synthesis of a PSGL-1 N-terminal glycopeptide containing tyrosine sulfate and alpha-O-linked sialyl Lewis X. *J. Am. Chem. Soc.* **2000,** *122* (17), 4241-4242.

25. Pratt, M. R.; Bertozzi, C. R., Syntheses of 6-sulfo sialyl Lewis X glycans corresponding to the L-selectin ligand "Sulfoadhesin". *Org. Lett.* **2004,** *6* (14), 2345-2348.

26. Baumann, K.; Kowalczyk, D.; Kunz, H., Total synthesis of the glycopeptide recognition domain of the P-selectin glycoprotein ligand 1. *Angew. Chem. Int. Ed.* **2008,** *47* (18), 3445-3449.

27. Hanashima, S.; Castagner, B.; Esposito, D.; Nokami, T.; Seeberger, P. H., Synthesis of a sialic acid alpha(2-3) galactose building block and its use in a linear synthesis of sialyl Lewis X. *Org. Lett.* **2007,** *9* (9), 1777-1779.

28. Otsubo, N.; Ishida, H.; Kiso, M., The first, highly efficient synthesis of spacer-armed O-glycans on GlyCAM-1 and PSGL-1: the counter-receptors for L- and P-selectin. *Tetrahedron Lett.* **2000,** *41* (20), 3879-3882.

29. Xia, J.; Alderfer, J. L.; Locke, R. D.; Piskorz, C. F.; Matta, K. L., Complex oligosaccharide investigations: Synthesis of an octasaccharide incorporating the dimeric Le(x) structure of PSGL-1. *J. Org. Chem.* **2003,** *68* (7), 2752-2759.

30. Huang, K. T.; Wu, B. C.; Lin, C. C.; Luo, S. C.; Chen, C. P.; Wong, C. H.; Lin, C. C., Multi-enzyme one-pot strategy for the synthesis of sialyl Lewis X-containing PSGL-1 glycopeptide. *Carbohydr. Res.* **2006,** *341* (12), 2151-2155.

31. Yu, B.; Yu, H.; Hui, Y. Z.; Han, X. W., Synthesis of a group of diosgenyl saponins by a one-pot sequential glycosylation. *Tetrahedron Lett.* **1999,** *40* (49), 8591-8594.

32. Boons, G. J., Strategies in oligosaccharide synthesis. *Tetrahedron* **1996,** *52* (4), 1095-1121.

33. Wang, Y. H.; Ye, X. S.; Zhang, L. H., Oligosaccharide assembly by one-pot multi-step strategy. *Org. Biomol. Chem.* **2007,** *5* (14), 2189-2200.

34. Wang, Y. H.; Zhang, L. H.; Ye, X. S., Oligosaccharide synthesis and library assembly by one-pot sequential glycosylation strategy. *Comb. Chem. High Throughput Screening* **2006,** *9* (1), 63-75.

35. Raghavan, S.; Kahne, D., A One-Step Synthesis of the Ciclamycin Trisaccharide. *J. Am. Chem. Soc.* **1993,** *115* (4), 1580-1581.

36. Demchenko, A.; Boons, G. J., A highly convergent synthesis of a hexasaccharide derived from the oligosaccharide of group B type III Streptococcus. *Tetrahedron Lett.* **1997,** *38* (9), 1629-1632.

37. Zhu, T.; Boons, G. J., A two-directional approach for the solid-phase synthesis of trisaccharide libraries. *Angew. Chem. Int. Ed.* **1998,** *37* (13-14), 1898-1900.

38. Wang, C. C.; Lee, J. C.; Luo, S. Y.; Fan, H. F.; Pai, C. L.; Yang, W. C.; Lu, L. D.; Hung, S. C., Synthesis of biologically potent alpha 1 -> 2-linked disaccharide derivatives via regioselective one-pot protection-glycosylation. *Angew. Chem. Int. Ed.* **2002,** *41* (13), 2360-2362.

39. Vohra, Y.; Vasan, M.; Venot, A.; Boons, G. J., One-pot synthesis of oligosaccharides by combining reductive openings of benzylidene acetals and glycosylations. *Org. Lett.* **2008,** *10* (15), 3247-3250.

40. Chen, X. T.; Sames, D.; Danishefsky, S. J., Exploration of modalities in building alpha-O-linked systems through glycal assembly: A total synthesis of the mucin-related F1 alpha antigen. *J. Am. Chem. Soc.* **1998,** *120* (31), 7760-7769.

41. Rencurosi, A.; Lay, L.; Russo, G.; Caneva, E.; Poletti, L., Glycosylation with trichloroacetimidates in ionic liquids: Influence of the reaction medium on the stereochemical outcome. *J. Org. Chem.* **2005,** *70* (19), 7765-7768.

42. Benakli, K.; Zha, C. X.; Kerns, R. J., Oxazolidinone protected 2-amino-2-deoxy-D-glucose derivatives as versatile intermediates in stereoselective oligosaccharide synthesis and the formation of alpha-linked glycosides. *J. Am. Chem. Soc.* **2001,** *123* (38), 9461-9462.

43. Cheng, H.; Cao, X. H.; Xian, M.; Fang, L. Y.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D. X.; Wang, P. G., Synthesis and enzyme-specific activation of carbohydrate-geldanamycin conjugates with potent anticancer activity. *J. Med. Chem.* **2005,** *48* (2), 645-652.

44. Tanaka, H.; Adachi, M.; Takahashi, T., Efficient synthesis of core 2 class glycosyl amino acids by one-pot glycosylation approach. *Tetrahedron Lett.* **2004,** *45* (7), 1433-1436.

45. Veeneman, G. H., Chemical synthesis of O-glycosides. *Carbohydrate Chemistry* **1998**, 98-174.

46. Zhu, T.; Boons, G. J., Intermolecular aglycon transfer of ethyl thioglycosides can be prevented by judicious choice of protecting groups. *Carbohydr. Res.* **2000,** *329* (4), 709-715.

47. Berces, A.; Whitfield, D. M.; Nukada, T.; do Santos Z, I.; Obuchowska, A.; Krepinsky, J. J., Is acyl migration to the aglycon avoidable in 2-acyl assisted glycosylation reactions? *Canadian Journal of Chemistry* **2004,** *82* (7), 1157-1171.

48. Sjolin, P.; Kihlberg, J., Use of fluorobenzoyl protective groups in synthesis of glycopeptides: beta-elimination of O-linked carbohydrates is suppressed. *J. Org. Chem.* **2001,** *66* (9), 2957-65.

49. Cato, D.; Buskas, T.; Boons, G. J., Highly efficient stereospecific preparation of Tn and TF building blocks using thioglycosyl donors and the Ph2SO/Tf2O promotor system. *J. Carbohydr. Chem.* **2005,** *24* (4-6), 503-516.

50. Cheshev, P. E.; Kononov, L. O.; Tsvetkov, Y. E.; Shashkov, A. S.; Nifantiev, N. E., Syntheses of Î±- and Î²-Glycosyl Donors with a Disaccharide Î²-D-Gal-(1 3)-D-GalNAc Backbone. *Russ. J. Bioorg. Chem.* **2002,** *28* (5), 419-429.

51. Schmidt, R. R.; Kinzy, W., Anomeric-Oxygen Activation for Glycoside Synthesis - the Trichloroacetimidate Method. *Adv. Carb. Chem. Biochem.* **1994,** *50*, 21-123.

52. Wang, Y.; Zhang, L.-H.; Ye, X.-S., Oligosaccharide synthesis and library assembly by one-pot sequential glycosylation strategy. *Combinatorial Chemistry & High Throughput Screening* **2006,** *9* (1), 63-75.

53. Codee, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A., Chemoselective glycosylations using sulfonium triflate activator systems. *Tetrahedron* **2004,** *60* (5), 1057-1064.

54. 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.

55. Demchenko, A. V.; Boons, G.-J., A Highly Convergent Synthesis of a Complex Oligosaccharide Derived from Group B Type III Streptococcus. *J. Org. Chem.* **2001,** *66* (8), 2547-2554.

56. 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* (36), 11700-11710.

57. Venot, A.; Swayze, E. E.; Griffey, R. H.; Boons, G.-J., Disaccharide mimetics of the aminoglycoside antibiotic neamine. *ChemBioChem* **2004,** *5* (9), 1228-1236.

58. Takano, Y.; Habiro, M.; Someya, M.; Hojo, H.; Nakahara, Y., Preparation of core 2 type tetrasaccharide carrying decapeptide by benzyl protection-based solid-phase synthesis strategy. *Tetrahedron Letters* **2002,** *43* (46), 8395-8399.

59. Tam, J. P.; Heath, W. F.; Merrifield, R. B., Mechanisms for the removal of benzyl protecting groups in synthetic peptides by trifluoromethanesulfonic acid-trifluoroacetic acid-dimethyl sulfide. *Journal of the American Chemical Society* **1986,** *108* (17), 5242-51.

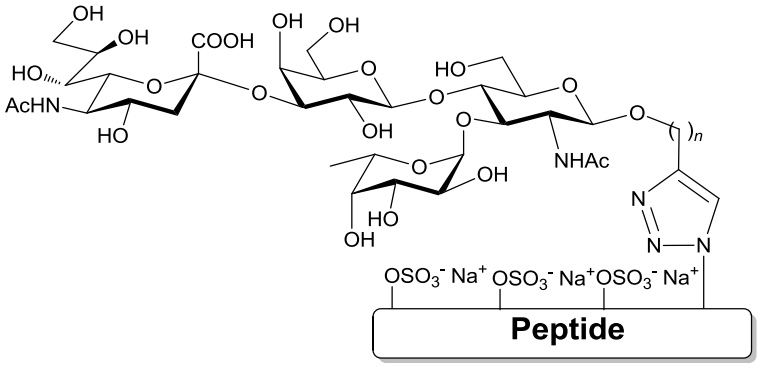
Chapter IV

Click Assisted Synthesis of Glycosulfopeptide Mimics of P-Selectin Glycoprotein Ligand-1*

*** Yusuf Vohra** and Geert-Jan Boons. To be submitted to *J. Am. Chem. Soc.*

4.1 Abstract

P-Selectin Glycoprotein Ligand-1 (PSGL-1), located on leukocytes, is a dimeric, mucin-type glycoprotein ligand known to play an important role in inflammation by binding to E-, P- and L-selectin. Inhibition of this binding by blocking PSGL-1 represents a promising therapeutic approach toward diseases in which inflammation has a destructive role (e.g. ischemia, venous thrombosis, hemorrhage, atherosclerosis, asthma, skin inflammation and autoimmune diseases). Glycosulfopeptides modeled on PSGL-1 have shown to inhibit P-selectin dependent leukocyte rolling *in vivo* in inflamed venules, suggesting that synthetic structures resembling the *N*-terminus of PSGL-1 represent a promising therapeutic target. A novel, highly convergent, completely synthetic route for the synthesis of glycosulfopeptide mimics of the signal sequence of PSGL-1 is presented for the first time. The method aims to provide sufficient quantities of the glycosulfopeptide for improved biological studies on the blocking of P-selectins and possible therapeutic use against chronic inflammation.



4.2 Research Objective

P-selectin and P-selectin glycoprotein ligand 1 are vascular adhesion molecules important for recruitment of leukocytes to the site of inflamed tissue. Glycosulfopeptides modeled on PSGL-1 have shown to inhibit P-selectin dependent leukocyte rolling *in vivo* in inflamed venules,[1-6] suggesting that synthetic structures resembling the *N*-terminus of PSGL-1 represent a promising therapeutic target. A 15-amino acid signal sequence with a threonine-linked hexasaccharide and three sulfated tyrosine residues has been shown to be the binding sequence of PSGL-1.

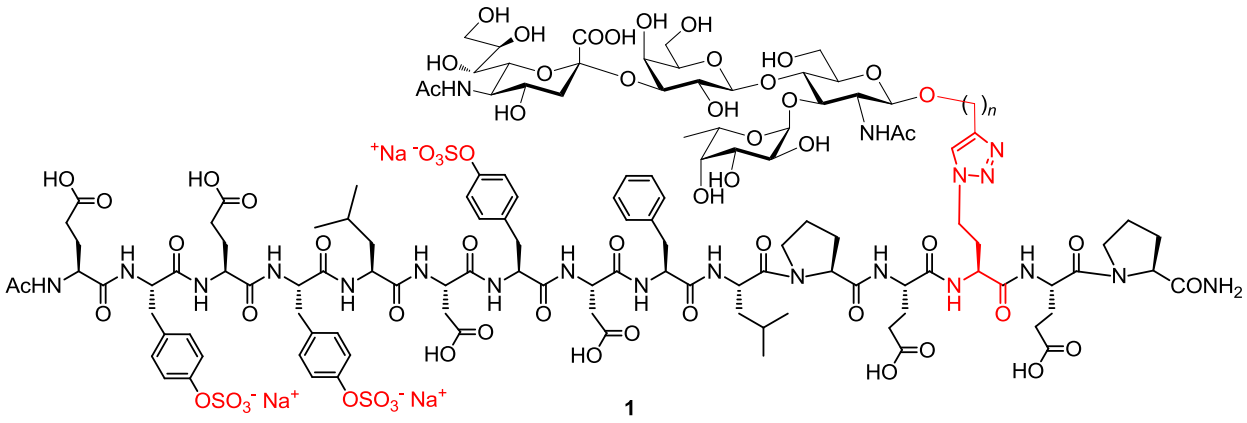


Figure 4.1 Target molecule

It has been suggested in many reports that inhibitors of selectins may possess therapeutic properties which can be applied for the treatment of a number of diseases. We have already demonstrated a highly convergent route to obtain the *O*-glycan of PSGL-1linked to threonine (Chapter **III**). Although this procedure provides an efficient route to obtain the *O*-glycan, the synthesis of the actual glycopeptide recognition domain of PSGL-1 represents a formidable challenge. A large amount of the glycosylated amino acid is required for application to the solid-phase synthesis of the glycopeptide.[7-8] Our objective is to minimize the effort of synthesizing this glycopeptide by reducing the amount of the *O*-glycan required. In the current chapter, we

report a novel, highly convergent, and completely synthetic route for the synthesis of glycosulfopeptide mimic of the signal sequence of PSGL-1 (Figure **4.1**). The primary aim of this method is to provide sufficient quantities of the glycosulfopeptide for increased and improved biological studies on the blocking of P-selectins and possible therapeutic use against chronic inflammation. Also, a number of analogs will be synthesized with relatively small effort and binding studies will be carried out to understand the effect of these modifications. We expect that the strategic principles employed in the synthesis could be further exploited to synthesize a library of PSGL-1 mimics for SAR studies.

4.3 Results and Discussion

As part of the program to prepare a library of PSGL-1 mimics, we envisioned a novel synthetic approach where glucosulfopeptide **1** can be synthesized by conjugating the SLe^x-oligosaccharide with the peptide *via* a Huisgen [3 + 2] cycloaddition reaction to form a triazole linkage. The peptide sequence, including the three sulfated tyrosine residues and an unnatural azidohomoalanine[9], rather than the native threonine residue, can be synthesized utilizing Fmoc-based microwave-assisted solid-phase peptide synthesis. The sialyl $Lewis^x$ (SLe^x) oligosaccharide, containing a terminal acetylene moiety at the reducing end, can be synthesized by coupling of SLe^x tetrasaccharide donor with propargyl alcohol (Figure **4.2**). The preparation of SLe^x tetrasaccharide can be achieved by utilizing previously reported chemoselective, orthogonal and one-pot multi-step reactions (Chapter **II**).[10-11] Thus, a one-pot procedure involving the reductive opening of benzylidene acetals and glycosylations could be employed to streamline the *O*-glycan synthesis. The methodology can be further improved by performing the majority of protecting group manipulations on the monosaccharide building blocks while leaving

fewer steps for the latter stages of synthesis, thus making it a highly convergent process. 2-Methyl naphthyl ether (NAM) groups can be utilized as the non-participating functionalities instead of benzyl ethers due to their compatibility with NHTroc and alkyne functionalities. Deprotection of NAM can be achieved using DDQ, unlike benzyl ethers which require catalytic hydrogenation using Pd/C and H_2(g).

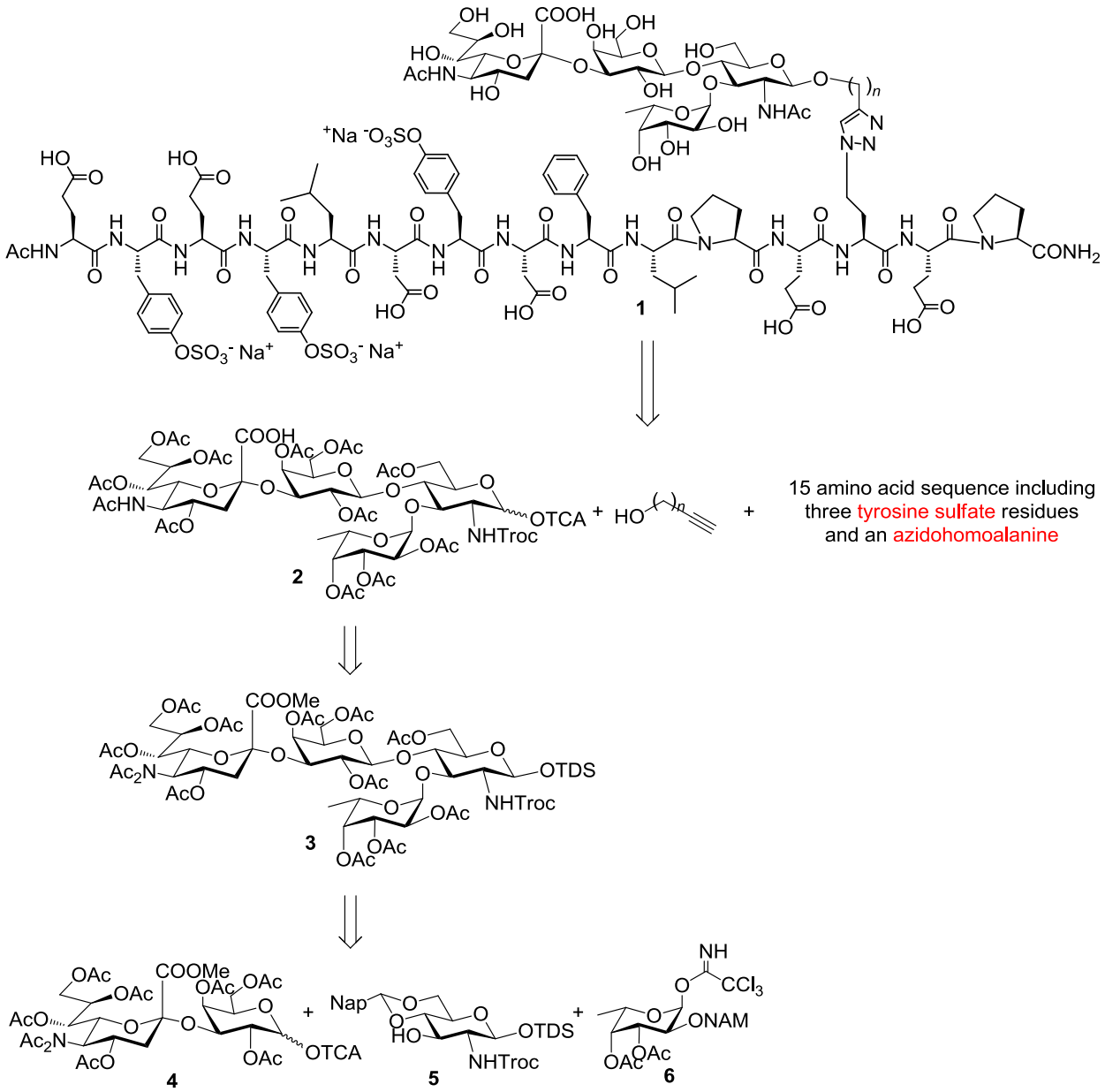
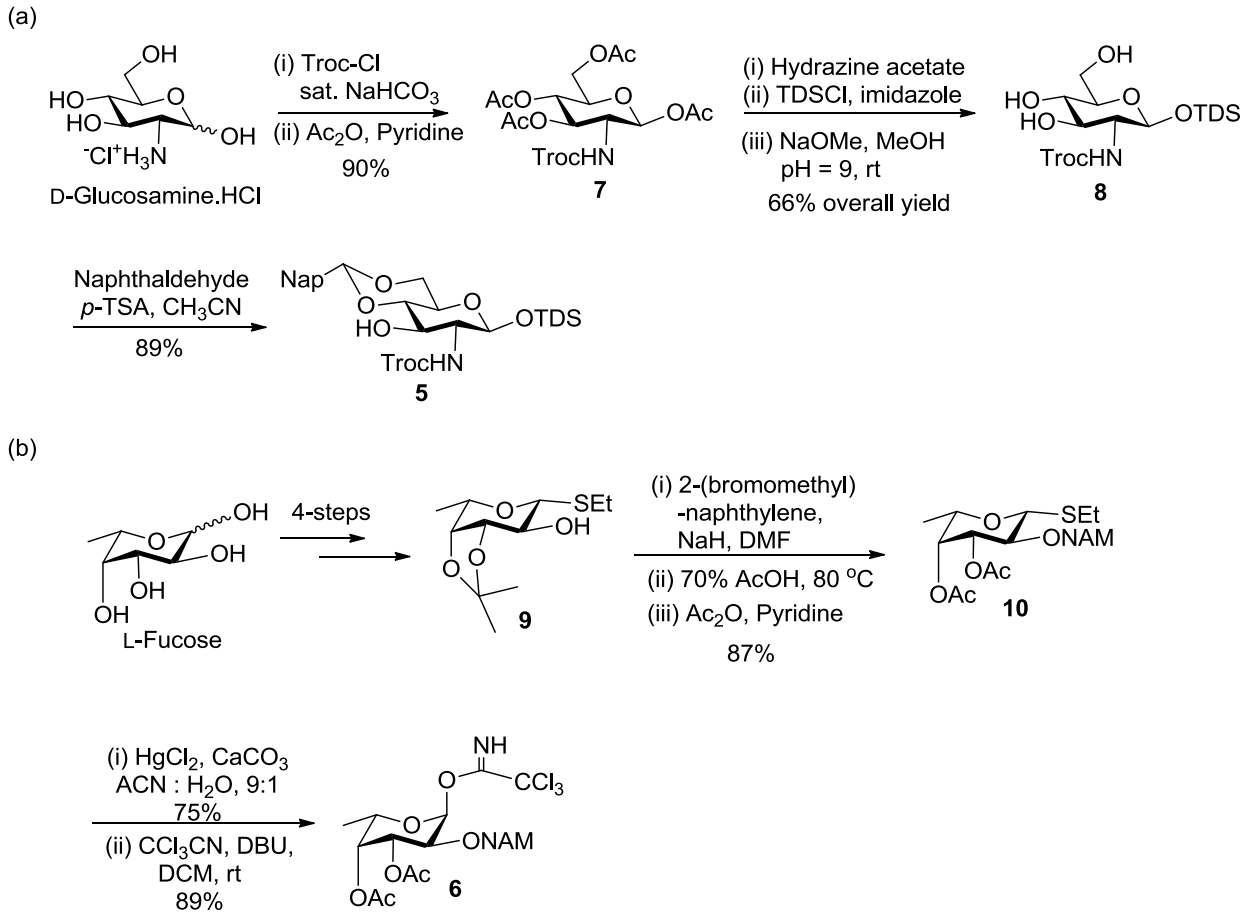


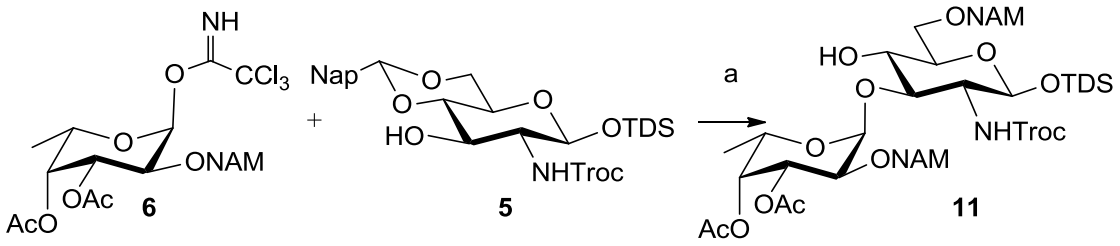
Figure 4.2 Retrosynthetic Analysis

The first stage of synthesis entailed the preparation of properly protected building blocks **5** and **6** (Scheme **4.1a**). Thus, compound **7** was obtained by reaction of D-glucosamine hydrochloride with trichloroethoxycarbonyl chloride in saturated sodium bicarbonate, followed by acetylation.[12] The anomeric acetyl group was removed by treatment with hydrazine acetate followed by thexyl dimethyl silyl (TDS) protection and removal of the C-3, C-4 and C-6 acetyls by reaction with sodium methoxide in methanol to give compound **8**. Finally, protection of the C-4 and C-6 alcohols was achieved by formation of naphthylidene acetal using naphthaldehyde and catalytic *p*-toluene sulfonic acid in acetonitrile to give glucosyl acceptor **5**.

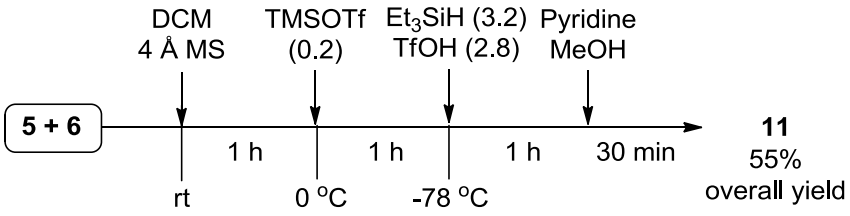


Scheme 4.1 Synthesis of building blocks **5** and **6**.

Fucosyl donor **6** was obtained in a facile manner from known compound **9**[13] (Scheme **4.1b**). Protection of the C-2 alcohol with a non-participation 2-methyl naphthyl ether was achieved by reaction of **9** with 2-(bromomethyl)-naphthylene and sodium hydride in DMF. Hydrolysis of the isopropylidene acetal followed by acetylation of the resulting diol gave compound **10**. Finally hydrolysis of the anomeric thioethyl group by mercuric chloride, followed by reaction with trichloroacetonitrile and catalytic DBU in dichloromethane gave the required fucosyl donor **6**.

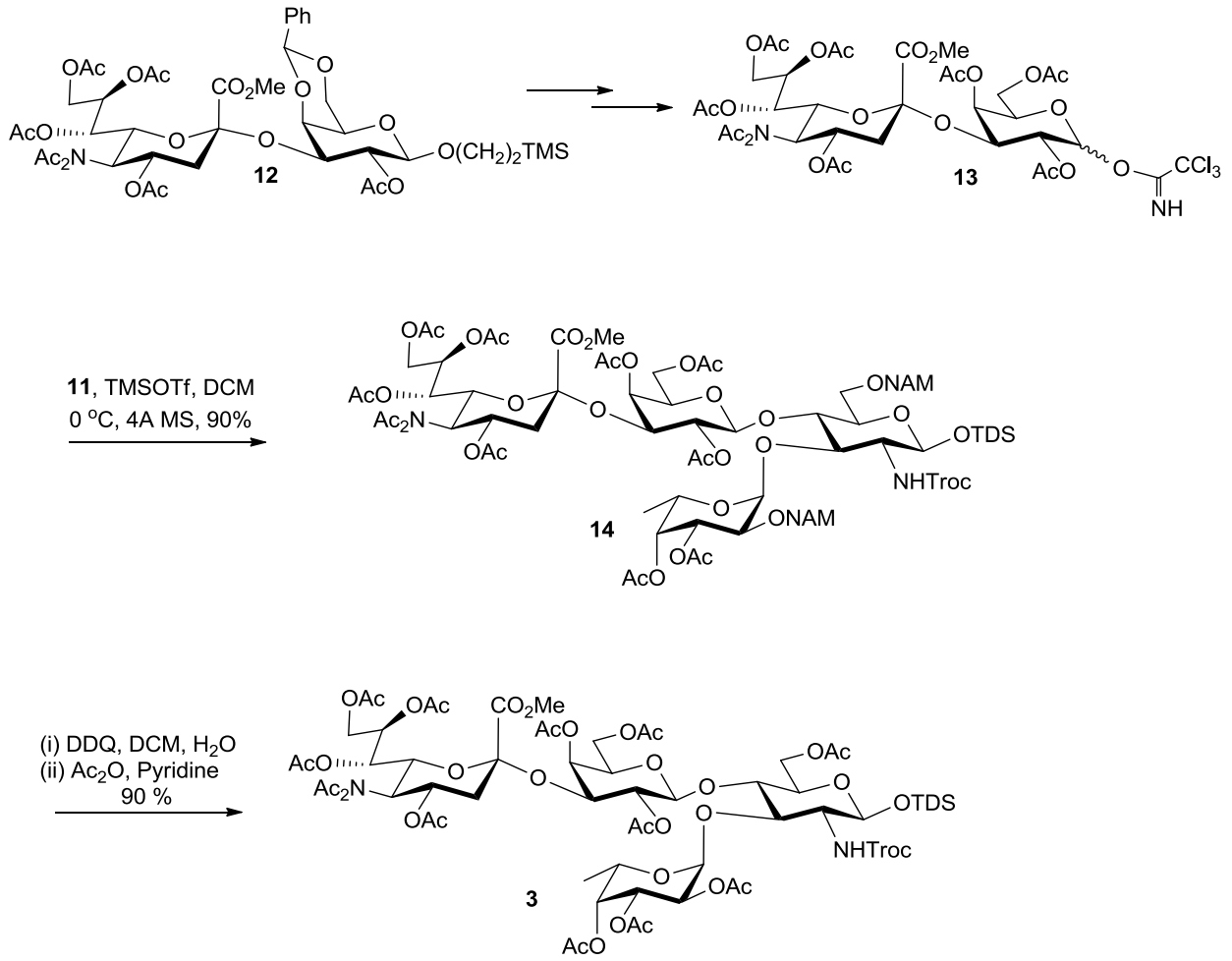


a) Reaction sequence for one-pot reaction:



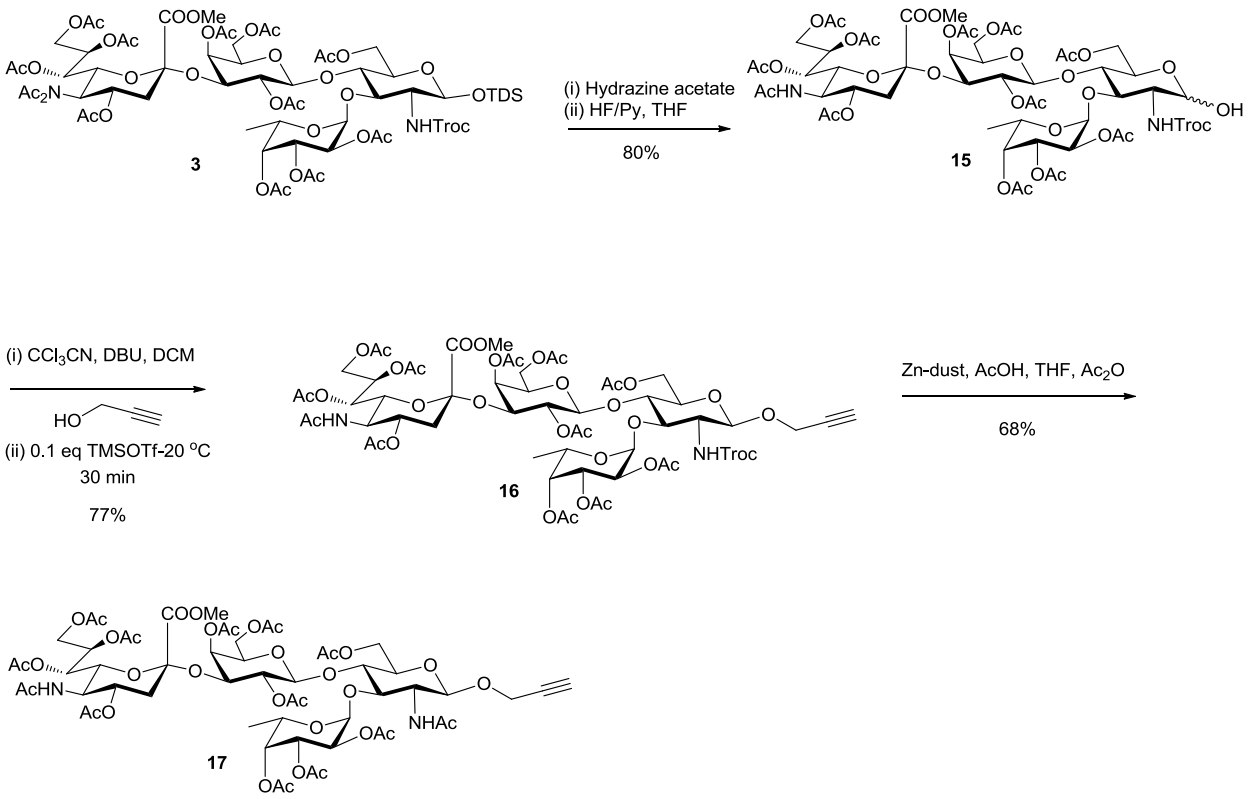
Scheme 4.2 One-pot synthesis of disaccharide **11**.

A TMSOTf-promoted glycosylation of glucosyl acceptor **5** with fucosyl tricholoroacetimidate donor **6** gave the corresponding disaccharide exclusively as the α-anomer in an excellent yield. Selective ring opening of the naphthylidene acetal of resulting disaccharide using triethylsilane and triflic acid furnished disaccharide acceptor **11**.[14] Formation of a small amount of disaccharide with loss of the anomeric silyl protecting group was also observed. This process was further streamlined by performing the glycosylation and the ring-opening reaction in one-pot as described in scheme **4.2**.[11]



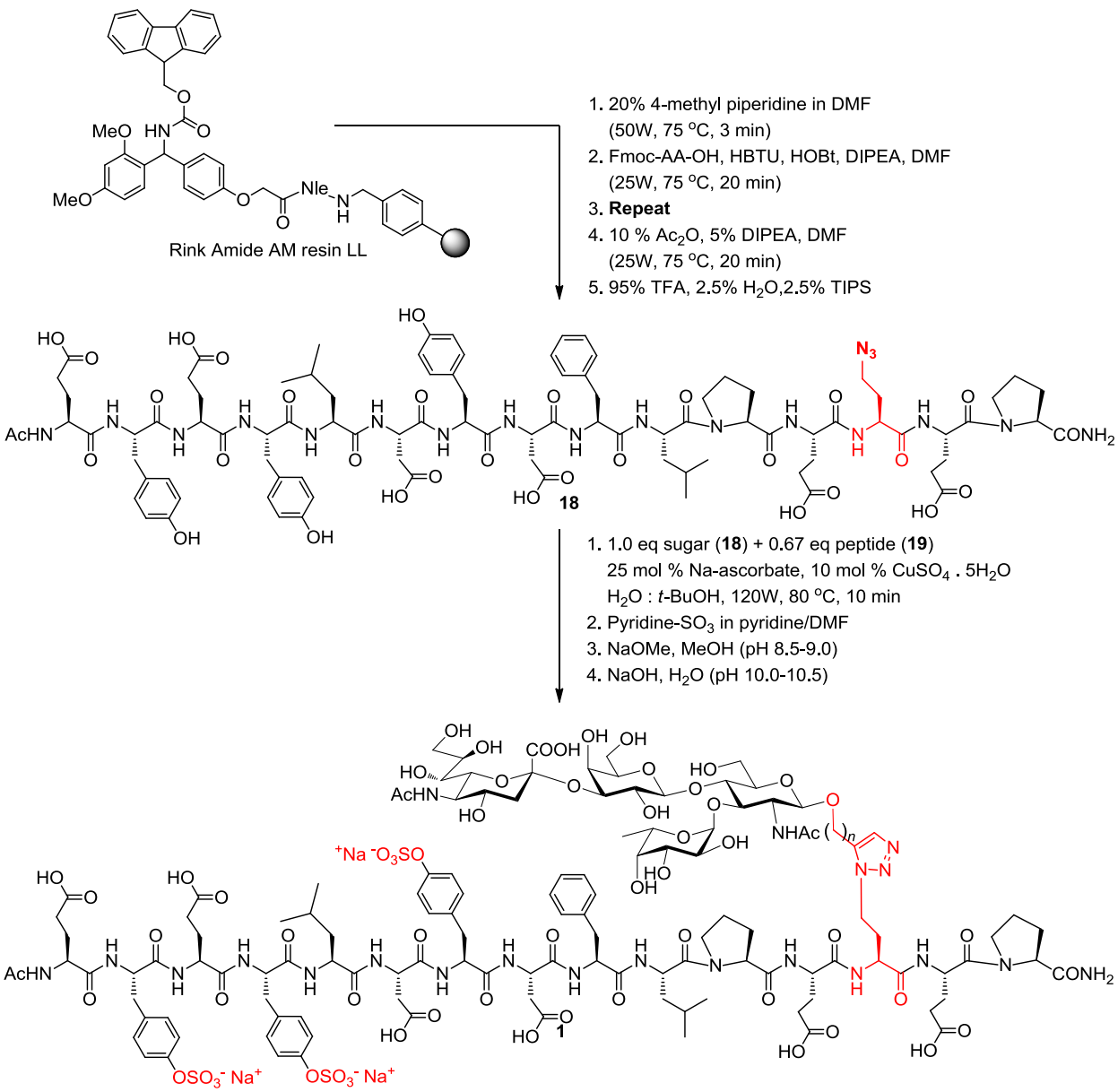
Scheme 4.3 Synthesis of SLe^x-tetrasaaccharide **3**.

The SLe^x tetrasaccharide **3** was obtained in a facile manner from **12**,[15] by first preparation of sialyl disaccharide donor **4** as described in chapter **II**.[10] A TMSOTf-promoted glycosylation of **11** with trichloroacetimidate donor **4** gave the SLe^x tetrasaccharide **14** in a yield of 90%. Removal of naphthyl methyl ethers using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) followed by acetylation using pyridine and acetic anhydride gave tetrasaccharide **3** in an overall yield of 90%.



Scheme 4.4 Synthesis of SLe^x-tetrasaaccharide with terminal acetylene **17**.

The next stage of synthesis entailed the preparation of SLe^x tetrasaccharide **17** for the coupling reaction with the peptide (Scheme **4.4**). Conversion of diacetate of the sialic acid residue to an acetamido derivative was achieved using hydrazine acetate. The resulting tetrasaccharide was subjected to 70% HF in pyridine with THF as solvent to give tetrasaccharide **15** in an overall yield of 80% over two steps. The conversion of anomeric alcohol to a trichloroacetimidate donor followed by glycosylation with propargyl alcohol in presence of catalytic TMSOTf gave compound **16** exclusively as β-anomer in an overall yield of 77%. Finally, tetrasaccharide **16** was subjected to Zn-dust in acetic acid and acetic anhydride to furnish SLe^x tetrasaccharide **17** in a yield of 68%. Lastly, compound **17** can be coupled with the peptide containing azidohomoalanine residue by Huisgen [3 + 2] cycloaddition reaction using copper(II) sulfate and sodium ascorbate to give the target compound **1**.



Scheme 4.5 Synthesis of target compound **1**.

The synthesis of the peptide **18** corresponding to the binding domain of PSGL-1 was carried out using microwave assisted Fmoc-based solid-phase peptide synthesis (Scheme **4.5**). Using a low loading rink amide AM resin the synthesis of a 15 amino acid sequence was carried out. An unnatural amino acid, azidohomoalanine, was used in place of threonine. Cleavage of the peptide from the resin and removal of side-chain protecting groups was accomplished by treatment with 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisopropyl silane. It is to be noted that thiol-based scavengers were avoided due to incompatibility with the azido functionality. The peptide was purified using RP-HPLC in an overall yield of 15%. Finally, a microwave-assisted Huisgen [3 + 2] cycloaddition reaction was performed using 1.0 eq. of SLe^x-tetrasaccharide **17** and 0.67 eq. of azido-peptide **18** in a mixture of H_2O/*t*-BuOH (1:1) containing 25 mol% sodium ascorbate and 10 mol% $CuSO_4$.[16] The resulting glycopeptide was carefully reacted with pyridine-SO_3 complex in pyridine/DMF (2:1) at 0 °C followed by treatment with sodium methoxide in methanol (pH 9) and subsequently with aqueous NaOH at pH 10.5. After neutralization with acetic acid, the resulting glycosulfopeptide **1** was obtained following purification by RP-HPLC.

4.4 Experimental Section

General remarks:

CH_2Cl_2 was distilled from CaH_2 prior to use in reactions. All the starting materials were kept *in vacuo* with P_2O_5 prior to use. Chemicals were purchased from Aldrich and Fluka and used without further purification. *N*-iodosuccinimide was used after recrystalization in dioxane/CCl_4. Column chromatography was performed on silica gel G60 (SiliCycle, 60-200μm 60 Å), reactions were monitored by TLC on Silicagel 60 F_{254} (EMD Chemicals Inc.). The compounds were detected by examination under the UV light and visualized by charring with 10% sulfuric acid in MeOH or cerium ammonium molybdate in 20% aq. sulfuric acid. Solvents were removed under reduced pressure at ≤ 30 ^{o}C. ^{1}H-NMR and HSQC spectra were recorded in $CDCl_3$ at 500 MHz on a Varian Inova spectrometer with tetramethylsilane as internal standard, unless otherwise stated. High-resolution mass spectra were obtained by using MALDI-ToF (Applied Biosystems 4700 Proteomics Analyzer) with 2,5-dihydroxybenzoic acid as matrix and the internal standards ultramark 1621 and PEG.

Ethyl 3,4-di-*O*-acetyl-2-*O*-(2-methyl-naphthyl)-6-deoxy-5-methyl-1-thio-β-L-fucopyranoside (10):

To a solution of compound **9** (2.2g, 8.87 mmol) in DMF (25 ml) under an argon atmosphere was added a 60% suspension of NaH in oil (461 mg, 11.52 mmol) and stirred at room temperature for 10 min. 2-(bromomethyl)-naphthylene (3.3g, 15.07 mmol) was added to the reaction mixture and stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 $^{\circ}$C followed by addition of MeOH (1 ml). The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford

white solid. To a solution of the resulting compound in AcOH (14 ml) was added H_2O (6 ml) and heated at 70 °C for 1 h. The reaction mixture was then concentrated *in vacuo* and co-evaporated with toluene (20 ml). The resulting residue was dissolved in pyridine (20 ml) and acetic anhydride (10 ml) and the reaction was stirred for 12 h at room temperature. The reaction mixture was cooled to 5 °C followed by addition of MeOH (5 ml). The solvent was removed by co-evaporation with toluene (3 x 25 ml). Silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) of the residue afforded compound **10** (3.3 g, 87%) as a white amorphous solid. Analytical data for **10**: R_f = 0.40 (Hexanes:EtOAc, 2:1, v:v); ^{1}H-NMR (300 MHz, $CDCl_3$) : δ = 7.82-7.25 (m, 7H, aromatic), 5.26 (bd, 1H, H-4), 5.07-5.01 (m, 2H, H-3, C*H*H-naphthylmethyl), 4.78 (bd, 1H, CH*H*-naphthylmethyl), 4.57 (d, 1H, *J* = 9.9 Hz, H-1), 3.80 (m, 1H, H-5), 3.73 (t, 1H, $J_{2,3} = J_{2,1}$ = 9.8 Hz, H-2), 2.84 (m, 2H, CH_2-thioethyl), 2.13 (s, 3H, COCH_3), 1.89 (s, 3H, COCH_3), 1.36 (t, *J* = 7.3 Hz, CH_3-thioethyl), 1.21 (d, J = 6.2 Hz, CH_3 fucose) ppm. ^{13}C from HSQC (75 MHz, $CDCl_3$) : δ = 85.59 (C-1), 76.80 (C-2), 76.04 (CH_2-naphthylmethyl), 74.54 (C-3), 73.53 (C-5), 71.27 (C-4), 25.81 (CH_2-thioethyl), 21.29 (2 x OAc), 17.02 (C-6), 15.26 (CH_3-thioethyl); HR-MALDI-ToF/MS: *m/z*: calc. for $C_{23}H_{28}O_6S$ $[M+Na]^+$: 455.1504; found 455.1509.

3,4-di-*O*-acetyl-2-*O*-(2-methyl-naphthyl)-6-deoxy-5-methyl-α-L-fucopyranosyl-trichloroacetimidate (6):

To a solution of compound **10** (3.0 g, 6.93 mmol) in acetonitrile (20 ml) and H_2O (5 ml) was added $HgCl_2$ (4.13 g, 15.25 mmol) and $CaCO_3$ (1.7 g, 17.32 mmol). The reaction mixture was stirred at room temperature for 12 h followed by addition of CH_2Cl_2 (50 ml), and washed with 10 % NH_4Cl solution (30 ml), water (30 ml) and brine (30 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo.* The residue was dissolved in dry

CH_2Cl_2 () followed by addition of trichloroacetonitrile (3.6 ml, 34.65 mmol) and 1,8-Diazabicyclo[5.4.0]-undec-7-ene (195 µL, 1.39 mmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated in *vacuo*. Silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) of the syrup afforded compound **6** (2.46 g, 67% over two steps, α-only) as a white amorphous solid. Analytical data for **6**: R_f = 0.45 (Hexanes:EtOAc, 2:1, v:v); ^{1}H-NMR (300 MHz, $CDCl_3$) : δ = 8.58 (s, 1H, N*H*), 7.83-7.39 (m, 7H, aromatic), 6.56 (d, 1H, $J_{1,2}$ = 4.2 Hz, H-1), 5.43-5.37 (m, 2H, H-3, H-4), 4.88-4.78 (m, 2H, CH_2-naphthylmethyl), 4.36 (m, 1H, H-5), 4.09 (dd, 1H, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 2.11 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.15 (d, J = 6.5 Hz, CH_3 fucose) ppm; HR-MALDI-ToF/MS: *m/z*: calc. for $C_{23}H_{24}Cl_3NO_7$ $[M+Na]^+$: 554.0516; found 554.0511.

Thexyldimethylsilyl 2-(2,2,2-trichloroethoxy)carbonyl amino-4,6-*O*-naphthylidene-2-deoxy-β-D-glucopyranoside (5):

To a mixture of compound **8** (2.0 g, 4.02 mmol) and naphthaldehyde (754 mg, 4.83 mmol) in dry acetonitrile (25 ml) was added p-toluene sulfonic acid monohydrate (38 mg, 0.20 mmol). The reaction mixture was stirred at room temperature for 4 h followed by addition of triethyl amine (100 µL). The reaction mixture was concentrated *in vacuo* followed by purification using silica gel column chromatography (Hexanes:EtOAc, 2:1, v:v) to afford compound **5** (2.27 g, 89%) as a white amorphous solid. Analytical data for **5**: R_f = 0.35 (Hexanes:EtOAc, 2:1, v:v); ^{1}H-NMR (300 MHz, $CDCl_3$) : δ = 7.80-7.31 (m, 7H, aromatic), 5.55 (s, 1H, C*H*-naphthylidene), 4.99 (bd, 1H, N*H*), 4.75 (d, 1H, J = 8.1 Hz, H-1), 4.59 (m, 2H, CH_2-Troc), 4.21 (m, 1H, H-6a), 4.00 (m, 1H, H-3), 3.72 (t, 1H, H-6b), 3.50 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.1 Hz, H-4), 3.40 (m, 1H, H-5), 3.27 (m, 1H, H-2), 2.78 (bs, 1H, O*H*), 1.52 (m, 1H,

$SiC(CH_3)_2CH(CH_3)_2$), 0.74 (m, 12H, $SiC(CH_3)_2CH(CH_3)_2$), 0.02 (bd, 6H, $Si(CH_3)_2$) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 102.70 (*CH*-naphthylidene), 96.37 (C-1), 82.07 (C-4), 75.70 (CH_2-Troc), 71.34 (C-3), 69.14 (C-6), 66.38 (C-5), 61.43 (C-2), 34.47 (*CH*-TDS), 20.72 (6 x TDS-CH_3), -1.02 (Si-CH_3), -2.67(Si-CH_3) ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{28}H_{38}Cl_3NO_7Si$ $[M+Na]^+$ calcd 656.1381; found 656.1386.

Thexyldimethylsilyl 3,4-di-*O*-acetyl-2-(2-methyl-naphthyl)-6-deoxy-5-methyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)carbonyl amino-6-*O*-(2-methyl-naphthyl)-2-deoxy-β-D-glucopyranoside (11):

A mixture of glycosyl acceptor **5** (82 mg, 0.13 mmol) and trichloroacetimidate donor **6** (90 mg, 0.17 mmol) in CH_2Cl_2 (2 ml) and 4 Å MS, was placed under an atmosphere of Ar and cooled to 0 °C. TMSOTf (0.026 mmol, 0.26M solution in DCM) was added and stirring at 0 °C was continued for 1 h. The reaction mixture was then cooled to -78 °C followed by addition of TfOH (32 µL, 0.36 mmol) and triethylsilane (66 µL, 0.41 mmol). The reaction mixture was then stirred at -78 °C for 1 hr. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 µL) and methanol (0.2 ml), diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (EtOAc - Hexanes gradient elution 5→40%) to afford compound **11** (71.5 mg, 55%) as an amorphous white solid. Analytical data for **11**: R_f = 0.35 (EtOAc:Hexanes, 1:2, v:v); ^1H-NMR (500 MHz, $CDCl_3$) : δ = 7.76-7.35 (m, 14H, aromatic), 5.26-5.19 (m, 3H, H-3', H-4', NH-Troc), 5.04 (bs, 1H, H-1'), 4.84 (bd, 1H, H-1), 4.75-4.65 (m, 4H, CH_2-naphthylmethyl, CH_2-Troc), 4.44 (m, 2H,

CH$_2$-naphthylmethyl), 4.33 (m, 1H, H-5'), 3.87-3.82 (m, 2H, H-3, H-2'), 3.73-3.64 (m, 2H, H-6a, H-6b), 3.51 (t, 1H, $J_{4,5} = J_{4,3}$ = 8.5 Hz, H-4), 3.41 (bs, 1H, H-5), 3.10 (bs, 1H, H-2), 2.00 (s, 3H, COC*H*$_3$), 1.89 (s, 3H, COC*H*$_3$), 1.50-1.40 (m, 1H, SiC(CH$_3$)$_2$C*H*(CH$_3$)$_2$), 1.02 (d, 3H, C*H*$_{3\text{fucose}}$), 0.76-0.73 (m, 12H, SiC(C*H*$_3$)$_2$CH(C*H*$_3$)$_2$), 0.00 (s, 6H, Si(C*H*$_3$)$_2$) ppm. ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 98.02 (C-1'), 95.29 (C-1), 82.74 (C-3), 74.92 (*CH*$_2$-naphthylmethyl), 74.61 (C-5), 74.10 (*CH*$_2$-naphthylmethyl, *CH*$_2$-Troc), 73.80 (C-2'), 71.77 (C-4'), 71.26 (C-4), 70.55 (C-3'), 70.44 (C-6), 66.28 (C-5'), 59.36 (C-2), 34.45 (SiC(CH$_3$)$_2$*CH*(CH$_3$)$_2$), 21.13, 20.83, 20.32, 18.80, 16.56, -1.03, -3.47 ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{49}H_{62}Cl_3NO_{13}Si$ $[M+Na]^+$ calcd 1028.2954; found 1028.2950.

Thexyldimethylsilyl [*O*-methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(3,4-di-*O*-acetyl-2-*O*-(2-methyl naphthyl)-6-deoxy-5-methyl-α-L-fucopyranosyl)-(1→3)]-*O*-[6-*O*-(2-methyl naphthyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (14):

A mixture of disaccharide acceptor **11** (45 mg, 0.045 mmol) and trichloroacetimidate donor **13** (50 mg, 0.052 mmol) in CH_2Cl_2 (2 ml) was placed under an atmosphere of argon and stirred at room temperature with 4Å MS for 1 h. The reaction mixture was then cooled to 0 ^oC. TMSOTf (4.0 μmol, 0.045M solution in CH_2Cl_2) was added and stirring was continued for 1 h at the same temperature. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 μL), diluted with CH_2Cl_2 (10 ml), filtered, and washed with sat. aq. $NaHCO_3$ solution (10 ml), water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The

residue was purified by silica gel column chromatography ($CHCl_3$:EtOAc, 1:1, v:v) to afford compound **14** (73 mg, 90%) as amorphous white solid. Analytical data for **14**: R_f = 0.25 (Acetone:Toluene, 1:3, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 7.81-7.41 (m, 14H, aromatic), 5.57-5.51 (m, 2H, H-4’’’, H-8’’’), 5.28-5.25 (m, 3H, H-1’’, H-4’’, H-3’’), 5.22 (d, 1H, *J* = 7.5 Hz, NH), 5.17 (m, 1H, H-7’’’), 5.05 (m, 1H, H-3’), 4.99 (m, 2H, H-5’’, H-1), 4.91 (bd, 2H, H-4’, H-1’), 4.88-4.72 (m, 5H, CH_2-naphthyl, C*H*H-naphthyl, CH_2CCl$_3$-Troc), 4.69 (m, 1H, H-2’), 4.58-4.52 (m, 2H, H-6’’’, C*H*H-naphthyl), 4.31 (t, 1H, $J_{5''',6'''} = J_{5''',7'''}$ = 10.2 Hz, H-5’’’), 4.23-4.17 (m, 3H, H-6a, H-6b, H-9a’’’, H-3), 4.08-4.00 (m, 2H, H-9b’’’, H-4’), 3.93-3.86 (m, 5H, H-5’, OCH_3, H-6a), 3.83 (m, 2H, H-6b, H-4), 3.47 (bd, 1H, H-5), 3.10 (m, 1H, H-2), 2.67-2.59 (m, 1H, H-3eq’’’), 2.34, 2.26 (2s, 6H, 2x NCOCH_3), 2.17-1.82 (9s, 27H, 9x COCH_3), 1.62 (m, 1H, H-3ax’’’), 1.52 (m, 1H, SiC(CH$_3$)$_2$C*H*(CH$_3$)$_2$), 1.18 (d, 3H, *J* = 6.6 Hz, CH_3 fucose), 0.76-0.72 (m, 12H, SiC(CH_3)$_2$CH(CH_3)$_2$), 0.07, 0.00 (2s, 6H, Si(CH_3)$_2$) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 99.87 (C-1’), 97.40 (C-1’’), 94.51 (C-1), 75.58 (C-5), 74.84 (*CH*$_2$-naphthyl), 74.48 (C-5’), 74.40 (C-4’), 74.37 (*CH*$_2$CCl$_3$-Troc), 74.35 (C-3), 73.50 (*CH*$_2$-naphthyl), 72.51 (C-4’’), 71.41 (C-4), 71.04 (C-4’), 70.43 (C-3’’),69.69 (C-6’’’), 68.71 (C-6), 68.10 (C-3’), 67.49 (C-4’’’), 67.48 (C-8’’’), 67.36 (C-7’’’), 64.66 (C-5’’), 62.21 (C-9’’’), 64.20 (C-6’), 61.60 (C-2), 56.32 (C-3’’’), 53.50 (O*CH*$_3$), 38.72 (C-3’’’), 31.00 (SiC(CH$_3$)$_2$*CH*(CH$_3$)$_2$), 28.63, 27.08 (2x NCO*CH*$_3$), 31.00 (SiC(CH$_3$)$_2$*CH*(CH$_3$)$_2$), 21.67, 21.35, 21.12, 21.00, 20.96, 20.92, 20.88, 20.80, 20.72 (9x CO*CH*$_3$), 20.49, 18.57 (SiC(*CH*$_3$)$_2$CH(*CH*$_3$)$_2$), 16.42 (CH_3 fucose), 1.56, 0.00 (Si(*CH*$_3$)$_2$) ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{83}H_{107}Cl_3N_2O_{34}Si$ $[M+Na]^+$ calcd 1831.5438; found 1831.5442.

Thexyldimethylsilyl [*O*-methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-non-ulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-6-deoxy-5-methyl-α-L-fucopyranosyl)-

(1→3)]-*O*-[6-*O*acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (3):

To a solution of compound **14** (70 mg, 0.038 mmol) in CH_2Cl_2 (2 ml) and H_2O (0.5 ml) was added DDQ (13 mg, 0.057 mmol) in dark and stirred vigorously at room temperature for 2 h. The reaction mixture was quenched with a buffer solution (0.7% Ascorbic acid + 1.3 % Citric acid + 1.9 % NaOH in H_2O, w/v) (0.5 ml), diluted with CH_2Cl_2 (20 ml), washed with water (10 ml), and brine (10 ml). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was dissolved in pyridine (10 ml) followed by addition of acetic anhydride (5 ml). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was cooled to 5 °C followed by addition of MeOH (5 ml). The solvent was removed by co-evaporation with toluene (3 x 25 ml). The residue was purified by silica gel column chromatography ($CHCl_3$:EtOAc, 1:1, v:v) to afford compound **3** (56 mg, 90%) as amorphous white solid. Analytical data for **3**: R_f = 0.20 (Acetone:Toluene, 1:3, v:v); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ = 5.47-5.40 (m, 2H, H-4''', H-8'''), 5.36 (d, 1H, *J* = 3.8 Hz, H-1''), 5.25 (bd, 1H, *J* = 2.9 Hz, H-4''), 5.13-5.10 (m, 2H, H-3'', H-7'''), 4.97-4.91 (m, 3H, H-2'', H-5'', H-4'), 4.81 (bt, 1H, $J_{2',1'} = J_{2',3'}$ = 8.1 Hz, H-2'), 4.72-4.67 (m, 2H, C*H*HCCl$_3$-Troc, H-6a), 4.64 (d, 1H, *J* = 9.3 Hz, H-1'), 4.52-4.46 (m, 4H, H-3', CH*H*CCl$_3$-Troc, H-6''', H-1), 4.33-4.22 (m, 2H, H-6a', H-5'''), 4.14-4.09 (m, 2H, H-6b', H-9a'''), 4.01-3.97 (m, 1H, H-9b'''), 3.95-3.91 (bq, 1H, H-6b), 3.85-3.78 (m, 5H, H-5', O*CH*$_3$, H-4), 3.75 (bt, 1H, $J_{3,2} = J_{3,4}$ = 9.4 Hz, H-3), 3.58 (bm, 1H, H-2), 3.39 (bm, 1H, H-5), 2.58 (m, 1H, H-3eq'''), 2.27, 2.20 (2s, 6H, 2x NCOC*H*$_3$), 2.13-1.85 (9s, 27H, 9x COC*H*$_3$), 1.51-1.46 (m, 2H, H-3ax''', SiC(CH$_3$)$_2$C*H*(CH$_3$)$_2$), 1.08 (d, 3H, *J* = 6.6 Hz, C*H*$_3$ fucose), 0.74-0.66 (m, 12H, SiC(C*H*$_3$)$_2$CH(C*H*$_3$)$_2$), 0.02, 0.00 (2s, 6H, Si(C*H*$_3$)$_2$) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) : δ = 100.31 (C-1'), 96.52 (C-1), 95.62 (C-1''),

75.45, 75.34 (CH_2CCl_3-Troc), 74.34 (C-3), 73.56 (C-5), 71.88, 71.77 (C-4''), 71.33 (C-5'), 70.32 (C-2'), 69.77, 67.98, 67.87, 67.31, 66.98, 64.53, 62.52, 62.08, 61.96, 61.74, 60.52 (C-2), 56.17 (C-5'''), 53.49 (OCH_3), 38.62 (C-3'''), 31.00 ($SiC(CH_3)_2CH(CH_3)_2$), 28.63, 27.08 (2x $NCOCH_3$), 21.86, 21.73, 21.34, 21.23, 21.12, 21.02, 20.99, 20.91, 20.88, 20.80, 20.72 (11x $COCH_3$), 20.18, 18.70 ($SiC(CH_3)_2CH(CH_3)_2$), 16.17 (CH_3 fucose), -1.43, -3.12 ($Si(CH_3)_2$) ppm. HR-MALDI-ToF/MS: *m/z*: for $C_{65}H_{95}Cl_3N_2O_{36}Si$ $[M+Na]^+$ calcd 1635.4397; found 1635.4401.

4.5 Conclusion

In conclusion, a glycosulfopeptide mimic of the signal sequence of P-selectin glycoprotein ligand-1 was synthesized using a highly convergent strategy. It is expected that the technology developed here will enable us to prepare not only more analogs of similar adhesion molecules but also other complex glycopeptides of biological and medical importance.

4.6 References:

1. Hicks, A. E. R.; Leppanen, A.; Cummings, R. D.; McEver, R. P.; Hellewell, P. G.; Norman, K. E., Glycosulfopeptides modeled on P-selectin glycoprotein ligand-1 inhibit P-selectin-dependent leukocyte rolling in vivo. *Faseb Journal* **2002,** *16* (9), 1461-+.
2. Hicks, A. E. R.; Nolan, S. L.; Ridger, V. C.; Hellewell, P. G.; Norman, K. E., Recombinant P-selectin glycoprotein ligand-1 directly inhibits leukocyte rolling by all 3 selectins in vivo: complete inhibition of rolling is not required for anti-inflammatory effect. *Blood* **2003,** *101* (8), 3249-3256.
3. Leppanen, A.; Mehta, P.; Ouyang, Y. B.; Ju, T. Z.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D., A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. *Journal of Biological Chemistry* **1999,** *274* (35), 24838-24848.
4. Hayward, R.; Campbell, B.; Shin, Y. K.; Scalia, R.; Lefer, A. M., Recombinant soluble P-selectin glycoprotein ligand-1 protects against myocardial ischemic reperfusion injury in cats. *Cardiovascular Research* **1999,** *41* (1), 65-76.
5. Kyriakides, C.; Favuzza, J.; Wang, Y.; Austen, W. G.; Moore, F. D.; Hechtman, H. B., Recombinant soluble P-selectin glycoprotein ligand 1 moderates local and remote injuries following experimental lower-torso ischaemia. *British Journal of Surgery* **2001,** *88* (6), 825-830.
6. Wang, K.; Zhou, X. R.; Zhou, Z. M.; Tarakji, K.; Qin, J. X.; Sitges, M.; Shiota, T.; Forudi, F.; Schaub, R. G.; Kumar, A.; Penn, M. S.; Topol, E. J.; Lincoff, A. M., Recombinant soluble P-selectin glycoprotein ligand-Ig (rPSGL-Ig) attenuates infarct size

and myeloperoxidase activity in a canine model of ischemia-reperfusion. *Thrombosis and Haemostasis* **2002,** *88* (1), 149-154.

7. Baumann, K.; Kowalczyk, D.; Kunz, H., Total synthesis of the glycopeptide recognition domain of the P-selectin glycoprotein ligand 1. *Angewandte Chemie-International Edition* **2008,** *47* (18), 3445-3449.

8. Baumann, K.; Kowalczyk, D.; Gutjahr, T.; Pieczyk, M.; Jones, C.; Wild, M. K.; Vestweber, D.; Kunz, H., Sulfated and Non-Sulfated Glycopeptide Recognition Domains of P-Selectin Glycoprotein Ligand 1 and their Binding to P- and E-Selectin. *Angewandte Chemie-International Edition* **2009,** *48* (17), 3174-3178.

9. van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G., Expanding the diversity of chemical protein modification allows post-translational mimicry. *Nature* **2007,** *446* (7139), 1105-1109.

10. Vohra, Y.; Buskas, T.; Boons, G. J., Rapid Assembly of Oligosaccharides: A Highly Convergent Strategy for the Assembly of a Glycosylated Amino Acid Derived from PSGL-1. *Journal of Organic Chemistry* **2009,** *74* (16), 6064-6071.

11. Vohra, Y.; Vasan, M.; Venot, A.; Boons, G. J., One-pot synthesis of oligosaccharides by combining reductive openings of benzylidene acetals and glycosylations. *Organic Letters* **2008,** *10* (15), 3247-3250.

12. Benakli, K.; Zha, C. X.; Kerns, R. J., Oxazolidinone protected 2-amino-2-deoxy-D-glucose derivatives as versatile intermediates in stereoselective oligosaccharide synthesis and the formation of alpha-linked glycosides. *Journal of the American Chemical Society* **2001,** *123* (38), 9461-9462.

13. Rencurosi, A.; Lay, L.; Russo, G.; Caneva, E.; Poletti, L., Glycosylation with trichloroacetimidates in ionic liquids: Influence of the reaction medium on the stereochemical outcome. *Journal of Organic Chemistry* **2005,** *70* (19), 7765-7768.

14. 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.

15. Demchenko, A. V.; Boons, G.-J., A Highly Convergent Synthesis of a Complex Oligosaccharide Derived from Group B Type III Streptococcus. *J Org Chem* **2001,** *66* (8), 2547-2554.

16. Miller, N.; Williams, G. M.; Brimble, M. A., Synthesis of Fish Antifreeze Neoglycopeptides Using Microwave-Assisted "Click Chemistry". *Organic Letters* **2009,** *11* (11), 2409-2412.

Chapter V

Conclusions

Glycoconjugates play critical roles in many physiological processes including cell recognition, proliferation and differentiation, fertilization, embryogenesis, and neuronal development. Many microbes, including viruses, bacteria, and their toxins, have evolved to exploit binding with cell-surface carbohydrates to cause infection. Protein-carbohydrate interactions have been implicated in a variety of diseases such as cancer metastasis, arteriosclerosis, thrombosis and arthritis. A thorough understanding of these interactions can lead to novel carbohydrate-based therapeutics. Despite the great potential for interfering with the above mentioned processes, the lack of pure and structurally well-defined carbohydrates and glycoconjugates represents a major obstacle to advances in glycobiology and glycomedicine. These compounds are often found in low concentrations and in micro-heterogeneous forms in nature, thus greatly complicating their isolation and characterization. The past couple of decades have seen a great improvement in chemical and enzymatic synthesis of structurally well-defined oligosaccharides. Mutant glycosidase enzymes have been developed which have made possible to synthesize oligosaccharides previously inaccessible due to the lack of corresponding glycosyltransferases. Chemoselective and orthogonal glycosylation techniques have allowed researchers an access to a number of fully synthetic oligosaccharides such as heparan sulfate, the tumor associated STn-antigen, blood group antigens (Le^x, Le^y) and Globo-H. Organic

carbohydrate synthesis has also allowed us to incorporate these carbohydrate fragments into vaccines with potential therapeutic applications.

To streamline the process of oligosaccharide synthesis, several groups are pursuing one-pot multi-step approaches. These approaches have been broadly classified based on chemoselective, orthogonal and pre-activation strategies for glycosylations and regioselective one-pot protection of monosaccharides. In order to further streamline the process of oligosaccharide synthesis, we developed a new class of one-pot reactions that addresses some of the shortcomings of the above approaches. We exploited the reaction conditions required for regioselective opening of benzylidene acetals. We envisoned that regioselective reductive opening of acetals using trifluoromethane sulfonic acid (TfOH) and triethyl silane (Et_3SiH) may be compatible with glycosylations using trichloroacetimidate donors. A number of trichloroacetimidate donors and glucosyl acceptors with benzylidene acetals on the C-4 and C-6 positions and a free alcohol were synthesized. Thus, a mixture of trichloroacetimidate donor and a glucosyl acceptor in dichloromethane was activated with catalytic TfOH at 0 °C to obtain the corresponding disaccharide. After a reaction time of 30 min, the reaction flask was cooled to -78 °C, followed by addition of excess TfOH and Et_3SiH resulting in the opening of benzylidene acetal over a period of 30 min to reveal a new alcohol on the disaccharide. After increasing the temperature of the reaction flask to 0 °C, another trichloroacetimidate donor dissolved in dichloromethane was added to the reaction flask to obtain the trisaccharide in less than 90 min. A range of disaccharides and trisaccharides, including the Le^x trisaccharide, were synthesized to demonstrate the diversity of this approach. The attraction of this approach is that it makes it possible to assemble branched oligosaccharides by a one-pot procedure, a task that cannot readily be accomplished by chemoselective, orthogonal, and iterative glycosylations.

As described previously, carbohydrates have been shown to play an important role in cell adhesion. P-selectin glycoprotein ligand 1 (PSGL-1) has clearly been demonstrated to mediate the adhesion of leukocytes to selectins under blood flow. P-selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl Lewis^x (SLe^x) moiety linked to a properly positioned core-2 *O*-glycan and three tyrosine sulfate residues. Previous attempts to chemically synthesize the glycopeptide recognition domain of PSGL-1 suffered from extensive replacement of protecting groups at advanced stages of the synthesis and poor regioselectivities in crucial glycosylation steps, compromising the overall yield of target compound. We have developed a highly convergent synthesis of the PSGL-1 oligosaccharide linked to threonine based on the use of trichoroacetimidate donors and thioglycosyl acceptors that give products that can immediately be employed in a subsequent glycosylation step without the need for protecting group manipulations. The longest linear sequence entailed only seven chemical steps and gave the target compounds in an excellent overall yield of 17%.

Although this procedure demonstrates an efficient route to obtain the *O*-glycan, the synthesis of the actual glycopeptide recognition domain of PSGL-1 represents a formidable challenge. A large amount of the glycosylated amino acid is required for application to the solid-phase synthesis of the glycopeptide. To address this issue, we envisioned a novel method that can provide a number of analogs of PSGL-1 rather quickly with considerably less effort. To achieve this, we exploited the Huisgen [3 + 2] cycloaddition reaction to conjugate the oligosaccharide with the peptide. The peptide was modified by using an azidohomoalanine residue in place of the natural threonine. Thus, a SLe^x oligosaccharide linked with a propargyl functionality was synthesized using the techniques described previously. A 15-amino acid peptide sequence, including the three tyrosine sulfate residues and an unnatural

azidohomoalanine, was synthesized by Fmoc-based microwave-assisted solid-phase peptide synthesis. The oligosaccharide was conjugated to the peptide by a microwave-irradiated Cu(I) catalyzed Huisgen [3 +2] cycloaddition reaction. A range of glycopeptides with varying chain lengths at the oligosaccharide reducing end could be synthesized by changing the alkynyl group. Finally, binding studies of these glycopeptides with P-selectin can be performed.