A MODULAR APPROACH TOWARD CHONDROITIN SULFATE OLIGOSACCHARIDES

by

Jo-Sette Lynn Wilkes

(Under the Direction of GEERT-JAN BOONS)

ABSTRACT

Chondroitin Sulfate (CS) belongs to a class of linear, highly charged, acidic polysaccharides known as glycosaminoglycans (GAGs). It is composed of β (1→4)-linked disaccharide units having a glucuronic acid (GlcA) β (1→3)-linked to a N-acetyl galactosamine (GalNac). CS is present on the cell surface and extracellular matrices of all connective tissues as protein complexes known as CS proteoglycans (CSPG). These molecules mediate various biological functions such as central nervous system development, signal transduction, morphogenesis, viral, and bacterial infections.

Though CS oligosaccharides are involved in a range of biological functions, little is known about the optimum structures of the CS oligosaccharides responsible for biological activity. Thus far, CS from natural sources have been the most

common way to obtain these oligosaccharides, but the heterogeneity and complexity of these oligosaccharides make the establishment of structure-activity studies (SAR) difficult. Therefore, the chemical synthesis of well-defined CS oligosaccharides libraries are necessary and will help elucidate the role of CS in biology as well as identify potential therapeutic applications.

To address this deficiency, a modular approach was developed for the parallel combinatorial synthesis of CS oligosaccharides. In this approach, a small number of selectively protected disaccharide building blocks were easily converted into glycosyl donors and acceptors for the preparation of a wide range of oligosaccharides. Herein, we report the synthesis of heterogeneous tetra-, hexa-, and, octasaccharide CS standards based on motifs that have been shown to act as cell surface receptors for the parasite *Plasmodium falciparum* causing pregnant women to become susceptible to malaria despite previous immunity.

INDEX WORDS: Glycosaminoglycans, Chondroitin sulfate, uronic acids, modular synthesis, and stereoselective synthesis.

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By

Jo-Sette Lynn Wilkes

B.S., North Carolina Central University. Durham, NC 2005

M.S., North Carolina Central University, Durham, NC 2009

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By

Jo-Sette Lynn Wilkes

Major Professor: Geert-Jan Boons

Committee: Robert Phillips
Joshua Sharp

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

To my awesome parents for their unfailing love and support. Also to my grandmother, who passed away during my doctoral studies, you may be gone, but your legacy of love and strength will live on through me. To Amadou, Sean, Oscar, Trayvon, Michael, Walter, Freddy, Eric, Sandra, John, Dontre, Tamir and the countless others we speak your names…

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ABBREVIATIONS

Å	Angstrom
Ac	Acetyl
AcOH	Acetic acid
Ar	Aromatic
BAIB	[bis(acetoxy)iodo]benzene
$BF_3.Et_2O$	Borontrifluoride Diethyletherate
Bn	Benzyl
Bz	Benzoyl
COSY	Correlation Spectroscopy
DBU	1,8-Diazabicycloundec-7-ene
DCE	Dichloroethane
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
dFBz	2,5-Difluorobenzoyl
DHB	2,5-Dihydroxybenzoic acid
DIC	*N,N'*-Diisopropylcarbodiimide
DMAP	4- *N,N'*-Dimethylaminopyridine

DMF ..... *N,N'*-Dimethylformamide

DTBMP 2,6-Di-*tert*-butyl-4-methyl-pyridine

Et_3SiH ..... Triethylsilane

EtOAc .....Ethylacetate

EtOH

..... Ethylalcohol

Gal ..... Galactose

GalN ..... Galactose amine

Glc ..... Glucose

h ..... hour

Hz .....Hertz

HSQC ..... Heteronuclear Single Quantum Coherence Spectroscopy

IAD ..... Intramolecular Aglycon Delivery

Lev ..... Levulinyl

Le^x ..... $Lewis^x$

Le^y ..... $Lewis^y$

mAb ..... Monoclonal antibody

MALDI-TOF ..... Matrix Assisted Laser Desorption Ionization Time of Flight

*m*CPBA .....*meta*-Chloroperbenzoic acid

MS ..... Molecular Sieves

mmol ..... Millimole

m/z .....Mass to charge ratio

NaH ..... Sodium hydride

Nap	2-Methylnaphthyl
NIS	*N*-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
Py	Pyridine
SEt	Thioethyl
STol	Thiotolyl
TEMPO	(2,2,6,6-Tetramethyl-Piperidin-1-yl)oxyl
Tf_2O	Trifluoromethanesulfonic Anhydride
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
TLC	Thin Layer Chromatography
TMS	Trimethylsilane
TMSOTf	Trimethylsilyl Trifluorometahnesulfonate
Troc	2,2,2-Trichloroethyloxycarbony

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 GLYCOSAMINOGLYCANS

Chondroitin sulfate belongs to a family of linear, polyanionic, heteropolysaccharides known as glycosaminoglycans (GAGs), which also includes heparan Sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronic acid (HA).[1-5] The majority of these complex carbohydrates are comprised of disaccharide repeating units of a hexuronic acid (β-D-glucuronic or α- L-iduronic acid) linked to a hexosamine (β-D-galactosamine or α-D-glucosamine) except KS. This GAG contains a galactose instead of a hexuronic acid moiety. These polysaccharides are present on all animal cell surfaces in the extracellular matrix (ECM). CS, DS, and HS are synthesized in the Golgi, where these biopolymers are individually O-linked covalently by serine residues to the core protein, forming a large polysaccharide-protein conjugate known as a proteoglycan (PG) [6,7] (Figure 1.1). KS differs from the GAGs described above because it may be N-linked by asparagine or O-linked to the core protein via serine or threonine. [8] On the other hand, HA is the only un-sulfated GAG and it is synthesized by integral plasma membrane synthase, which secretes the nascent chain instantly [9] (Figure1.1).

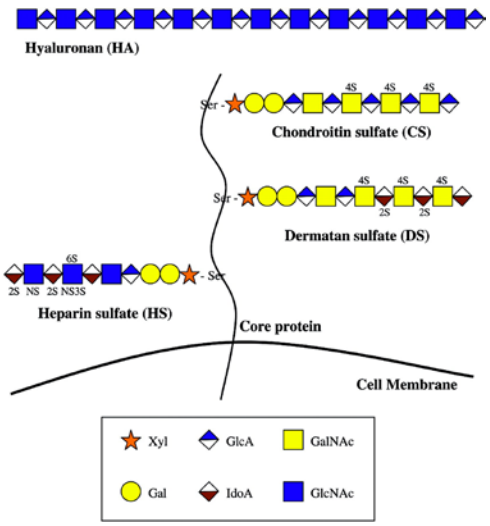


Figure 1.1: Structure of Glycosaminoglycans

Often referred to as mucopolysaccharides due to their viscous and lubricating properties, GaGs were initially dismissed as an “inert glue” surrounding cells.[10] Presently, it is known that the functioning of every mammalian physiological system depends on the regulated expression of GAGs.[11] Due to their acidic character, these polysaccharides interact with a myriad of proteins such as growth factors, cytokines, and morphogens. These interactions in turn cause them to be involved in complex biological roles such as signal transduction, pathway regulation, and lipid metabolism.[12,13] Aberrations in GAG expression are also associated with pathological processes like cancer, inflammation, and microbial pathogenesis.[14-16]

1.2 CHONDROITIN SULFATE

CS may be most known for its use as a dietary supplement for the treatment of osteoarthritis. However, it is used as an additive in food, and it is also found in products like eye drops as well as cosmetics. This biopolymer is present in both vertebrates and invertebrates where it is ubiquitously distributed on cell surfaces as well as within the extracellular and pericellular matrices. [17-20] The basic structure of the CS disaccharide unit was first reported in 1913, in which glucuronic acid (GlcA) was identified. Two years later the structure of the N-acetylgalactosamine (GalNAc) was also reported[21]. We now know that CS is composed of repeating β (1→4)-linked disaccharide units having a GlcA β (1→3)-linked to a GalNac [6,22,23] (Figure 1.2). A chain of CS can consist of between 40 to 100 disaccharide subunits.[24] Thes polysaccharides can be sulfated at the C-4 and C-6 position of the GalNac moiety or the C-2 and C-3 positions of the GlcA moiety. The chondroitin backbone can be modified by various sulfotransferases during biosynthesis. This results in five distinct CS disaccharide motifs: **CS-O** [GlcA-GalNAc] is non-sulfated; **CS- A** [GlcA-GalNAc-(4S)] is sulfated at the C-4 of the GalNac; **CS-C** [GlcA-GalNAc- (6S)] is sulfated at the C-6 of the GalNac; **CS-D** [GlcA-(2S)-GalNAc-(6S)] is di- sulfated at the C-2 of the GlcA and the C-6 of the GalNac; and finally **CS-E** [GlcA- GalNAc-(4S,6S)] is di-sulfated at the C-4 and C-6 of the GalNac. Other

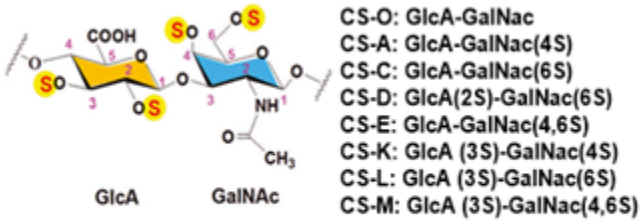


Figure 1.2: Common CS sulfation patterns found in nature

known disaccharide motifs involve sulfation at the C-3 of the GlcA and are designated as **CS-K**, **CS-L**, and **CS-M** [25, 26] (Figure 1.2). Each CS polysaccharide is attached to a serine residue of a core protein by the tetrasaccharide sequence of Xyl-Gal-Gal-GlcA. These glycoproteins are referred to as a chondroitin sulfate proteoglycan (CSPG), and their molecular weights range between 80 – 3,500 kDa [6].

1.3 BIOSYNTHESIS OF CHONDROITIN SULFATE

The biosynthetic pathway of CS is non-template driven and begins in the endoplasmic reticulum (ER) and finishes in the Golgi apparatus. The assembly of CS, DS, and HS GAGs are all initiated by the synthesis of a common tetrasaccharide linkage region (GlcAβ1–3Galβ1–3Galβ1–4Xylβ1–O-Ser) .[6, 22, 23] This region is O- linked covalently to the serine residues present on core proteins. As illustrated in Figure 1.3, the common linkage region is assembled by the individual addition of the monosaccharide units by their corresponding glycosyltransferases. Firstly, xylose (Xyl) is added by xylosyltransferase (XylT) in the ER[27,28]. Subsequently, two galactoses (Gal) are added by β-1,4-galactosyltransferase I (GalT-I)[29,30] and β-1,3-galactosyltransferase II (GalT-II) respectively in the media/trans-Golgi. [31] Finally, GlcA is added by GlcAT-I (β-1,3-glucuronyltransferase I) also in the medial/trans-Golgi regions.[32-34] Additionally, the linkage region is frequently modified by 2-phosphorylation of the Xyl and sulfation at C-6 of the first Gal and di-sulfation of the C-4 and C-6 position of the second Gal.[35] The enzymes responsible for the phosphorylation and sulfation have been identified as FAM20B and chondroitin 6-*O*-sulfotransferase-1 (C6ST-1),

respectively. [36, 37] It is still unclear what role these modifications play during biosynthesis, but recent observations suggest they may play a role in initiating CS synthesis over that of another GaG such as HS.

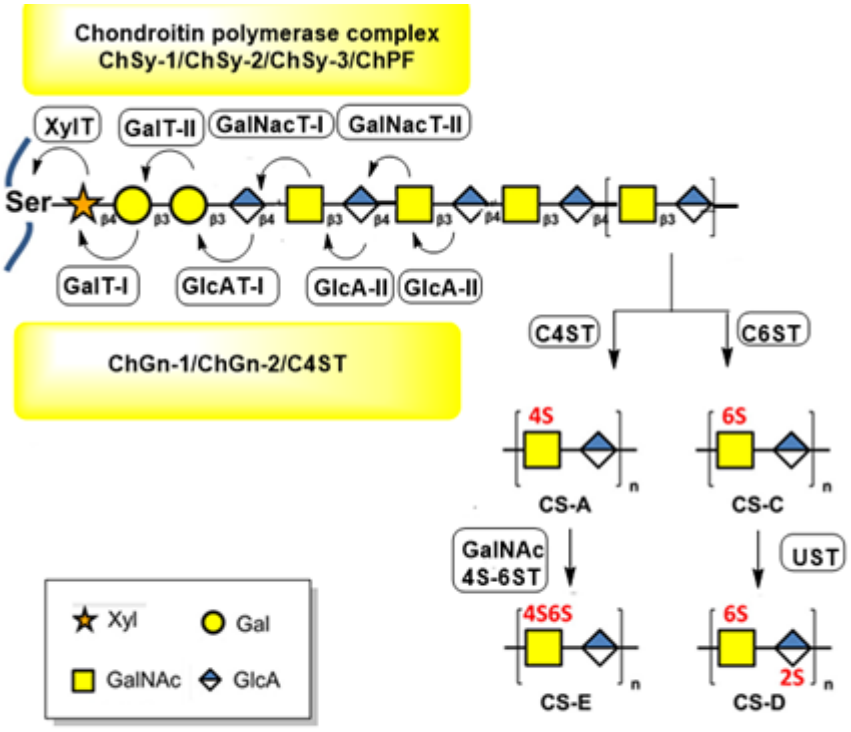


Figure 1.3: The Biosynthesis of CS

The biosynthesis of the CS backbone is triggered by the first transfer of a GalNac moiety by GalNAc transferase I (GalNAcT-I) to the non- reducing end of the GlcA. Subesequently, the polymerization of the backbone by alternating additions of GlcA and GalNac are catalyzed synergistically by a manifold of enzymes including; GalNAc transferase (GalNAcT-II), GlcA transferase (GlcAT-II), chondroitin synthases (ChSy-1, ChSy-2, ChSy-3) , CS N-acetyl-galactosaminyl transferases I and II (ChGn-I & ChGn-2), and chondroitin sulfate polymerizing factor (ChPF) [38-42] (Figure 1.3).

There have been seven sulfotransferases identified as being responsible for the sulfation of CS and DS polysaccharide chains. [43] These transferases catalyze the transfer of a sulfo group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the corresponding sulfation sites on the GalNAc and GlcA moieties. The non-sulfated backbone appears to go initially through a “4-O-sulfation” or a “6-O-sulfation” pathway. 4-O-sulfation of the GalNAc moieties are catalyzed by phylogenetically related chondroitin 4-O-sulfotransferases (C4ST-1, C4ST-2, C4ST-3) [44-47] which are responsible for all CS-A subunits. In the 4-O-sulfation pathway, CS-A acts as a precursor to subsequent 6-O sulfation by GalNAc 4-sulfate 6-O-sulfotransferase (GalNac4S-6ST) rendering the di-sulfated CS-E subunit. On the other hand, sulfation of the GalNAc residues at the 6-O position are catalyzed by chondroitin 6-O-sulfotransferase-1 (C6ST-1) giving subunit CS-C. It then serves as an acceptor substrate for 2-O-sulfation of the GlcA by Uronyl 2-O-sulfotransferase (UST) affording CS-D (Figure 1.3).

It has been observed that aberrations, such as the mutation of specific enzymes, during biosynthesis may cause physical abnormalities. For example, patients with peripheral neuropathies such as Bell’s palsy and hereditary motor and sensory neuropathy (HMSN) are caused by mutations in the ChGn-1 enzyme[48]. Also, mutations in the ChSy-1 transferase plays an important role in skeletal development in syndromic recessive preaxial brachydactylies. This causes limb malformations, short stature and hearing loss[49,50] Spondyloepiphyseal dysplasia (SED) is a group of disorders with primary involvement of the vertebrae and epiphyseal centers. As a result people with this disorder have short-trunk

disproportionate dwarfism. This is associated with a mutation of sulfotransferase C6ST-1.[51]

1.4 CHONDROITIN SULFATE'S ROLE IN MAMMALIAN PHYSIOLOGY

CS proteoglycans participate in a myriad of essential biological processes such as the development and repair of the central nervous system (CNS),[52-55] regulation of cells behaviors[56,57], and cellular recognition.[58-60] These critical biological activities seem to be conducted by the finely tuned repartition of negative charges present on the sulfate and carboxylate groups.[61]

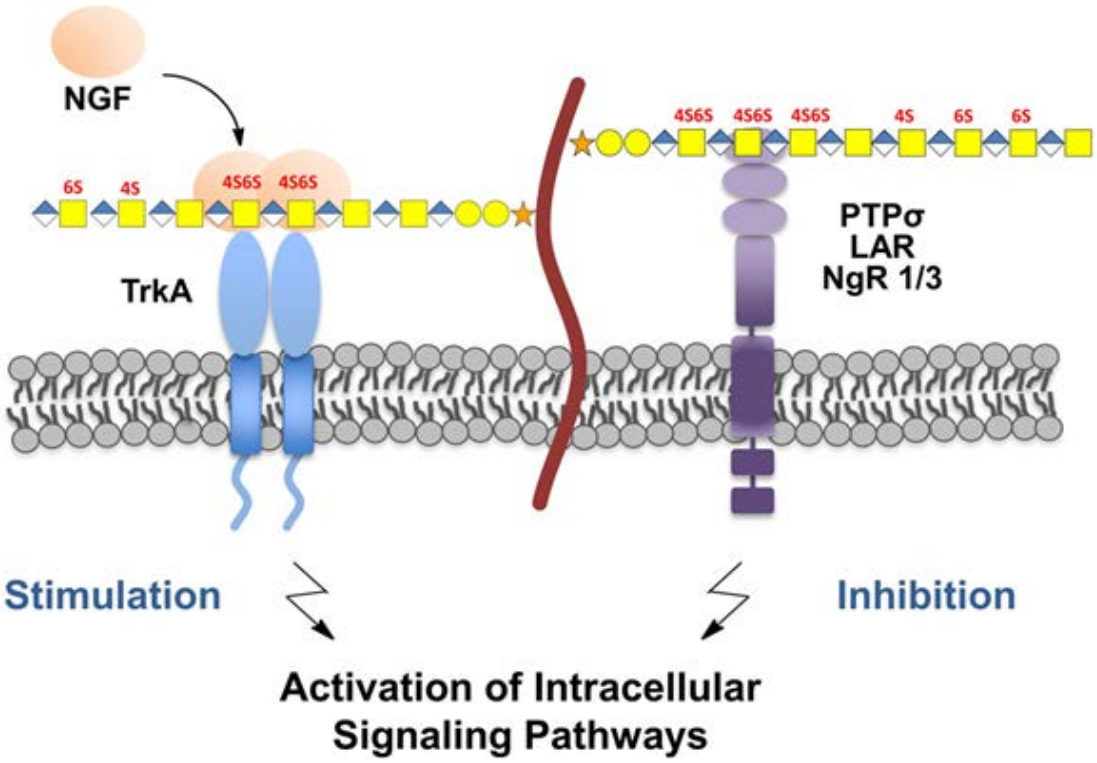


Figure 1.4: Modulation of intracellular signaling pathways by CS[65]

CS's Role in the Central nervous system:

CSPGs are a major component of the extracellular matrix (ECM) where they provide structural support and modulate neuronal activity.[62,63] As of now, there are at least 16 CSPGs identified in the nervous system. [64] The most abundant CSPGs are members of the lectican family, which is comprised of aggrecan, brevican, neurocan, and versican.[65] The CS side chains present on these proteoglycans interact with a plethora of growth factors, chemokines and guidance molecules in the developing brain. In turn, they modulate the availability of these factors to cells. While certain fibroblast growth factors bind to highly sulfated CS chains. Guidance proteins such as slit2, netrin1, and ephrinA1 appear to bind to CS chains in a sulfate-dependent manner.[66] CSPGs are also thought to participate in the myelination of developing CNS axons, because during postnatal development, the proteoglycan brevican is expressed by premature oligodendrocytes concurrently with the time axon fibers ensheathed.[67]

Uniquely, CS chains present in the CNS can have stimulatory as well as inhibitory effects. These contradictory functions are thought to arise from the structural diversity of CS chains (Figure 1.4). Neurite growth can be stimulated by the localization of soluble ligands like growth factors to the cell surface and mediate interactions with their respective receptors. For example, nerve growth factor (NGF) signaling and neurite outgrowth are enhanced when CS-E is present on the cell surface.[68] On the other hand, CS chains can inhibit neurite outgrowth by interacting directly with transmembrane receptors such as PTPσ and affect intracellular signaling. [69, 70]

One increasing area of interest is the role of these polysaccharides in traumatic CNS injury. It has been discovered that the glial scar is composed mainly of CSPGs.[71] Recent discoveries suggest that the major cause of regeneration failure is due to the upregulation of these CSPGs. For instance, chondroitinase ABC (ChABC), which degrades CS-GAGs, has been shown to stimulate both functional and structural recovery.

Cancer and CS:

Recent discoveries have suggested that CS is expressed on most human cancer cells.[72] Therefore it plays an integral part in tumor progression and metastasis by regulating cell adhesion, migration, invasion, and growth. [73-78] CS appears to mediate the signaling pathways of this pathological event in a sulfation dependent manner. As illustrated in Figure 1.5(A) polysaccharide chains of CS-A and CS-E can bind to specific growth factors and form a complex with the growth factor receptors. In turn, this conglomerate causes the activation of the mitogen-activated protein kinase (MAPK) pathway promoting malignant transformation. [79] The role of selectins in cancer cell adhesion and metastasis have been well documented.[80,81] Interactions with CS and selectins also depend on the type of sulfation pattern present. Studies have provided evidence that CS-E binds selectively with L-selectin and P-selectin (Figure 1.5B). [82] This motif is currently garnering much attention and is being evaluated as a P-selectin ligand and as a potential prognostic biomarker in ovarian cancer.[83] Metalloproteinases (MMPs) are a family of zinc-containing endopeptidases that degrade components of the extracellular matrix. MMPs promote tumor invasion, but also affect tumor cell

behavior which leads to cancer progression.[84] The activation of MMP2, a principle degradation enzyme in the metastatic cascade is dependent on the type of sulfation. Hence, CS-A side chains of melanoma chondroitin sulfate proteoglycans (MCSP) form a complex with pro-MMP2 and MT3-MMP promoting metastasis. [85] As depicted in Figure 1.5(C) the expression of MCSPs also increase the function of the transmembrane receptor integrin.[86] Thus activating the extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) pathway, which stimulates tumor growth and motility.

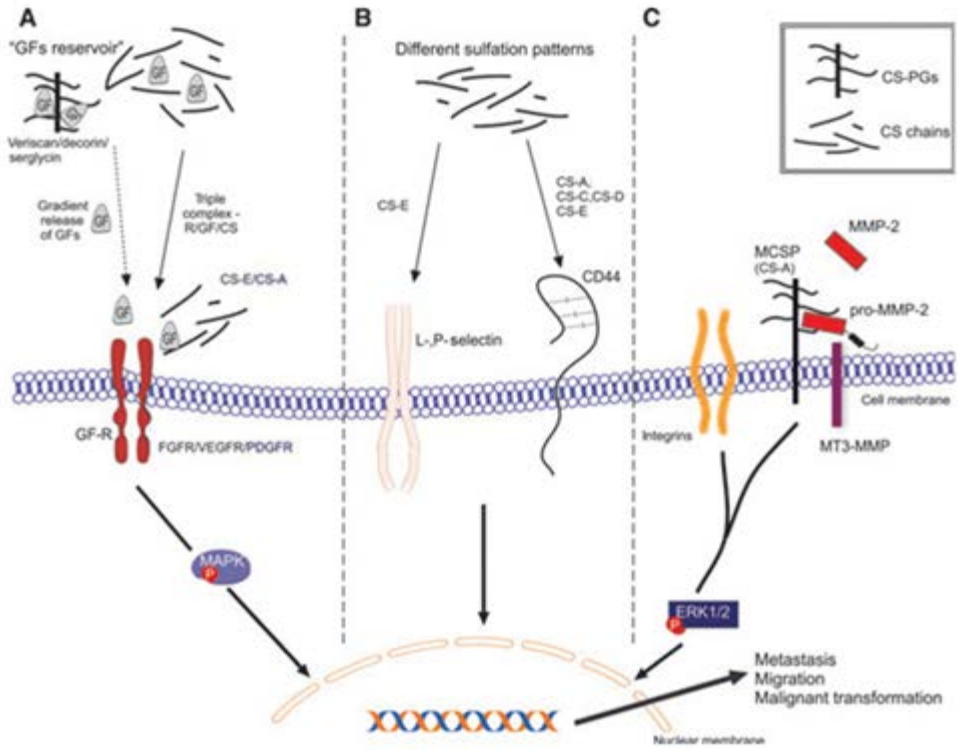


Figure 1.5: CS-mediated signaling pathways in Cancer [79]

CS chains as cell surface receptors for pathogens:

Many foreign pathogens such as viruses, parasites, and bacteria, exploit the adhesion properties of cell surface CS polysaccharides. For example, the parasite *Plasmodium falciparum* causes malaria in women who have already obtained immunity. Known as Pregnancy-associated malaria (PAM), this pathogenesis is mediated by low sulfated CS-A found in the placenta. Where it binds to the protein variant surface antigen 2-chondroitin sulfate A (VAR2CSA) present on infected red blood cells. More specifically, CS-A polysaccharides bind to the Duffy binding – like 3x and 6 (DBL3x & DBL6) domains of VAR2CSA with micromolar affinity as illustrated in figure 1.6. [87]

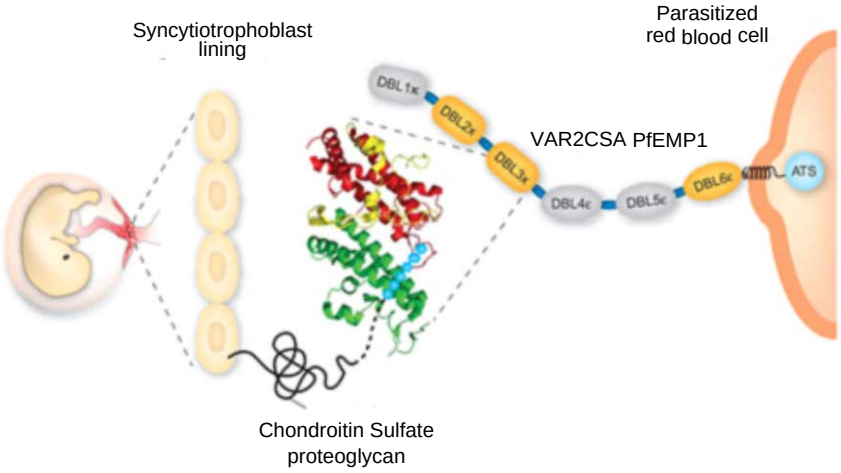


Figure 1.6: Low sulfated CS-A found in the placenta bind to the protein VAR2CSA present on parasitized red blood cells in pregnancy associated malaria[88]

In the case of the herpes simplex virus (HSV) it has been observed that CS chains composed mostly of CS-E motifs act as cell surface receptors. [88] Sog9 that are deficient C4ST-1, the enzyme responsible for the sulfation of the CS-E motifs,

are less susceptible to viral invasion. However susceptibility is increased when C4ST-1 is added. The observation suggests that C4St-1 expression mediates this pathogenesis.

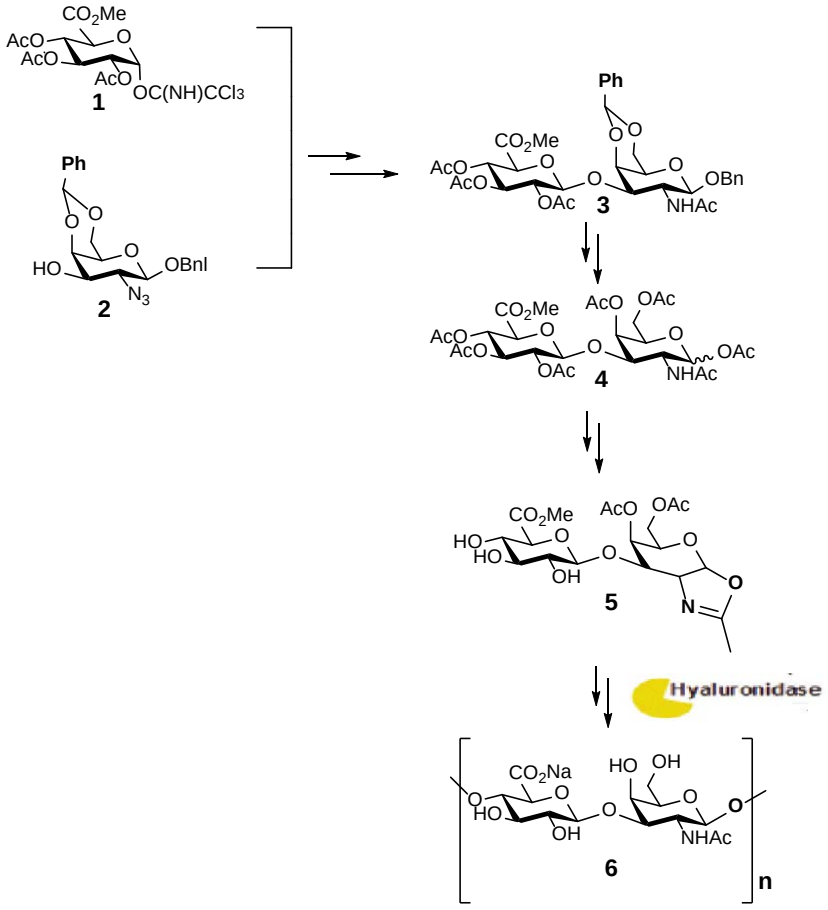
1.5 ENZYMATIC AND CHEMICAL APPROACHES SYNTHESIS OF CS

Even though CS plays a critical role in several biological processes, very little is known about the structural characteristics responsible. This is due to a lack of pure defined CS standards. Most commercial sources of CS are derived from animal sources that possess a combination of different sulfation patterns as well as lengths. Over the past decade, an increased number of enzymatic, as well as chemical synthesis toward CS standards, have been reported.

Enzymatic synthesis:

While chemical synthetic approaches toward CS standards are well established, enzymatic routes are an attractive alternative. Kobayashi and co-workers reported the enzymatic synthesis of CS-O and CS-A polysaccharides by hyaluronidase (HAase) catalyzed polymerization.[89, 90] This method was based on the concept they coined “transition state analog substrate” (TSAS), where a monomer was designed with a structure that mimicked the transition state of the enzyme-catalyzed hydrolysis. HAase is an endo-β-N-acetylhexosaminidase that catalyzes the glycosidic bond cleavage of HA and CS *in vivo*. However invitro it catalyzes bond formation by transglycosylation. In this approach, the coupling of monosaccharides **1** and **2** afforded critical intermediate **3** (Scheme1.1)**.** At this

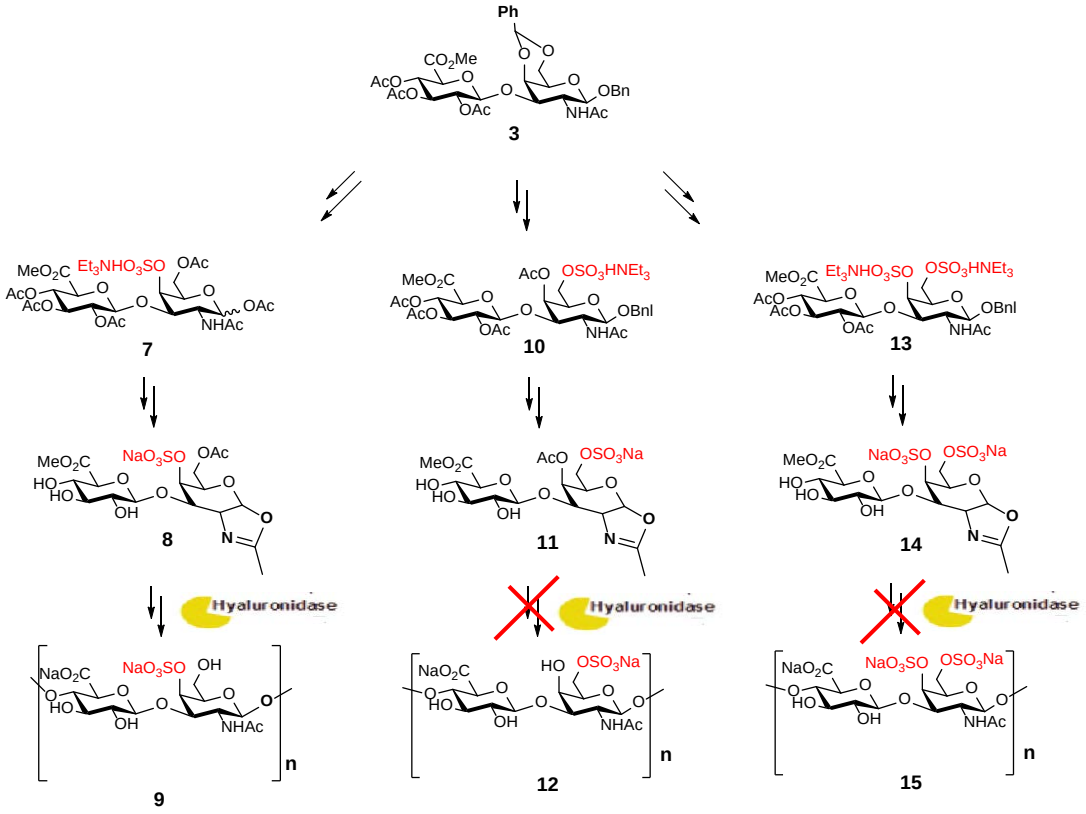
point, the synthesis diverted into two different pathways. In the first pathway, the benzylidene was removed by exposure to acidic conditions followed by acetylation.



Scheme 1.1: Enzymatic polymerization of CS-O with hyaluronidase

Intermediate **4** was treated with trimethyl silyl triflate followed by acetyl removal to afford substrate monomer **5.** Then, they polymerized the non-sulfated monomer in the presence of HAase to afford polymer **6.** In the second pathway after benzylidene removal, the 6-hydroxyl group was acetylated, and the 4-hydroxyl group was sulfonated to provide 4-O-sulfated disaccharide **7**. The 1-O-benzyl group was removed by hydrogenation using palladium (II) hydroxide-charcoal followed by treatment with p-toluenesulfonyl chloride and hydrolyzation by

aqueous sodium hydroxide–methanol mixed solution to give **8.** Subsequently, the sulfated monomer was polymerized by treatment with HAase to render **9**. The polymerization reactions catalyzed by both ovine testicular HAase (OTH) and bovine testicular HAase (BTH) proceeded smoothly at 30 ^{0}C in the pH range 6.0 to 8.5 with total control of regioselectivity and stereochemistry. However, this approach was limited and not able to produce a variety of sulfated standards as illustrated in Scheme 1.2.



Scheme 1.2: Enzymatic polymerization of CS with hyaluronidase

Sugiura and co-workers developed a stepwise elongation of non-sulfated chondroitin by generating two K4CP mutant immobilized enzyme beads.

Expressed by *Esherichia* Coli, the CS polymerase K4CP consists of two active glycosyltransferase domains. D-GlcA and D-GalNAc monosaccharides units were

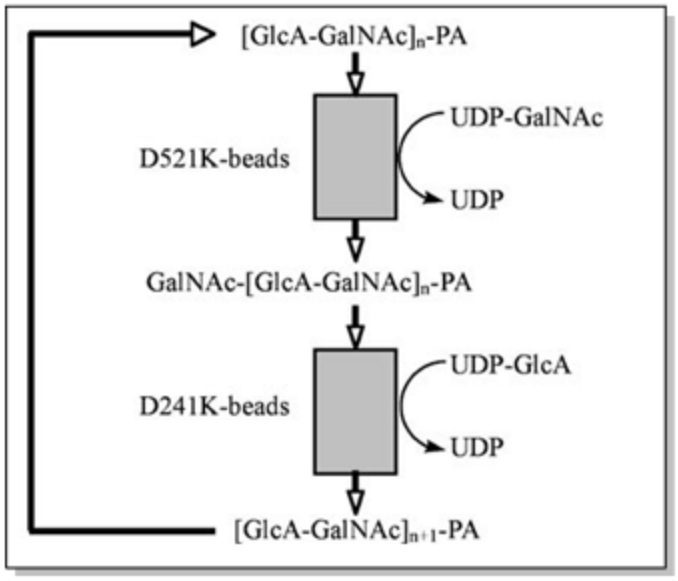
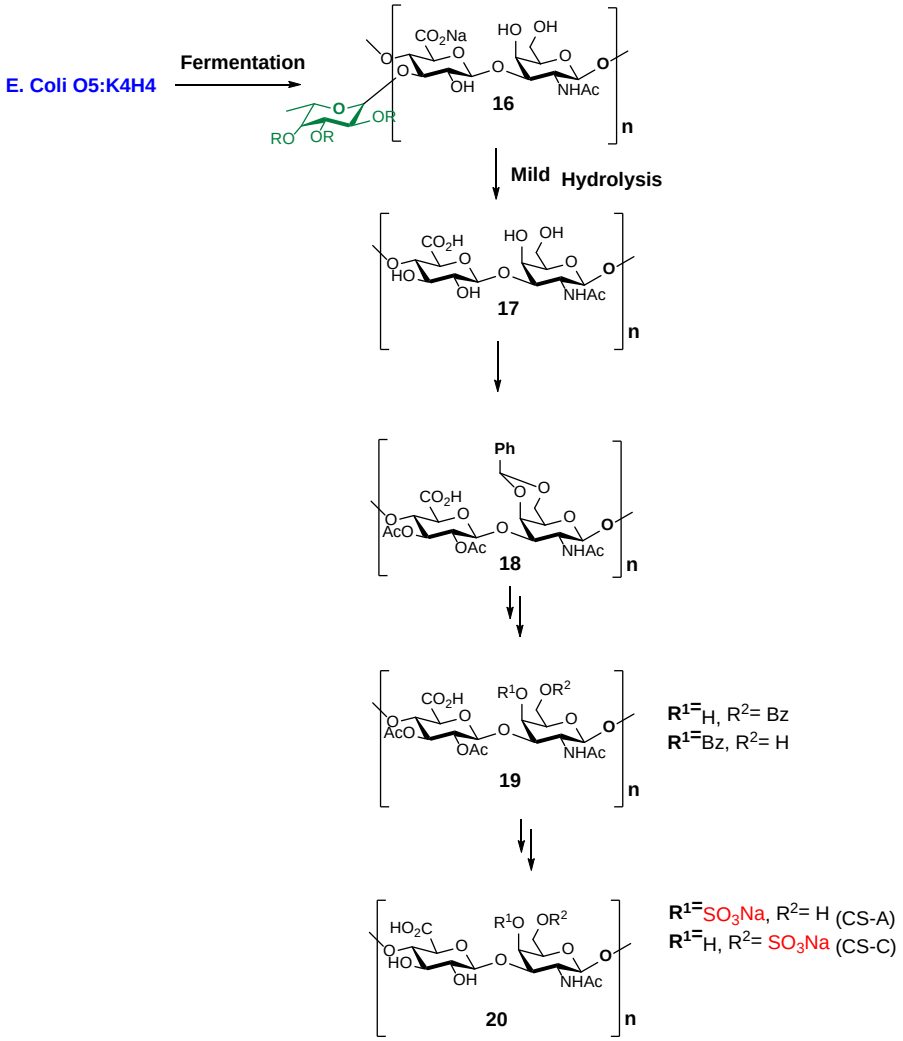


Figure 1.7: Schematic of stepwise synthesis of CS with immobilized beads[91]

alternatively transferred by this enzyme from UDP-D-GalNac and UDP-D-GlcA donors to the non-reducing end of a chondroitin chain acceptor.[92] This group exploited the duality of this enzyme by accomplishing mutations that nullified one glycosyltransferase activity while maintaining the other. The two mutant enzymes, D-251K (GalNAc-T) which transferred the D-GalNAc residue and D-241K (GlcA-T), which transferred the D-GlcA residue, were immobilized on agarose beads. Well defined non-sulfated oligosaccharides ranging from lengths of 7 to 16 were obtained by sequential reactions of UDP-D-GalNAc with D521K-immobilized beads, or UDP-D-GlcA and enzyme D421K-immobilized beads in 1 mL of the buffer with pH of 7.2 at 30 ^{0}C until elongation was complete.



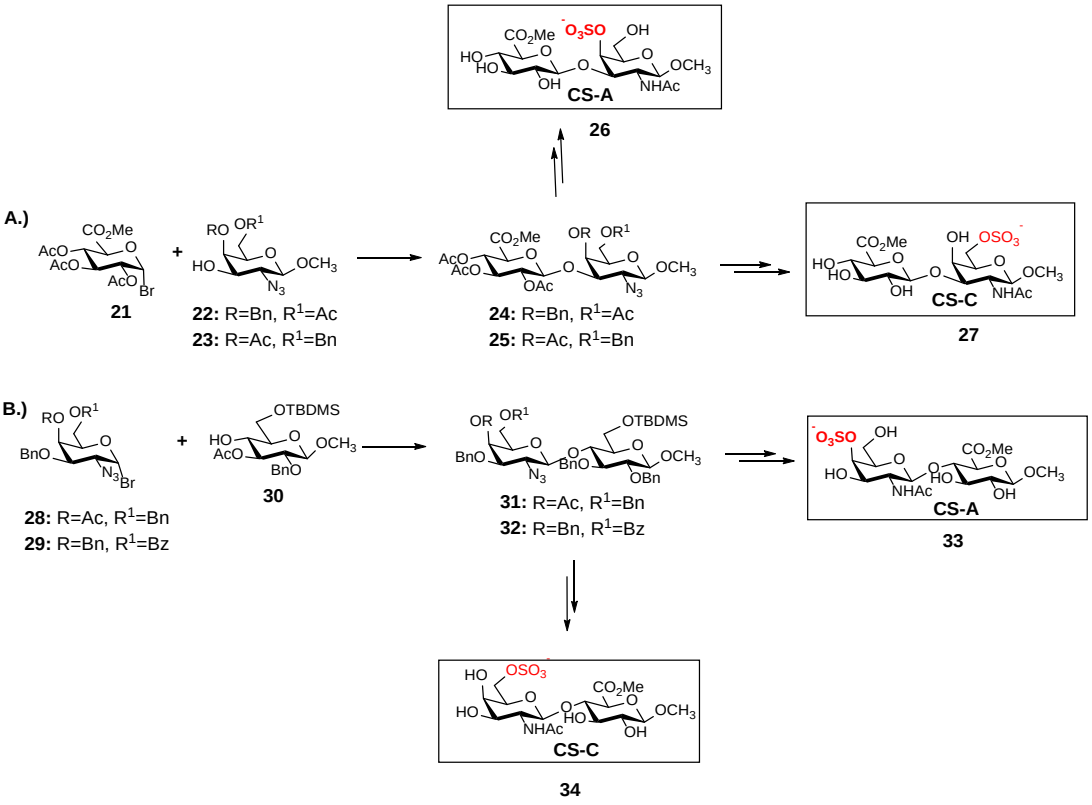
Scheme 1.3: Microbiological–Chemical Strategy to Produce Chondroitin Sulfate A,C

Using a microbiological-chemical strategy, Bedini and co-workers reported the synthesis of heterogeneous CS polysaccharide mostly composed of CS-A and CS-C. The fermentation of E. Coli O5:K4H4 biosynthesizes capsular polysaccharide with a Chondroitin backbone containing β -fucosylation at the O-3 position of the GlcA residues **16**.[92] After de-fucosylation by mild hydrolysis the 4-O and the 6 -O positions of **17** were protected by a benzylidene. This protection sequence enabled selective sulfation followed by acylation of the remaining free hydroxyl groups. Polysaccharide **18** was subjected to oxidative cleavage

conditions by treatment with $NaBrO_3/Na_2S_2O_4$ in an ethyl acetate/water mixture. However, these conditions had low selectivity and rendered polysaccharide **19** composed of a mixture O-4 and O-6 benzylation. Sulfates were introduced and followed by subsequent alkaline deacylation yielding CS-A/CS-C polysaccharide **20.**

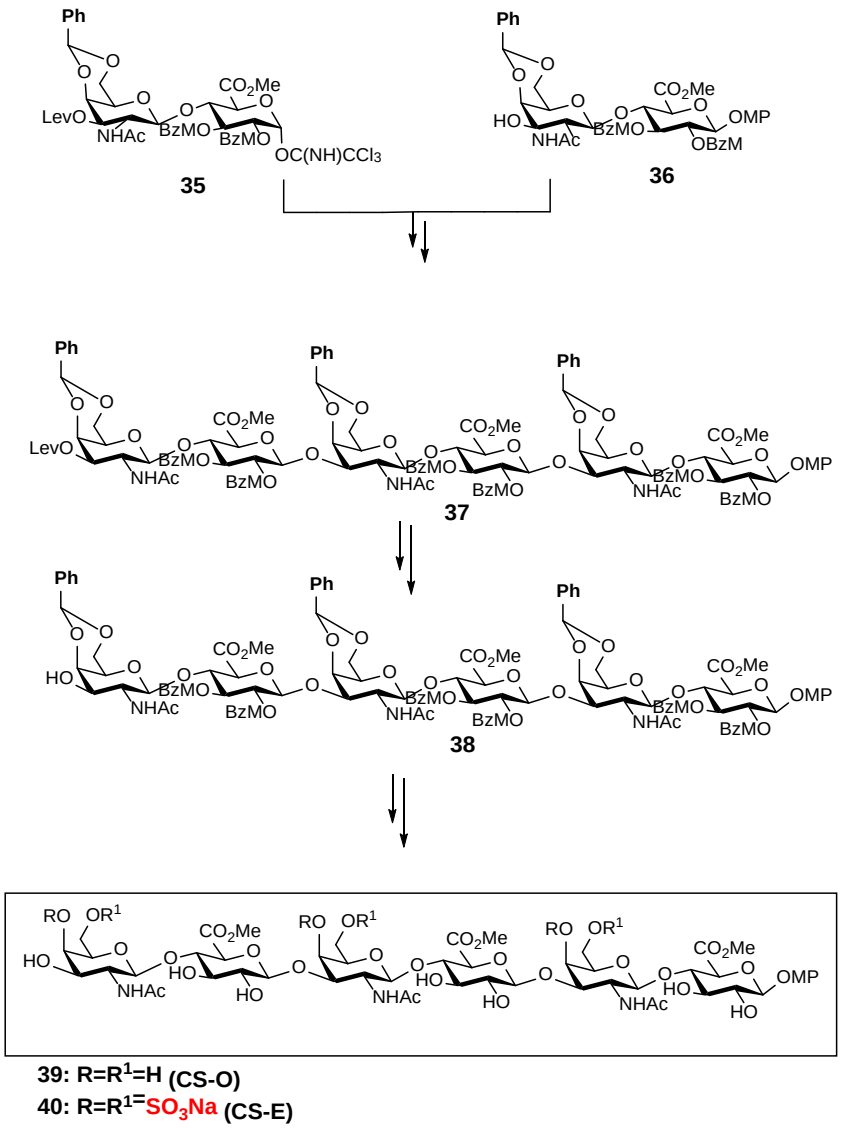
Chemical synthesis:

Earlier synthetic approaches toward CS standards focused on the synthesis of CS-A and CS-C disaccharides. Jacquinet and co-workers utilized a glucuronic acid bromide donor **21** and 2-azido-2deoxy galactose acceptors **22** or **23** (Scheme 1.4).[93] The monosaccharide units were glycosylated rendering β-GlcA-(1, 3)-GalNac disaccharides **24** and **25**. After deprotection, 4-O and 6-O sulfation was followed by deacetylation CS-A **26** or CS-O **27** disaccharides were rendered. Comparably, Sinay and co-workers employed 2-azido-2deoxy galactose bromides **28**, **29** and glucose acceptor **30** to provide disaccharides **31** and **32** (Scheme 1.4).[94] In this case, oxidation took place at the disaccharide stage upon de-6-O-silylation. Subsequent deprotection and sulfation provided β-GalNac- (1, 4) - GlcA disaccharides **33** and **34**.



Scheme 1.4: Seminal synthesis of CS-A and CS-C disaccharides

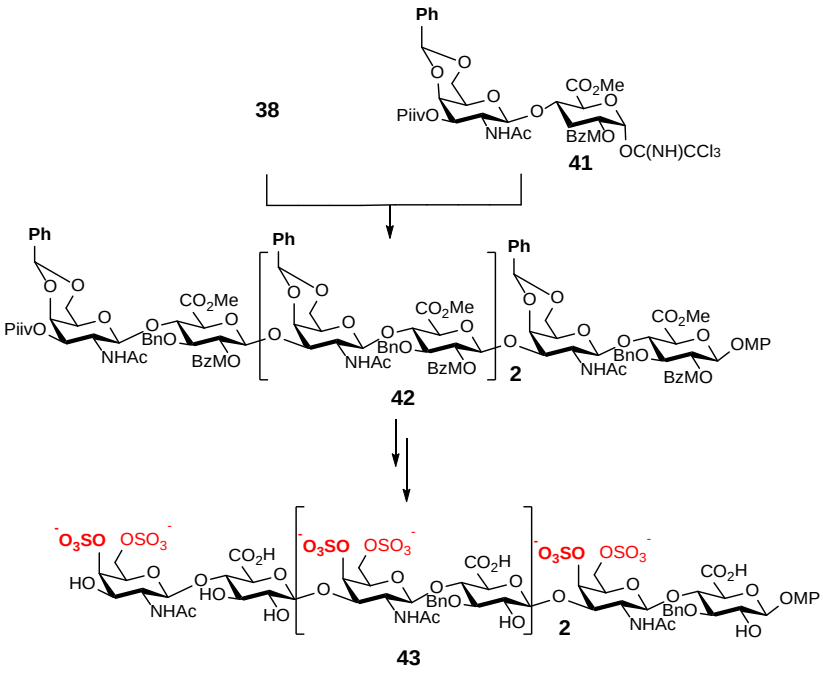
In 2004, Tamura and co-workers utilized glycosyl donor **35** as well as acceptor **36** with a GalNac-(1, 4)-GlucA motif to synthesize fully protect hexasaccharide **37** (Scheme 1.5) **.**[95] While azido protection of the galactosamine was explored, the reduction of several azides followed by subsequent acetylation resulted in very



Scheme 1.5: Tamura synthesis of CS-E hexasaccharides

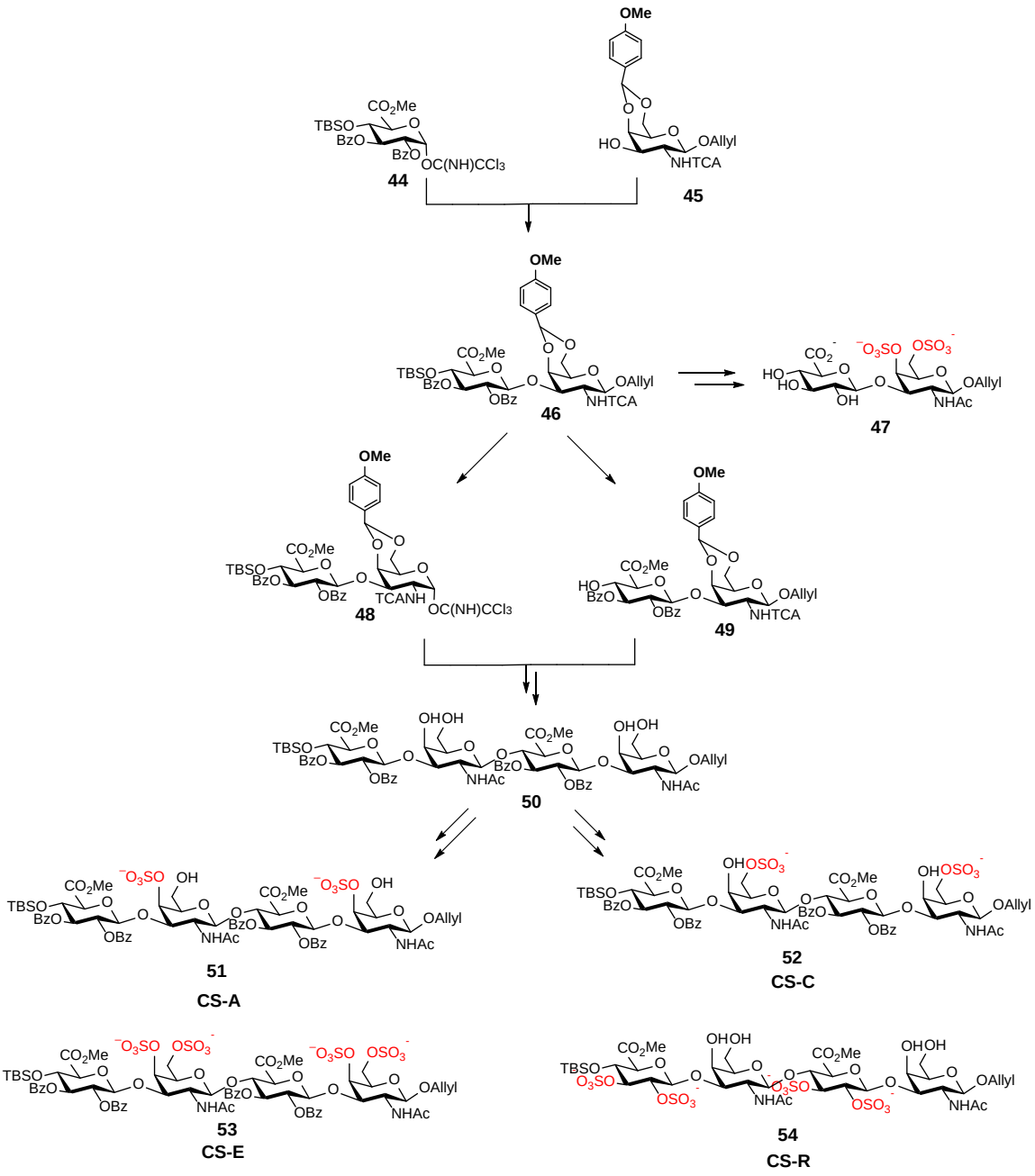
low yields less than 10%. After benzylidene removal and saponification the non-sulfated CS-O **39** was yielded. In the case of the sulfated hexasaccharide, benzylidene removal was followed by di-sulfation of the C-4 and C-6 position. Saponification was performed rendering the CS-E hexasaccharide **40** at a 57% yield. Furthermore in 2008, they were able to use this same approach in the synthesis of an octasaccharide (Scheme 1.6).[96] The glycosylation of

hexasaccharide acceptor **38** and disaccharide donor **41** gave the fully protected octasaccharide **42.** Using the previously established procedures of deprotection and sulfation CS-E octasaccharide **43** was produced.



Scheme 1.6: Tamura synthesis of CS-E octasaccharide

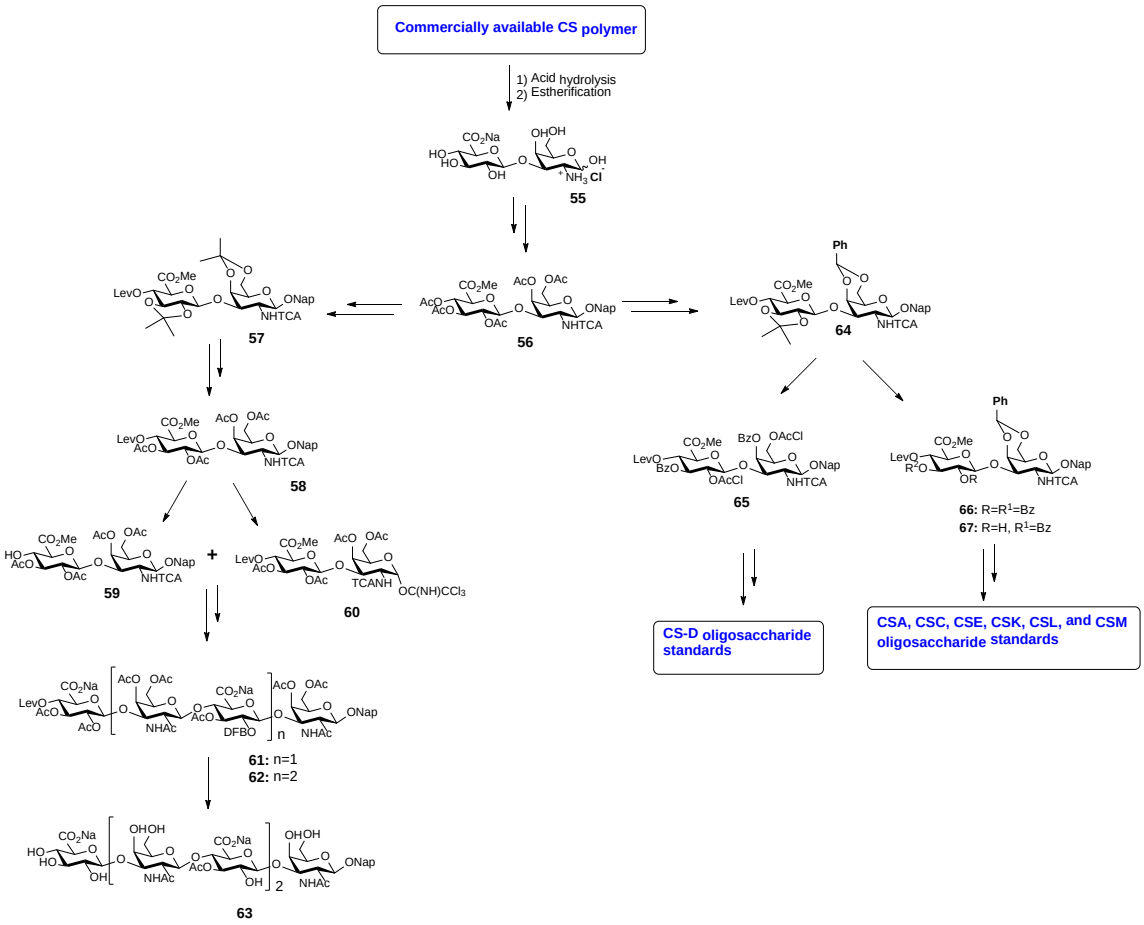
The research group of Hsieh-Wilson has made several important contributions toward the synthesis of CS standards in the elucidation of possible "sulfo" codes responsible for different biological processes. From 2004 to 2006 [97, 98] this group reported the synthesis of di- and tetrasaccharide motifs CS-A, CS-C, CS-E and an unnatural tetrasaccharide designated as CS-R to probe the



Scheme 1.7: Hsieh-Wilson synthesis of CS-A, CS-C, CS-E, and CS-R tetrasaccharides

interactions of CS and different growth factors in the regulation of neuronal growth. Starting from the coupling of the monosaccharide donor **44** and acceptor **45** common disaccharide **46** was rendered (Scheme 1.7). It was then transformed into the corresponding glycosyl donor **48** by the removal of the anomeric allyl by

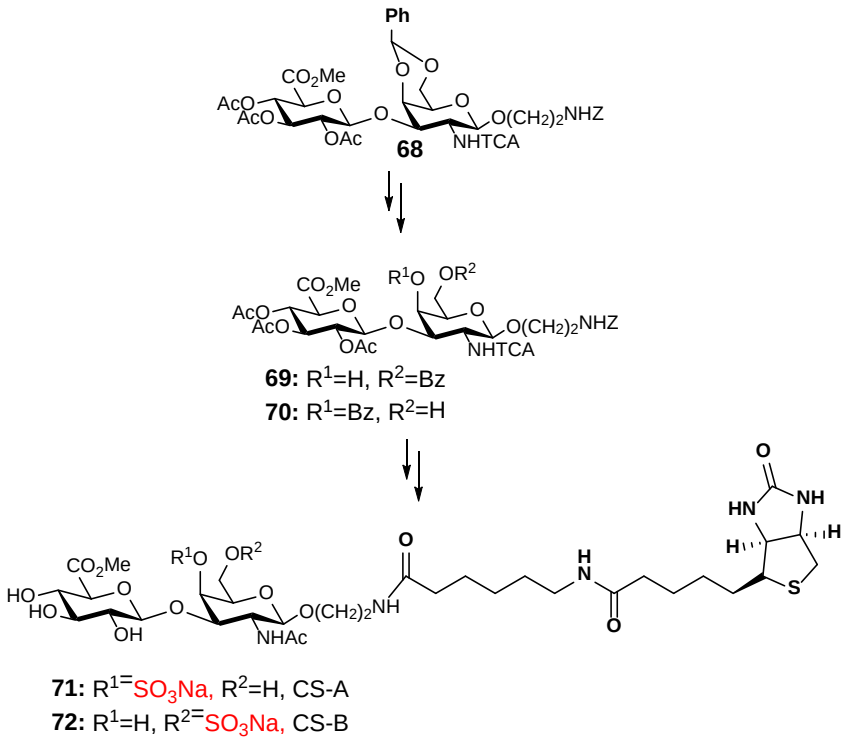
treatment with Grubbs' second-generation catalyst followed by imdoylation. The glycosyl acceptor **49** was produced by desilylation of the C-4**.** Unlike the aforementioned synthetic approaches, a trichloroacetyl group was utilized as a neighboring group participating auxiliary to ensure β-stereoselectivity of the fully protected tetrasaccharide. Transformation of the trichloroacetyl into the acetamido by radical reduction was followed by the oxidative cleavage of the para-methoxybenzylidene revealing tetrol **50.** The CS-A tetrasaccharide was synthesized by selective benzoylation of the C-6 hydroxyl with benzoyl cyanide, followed by the sulfation of the C-4 position, deprotection, and saponification gave **51** at a 49%. The precursor for CS-C was sulfated by mild conditions selectively sulfating the more readily reactive primary alcohol at C-6. In contrast, the CS-E precursor was treated with vigorous conditions to ensure di-sulfation. After silyl deprotection and saponification CS-C and CS-E tetrasaccharides, **52** and **53** were produced. They utilized the same protocol to synthesize CS-E disaccharide **47**. Finally, CS-R tetrasaccharide **54** was obtained after benzylidene acetal formation, saponification, sulfation of the C-3 and C-4 of the GlcA, and desilylation. This lab was the first to evaluate CS - protein interactions by microarray.



Scheme 1.8: Jacquinet semi-synthetic approach toward chondroitin oligomers and precursors

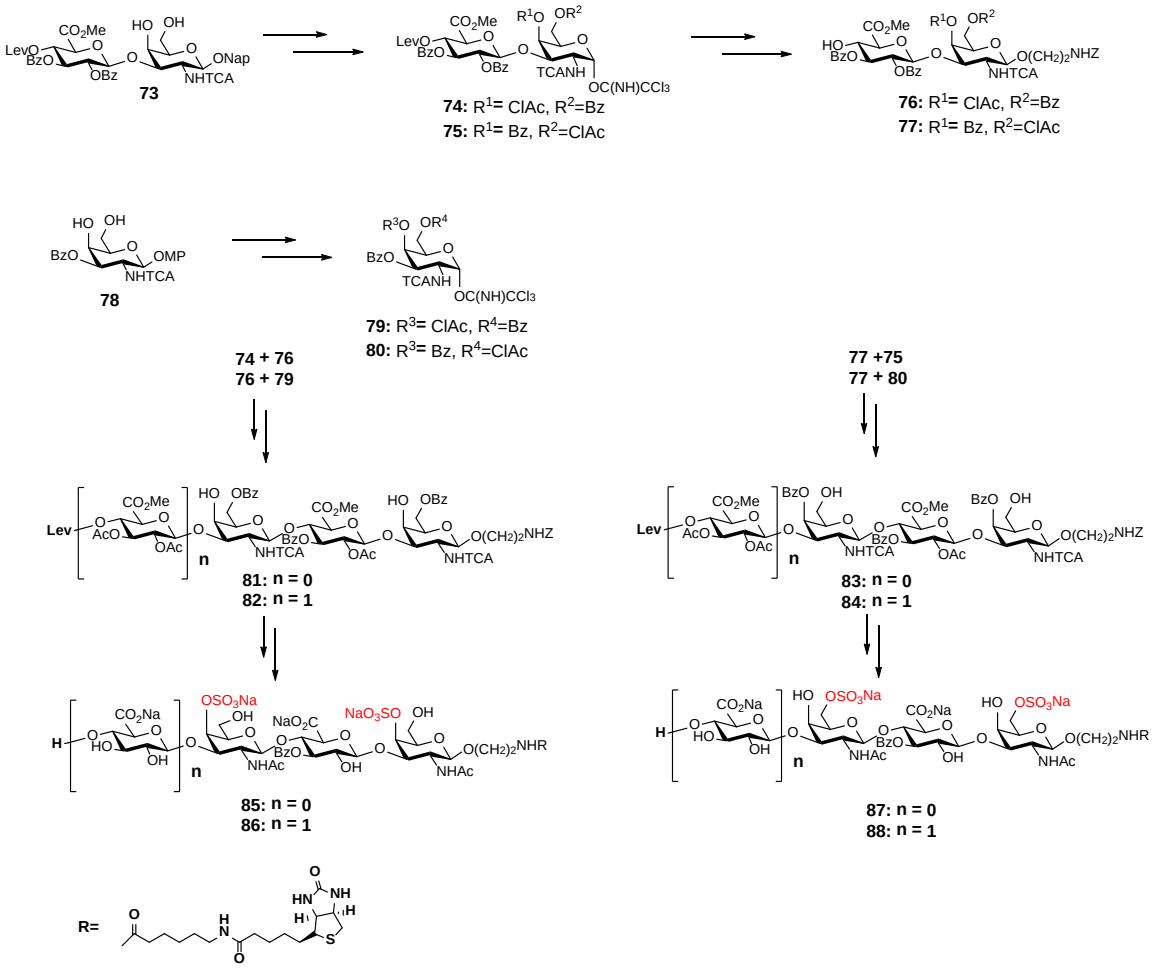
Over the past decade, Jacquinet and co-workers have described the synthesis of a wide range of size-defined oligomers. In, 2006 they reported the synthesis of a non-sulfated hexasaccharide along with the precursor disaccharide building blocks for all known sulfated motifs.[99] This synthetic approach was unique because instead of starting from monosaccharide units, this strategy utilized the hydrolysis of a commercially available CS polymer. Using known acid hydrolysis procedures followed by desulfation, N-deacetylation, esterification disaccharide **55** was rendered. After eleven chemical transformations key disaccharide **56,** was produced bearing a TCA participating group at C-2 and a 2-naphthylmethyl (NAP)

group at the anomeric position. To obtain the non-sulfated chondroitin oligomers (Scheme 1.8), disaccharide **57** was prepared by deacetylation followed by the isopropylidene protection and levulinoylation of the C-4 of the GlcA. Acid Hydrolysis followed by acetylation afforded **58** which was transformed into glycosyl acceptor **59** and glycosyl donor **60.** Fully protected tetrasaccharide **61** and hexasaccharide **62** were produced repetitiously, by consecutive glycosylations catalyzed by TMSOTf and delevulinoylation steps. After transformation of the N-trichloroacetyl groups to N-acetyl followed by subsequent saponification the non-sulfated chondroitin hexasaccharide **63** was provided. The synthesis of the disaccharide building blocks for the sulfated oligosaccharides began with common disaccharide **64.** The presence of two different acetals allowed the selectivity of the two sugar units. **65** was utilized as the precursor for CS-D oligosaccharides, while **66** or **67**were the common building blocks for CS-A, CS-C, CS-E, CS-K, CS-L, and CS-M. In 2009, this team reported the first preparation of these size-defined oligomers utilizing this semi-synthetic approach.[100]



Scheme 1.9: Jacquinet synthesis of biotinylated CS disaccharides

More recently, Jacquinet and co-workers also described the synthesis of biotinylated homo- and heterogeneous C-4 and C-6 sulfated CS oligomers (Scheme 1.9). [101] Disaccharide targets were derived from the common disaccharide **68.** Modifications involved a one–pot procedure that called for the treatment of the diol with trimethyl orthobenzoate in the presence of a catalytic amount of 10-camphorsulfonic acid (CSA) gave the 4, 6-orthoester. This intermediate was treated with acetic conditions to give the C-6 and C-4 benzoyl derivatives **69** and **70** in approximately 1:1 ratio (35% and 37%). Using established deprotection, sulfation, and biotinylation protocols targets **71** and **72** were produced. The synthesis towards homogenous tri- and tetrasaccharides involved the synthesis of six disaccharide building blocks; trichloroimidate disaccharide

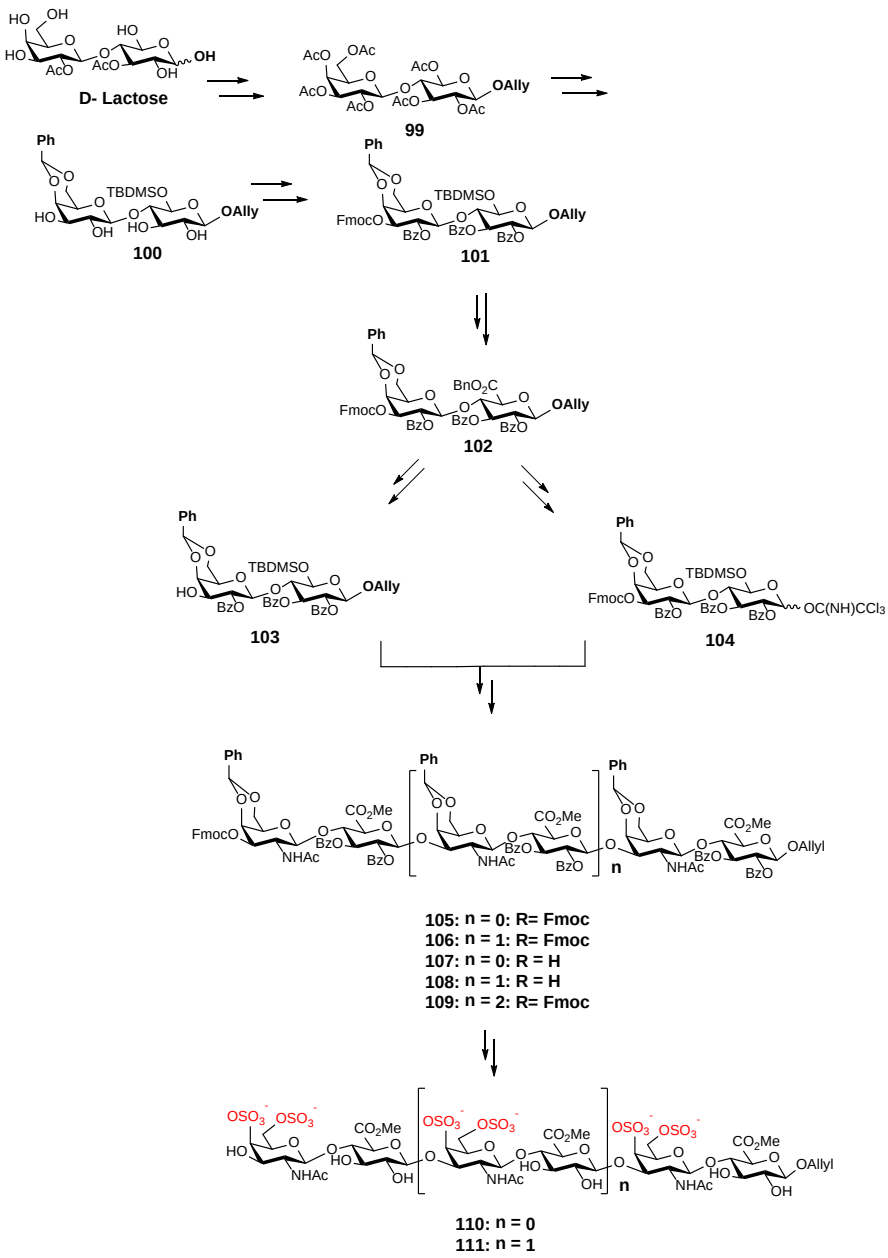


Scheme 1.10: Jacquinet synthesis of homogeneous biotinylated CS tri- and tetrasaccahrides

donors (**74** , **75**) disaccharide acceptors (**76** , **77)**, and monosaccharide donors (**79**, **80)**. Here again, they utilized the one-pot conditions previously described. These common building blocks possessed chloroacetate groups for the introduction of sulfate groups. The glycosylations performed were either “2 + 1” or “2 + 2” as shown in Scheme 1.1O. Subsequent removal of chloroacetates, sulfation, saponification and biotinylation rendered trisaccharides **85** and **87** as well as tetrasaccharides **88** and **86.** Employing the same route, biotinylated heterogeneous tri-and tetrasaccharides **95-98** were synthesized this was the first

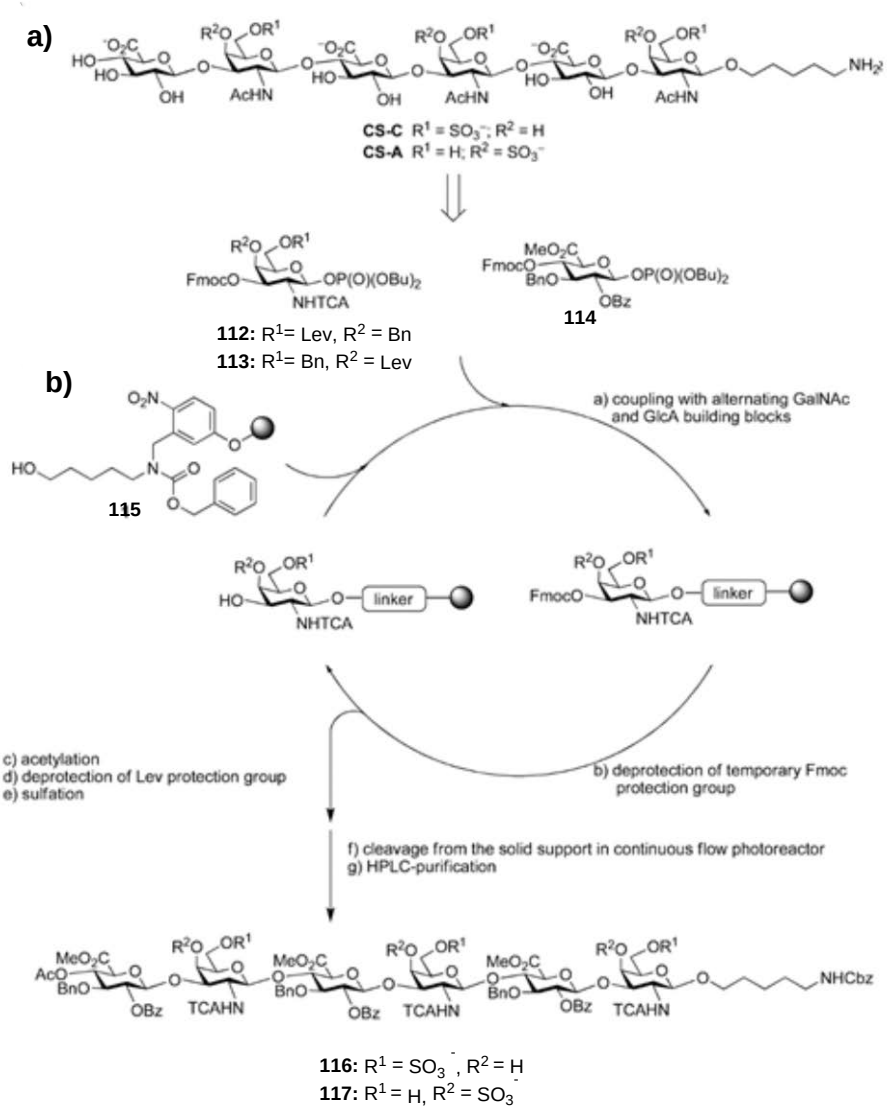
publication to report a synthetic approach towards heterogeneous CS standards (Scheme1.11).

Scheme 1.11: Jacquinet synthesis of heterogeneous biotinylated CS tri- and tetrasaccahrides



Scheme 1.12: Despras synthesis of tetra- and hexasaccharide analogues

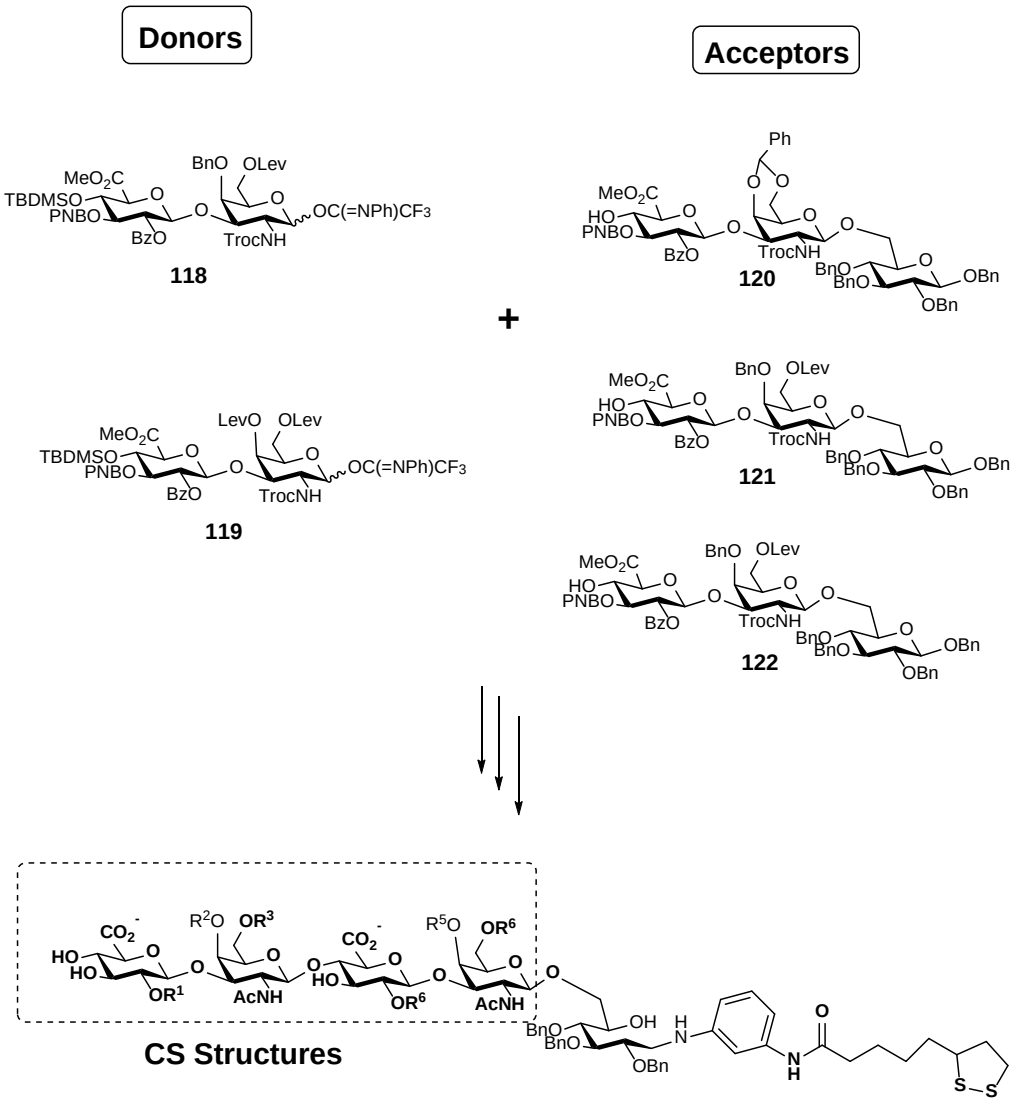
Despras *et al*. described the preparation of a library di- to hexasaccharide CS-E standards in a rapid manner to establish structure–activity relationships.[102] This synthesis began with peracetylated allyl-β-lactose **99** derived from commercially available and easily accessible D-lactose. After deacetylation had followed by selective benzylidene formation, the free C-6 was silylated temporarily to give **100**. This tetrol was selectively protected at the C-3 by Fmoc-Cl and sym-collidine to generate a hindered acylating agent *in situ*. This was followed by benzoylation to render disaccharide **101**. After selective deprotection of the silyl in the presence of Fmoc by TMSOTf at low temperature, the resulting primary alcohol was oxidized using TEMPO/BAIB followed by esterification to produce **102.** This key intermediate was transformed into the respective glycosyl acceptor and donor **103** and **104**. Repetitive glycosylations mediated by TMSOTf gave a library of **105**-**109**. CS -E analogs were prepared from tetra and hexasaccharide **107** and **108**. Previous Fmoc protection was replaced by an acetyl group to avoid unwanted sulfation. Moreover, the benzylidene acetals were removed, and the free hydroxyls were sulfated. The final CS-E tetrasaccharide **110** and hexasaccharide **111** were obtained after direct saponification using KOH.



Scheme 1.13 : a) Retrosynthesis of CS oligosaccharide standards on solid support b) Automated synthesis of CS hexasaccharides[103]

A solid phase synthesis towards CS- A and CS-C standards were reported by Seeberger and co-workers.[103] Starting with the synthesis of differently protected galactosamine phosphates (**110**, **111**) and a glucuronic acid phosphate (**112**) building blocks. Utilizing functionalized photocleavable linker **113,** CS-C hexasaccharide **114** and was successfully achieved using building blocks **110** and

112 in 16 steps over three days, with a yield averaging 88% yield per step. Furthermore building blocks **111** and **112** were employed to generate CS-A hexasaccharide **115** averaging 86% yields per step. As illustrated in Scheme 1.13, for the assembly of the carbohydrate backbones, each glycosylation cycle was performed three times and catalyzed by TMSOTf at -15^0 C. Piperidine was used to remove the temporary Fmoc protecting group on the non-reducing end sugar. Before sulfation, the terminal Fmoc protecting group was replaced by a stable acetyl protecting group to avoid unwanted reactions. All of the levulinoyl esters were then selectively removed with buffered hydrazine. Sulfation required incubation with a sulfur trioxide pyridine complex in pyridine and DMF at 50^0C. The hexasaccharides were cleaved from the solid support by exposure to UV light. However, a straightforward procedure for the final deprotection remains to be developed in order to obtain the target hexasaccharide structures.



123. CS-C+E (GlcA-GalNAc6S-GlcA-GalNAc4S6S)
124. CS-D+T (GlcA2S-GalNAc6S-GlcA2S-GalNAc4S6S)
125. CS-E+A (GlcA-GalNAc4S6S-GlcA-GalNAc4S)
126. CS-E+C (GlcA-GalNAc4S6S-GlcA-GalNAc6S)
127. CS-E+E (GlcA-GalNAc4S6S-GlcA-GalNAc4S6S)

Scheme 1.14. Suda Synthesss of CS tetrasaccharide structures containing 4,6-disulfate patterns

More recently, the Suda lab synthesized heterogeneous CS tetrasaccharides from combinations of trifluoroimidate donors **118**,**119** and trisaccharide acceptors **120 -122** (Scheme 1.14).[104] Unlike previous studies, *N*-trichloroethoxycarbonyl (Troc) protection, was reported for the galactosamine moiety and ensured β-selective glycosylation. The library of compounds

synthesized were immobilized onto gold-coated chips to prepare “array-type sugar chips”. The binding affinities of specific proteins were evaluated by surface plasmon resonance (SPR) imaging biosensor.

In general, there have been various chemical syntheses toward CS oligosaccharide standards. Although elegant, these strategies have not been employed for the preparation of a library of larger and more complex CS oligosaccharides. In chapters II, III, and IV we describe a modular approach that employs a set of properly protected disaccharide building blocks that resemble the different disaccharide motifs found in nature. These building blocks can repeatedly be used for the preparation of multiple targets and have the potential to provide a library of CS oligosaccharides for structure-activity relationship studies.

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Chapter II

INVESTIGATING THE REACTIVITY OF N-ACETYL GALACTOSAMINE ACCEPTORS AND GLUCURONIC ACID DONORS IN THE SYNTHESIS OF DISACCHARIDE BUILDING BLOCKS

2.1 ABSTRACT

The most common CS motifs involve sulfation at the C-4 or C-6 positions of the GalNAc. However, CS can also be sulfated at the C-2 or C-3 of the GlcA moiety. These polysaccharides have been implicated in a myriad of physiological as well as pathological processes. It has been difficult to elucidate the structures of the CS involved in specific biological activities, due to a lack of CS oligosaccharide standards. To address this deficiency, a modular approach was developed utilizing key disaccharide building blocks based on the most common chondroitin motifs found in nature.

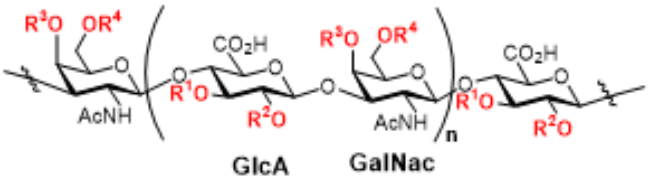
2.2 INTRODUCTION

As described in Chapter I, CS plays a role in a plethora of physiological events like cytokinesis, morphogenesis, central nervous system development, wound repair, growth factor signaling, and cell division.[1-3] However, CS has also been implicated in pathological conditions such as cancer, peripheral neuropathy, and glial scar formation after spinal cord injury. Due to a lack of CS oligosaccharide standards, it has been difficult to establish the structural requirements of the CS

structures responsible for these specific biological activities. Most efforts to chemically synthesize CS have focused on one-compound-at-the-time synthesis.[4] Small libraries of 4-*O* and 6-*O* sulfated di- tetra- and hexasaccharide standards have been prepared by regioselective sulfation or protection of the C4, 6-diol of GalNAc.[5-8] Although elegant, such a strategy cannot be employed for the preparation of a library of larger and more complex CS oligosaccharides. We report here a robust modular approach that employs a set of properly protected disaccharide building blocks that resemble the different disaccharide motifs found in nature (Figure 2.1). These building blocks can repeatedly be used for the preparation of multiple targets and have the potential to provide a library of CS oligosaccharides for structure-activity relationship studies.

2.3 RESULTS AND DISCUSSION

CS is composed of β (1→4)-linked disaccharide units having a GlcA β (1→3)-linked to GalNac [9] (Figure 2.1). Additionally, O-sulfation can occur at the C-4 and C-6 hydroxyl positions of the GalNAc, or at the C-2 hydroxyl position of the GlcA. In rare cases, sulfation may take place at the C-3 hydroxyl position of the GlcA. This allows for at least 16 possible combinations of sulfated disaccharide subunits. An amalgamation of these different combinations creates the potential for extreme structural diversity.



CSO: R^1=H , R^2=H, R^3=H, R^4=H
CSA: R^1=H , R^2=H, R^3=SO_3, R^4=H
CSC: R^1=H , R^2=H, R^3=H, R^4=SO_3
CSD: R^1=H , R^2=SO_3, R^3=H, R^4=SO_3
CSE: R^1=H , R^2=H, R^3=SO_3, R^4=SO_3
CSK: R^1=SO_3 , R^2=H, R^3=SO_3, R^4=H
CSL: R^1=SO_3 , R^2=H, R^3=H, R^4=SO_3
CSM: R^1=SO_3, R^2=H, R^3=SO_3, R^4=SO_3

Figure 2.1 Common CS sulfation motifs found in nature

This modular approach was developed for the parallel combinatorial synthesis of the most common chondroitin motifs: **CS-O** (GlcA-GalNAc), **CS-A** (GlcA-GalNAc (4S)), **CS-C** (GlcA-GalNAc (6S)), **CS-D** (GlcA (2S) –GalNAc (6S)), and **CS-E** (GlcA-GalNAc (4,6S)). The key to this approach is the synthesis of a somewhat small number of selectively protected disaccharide building blocks that can easily be converted into glycosyl donors and acceptors (Scheme 2.1). The set of protecting groups selected should meet the following requirements:

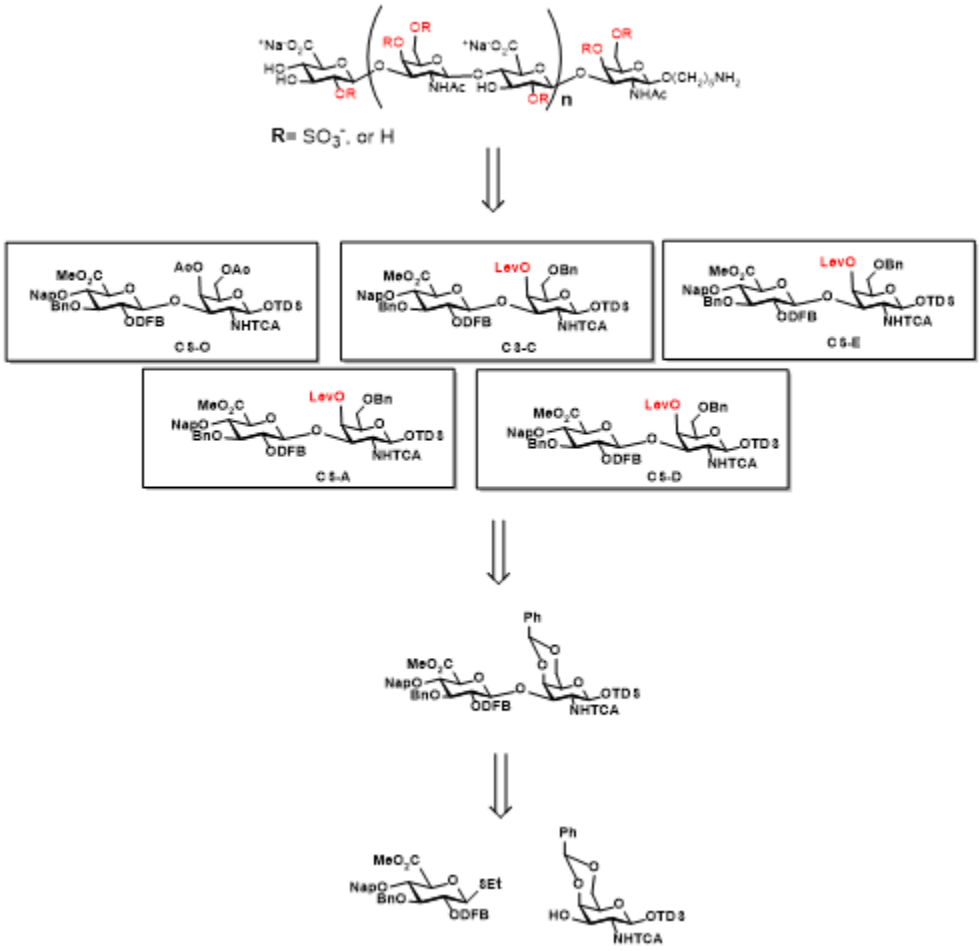
(i) The C-2 hydroxyl-protecting groups of the GlcA moieties should allow the stereoselective introduction of 1,2 trans-glycosidic linkages.

(ii) The C-2 amino group of the GalNac derivative needs to be derivatized in such a way that ensures 1,2-trans-glycosidic linkages during the elongation of the oligosaccharides.

(iii) Temporary protecting groups should be employed at the anomeric center of the GalNac and C-4 of the GlcA to prepare selectively glycosyl donors and acceptors.

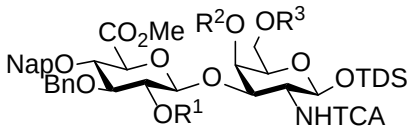
(iv) A protecting group is required that can be selectively removed to reveal hydroxyls for sulfation.

(v) The removal of the permanent protecting groups should be compatible with the presence of base- and acid-labile sulfate esters.



Scheme 2.1: Retrosynthetic Analysis

The protecting group strategy that we developed for the disaccharide modules is summarized in Figure 2.2. Since there is a (1→3) –β- linkage between the GlcA and the GalNac, the GlcA of the disaccharides must carry an ester protecting group at the C-2 position. Studies have shown that during glycosylations these groups ensure the formation of 1,2-trans-glycosides by neighboring group participation (Scheme 2.2).[10, 11] Previously, we have observed that an acetyl ester at C-2 led to the formation of ortho-esters during glycosylations. It is well known that ortho-ester formation can be circumvented by employing a benzoyl or pivaloyl protecting

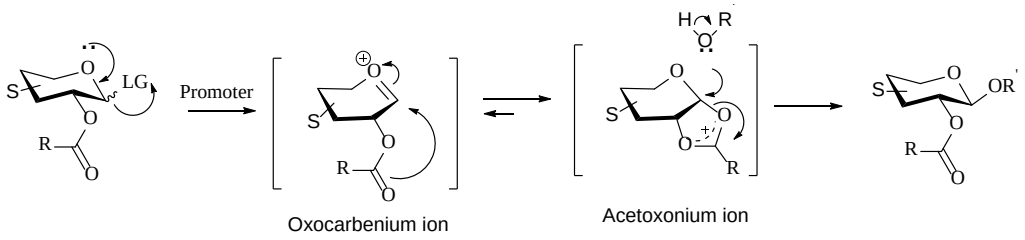


Sulfated	**Unsulfated**
R^1 = Lev	R^1 = Ac, Bn
R^2 = Lev	**R^2 =** Ac, Bn
R^3 = Lev	**R^3 =** DFB

Figure 2.2: Orthogonal protecting groups for modular disaccharide building blocks

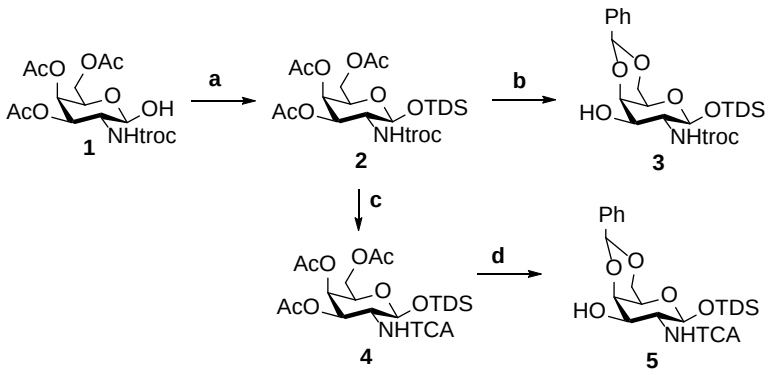
group.[12] However, for CS synthesis, these functionalities cannot be used as permanent protecting groups because their removal require strong basic conditions, and from previous experience in our lab led to the hydrolysis of O-sulfates. To address this problem, we employed 2, 5 difluorobenzoyl esters because this protecting group will perform neighboring group participation efficiently, but can also be cleaved easily by basic conditions.[13] Since CS is composed of repeating β (1→4)-linked disaccharide, C-2 amino group protection must also bear a neighboring group participation functionality. A couple of groups

were evaluate, howbeit trichloroacetimdo (TCA), an established neighboring group participating auxiliary,[14] was utilized in the synthesis of these CS disaccharide building blocks.



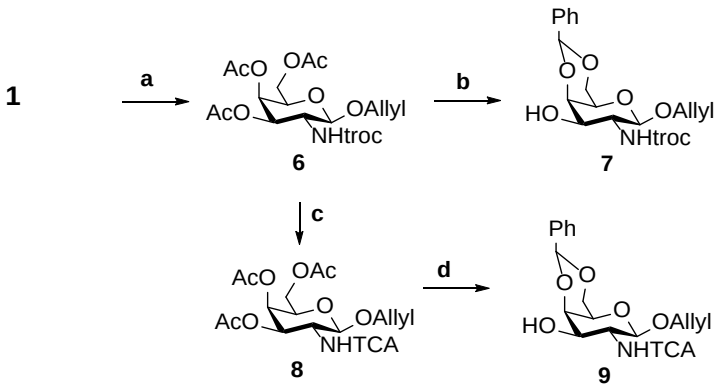
Scheme 2.2: Neighboring group participation by C-2-O-acyl functionality to form 1,2-trans-glycosides.

The anomeric position was protected with the orthogonal protecting group dimethyl thexylsilane (TDS), and this functionality can easily be removed by treatment with HF in pyridine without affecting the other protecting groups. Though 9-fluorenylmethyl carbamate (Fmoc) was examined, 2- naphthyl methyl ether (Nap) was employed for protection at the C-4. Nap is unique because it can be selectively removed by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).[15] On the other hand, it can be a permanent protecting group and be removed by hydrogenation, which is the last step of deprotection. Finally, levulinoyl esters (Lev) were used for those hydroxyls that needed sulfation. These Lev groups can be selectively cleaved with hydrazine buffered with acetic acid.[16]



Scheme 2.3: a)TDSCl, Imidazole, DMF, 71% b) i.Guanidine HCl/NaOme, 62%, ii.$PhCH(OMe)_2$, PTSOH, CH_3CN, 62% c) i. Zn, AcOH, ii. TCACl, NEt_3 ,DCM 78% d) i. 25% ammonia, MeOH, 92% ii. $PhCH(Ome)_2$,PtsOH,CH_3CN, 54%

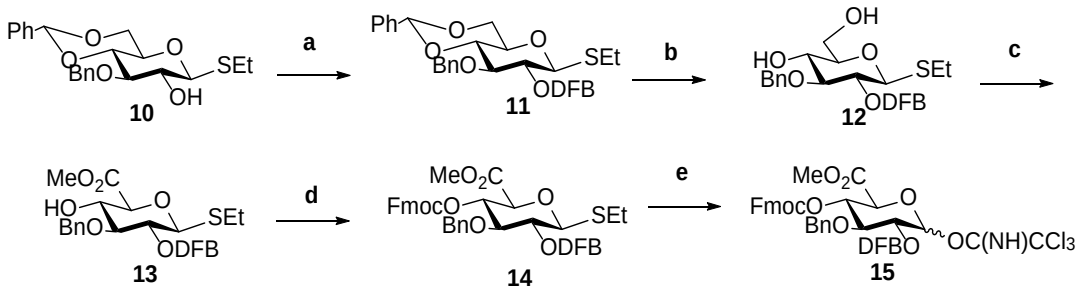
During this synthetic approach, we constructed several differently substituted GalNac monomeric building blocks as acceptors (Schemes 2.3 & 2.4). Herein, we evaluated acceptors with TDS and Allyl protection at the anomeric position, as well as Troc and TCA protection of the C-2 amine. Starting from the known hemiacetal **1** a TDS group was introduced with treatment with TDS-Cl and imidazole in DMF giving common intermediate **2** in a 71% yield. At this point the synthesis diverted into two different pathways rendering two different acceptor



Scheme 2.4: a) i.Cl_3CCN, DBU, DCM ii. AllyOH,TMSOTf 75% b) i.Guanidine HCl/NaOme,MeOH, 90%, ii.$PhCH(OMe)_2$,PTSOH, CH_3CN, 61% c) i. Zn, AcOH, ii. TCACl, NEt_3 ,DCM 78% d) i. 25% ammonia, MeOH, 92% ii. $PhCH(Ome)_2$,PtsOH,CH_3CN, 67%

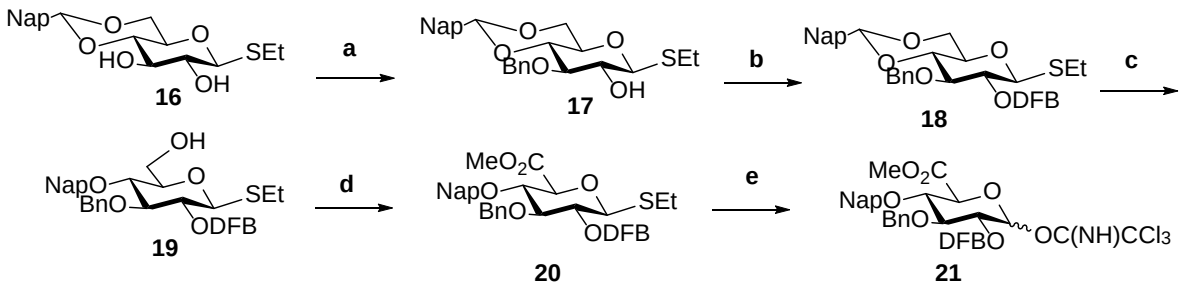
moieties. The initial pathway started with acetyl removal with a sodium methoxide solution buffered with guanidine to prevent removal of the base liable Troc.[17] This was followed by benzylidene protection with benzaldehyde dimethyl acetal in acetonitrile to get acceptor **3** in a 62% yield. On the other hand, the second pathway began with the transformation of Troc into TCA. Hence, the Troc present of compound **2** was reduced to the free amine by zinc in acetic acid.[18] This intermediate was then treated with trichloroacetyl chloride[19] in DCM to render **4** in a 78% yield. Since TCA is less base labile than Troc, acetyl removal was achieved with 25% ammonia in methanol, followed by benzylidene protection to give acceptor **5** at 54% yield. Additionally, two acceptors containing allyl anomeric protection were synthesized. Starting again with common intermediate **1,** a trichloroacetimidate was formed by treatment with Cl_3CCN and hindered base DBU in DCM. The resulting imidate was coupled with allyl alcohol giving **6** in a 75% yield. This synthesis was also diverted into two pathways. In the initial pathway, acetyl removal was followed by benzylidene protection which yielded acceptor **7** in

a 61% yield. In the alternative route, Troc was transformed into TCA at 78% yield and followed by acetyl removal. The C-4 and C-6 were protected by a benzylidene as previously described giving **9** in a 76% yield**.**



Scheme 2.5: a) DFBCl, DMAP, Pyr., 78% b) PtsOH, CH_3CH_2SH,DCM, 72% c) i. TEMPO, BAIB, ii.THF, CH_2N_2, 42%d) FmocCl, DMAP, Pyr, DCM, 91% e) i.NBS, H_2O, Acetone, ii. Cl_3CCN, NaH, DCM

Along with the four acceptors, there were also four differently protected GlcA monomeric building blocks evaluated as glycosyl donors (Scheme 2.5 & 2.6). Herein, we explored C-4 protection with either the carbamate Fmoc or Nap ether. Furthermore, we also observed the differences in glycosylation yields with thioglycosides versus trichloroimidates. Starting with DFB protection of the C-2 position of known alcohol **10** by DFB-Cl and a catalytic amount of DMAP in

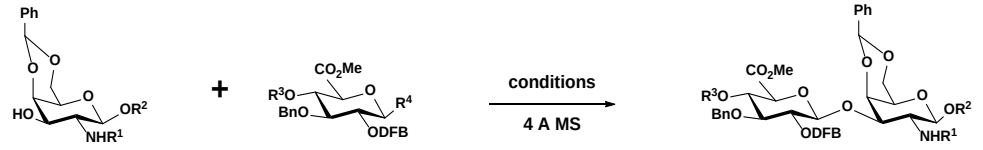


Scheme 2.6: a) i.(Bu2Sn)O, MeOH ii. BnBr, CsF, DMF, 55% b) DFBCl, DMAP, Pyr., 79% c)Cl_2PhB, Et_3SiH, DCM, -78^{O}C, 68% d) i. TEMPO, BAIB , ii.THF, CH_2N_2, 64% d) i.NBS,H_2O, Acetone, 64%, e) Cl_3CCN, NaH, DCM

pyridine rendering **11** at 78%. This was followed by benzylidene removal with treatment with PtsOH and ethanethiol in DCM. The acid functionality was introduced by utilizing the regioselective C-6 oxidation reaction on diol **12** with 2,2,6,6-tetramethyl-1-piperdinyloxy (TEMPO) and bis acetoxy iodo benzene (BAIB).[20] Subsequent methylation with freshly prepared diazomethane gave methyl ester **13** at 42% yield. Despite trying different conditions to circumvent the oxidation of the sulfur molecule present, sulfone and sulfoxide side products were observed. Next, the C-4 position was protected with Fmoc by treatment with Fmoc-Cl, pyridine, a catalytic amount of DMAP in DCM to give thioglycoside donor **14** in a 91% yield. The imidate donor was easily obtained by hydrolysis of the SEt group with NBS in a mixture of acetone and H_2O to give a lactol.[21] This intermediate was transformed into a trichloroacetimidate by treatment with trichloroacetonitrile and sodium hydride in DCM to yield donor **15**. The synthesis of donors possessing a Nap at C-4 started with Nap acetal protected **16**. This diol underwent selective benzylation of the C-3 hydroxyl of the resulting compound by treatment with dibutyl tin oxide followed by reaction with benzyl bromide in the presence of CsF in DMF to give **17** in 55% yield. This was followed by DFB protection which rendered **18** in

79% yield. This 4,6-naphthalidene acetal was reductively opened using dichlorophenylborane and Et_3SiH in DCM at -78 ^oC to give compound **19** having a C-6 hydoxyl and C-4 benzyl ether in 68% yield.[22] Tempo- Baib oxidation followed by methylation rendered thioglycoside **20** at 64 % yield. SEt hydrolysis followed by trichloroacetimidate formation as previously described gave donor **21.**

Table 2.1: Evaluated different acceptors and donors



3. R^1 = Troc, R^2= TDS
5. R^1 = TCA, R^2= TDS
7. R^1 = Troc, R^2= Allyl
9. R^1 = TCA, R^2= Allyl

14. R^3 = Fmoc, R^4=SEt
15. R^3= Fmoc, R^4= $OC(NH)CCl_3$
20. R^3= Nap, R^4=SEt
21. R^3 = Nap, R^4= $OC(NH)CCl_3$

22. R^1 = Troc, R^2= TDS, R^3 = Fmoc
23. R^1 = Troc, R^2= Allyl, R^3 = Fmoc
24. R^1 = TCA, R^2= Allyl, R^3 = Fmoc
25. R^1 = TCA, R^2= TDS, R^3 = Fmoc
26. R^1 = TCA, R^2= TDS, R^3 = Nap
27. R^1 = Troc, R^2= TDS, R^3 = Nap

Entry	Accepter	Donor	Conditions	Product	Yield%
1	**3**	**14**	TfOH, NIS, DCM,0^0C	**22**	11
2	**3**	**15**	TMSOtf, DCM, -35^oC	**22**	35
3	**7**	**15**	TMSOtf, DCM, -35^oC	**23**	36
4	**9**	**15**	TMSOtf, DCM, -35^oC	**24**	46
5	**5**	**15**	TMSOtf, DCM, -35^oC	**25**	29
6	**5**	**21**	TMSOtf, DCM, -35^oC	**26**	60
7	**5**	**20**	TfOH, NIS, DCM,0^0C	**26**	74
8	**3**	**20**	TfOH, NIS, DCM,0^0C	**27**	54

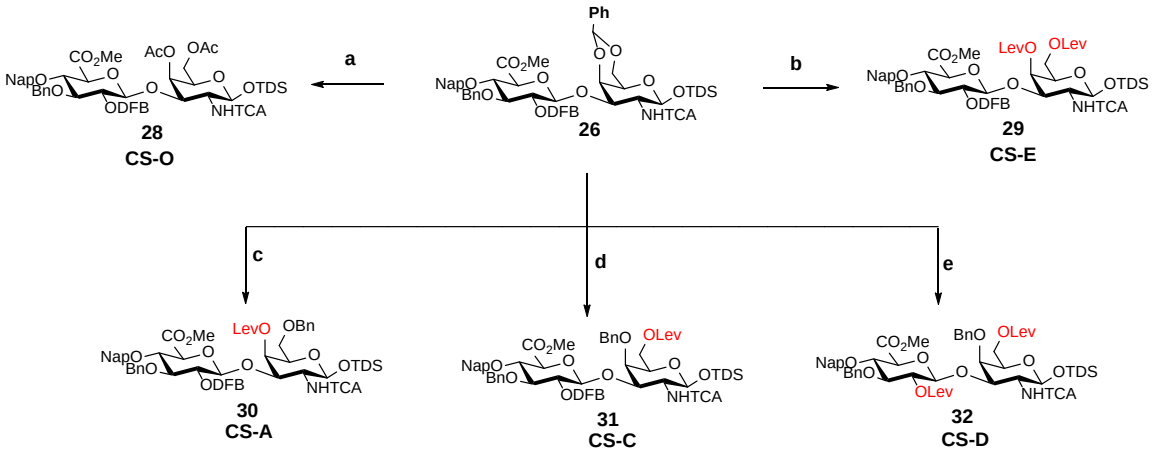
A common disaccharide intermediate that can easily be modified into the different CS motifs is crucial for this approach to work. However, finding the perfect combination of monosaccharide acceptors and donors proved to be an arduous task. Table 2.1 is just a glimpse of the glycosylations attempted, but these entries

summarize the observations that gave us the most insight on how to proceed further. The first glycosylation attempted was with acceptor **3** and thioglycoside donor **15.** Disaccharide **24** was recovered at a negligible 11% yield. In an attempt to increase this poor yield we decided to utilize trichloroimdate donor **16**. This increased the yield by threefold to 38%. Since this new donor appeared to increase the yield we focused our attention on optimizing the acceptor. During glycosylation we noticed a side product in which the TDS had been removed. So we investigated an acceptor with an Allyl at the anomeric center **7** using the same conditions. Coupling this new acceptor with **16** yielded disaccharide **25** at 36% yield. The next change made was transforming the Troc into a TCA. Previous works had proven this group as a powerful auxiliary. The glycosylation of acceptor **9** and donor **16** produced disaccharide **26** at 46% yield. Although this was an improvement, test trials removing the allyl group were not consistent, so we decided to synthesize accepter **5** bearing TDS anomeric protection with TCA amino protection**.** However, the glycosylation with imidate donor **16** yielded disaccharide **27** at a meager 29% yield. Now we turned our attention again toward modifying the donor. We postulated that the electron withdrawing carbamate functionality, Fmoc at the C-4 position, was affecting the reactivity of the molecule. Therefore, we replaced it with the electron donating Nap ether. The glycosylation between acceptor **5** and trichloroimidate donor **23** rendered disaccharide **28** at a 60% yield. This was a marked improvement over previous yields. Since the thioglycoside donor **22** was on hand, we evaluated it as well, and surprisingly this combination gave the best yield at 71%. Additionally, we evaluated the coupling of

donor **22** with the initial acceptor **3**, bearing NHTroc protection. Interestingly, the yield was also improved to 54%. Due to our observations, we concluded that the electron donating Nap ether at the C-4 position of the GlcA moiety improved the reactivity of the donor, playing the most significant role in the optimization of this glycosylation.

Since entry 7 gave the best results, disaccharide **26** was then modified into disaccharide precursors based on the common sulfate motifs found in nature (Scheme 2.7). Free hydroxyls O-4 and O-6 were revealed by treatment with TFA in a mixture of DCM and H_2O. In the case of the module for CS-O, the diol was acetylated with acetyl anhydride and a catalytic amount of DMAP in pyridine rendering the CS-O precursor compound **28** in 81% yield**.** However in the case oof the disulfated CS-E, this intermediate was levulinoylated by levulinyl anhydride and a catalytic amount of DMAP in pyridine yielding **29** at 72% yield. Since CS-A involves sulfation at the C-4 position of the GalNac, the benzylidene of **26** was opened reductively with TfOH and triethylsilane in DCM at -78^{o}C.[22] This procedure regioselectively gave a free hydroxyl at C-4. This alcohol was subsequently levulinoylated to give CS-A precursor **30** at an 83% yield. CS-C involves sulfation at the C-6 position of the GalNac. Therefore, disaccharide **26** was open reductively to selectively give a free hydroxyl at the C-6 of the GalNac. In this case dichlorophenyl borane and triethylsilane in DCM at -78^{o}C were the conditions used. This intermediate was treated with levulinoyl anhydride to render the CS-C precursor **31** at 69% yield. Finally, the same intermediate with a free hydroxyl at C-6 was treated with a catalytic amount of sodium methoxide to remove the ester

DFB. This diol was subsequently levulinoylated giving precursor CS-D **32** at a yield of 50%.



Scheme 2.7: a) i. TFA:DCM:H_2O i. Ac_2O, DMAP, Pyr 81% b) i. TFA:DCM:H_2O ii. Lev_2O, DMAP,72% c) i. TfOH, Et_3SiH, DCM,-78^{0}C ii. Lev_2O,DMAP,83% d) i. $PhBCl_2$, Et_3SiH, DCM, -78^{0}C ii. Lev_2O, DMAP,69% e) i. $PhBCl_2$, Et_3SiH, DCM, -78^{0}C ii. NaMOe, MeOH iii Lev_2O, DMAP,50%

2.4 CONCLUSION

Herein, we described an efficient route for the synthesis of disaccharide modules based on the common CS motifs found in nature. This was done by the coupling of strategically protected GlcA donor and GalNac acceptor monomeric building blocks. In Chapter III we describe how some of these disaccharide modules were easily modified and transformed into glycosyl donors and acceptors to build a robust library of homogenous as well as heterogeneous CS oligosaccharides.

2.5 EXPERIMENTAL SECTION

General procedures:

All moisture sensitive reactions were performed under an argon atmosphere by using vacuum dried glassware. All commercial materials were used without purification, unless otherwise noted. CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Toluene, DMF, diethylether, methanol and THF were purchased anhydrous and used without further purification. Molecular sieves (4Å) were flame activated *in vacuo* prior to use. All reactions were performed at room temperature unless specified otherwise. TLC-analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV-absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24} \cdot H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150°C. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 mm, 60Å) or on Bondapak C-18 (Waters). 1H and 13C NMR spectra were recorded on a Varian inova-300 (300/75 MHz), a Varian inova-500 (500/125 MHz) and a Varian inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d =doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances)coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basis of 1HNMR, 13C NMR, COSY and HSQC experiments.

Optical rotations were measured using a Jasco P-1020 polarimeter. Mass spectra were recorded on an Applied Biosystems 4700 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxybenzoicacid (DHB) and ultramark 1621 as the internal standard. The ESI-MS spectra were recorded on 9.4 T Bruker Apex Ultra QeFTMS (Billerica, MA) mass spectrometer.

Dimethyl thexylsilane 3,4,6-tri-*O*-acetyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino-*β*-D-galactopyranoside (2)

Dimethyl thexylsilane chloride (11.60ml,71.6mmol) and imidazole (6.60g, 97.50mmol) were added to a solution of 3,4,6-triacetate **1** (18.00 g, 37.50 mmol) in anhydrous DMF (90ml). The solution was stirred at rt overnight. Upon completion, the reaction was diluted with EtOAc and the solvents were washed with H_2O, saturated sodium bicarbonate and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 4:1→3:1) afforded **2** (16.52g, 26.55mmol, 71%).^{1}H NMR (300 MHz, $CDCl_3$): δ 5.21 (dd, J = 3.5, 1.3 Hz, 1H, H-4), 5.02 (d, J = 10.7 Hz, 1H, H-3), 4.93 (d, J = 8.6 Hz, 1H, NH), 4.65 – 4.42 (m, 3H, H-1,(Cl_3CCH_2OC=O)), 4.06 – 3.90 (m, 2H, H-6a, H-6b), 3.79 – 3.70 (m, 1H, H-5), 3.70 – 3.57 (m, 1H, H-2), 2.02 (d, *J* = 2.8 Hz, 3H, $_3$HC), 1.90 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 1.53 – 1.42 (m, 1H), 0.78 – 0.65 (m, 12H, ($CCH(CH_3)_4$), 0.03 (s, 3H, $SiCH_3$), -0.00 (d, *J* = 2.8 Hz, 3H, $SiCH_3$). ^{13}C NMR (300 MHz, $CDCl_3$) δ 96.17, 74.84, 74.64, 70.65, 69.53, 66.75, 61.67, 54.88, 33.83, 20.79, 20.72, 20.70, 20.04, 18.82. HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{38}Cl_3NO_{10}Si$ $[M+Na]^+$: 644.1228 ; found: 644.6901

Dimethyl thexylsilane 2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-4,6-*O*-benzylidene-β-D-galactopyranoside (3)

Compound **2** (2.00g, 3.21mmol) was added to a Guanidine/Guanidinium Chloride NaOMe solution (90ml) , and was stirred at rt for 30min. Upon completion the reaction was quenched by Dowex 50X8-200, which was added and allowed to stir an additional 30 min. The mixture was filtered and the solvent removed *in vacuo*. The triol intermediate was suitable for the next step without purification**.** Camphor sulfonic acid (.362g, 15.60mmol) was added to a solution of benzaldehyde dimethyl acetal (3.05, 20.51 mmol) and the triol (7.76g, 15.60mmol) in anhydrous CH_3CN (40ml). The solution was stirred at room temperature overnight. The reaction was then diluted with EtOAc and washed with H_2O, saturated sodium bicarbonate, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (hexane/EtOAc 2:1) yielded **3** (5.93g, 10.14mmol, 62%) ^1H NMR (300 MHz, $CDCl_3$): δ 7.41 – 7.15 (m, 5H, CH Aromatic), 5.40 (s, 1H, PhCH), 4.88 (s, 1H, NH), 4.63 (d, *J* = 7.9 Hz, 1H, H-1, β), 4.52 (s, 2H,(Cl_3CCH_2)), 4.11 (dd, *J* = 12.4, 1.5 Hz, 1H, H-6a), 4.03 (d, *J* = 3.5 Hz, 1H, H-4) , 3.99 – 3.94 (m, 1H, H-6b), 3.94 – 3.87 (m, 1H, H-3), 3.82-3.70 (m, 1H, H-3), 3.47-3.39 (m, 1H, H-2), 3.31 (dd, *J* = 3.3, 1.7 Hz, 1H, H-5), 2.56 (s, 1H, OH), 1.57 – 1.41 (m, 1H, CH), 0.76 – 0.64 (m, 12H,($CCH(CH_3)_4$), 0.05 (s, 2H,$SiCH_3$), 0.00 (s, 2H, $SiCH_3$).^{13}C NMR (75 MHz, $cdcl_3$) δ 128.80, 126.62, 101.60, 95.75, 75.30, 74.98, 70.79, 70.61, 70.08, 69.61, 69.42, 66.68, 33.93, 20.37, 18.81. HR

MALDI-TOF MS: m/z: calcd for $C_{24}H_{36}Cl_3NO_7Si$ $[M+Na]^+$: 606.1224; found: 606.2659

Dimethyl thexylsilane 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-trichloracetamido-*β*-D-galactopyranoside (4)

Zinc dust (71.29 g, 1090.00mmol) was added to a solution of **1** (24.66 g, 38.90 mmol) in AcOH (294 ml) and allowed to stir for 2hr. Upon completion the amine was filtered over celite and then co-evaporated with toluene and dried under vacuum overnight. The crude amine was dispersed in DCM (200 ml) and cooled to 0 ^{0}C. Trichloroacetyl chloride (21.83 ml, 120.10 mmol) and TEA (32.57 ml, 320.00mmol) were added and the reaction was complete in 30min. It was then diluted with DCM and was washed with saturated sodium bicarbonate and water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 2:1) yielded Allyl 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-trichloracetamido-*β*-D-galactopyranoside **4** (17.93g, 30.34 mmol, 78%)^{1}H NMR (300 MHz, $cdcl_3$) δ 6.60 (d, *J* = 8.9 Hz, 1H, NH), 5.38 (d, *J* = 2.9 Hz, 1H, H-4), 5.26 (dd, *J* = 11.3, 3.4 Hz, 1H, H-3), 4.90 (d, *J* = 7.9 Hz, 1H, H-1β), 4.22 – 4.00 (m, 3H, H-6a, H-6b, H-2), 3.93 (t, *J* = 6.8 Hz, 1H, H-5), 2.18 (s, 3H, CH3CO), 2.05 (s, 3H, CH3CO), 1.99 (s, 3H, CH3CO), 1.62 (m,1H, CH), 0.87-0.84 (m, 12H, $(CCH(CH_3)_4$), 0.23 – 0.09 (m, 6H, $Si(CH_3)_2$).^{13}C NMR (75 MHz, $cdcl_3$) δ, 95.77, 95.72, 71.04, 69.56, 69.38, 66.91, 61.77, 55.24, 34.47, 21.10, 21.02, 20.87, 20.15, 19.01, -1.35,

-2.74. . HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{36}Cl_3NO_9Si$ $[M+Na]^+$: 614.1123; found: 614.1096

Dimethyl thexylsilane 2-deoxy-2-trichloroacetamido-4, 6-*O*-benzylidene-β-D-galactopyranoside (5)

The acetylated compound **4** (2.11 g, 5.8 mmol) was taken up in anhydrous MeOH (21.4 mL) and 28% aqueous ammonia (2.14 mL). The mixture was stirred overnight and concentrated *in vacuo*. After a quick column this triol intermediate was taken directly to the next step. Camphor sulfonic acid (.249g, 1.07 mmol) was added to a solution of benzaldehyde dimethyl acetal (1.61ml, 10.70 mmol) and the triol (2.50 g, 5.37 mmol) in anhydrous CH_3CN (20ml). The solution was stirred at room temperature overnight. Upon completion, the reaction was diluted with EtOAc and washed with H_2O, saturated sodium bicarbonate, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 1:1) yielded **5** (1.61g, mmol, 54%). ^{1}H NMR (300 MHz, cdcl$_3$) δ 7.65 – 7.29 (m, 5H, CH Aromatic), 6.79 (d, *J* = 7.6 Hz, 1H, NH), 5.58 (s, 1H, PhC*H*), 4.99 (d, *J* = 7.9 Hz, 1H, H-1 β) , 4.32 – 4.25 (m, 1H, H-6a), 4.23 (d, *J* = 3.7 Hz, 1H, H-4), 4.14 – 4.02 (m, 2H, H-6b, H-3), 3.88 – 3.75 (m, 1H, H-2), 3.51(s, 1H, H-5) 2.73 (d, *J* = 10.4 Hz, 1H, OH), 1.72 – 1.58 (m, 1H, CH), 0.90 – 0.81 (m,12H,(CCH(CH_3)$_4$), 0.25 – 0.13 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, cdcl$_3$) δ 129.01, 126.63, 101.62, 95.34, 95.32, 75.19, 69.54, 69.43, 69.38, 69.38, 66.87, 58.97, 34.17, 20.14, 18.90, -1.31, -2.67. . HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{34}Cl_3NO_6Si$ $[M+Na]^+$: 576.1119; found: 576.7151

Allyl 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-[[(2, 2, 2-trichloroethoxy) carbonyl] amino]-*β*-D-glucopyranoside (6)

Trichloroacetonitrile (2.08ml, 10.00mmol) and DBU (.119ml, 0.76mmol) were added to a solution of **1** (2.00g, 4.17 mmol) in anhydrous DCM (20ml). After the solution was stirred at room temperature for 2hr, the solvent was concentrated *in vacuo* and the residue was chromatographed on silica gel (hexane/EtOAc 2:1). Then TMSOTf (.052 ml, .190mmol) was added to a solution of the above imidate (1.87g, 2.99mmol) along with allyl alcohol (1.02 ml, 20.51mmol) in anhydrous DCM (30ml). The reaction was stirred at -20^{0}C for 1hr, and upon completion the reaction was quenched with TEA and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 2:1) provided glycoside **6** (1.14 g, 75%) ^{1}H NMR (300 MHz, cdcl$_3$) δ 5.88 (ddd, *J* = 22.0, 10.9, 5.7 Hz, 1H, CH=CH$_2$), 5.38 (d, *J* = 2.8 Hz, 1H, H-4) 5.34 – 5.17 (m, 3H, CH=CH$_2$, H-3), 5.01 (s, 1H, NH), 4.80 – 4.61 (m, 3H, (Cl$_3$CCH$_2$) H-1), 4.38 (dd, *J* = 12.9, 5.0 Hz, 1H, CH$_{2a}$CH), 4.24 – 4.06 (m, 3H, H-6a, H-6b, CH$_{2b}$CH), 3.90 (t, *J* = 6.6 Hz, 1H, H-5), 3.86 – 3.76 (m, 1H, H-2), 2.15 (s, 3H, CH$_3$), 2.06 (s, 3H, CH$_3$), 2.00 (s, 3H,CH$_3$).^{13}C NMR (75 MHz, cdcl$_3$) δ 118.79, 117.67, 100.00, 74.72, 70.70, 70.66, 70.57, 69.94, 66.68, 61.75, 53.37, 20.74, 20.66, 20.72. HR MALDI-TOF MS: m/z: calcd for C$_{18}$H$_{24}$Cl$_3$NO$_{10}$ [M+Na] $^+$: 542.0363; found: 542.0929

Allyl 2-deoxy-2-[[(2, 2, 2-trichloroethoxy) carbonyl] amino]-4, 6-*O*-benzylidene-β-D-galactopyranoside (7) A solution of **6** (5.00g, 12.69mmol) in a Guanidine/Guanidinium Chloride NaOMe solution (50ml) was stirred at ambient temp for 30min. To quench the reaction Dowex 50X8-200 was added and allowed

to stir an additional 30 min. The mixture was filtered and the solvent removed *in vacuo* to afford the triol. This compound was suitable for the next step without purification. Camphor sulfonic acid (.292g, 1.26 mmol) was added to a solution of benzaldehyde dimethyl acetal (2.47ml, 16.02 mmol) and the triol (5.00g, 12.69 mmol) in anhydrous CH_3CN (40ml). The solution was stirred at room temperature overnight. Upon completion, the reaction was diluted with EtOAc and washed with water, saturated sodium bicarbonate, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (hexane/EtOAc 2:1) yielded **7** (3.76g, 7.81mmol 61%) ^{1}H NMR (300 MHz, $cdcl_3$) δ 7.66 – 7.29 (m, 5H, CH Aromatic), 6.01 – 5.80 (m, 1H, C*H*=CH_2), 5.58 (s, 1H, PhC*H*), 5.39 – 5.08 (m, 2H, CH=C*H*$_2$), 4.78-4.62 (m, 3H,(Cl_3CCH_2) , H-1), 4.47 – 4.31 (m, 2H, H-6a,), 4.29 – 4.19 (m, 1H, H-4), 4.16 – 3.95 (m, 3H, H-6b,H-3), 3.69 (s, 1H, H-2), 3.49 (dd, *J* = 3.1, 1.7 Hz, 1H, H-5), 2.76 (s, 1H, OH). ^{13}C NMR (75 MHz, $cdcl_3$) δ 133.78, 128.61, 126.43, 118.31, 117.90, 117.87, 101.53, 99.48, 75.34, 74.80, 70.59, 69.72, 69.68, 69.59, 69.48, 67.03, 56.10. HR MALDI-TOF MS: m/z: calcd for $C_{19}H_{22}Cl_3NO_7$ $[M+Na]^+$: 504.0360 ; found: 504.5380

Allyl 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-trichloracetamido-*β*-D-galactopyranoside (8)

Zinc dust (1.66 g, 22.2 mmol) was added to a solution of **6** (4.157 g, 8.01 mmol) in AcOH (60.67 ml) and was allowed to stir for 2hr. Upon completion the amine was filtered over celite and then co-evaporated with toluene and dried under vacuum overnight. The crude amine was dispersed in 40ml DCM and cooled to 0 ^{0}C. Trichloroacetyl chloride (5.66 ml, 50.5) and TEA (9.39ml, 67.40) were added.

According to the TLC, the reaction was completed in 30min. It was then diluted with DCM, saturated sodium bicarbonate and water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 2:1) yielded **8** (3.08g 6.28mmol, 78%)^{1}H NMR (300 MHz, DMSO) δ 8.97 (d, *J* = 9.1 Hz, 1H, NH), 5.82 (ddt, *J* = 17.1, 10.4, 5.2 Hz, 1H, $CH=CH_2$), 5.30 – 5.16 (m, 3H, H-4, H-3,Ha, $CH=CH_2$), 5.11 (ddd, *J* = 10.4, 3.3, 1.5 Hz, 1H, Hb, $CH=CH_2$), 4.70 (d, *J* = 8.4 Hz, 1H, H-1, β), 4.26 – 4.16 (m, 1H, $CH_{2a}CH$), 4.13 – 3.87 (m, 6H, $CH_{2b}CH$, H-6a, H-6b, H-2, H-5), 2.11 (s,3H, CH_3), 1.98 (s, 3H, CH_3), 1.87 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 99.87, 70.59, 70.52, 70.41, 69.73, 69.61, 66.97, 62.00, 56.27, 52.36, 21.14, 21.06, 20.94. HR MALDI-TOF MS: m/z: calcd for $C_{17}H_{22}Cl_3NO_9$ $[M+Na]^+$: 512.0258; found: 512.1335

Allyl 2-deoxy-2-trichloroacetamido-4, 6-*O*-benzylidene-β-D galactopyranoside (9)

A solution of **8** (2.11 g, 5.8 mmol) in anhydrous MeOH (21.4 mL) and 28% aqueous ammonia (2.14 mL) was stirred overnight and concentrated *in vacuo*. This compound was taken directly to the next step. Camphor sulfonic acid (.090g, .39 mmol) was added to a solution of benzalaldehyde dimethyl acetal (.837ml, 7.90 mmol) and the triol (1.44 g, 3.90 mmol) in anhydrous CH_3CN (40ml). The solution was stirred at room temperature overnight. Upon completion, the reaction was diluted with EtOAc and washed with H_2O, saturated sodium bicarbonate, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 1:1) yielded **9** (1.18g, 2.61 mmol, 67%) ^{1}H NMR (300 MHz, $cdcl_3$) δ 7.58 – 7.30 (m, 5H, CH Aromatic), 6.86 (d, *J* = 6.8 Hz,

1H, NH), 5.89 (ddd, *J* = 16.7, 11.0, 6.2 Hz, 1H, $CH=CH_2$), 5.60 (s, 1H, PhCH), 5.29 (dd, *J* = 17.2, 1.6 Hz, 1H, $CH=CH_{2a}$), 5.20 (dd, *J* = 10.3, 1.5 Hz, 1H, $CH=CH_{2b}$), 4.86 (d, *J* = 8.3 Hz, 1H, H-1,β), 4.46 – 4.30 (m, 2H, H-6a, $CH_{2a}CH$), 4.28-4.18 (m, 2H, H-4, H-3), 4.16 – 3.99 (m, 2H, , H-6b, $CH_{2b}CH$), 3.88-3.76 (m, 1H, H-2), 3.54 (s, 1H, H-5), 2.76 (d, *J* = 10.3 Hz, 1H, OH). ^{13}C NMR (75 MHz, $cdcl_3$) δ 128.82, 126.61, 118.31, 118.20, 118.18, 118.07, 101.49, 98.61, 98.61, 75.13, 69.49, 69.48, 69.37, 69.32, 69.23, 66.83, 57.04. HR MALDI-TOF MS: m/z: calcd for $C_{18}H_{20}Cl_3NO_6$ $[M+Na]^+$: 474.0254; found: 474.5074

Ethyl 4,6-*O*-(2-benzylidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (11)

2,5-difluorobenzoyl chloride (0.593 mL, 4.78 mmol) and DMAP (.039g, 0.318 mmol) were added to a stirred solution of **10** (1.28 g, 3.18 mmol) in DCM (15 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in toluene) to afford compound **11** (1.35 g, 78%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 – 7.07 (m, 13H, CH Aromatic), 5.77 (s, 1H, PhC*H*), 5.33 (dd, *J* = 10.1, 8.3 Hz, 1H, H-2), 4.89 (d, *J* = 11.9 Hz, 1H, PhC*H*H), 4.70 (d, *J* = 11.9 Hz, 1H, PhCH*H*), 4.63 (d, *J* = 10.1 Hz, 1H, H-1), 4.46 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.98 – 3.85 (m, 3H, H-6b, H-4, H-3), 3.69 – 3.52 (m, 1H, H-5), 2.78 (m, 2H, SCH_2CH_3), 1.25 (t, *J* = 7.5 Hz, 3H, SCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.3, 128.2, 126.7, 125.7, 123.9, 121.7, 118.7, 118.6, 101.7,

84.3, 81.9, 79.7, 74.6, 74.6, 72.7, 71, 68.9, 24.2, 15.0; HR MALDI-TOF MS: m/z: calcd for $C_{29}H_{28}F_2O_6S$ $[M+Na]^+$: 565.1575; found 565.1459.

Ethyl-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (12)

.340g (.369 mmol) of p-Toluenesulfonic acid and 4.16 ml (53.69 mmol) of ethanethiol were added to a stirred solution of 4.85 (8.95 mmol) **11**. After 2h TLC indicated complete consumption of the starting material (~2 h). The reaction was quenched with TEA, concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (50% EtOAc in hexane) to yield **12** (2.82 g, 72%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.77 – 6.89 (m, 8H, CH Aromatic), 5.39 – 5.12 (m, 1H, H-2), 4.84 – 4.62 (m, 2H, $PhCH_2$), 4.57 (d, *J* = 10.0 Hz, 1H, H-1), 4.04 – 3.62 (m, 5H, H-6ab, H-4, H-5), 3.46 (ddd, *J* = 8.9, 5.0, 3.4 Hz, 1H, H-3), 2.71 (qd, *J* = 7.4, 2.3 Hz, 2H, SCH_2CH_3), 2.50 – 2.36 (m, 1H, OH), 2.05 (t, *J* = 6.6 Hz, 1H, OH), 1.24 (t, *J* = 7.5 Hz, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $cdcl_3$) δ118.49, 128.14, 118.43, 72.68, 74.84, 74.86, 83.64, 62.63, 62.64, 70.52, 83.87, 79.45, 24.12,14.76 HR MALDI-TOF MS: m/z: calcd for $C_{22}H_{24}F_2O_6S$ $[M+Na]^+$: 477.1262; found 477.4984.

Ethyl-3-O-benzyl-2-O-difluorobenzoyl-1-thio-β-D--glucopyranosyluronate (13) Tempo (78 mg, .50 mmol) and Baib (1.771 g, 2.50 mmol) were added to a vigorously stirred mixture of **12** (1.105 g, 2.50 mmol) in 10 ml of DCM and 5ml H_2O. Stirring was continued for ~45 min when the TLC indicated complete conversion of the starting material to a spot of lower Rf. The reaction mixture was quenched by the addition of 10 % aqueous $Na_2S_2O_3$. The mixture was extracted with EtOAc, and the combined aqueous layers were back-extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and filtered, and the filtrate was

concentrated *in vacuo*. The oily residue was dissolved in EtOAc and treated with an excess of freshly prepared ethereal solution of diazomethane until the reaction mixture remained yellow. The excess diazomethane was quenched by the addition of AcOH until the reaction mixture became colorless. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (20% EtOAc in hexane) to yield **13** (520 mg, 42%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.63 – 6.88 (m, 8H, CH Aromatic), 5.25 (ddd, *J* = 9.9, 9.1, 0.7 Hz, 1H, H-2), 4.92 – 4.65 (m, 2H, $PhCH_2$), 4.57 (d, *J* = 10.1 Hz, 1H, H-1), 4.13 – 3.89 (m, 2H, H-4, H-5), 3.83 (s, 3H, OCH_3), 3.78 – 3.64 (m, 1H, H-3), 2.84 – 2.60 (m, 2H, SCH_2CH_3), 1.24 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $cdcl_3$) δ (.520g, 42%) 118.43, 121.55, 125.79, 128.04, 118.41, 71.77, 74.86, 74.84, 84.15, 72.09, 77.85, 52.84, 50.60, 82.32, 24.13,14.69; HR MALDI-TOF MS: m/z: calcd for $C_{23}H_{24}F2O_7S$ $[M+Na]^+$: 505.1211; found 505.1336.

Ethyl-4-*O*-(2-methyl-fluorenylmethoxycarbonyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D--glucopyranosyluronate (14)

To a solution of compound **13** (1.89 g, 3.92 mmol) in DCM at 0 ^{o}C was added Fmoc-Cl (1.49 g, 5.87 mmol), Pyridine (1.58 ml, 19.58) and DMAP (.049g, .392 mmol). The reaction mixture was brought to room temperature, and allowed to stir overnight. TLC indicated complete consumption of the starting material. After quenching the reaction with methanol the mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate and brine (50 mL). The organic phase was dried over MgSO4, filtered, and concentrated *in vacuo*. The

residue was chromatographed over silica gel using a gradient of hexanes and EtOAc to give compound **14** (2.14 g, 78%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.83 – 7.02 (m, 20H, CH Aromatic), 5.34 (dd, *J* = 18.0, 8.5 Hz, 1H, H-2), 5.14 (t, *J* = 9.6 Hz, 1H, H-4), 4.79 – 4.55 (m, 3H, FmocCH_2, H-1), 4.52 – 4.30 (m, 3H, PhCH_2), 4.24 (t, *J* = 7.1 Hz, 1H, H-5), 4.11 (d, *J* = 10.0 Hz, 1H, H-3), 3.94 (t, *J* = 9.1 Hz, 1H), 3.71 (s, 3H,OCH_3), 2.89 – 2.60 (m, 2H, SC*H*$_2$$CH_3$), 1.25 (t, *J* = 7.4 Hz, 3H, SCH_2C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) 127.93, 127.91, 127.24, 125.69, 125.68, 125.1, 124.96, 121.57, 120.08, 118.59, 118.42, 83.69, 80.36, 76.33, 76.32, 75.14, 74.71, 74.69, 71.68, 70.43, 70.38, 52.84, 50.64, 46.59, 29.65, 23.94,14.63. $C_{38}H_{34}F_2O_9S$ HR MALDI-TOF MS: $[M+Na]^+$: 727.1892; found 727.1274.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-1-thio-β-D-glucopyranoside (17)

A solution of ethyl 4, 6-*O*-(2-napthalidene)-1-thio-β-D-glucopyranoside **16** (8 g, 22 mmol) and dibutyltin oxide (6.5g, 26.5 mmol) in methanol (80 mL) was refluxed for 4 h to produce a clear mixture. The solvent was then evaporated and the resultant residue was dissolved in DMF (80 mL) followed by the addition of BnBr (4 mL, 33 mmol) and CsF (4g, 26.5 mmol). The mixture was stirred at room temperature overnight, concentrated and the residue was purified by flash chromatography over silica gel (40 % EtOAc in toluene) to afford compound **17** (5.49 g, 55%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.26 (m, 12H, CH Aromatic), 5.73 (s,1H, NapC*H*), 4.99 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.84 (d, *J* = 11.7 Hz, 1H, PhCH*H*), 4.48 (d, *J* = 9.6 Hz, 1H, H-1), 4.41 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.89 – 3.67 (m, 3H, H-6b, H-3, H-4), 3.66 – 3.50 (m, 2H, H-5, H-2), 2.76 (q, *J* = 7.4 Hz, 2H, SC*H*$_2$$CH_3$), 2.54 (d, *J*

= 2.0 Hz, 1H, O*H*), 1.32 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.4, 128.3, 128.2, 126.6, 125.7, 123.9, 101.8, 87.0, 81.6, 75, 75.0, 73.3, 71.1, 69, 24.9, 15.3; HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{28}O_5S$ $[M+Na]^+$: 475.1555; found 475.1537.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (18)

2,5-difluorobenzoyl chloride (0.82 mL, 6.63 mmol) and DMAP (108 mg, 0.88 mmol) were added to a stirred solution of **17** (2 g, 4.42 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in toluene) to afford compound **18** (2.06 g, 79%); ^1H NMR (300 MHz, $CDCl_3$) δ 7.98 – 7.07 (m, 15H, CH Aromatic), 5.77 (s, 1H, NapC*H*), 5.33 (dd, *J* = 10.1, 8.3 Hz, 1H, H-2), 4.89 (d, *J* = 11.9 Hz, 1H, PhC*H*H), 4.70 (d, *J* = 11.9 Hz, 1H, PhCH*H*), 4.63 (d, *J* = 10.1 Hz, 1H, H-1), 4.46 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.99 – 3.83 (m, 3H, H-6b, H-4, H-3), 3.68 – 3.55 (m, 1H, H-5), 2.74 (m, 2H, SCH_2CH_3), 1.25 (t, *J* = 7.5 Hz, 3H, SCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.3, 128.2, 126.7, 125.7, 123.9, 121.7, 118.7, 118.6, 101.7, 84.3, 81.9, 79.7, 74.6, 74.6, 72.7, 71, 68.9, 24.2, 15.0; HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{30}F_2O_6S$ $[M+Na]^+$: 615.1629; found 615.1699.

Ethyl 4-*O*-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (19)

Compound **18** (287 mg, 0.48 mmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (0.2 mL, 1.44 mmol) and dichlorophenylborane (0.2 mL, 1.63 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and TEA (0.5 mL). The resulting mixture was diluted with DCM and washed with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **19** (195 mg, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 6.91 (m, 15H, CH Aromatic, 5.19 (t, *J* = 9.5 Hz, 1H, H-2), 4.94 (d, *J* = 11.1 Hz, 1H, NapC*H*H), 4.79 (d, *J* = 3.4 Hz, 1H, PhC*H*H), 4.75 (d, *J* = 3.6 Hz, 1H, PhC*H*H), 4.64 (d, *J* = 11.3 Hz, 1H, NapC*H*H), 4.49 (d, *J* = 10.0 Hz, 1H, H-1), 3.94 – 3.75 (m, 2H, H-3, H-6a), 3.75 – 3.64 (m, 2H, H-4, H-6b), 3.44 (ddd, *J* = 9.7, 4.7, 2.6 Hz, 1H, H-5), 2.72 – 2.55 (m, 2H, SC*H*$_2$CH$_3$), 1.17 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 128.1, 128, 127, 126.2, 126, 125.8, 121.5, 118.5, 118.4, 84.4, 83.7, 79.9, 77.8, 75.5, 73.1, 62.2, 24.5, 15.3; HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{32}F_2O_6S$ $[M+Na]^+$: 617.1785; found 617.1741.

Ethyl-4-*O*-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D--glucopyranosyluronate (20)

Tempo (.099g, .636 mmol) and Baib (2.25g, 6.9 mmol) were added to a vigorously stirred mixture of **19** (1.887 g, 3.18 mmol) in DCM (10ml) and H_2O (5ml). Stirring was continued for ~45 min when the TLC indicated complete conversion of the

starting material to a spot of lower Rf. The reaction mixture was quenched by the addition of aqueous 10% $Na_2S_2O_3$ (10 mL). The mixture was extracted with EtOAc (2 × 10 mL), and the combined aqueous layers were back-extracted with EtOAc (10 mL). The combined organic layers were dried over MgSO4 and filtered, and the filtrate was concentrated in vacuo. The oily residue was dissolved in THF (0.1 M) and treated with an excess of freshly prepared ethereal solution of diazomethane until the reaction mixture remained yellow. The excess diazomethane was quenched by the addition of AcOH until the reaction mixture became colorless. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (20% EtOAc in hexane) to yield (1.257 g, 64%) methyl ester **20**. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.89 – 6.97 (m, 15H, CH Aromatic), 5.31 (t, *J* = 9.5 Hz, 1H, H-2), 4.94 (d, *J* = 11.1 Hz, 1H, NapC*H*H), 4.85 – 4.77 (m, 2H, PhC*H*H, NapC*H*H), 4.70 (d, *J* = 11.4 Hz, 1H, PhC*H*H), 4.56 (d, *J* = 10.0 Hz, 1H, H-1'), 4.02 (dd, *J* = 4.5, 1.0 Hz, 2H, H-4, H-5), 3.87 (dt, *J* = 8.9, 4.4 Hz, 1H, H-3), 3.70 (s, 3H, OCH_3), 2.72 (m, 2H, SCH_2CH_3), 1.23 (t, *J* = 7.5 Hz, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) 14.9, 24.12, 52.78, 72.34, 75.39, 75.45, 75.5, 79.01, 83.59, 84.24, 118.54, 118.61, 118.91, 121.8, 125.98, 126.16, 126.34, 127.1, 128.11, 128.19; HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{32}F_2O_7S$ $[M+Na]^+$: 645.1837; found 645.1926

Dimethyl thexylsilane (methyl-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-O-(2-methylnaphthyl) -β-D-glucopyranosyluronate)-(1→3)-4,6-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (26)

Acceptor **5** (2.58g, 4.65 mmol) and thioglycoside donor **21** (3.47g, 5.59 mmol) were combined in a flask, and coevaporated with toluene (3 × 3 mL). Powdered freshly activated 4 Å molecular sieves were added, and the mixture was stirred for 30 min at ambient temperature and then cooled to 0 °C. NIS (1.57g, 6.98 mmol) and TfOH (41µl, 465 µmol) were added to the mixture, and stirring was continued until TLC indicated disappearance of the glycosyl donor (~30 min). The reaction mixture was quenched by addition of TEA and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/EtOAc 7:3) to afford **26** (3.86, 74%) ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.88 – 6.99 (m, 20H. CH Aromatic), 6.92 (d, *J* = 7.0 Hz, 1H, NHTCA), 5.60 (s, 1H,PhCH), 5.33 (t, *J* = 6.9 Hz, 1H, H-2'), 5.25 (d, *J* = 7.8 Hz, 1H, H-1), 5.01 (d, *J* = 6.6 Hz, 1H, H-1'), 4.85 – 4.58 (m, 5H, NapC*H*$_2$, PhC*H*$_2$, *H-3*), 4.50 (d, *J* = 3.5 Hz, 1H, C-4), 4.29 – 4.03 (m, 4H, H-6a, H-4', H-5',H-6b), 3.82 – 3.64 (m, 5H, OCH_3,H-2, H-3'), 3.47 (d, *J* = 5.2 Hz, 1H, H-5), 1.72-1.55 (1H,$(CH_3)_2CH$) 0.95 – 0.78 (m, 12H, $HCC_2(CH_3)_4$), 0.16 (d, *J* = 16.4 Hz, 6H, $Si(CH_3)_2$^{13}C NMR (75 MHz, $CDCl_3$) 19.88, 33.92, 50.29, 52.53 57.3, 66.48, 69.26, 69.3, 72.75, 73.19, 73.85, 74.21, 74.97, 75.3, 78.99, 82.08, 93.82, 99.77, 100.48, 118.15, 118.33, 121.69, 125.66, 126.13, 126.51, 127.91, 127.92, 128.32. HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{32}F_2O_7S$ [M+Na]+ : 1138.5108 ; found 1138.1508

Dimethyl thexylsilane (methyl-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (28)

A solution of compound **26** (1.29 g, 1.16 mmol) in a mixture of DCM/TFA/H_2O (10/1/0.1, v/v/v, 15 mL) was stirred at ambient temperature for 15 min. The mixture was concentrated *in vacuo* and the residue was coevaporated with toluene. The diol obtained was subsequently taken up in 15ml of pyridine and treated with 15 ml of acetic anhydride and allowed to stir overnight. Upon completion the reaction was diluted with DCM then washed with 0.1 M HCl aq., sat. $NaHCO_3$ aq. and H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc 8:2) to afford **28** (.998, 81%) ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.87 – 7.00 (m, 15H, CH Aromatic), 6.77 (d, *J* = 7.2 Hz, 1H, N*H*TCA), 5.42 (d, *J* = 3.4 Hz, 1H, H-4^a), 5.20 (t, *J* = 8.7, 7.3 Hz, 1H,H-2^b), 5.12 (d, *J* = 7.9 Hz, 1H, H-1^a), 4.89 (d, *J* = 11.1 Hz, 1H, NapC*H*H), 4.82 – 4.70 (m, 3H, PhC*H*H, NapC*H*H, H-1^b), 4.69 – 4.57 (m, 2H, PhC*H*H, H-3^a), 4.18 – 3.95 (m, 4H, H-6ab,H-4^b, H-5^b), 3.91 – 3.81 (m, 1H, H-5^a), 3.79 – 3.66 (m, 4H, OC*H*$_3$, H-3^b), 3.50 (dt, *J* = 10.8, 7.5 Hz, 1H. H-2^a), 2.10 (s, 3H, CH_3C=O), 2.04 (s, 3H, ,CH_3C=O), 1.66-1.55 (1H,$(CH_3)_2CH$), 0.91 – 0.71 (m, 12H, $HCC_2(CH_3)_4$), 0.11 (d, *J* = 10.4 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $cdcl_3$) δ 19.87, 20.66, 20.73, 52.59, 57.98, 62.64, 68.96, 71.08, 71.34, 72.27, 74.04, 74.87, 74.9, 75.03, 79.22, 81.7, 93.66, 100.02, 118.21, 118.3, 121.66, 124.15, 125.91, 126.13,

126.87,127.92 127.94 HR MALDI-TOF MS: m/z: calcd for $C_{52}H_{60}Cl_3F_2NO_{15}Si$ [M+Na]+ : 1134.2766; found 1134.3303

Dimethyl thexylsilane (methyl-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-4,6-di-*O*-levulinoyl-2-deoxy-2 trichloroacetamido-β-D-galactopyranoside (29)

A solution of compound **26** (215 mg, 176 µmol) in a mixture of DCM/TFA/H_2O (10/1/0.1, v/v/v, 15 mL) was stirred at ambient temperature for 15 min. The mixture was concentrated *in vacuo* and the residue was coevaporated with toluene. The diol (198 mg, 192 µmol) was taken up in pyridine (3ml) and levulinyl anhydride (412mg, 192 µmol) along with DMAP (4.6 mg,38.4 µmol) were added. After being stirred overnight the reaction was diluted with DCM then washed with 0.1 M HCl aq., sat. $NaHCO_3$ aq. and H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **29** (170mg, 72%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.88 – 7.06 (m, 15H, CH Aromatic), 6.80 (d, *J* = 7.3 Hz, 1H, N*H*TCA), 5.43 (d, *J* = 3.5 Hz, 1H, , H-4^a), 5.21 (dd, *J* = 8.9, 7.4 Hz, H-2^b), 5.11 (d, *J* = 7.9 Hz, 1H, H-1^a)), 4.91 (d, *J* = 11.1 Hz, 1H, NapC*H*H), 4.85 – 4.72 (m, 3H, PhC*H*H, NapC*H*H, H-1^b), 4.68 (d, *J* = 11.4 Hz, 1H, PhC*H*H), 4.60 (dd, *J* = 10.9, 3.6 Hz, 1H, H-3), 4.17 (dd, *J* = 11.5, 5.1 Hz, 1H, H-6a), 4.12 – 3.96 (m, 3H, H-6b,H-4^b, H-5^b), 3.90 – 3.83 (m, 1H, H-5^b), 3.76 (d, *J* = 5.3 Hz, 4H, OC*H*$_3$, H-3^b), 3.57 – 3.47 (m, 1H, H-2^a), 3.01 – 2.39 (m, 8H,CH_2-Lev), 2.30 – 2.16 (m, 6H, C*H*$_3$), 1.64 - 1.58 (m, 1H, ,$(CH_3)_2CH$), 0.94 – 0.74 (m, 12H, $HCC_2(CH_3)_4$), 0.14 (d, *J* = 17.7 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, cdcl$_3$) δ 127.94, 127.72, 127.06, 126.01, 125.88,

121.55, 118.33, 118.28, 99.92, 93.89, 81.67, 79.26, 75.15, 74.98, 74.95, 74.32, 73.63, 72.16, 71.28, 69.16, 62.44, 62.43, 58.25, 52.77, 38.62, 38, 37.98, 37.86, 33.96, 29.88, 28.96, 28.1, 19.85, -2.52, -3.17. HR MALDI-TOF MS: m/z: calcd for $C_{58}H_{68}Cl_3F_2NO_{17}Si$ [M+Na]+ : 1246.6038; found 1246.5692

Dimethyl thexylsilane (methyl-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy4-O-levulinyl-2-trichloroacetamido-β-D-galactopyranoside (30)

Disaccharide **26** (1.30g, 1.16 mmol) was dissolved in DCM (40 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^oC), triethylsilane (0.534 mL, 1.44 mmol) and TfOH (0.534 mL, 6.80 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and TEA (0.5 mL). The resulting mixture was diluted with DCM and washed with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The residue was carried to the next step without purification. To a solution of the alcohol (1.092, .979 mmol) in pyridine (15ml), were added levulinyl anhydride (1.04g, .489 mmol) and DMAP (23 mg, .195 mmol). After being stirred overnight, the reaction was diluted with DCM then washed with 0.1 M HCl aq., sat. $NaHCO_3$ aq. and H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **30** (1.00, 83%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.86 – 6.99 (m, 20H, CH Aromatic), 6.76 (d, *J* = 7.3 Hz, 1H, N*H*TCA), 5.50 (d, *J* = 3.3 Hz, 1H, H-4^a), 5.18 (dd, *J* = 8.8, 7.4 Hz, 1H, H-2^b), 5.08 (d, *J* = 7.9 Hz, 1H, H-1^a), 4.90 (d, *J* = 11.0 Hz, 1H, NapC*H*H), 4.83 – 4.71 (m, 3H, PhC*H*H, NapC*H*H, H-

1[b]), 4.65 (d, *J* = 11.4 Hz, 1H,), 4.56 (dd, *J* = 10.9, 3.5 Hz, 1H, H-3[a]), 4.48 (s, 2H, PhC*H*H), 4.11 – 3.94 (m, 2H, H-4[b], H-5[b]), 3.83 – 3.71 (m, 2H, H-5[a], H-3[b]), 3.69 (s, 3H, OCH_3), 3.51 (dd, *J* = 12.5, 4.7 Hz, 3H, H-2[a], H-6ab), 2.91 – 2.48 (m, 4H, CH_2-Lev, 2.18 (s, 3H, CH_3), 1.71 - 1.54 (m, 1H, ,$(CH_3)_2$C*H*), 0.90 – 0.70 (m, 12H, $HCC_2(CH_3)_4$), 0.12 (d, *J* = 13.5 Hz, 6H,Si(CH_3)$_2$). ^{13}C NMR (75 MHz, cdcl$_3$) δ 128.04, 127.95, 127.95, 126.02, 100.06, 93.87, 75.09, 75.07, 74.88, 74.23, 73.59, 73.54, 72.78, 69.44, 68.8, 58.48, 52.55, 37.98, 33.81, 29.91, 28.06,19.88, -2.52,-3.17. HR MALDI-TOF MS: m/z: calcd for $C_{58}H_{68}Cl_3F_2NO_{17}Si$ [M+Na]+ : 1238.6278; found 1238.2012

Dimethyl thexylsilane (methyl-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-4-O-benzyl-2-deoxy-6-O-Levulinyl-2-trichloroacetamido-β-D-galactopyranoside (31)

Disaccharide **26** (100 mg, 89 µmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (42 µL, 268 µmol) and dichlorophenylborane (35 µL, 268 µmol) were added. After 3 min, the reaction was quenched by the addition of MeOH (1 mL) and TEA (0.5 mL). The resulting mixture was diluted with DCM and washed with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The residue was carried to the next step without purification. To a solution of the diol in pyridine (2ml) were added levulinyl anhydride (96 mg, 484 µmol) and DMAP (1 mg, 8.9 µmol), After being stirred overnight the reaction was diluted with DCM then washed with 0.1 M HCl aq., sat. $NaHCO_3$ aq. and H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash

chromatography over silica gel (20% EtOAc in hexane) to afford compound **31** (75 mg, 69 %)^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.88 – 7.04 (m, 20H, CH Aromatic), 6.84 (d, *J* = 6.8 Hz, 1H, N*H*TCA), 5.40 (t, *J* = 8.4 Hz, 1H, H-2^{b}), 5.13 (dd, *J* = 7.9, 2.1 Hz, 1H, H-1^{a}), 4.93 (d, *J* = 11.2 Hz, 2H, NapC*H*H, PhC*H*H), 4.87 – 4.77 (m, 3H, PhC*H*$_2$,H-1^{b}), 4.75 – 4.61 (m, 3H, NapC*H*H, PhC*H*H, H-3^{a}), 4.19 (dd, *J* = 11.2, 7.5 Hz, 1H, H-6a), 4.08 (q, *J* = 2.1 Hz, 2H, H-4^{b}, H-5^{b}), 4.00 (dd, *J* = 11.3, 4.9 Hz, 1H, H-6b), 3.94 (d, *J* = 3.0 Hz, 1H, H-4^{a}), 3.83 (dd, *J* = 8.7, 4.4 Hz, 1H, H-3^{b}), 3.76 (d, *J* = 2.3 Hz, 3H, OC*H*$_3$), 3.68 (q, *J* = 7.6, 6.3 Hz, 1H, H-5^{a}), 3.55 (dt, *J* = 10.0, 7.4 Hz, 1H, H-2^{a}), 2.73-2.51 (m, 4H, C*H*$_2$-Lev), 2.20 (d, *J* = 2.2 Hz, 3H, C*H*$_3$-Lev), 1.72 – 1.51 (m, 1H, (CH$_3$)$_2$C*H*), 0.93 – 0.69 (m, 12H, HCC$_2$(C*H*$_3$)$_4$), 0.11 (dd, *J* = 14.9, 2.2 Hz, 6H, Si(C*H*$_3$)$_2$).^{13}C NMR (126 MHz, cdcl$_3$) δ 128.85, 128.31, 128.00, 127.81, 127.96, 126.7, 126.09, 125.4, 123.56, 121.5, 118.52, 118.43, 100.99, 93.52, 81.71, 79.86, 77.07, 75.42, 75.16, 75.21, 75.25, 75.16, 74.84, 74.8, 74.34, 73.8, 72.5, 72.23, 63.45, 63.48, 58.57, 52.69, 50.07, 37.93, 33.89, 29.85, 29.72, 27.79, 20.07, 18.61, 17.3, 16.01, 14.32, -2.49,-3.25. HR MALDI-TOF MS: m/z: calcd for $C_{60}H_{68}Cl_3F_2NO_{15}Si$ [M+Na]+ : 1238.6278; found 1238.4652.

Dimethyl thexylsilane (methyl-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-4-O-benzyl-2,6-O-Levulinyl-2-trichloroacetamido-β-D-galactopyranoside (32)

Disaccharide **26** (100 mg, 89 µmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (42 µL, 268 µmol) and dichlorophenylborane (35 µL, 268 µmol) were added. After 3 min, the reaction was quenched by the addition of MeOH (1 mL) and TEA (0.5 mL). The

resulting mixture was diluted with DCM and washed with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The residue was carried to the next step without purification. The alcohol was modified further by treatment with a catalytic amount of NaOMe in MeOH. Upon completion the reaction was concentrated *in vacuo* and the crude diol was taken up in pyridine (2ml) and levulinyl anhydride (192 mg, 96 µmol) and DMAP (1 mg, 8.9 µmol), were added. The reaction was allowed to stir overnight and upon completion was diluted with DCM then washed with 0.1 M HCl aq., sat. $NaHCO_3$ aq. and H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) rendering disaccharide **32** (52 mg, 50 %) . ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.86 – 7.02 (m, 17H, CH Aromatic), 6.80 (d, *J* = 7.3 Hz, 1H, N*H*TCA), 5.42 (d, *J* = 3.5 Hz, 1H, , H-4^a), 5.23 (dd, *J* = 8.9, 7.4 Hz, H-2^b), 5.13 (d, *J* = 7.9 Hz, 1H, H-1^a)), 4.92 (d, *J* = 11.1 Hz, 1H,NapC*H*H), 4.86 – 4.72 (m, 3H, PhC*H*H, NapC*H*H, H-1^b), 4.65 (d, *J* = 11.4 Hz, 1H, PhC*H*H), 4.61 (dd, *J* = 10.5, 3.6 Hz, 1H, H-3), 4.16 (dd, *J* = 11.5, 5.1 Hz, 1H, H-6a), 4.12 – 3.96 (m, 3H, H-6b,H-4^b, H-5^b), 3.90 – 3.83 (m, 1H, H-5^a), 3.73 (d, *J* = 5.3 Hz, 4H, OC*H*$_3$, H-3^b), 3.55 – 3.46 (m, 1H, H-2^a), 3.01 – 2.39 (m, 8H,C*H*$_2$-Lev), 2.32 – 2.16 (m, 6H, C*H*$_3$-Lev), 1.65 - 1.59 (m, 1H, $(CH_3)_2CH$), 0.94 – 0.74 (m, 12H, $HCC_2(CH_3)_4$), 0.15 (d, *J* = 17.7 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $cdcl_3$) δ 13C NMR (75 MHz, cdcl3) δ 128.06, 127.91, 126.63, 126.13, 102.02, 101.38, 93.51, 92.98, 81.09, 79.80, 75.18, 74.80, 74.51, 74.16, 74.10, 71.99, 63.58, 63.51, 58.45, 52.55, 37.82, 29.80, 29.67, 27.73,19.91. HR

MALDI-TOF MS: m/z: calcd for $C_{58}H_{72}Cl_3NO_{16}Si$ [M+Na]+ : 1196.3686; found 1196.4645

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Chapter III

A MODULAR APPROACH TOWARDS HETEROGENEOUS CS OLIGOSACCHARIDE STANDARDS

3.1 ABSTRACT

Pregnancy-associated malaria is caused by the sequestering of *Plasmodium falciparum* in the placenta. CS-A found in the placenta acts as receptors and bind to the infected red blood cells allowing this parasite to elude any preexisting immunity. It has been observed that a dodecasaccharide with a minimum of two CS-A disaccharide moieties is sufficient for maximum IRBC binding. Here we report the synthesis of tetra- and hexasaccharides containing both CS-A and CS-O motifs to provide structural information towards the identification of the optimal di-sulfated dodeca-isomer.

3.2 INTRODUCTION

To date, the overwhelming majority of synthetic approaches have been toward homogeneous oligosaccharides with very few reporting the synthesis of heterogeneous tetrasaccharides.[1,2] The sequencing of CSPG bikunin showed that the simplest proteoglycan bears a side chain composed of a complex mixture of CS-A and CS-O.[3] This indicates that the polysaccharides involved in biological activity could very possibly be a mixture of different types of CS. Pregnancy-

associated malaria (PAM) is an example of a pathological event that is mediated by heterogeneous CS.[4]

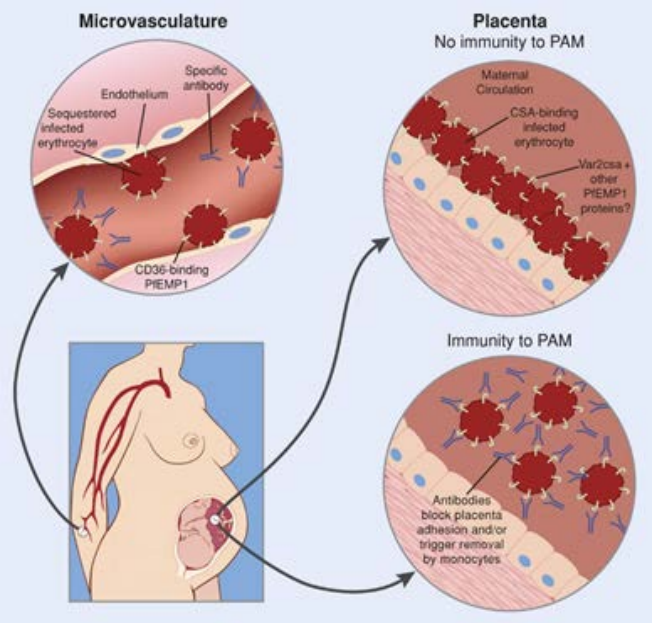


Figure 3.1: Binding of infected erythrocytes to microvasculature endothelium versus placenta.[11]

Malaria is the leading cause of morbidity and mortality in developing countries. According to the World Health Organization, there were 214 million cases in 2015 and 438,000 deaths[5]. Malaria is caused by Plasmodium parasites, the most virulent being *Plasmodium falciparum* which is responsible for >90% of all malarial deaths.[6] In malaria-endemic countries, adults have usually gained immunity. However, pregnant women become susceptible again. This phenomenon is known as pregnancy-associated malaria (PAM). In pregnant women, the parasite *Plasmodium falciparum* appears to take advantage of the availability of a new organ, the placenta. This allows the parasite to allude the

preexisting protective immunity and sequester in the placenta.[7] As demonstrated in Figure 3.1, this parasite sequesters in the microvascular of various organs by targeting multiple receptors, including CD36 and ICAM1 on the surface of endothelial cells, contributing to severe pathogenesis [8-16]. In the case of PAM, CS-A present in the placenta act as receptors that bind to infected red blood cells (IRBC) (Figure 3.1). Women who acquire PAM suffer from severe anemia, premature delivery, stillbirths, and even death. Despite the enormous societal and economic burden, currently, there is no target specific treatment for PAM. Studies by Dowda and co-workers have shown that a dodecasaccharide with a minimum of two CS-A disaccharide moieties is sufficient for maximum IRBC binding.[17] The structural requirements crucial for this binding are a CS-A disaccharide present at or proximal to the non-reducing end. Also the position and steric orientation of the carboxyl groups present at the non-reducing end (Figure 3.2). They also observed that dodecasaccharides with one, five, and six CS-A residues were not as efficient inhibitors as those containing two, three, and four CS-A motifs. This indicated that non-sulfated residues are also important for interaction with IRBC.[18] Identifying the optimal di-sulfated 12-mer isomer is a crucial step in developing therapeutic inhibitors of IRBC-C4S binding.

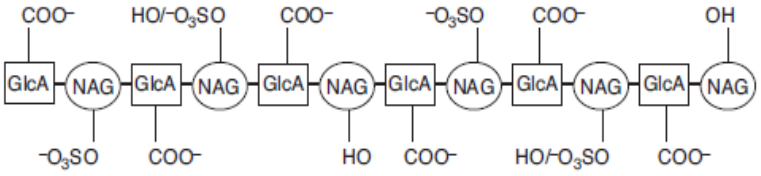
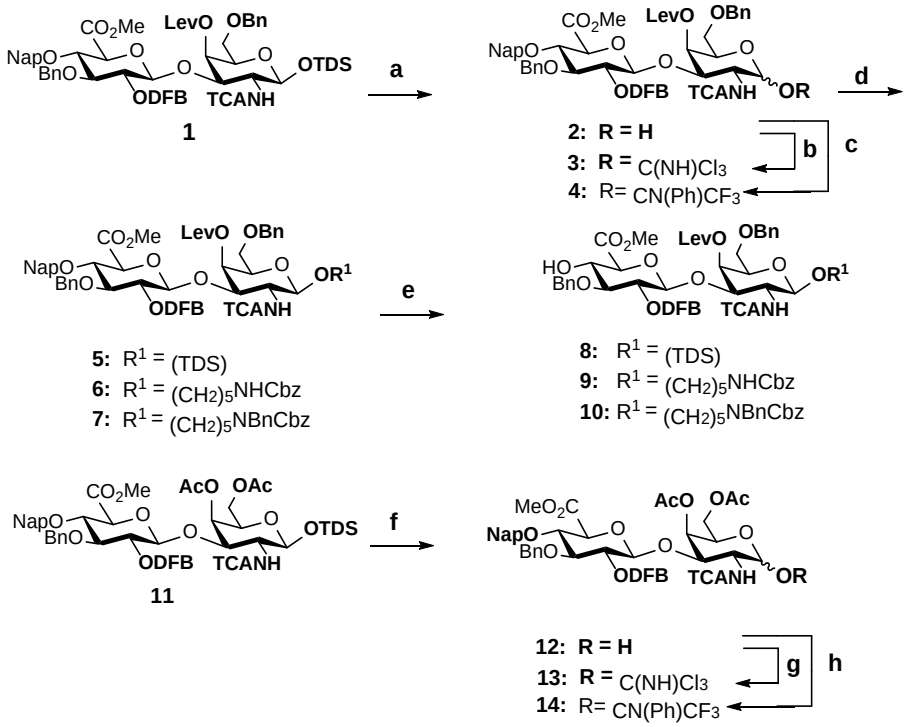


Figure 3.2: Schematic of oligosaccharide suggested to be responsible for pregnancy associated malaria[17].

Toward the goal of identifying the critical structural elements of low sulfated CS-A 12-mers, we have synthesized a library of tetra-, hexa-, and octa-oligosaccharides containing both CS-A and CS-O motifs. Assessment of the inhibitory capacity of these oligomers will provide structural information and SAR, which will help in designing effective therapeutic molecules.

3.3 RESULTS AND DISCUSSION

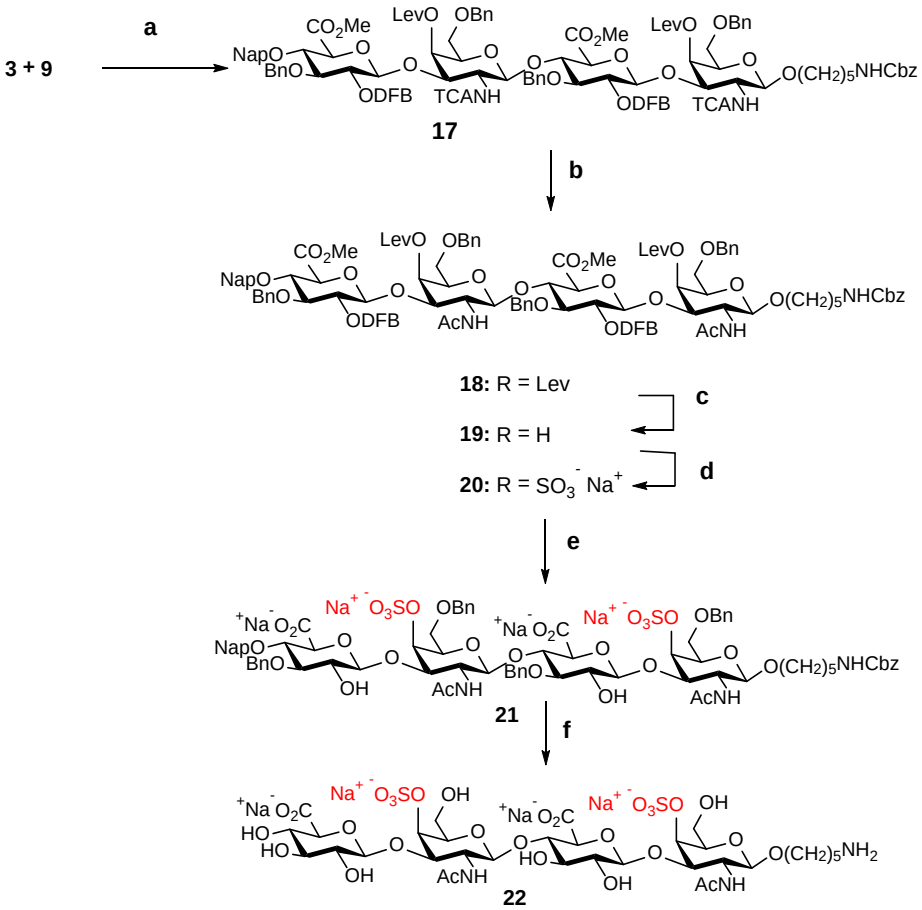
In this modular synthesis, CS-O and CS-A disaccharide building blocks were utilized to build tetra-, hexa- and ultimately octa- oligosaccharide standards. Thus, glycosyl donors were prepared by the removal of the anomeric TDS of **1** with HF in pyridine to give a lactol (Scheme 3.1), which was then converted into either trichloroacetimidate **3** or trifluoro-N-phenylacetimidate **4.** The compounds were equipped with an anomeric aminopentyl spacer, which provides an amine functional group for conjugation to a solid surface, which, for example, is required for microarray technology development.[19] Therefore, a mono-substituted or di-substituted linker was attached via glycosylation with TMSOtf in DCM rendering disaccharides **6** and **7** at 73% and 98% percent yields respectively. Then Nap was removed selectively by oxidative cleavage with 2,3-Dichloro-5,6-dicyano-1,4-



Scheme 3.1: a) HF, Pyr, 0° C, 73% b) $F_3C(NPh)Cl$, DBU, DCM c) $BnCbzN(CH_2)_5OH$ or $BnCbzN(CH_2)_5OH$, TMSOtf,DCM, 73% and 93%, 0 ^{0}C d) DDQ, 4:1 DCM: MeOH e) HF, Pyr, 0° C, 73% f) $F_3C(NPh)Cl$, NaH,DCM,90%

benzoquinone (DDQ) in a 4:1 mixture of methanol and MeOH giving acceptors **9** and **10** at 56% and 63% yields respectively.[20] Disaccharide **8** containing no spacer was also evaluated as a glycosyl acceptor. Two more donors were synthesized by TDS removal of **11** to form lactol **12.** Using standard protocol, trichloroacetimidate **13** and trifluoro-N-phenylacetimidate **14** were obtained and evaluated as glycosyl donors.

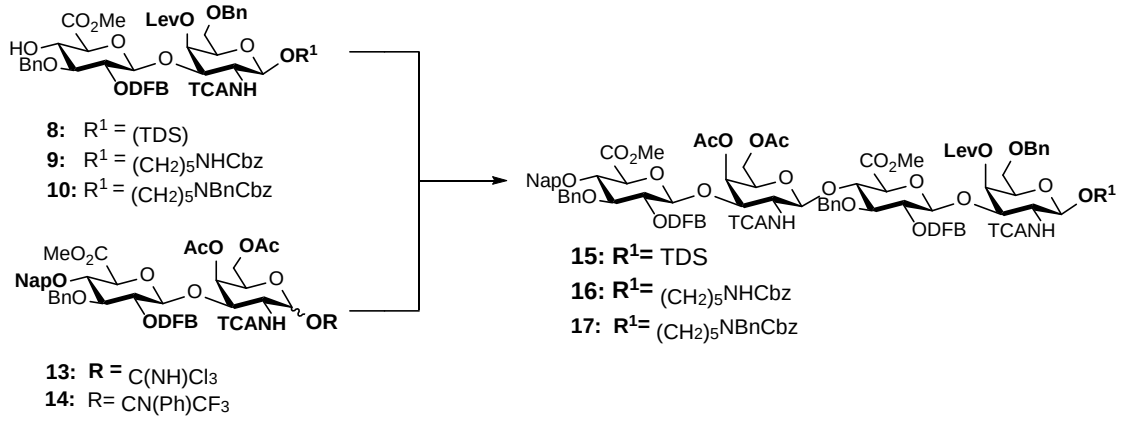
We initially synthesized a CSA-CSA tetrasaccharide, by first coupling glycosyl acceptor **3** with donor **9** to get tetrasaccharide **17** at 48 % yield (Scheme 3.2). Then the tetrasaccharide was transformed into the acetamido by treatment with Zn-Cu couple followed by selective Lev removal by hydrazine acetate. Subsequent



Scheme 3.2: a) TfOH, DCM, 48% b.) ZnCu, AcOH c) $NH_2NH_2 \cdot HOAc$, toluene/MeOH d) $SO_3 \cdot Py$, DMF, 67% e) 1. LiOH, H_2O_2, THF / 0.1 M NaOH f) $Pd(OH)_2$, MeOH, 63%

sulfation at the C-4 position proved to be sluggish but successfully yielded **20** at 67% yield. The di-sulfated tetrasaccharide was treated initially with LiOH and H_2O_2 in THF for the saponification of the methyl esters. During this reaction the pH was monitored to prevent any beta-elimination. Then the mixture was treated with 0.1 M NaOH to remove all esters present. Finally, **21** was subjected to hydrogenation conditions rendering the fully deprotected homogeneous CS-A tetrasaccharide **22**

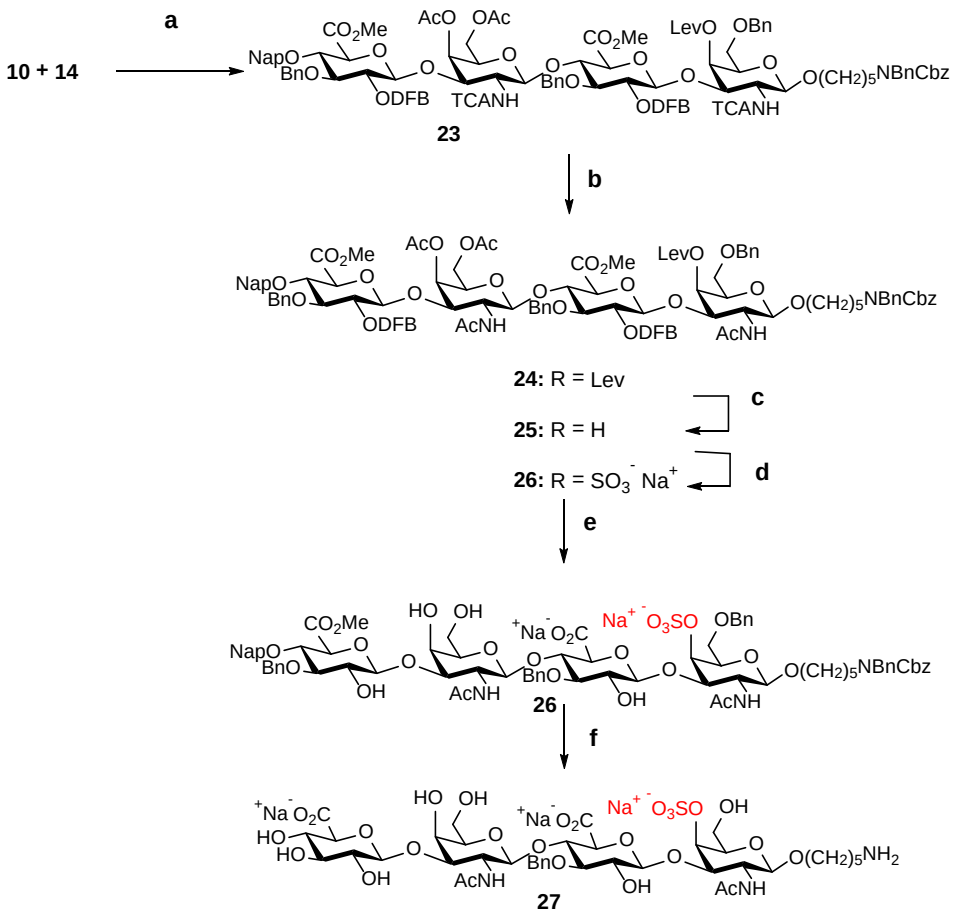
Table 3.1: Evaluation of different dissaccharide donors and acceptors for heterogeneous tetrasaccharide



Entry	Acceptor	Donor	Conditions	Product	Yield %
1	**8**	**13**	TfOH (.3 eq) , DCM, 0^0C	15	Trace
2	**8**	**14**	TfOH (.3 eq) , DCM, 0^0C	15	38
3	**9**	**13**	TfOH (.3 eq) , DCM, 0^0C	16	Trace
4	**9**	**14**	TfOH (.3 eq) , DCM, 0^0C	16	25
5	**10**	**14**	TfOH (.3 eq) , DCM, 0^0C	17	30
6	**10**	**14**	TfOH (1 eq) , DCM, -35^0C	17	65

at 63% yield.

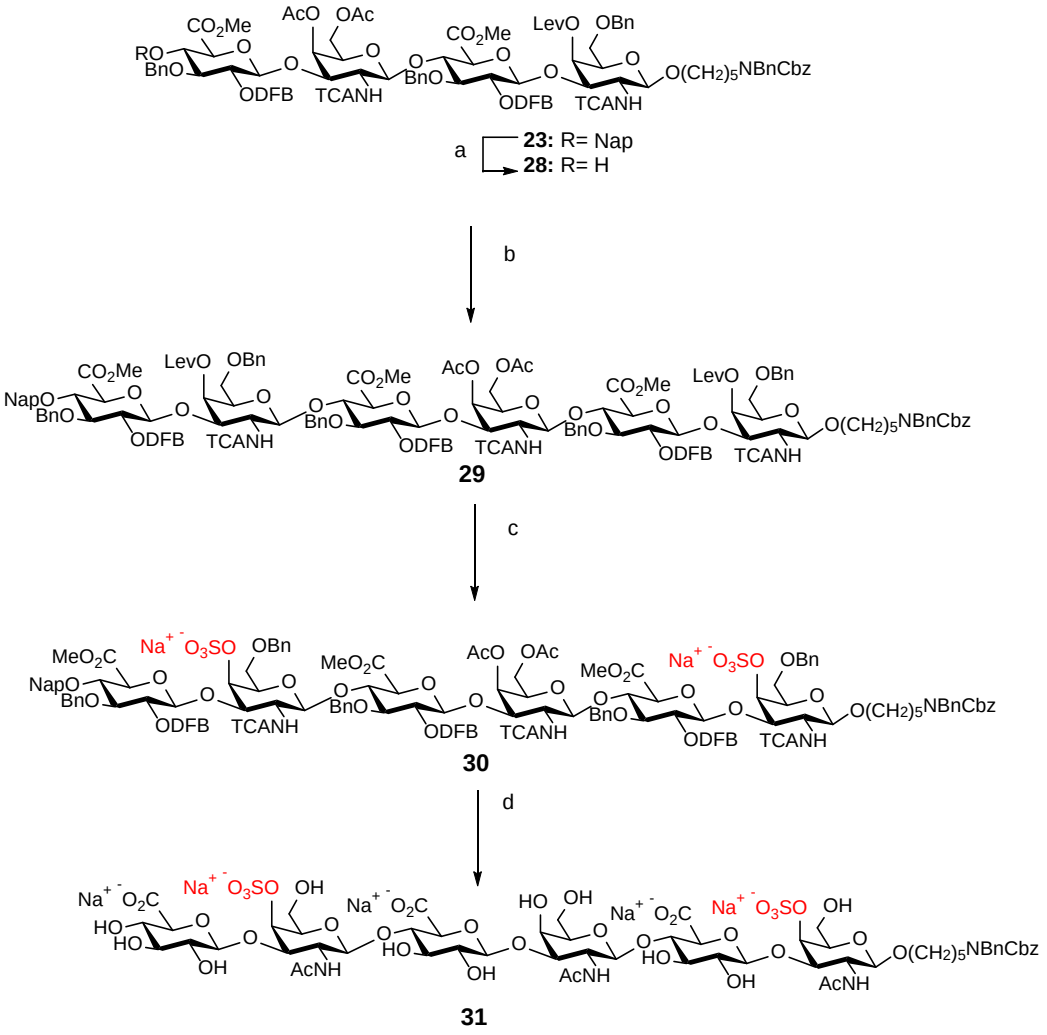
Although the synthesis was straightforward for the CSA-CSA target, finding the perfect combination of donor and acceptor for the heterogeneous CSO-CSA tetrasaccharide proved to be a challenge. Table 3.1 shows a few of many glycosylations attempted. The combination of non-linkered acceptor **8** and trichloroacetimidate **13** only gave a trace of the desired tetrasaccharide. Therefore, the more stable trifluoro-Nphenylacetimidate **14** was explored and improved the glycosylation to 38%. However, a side product with TDS cleaved off was observed. So linkered acceptor **9,** with a monosubstituted amine linker**,** was synthesized to circumvent the removal of the acid sensitive anomeric TDS. The addition of a spacer also added a handle for further exploration in the future. Howbeit, this addition did decrease the yield to 25%. To further improve this coupling, the next variable that was changed was the glycosylation conditions. According to previous HS synthesis in our lab, difficult glycosylations involving a trifluoro-



Scheme 3.3: a) TfOH, DCM, 71%b) ZnCu, AcOH, 65^{0}C, 72% b) NH_2NH_2 . HOAc, toluene/MeOH, 58% c) SO_3. Py, DMF, 35% d) 1. LiOH, H_2O_2, THF / 0.1 M NaOH, 72% e) $Pd(OH)_2$, MeOH, 73%

N-phenylacetimidate were optimized by increasing the equivalence of the acid promoter to 1 equivalence. So in this case, the TfOH promoter was increased from .3 to 1 equivalence. Since the amount of acid was increase the temperature was lowered from 0^{0}C to -35^{0}C to prevent any degredation or side reactions. Also out of concern of activating the secondary amine present on the linker, acceptor **10** was synthesized possessing a di-substituted linker. These changes appeared to increase the yield more than two-fold to 71 %. Now with tetrasaccharide **23** in hand, the sulfation and deprotection proceeded according to established procedures

shown in Scheme 3.3. Heterogeneous CSO-CSA tetrasaccharide **27** was rendered in a 47% yield.



Scheme 3.4: a) DCM:PBS buffer 4:1, 47% b) **12,** TfOH, DCM, 80% c) i.NH_2NH_2 HOAc, toluene/MeOH ii 6M SO_3 TMA, DMF, 98% d) LiOH, H_2O_2, THF / 0.1 M NaOH f) $Pd(OH)_2$ PBS buffer

Next, we attempted the synthesis of the CSA-CSO-CSA hexasaccharide. Firstly the tetrasaccharide acceptor was obtained by Nap removal to give **28** in 47% yield. Surprisingly the glycosylation of tetrasaccharide acceptor **28** and trifluoro-Nphenylacetimidate **4** resulted in fully protected hexasaccharide **29** in a very acceptable 80% yield. However, the initial attempt at transforming the trichloroacetamido into the acetamido by treatment with Zn-Cu couple in acetic acid did not go to completion. Jacquinet recently reported a similar result in a synthesis of a CS-E hexasaccharide.[21] So after several attempts, an alternative synthetic pathway was devised. In the alternative route, the removal of the trichloroacetamido was done with basic conditions simultaneously with ester removal (Scheme 3.4). So in the new pathway, Lev removal was first, which went according to the established protocol, this was followed by the introduction of sulfate groups which proved more difficult than the tetrasaccharide counterparts. After exploring several conditions, it was observed that using a very concentrated solution of the more stable sulfur trioxide trimethylamine complex at 65^{o}C overnight in DMF successfully rendered **30** at an excellent 98% yield**.** The sulfated hexasaccharide was saponified, and all esters, as well as trichloroacetamidos, were removed after approximately 72hr. MS taken periodically during this reaction showed that the removal of the TCA groups was very slow. Therefore, we surmised that the long reaction time was mainly due to the impressive stability of this protective group in basic conditions. Subsequently, the free amine residues were

selectively acylated by treatment with acetic anhydride. This compound was directly subjected to hydrogenation conditions in PBS buffer to avoid any methylation of the free amine linker. This reaction took five days, but the fully deprotected CSA-CSO-CSA heterogeneous hexasaccharide **31** was produced.

3.4 CONCLUSION

Herein we report the synthesis of a small library of tetra- and hexasaccharide CS standards. The structures of these targets were based on low sulfated CS fragments observed as responsible for the sequestering of *Plasmodium falciparum*-infected red blood cells inside in the placenta. These standards will be used to elucidate the definite structures responsible for this pathogenesis as well as lead to possible therapeutic vaccines.

3.5 EXPERIMENTAL SECTION

General procedures:

All moisture sensitive reactions were performed under an argon atmosphere by using vacuum dried glassware. All commercial materials were used without purification, unless otherwise noted. CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Toluene, DMF, diethylether, methanol and THF were purchased anhydrous and used without further purification. Molecular sieves (4Å) were flame activated in *vacuo* prior to use. All reactions were performed at room temperature unless specified otherwise. TLC-analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV-absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24} \cdot H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150°C. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 mm, 60Å) or on Bondapak C-18 (Waters). 1H and 13C NMR spectra were recorded on a Varian inova-300 (300/75 MHz), a Varian inova-500 (500/125 MHz) and a Varian inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d =doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances),coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basisof 1HNMR, 13C NMR, COSY and HSQC experiments. Optical rotations were measured using a Jasco P-1020 polarimeter. Mass spectra

were recorded on an Applied Biosystems 4700 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxybenzoicacid (DHB) and ultramark 1621 as the internal standard. The ESI-MS spectra were recorded on 9.4 T Bruker Apex Ultra QeFTMS (Billerica, MA) mass spectrometer.

General Procedure for Silyl Ether Cleavage:

100mg of the substrate was added to 1.5 ml of pyridine and .75ml HF-Pyr. After stirring for 18 h, the mixture was then diluted with DCM and washed with 10% $CuSO_4$ aq., water, saturated aqueous sodium bicarbonate, and brine. The organic phase was dried (MgSO4) and filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed over silica gel using a gradient of hexanes and EtOAc to give pure lactol.

General procedure for preparation of Trichloroacetimidates:

To a solution of the lactol in DCM (2 mL for 0.08 mmol) was added trichloroacetonitrile (5 equiv) and DBU (.1 equiv). After stirring at room temperature for 1.5 h, the reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was chromatographed over silica gel using a mixture of hexanes and EtOAc containing 0.01% pyridine to yield a trichloroacetimidate donor.

General procedure for preparation of Trifluoroacetimidate:

To a solution of the lactol in DCM (2 mL for 0.08 mmol) was added trichloroacetonitrile (5 equiv) and NaH (1 equiv). After stirring at room temperature for 1.5 h, the reaction mixture was filtered and the filtrate concentrated in vacuo.

The residue was chromatographed over silica gel using a mixture of hexanes and EtOAc containing 0.01% pyridine to yield a trichloroacetimidate donor.

General procedure of cleavage of Nap ethers:

Di - or tetrasaccharide starting materials were dissolved in a mixture of DCM and MeOH (4/1, v/v, 5ml for 400mg). DDQ (3 equiv.) was added, and the mixture was allowed to stir until TLC analysis indicated the disappearance of starting material (~5 h). The reaction mixture was diluted with DCM (30 mL), washed with water, saturated aqueous sodium bicarbonate, and brine, dried (MgSO4), and filtered. The filtrate was concentrated, and the residue was purified by silica gel column chromatography using a gradient of hexanes or toluene and EtOAc to afford the product.

General procedure for reduction of N-trichloroacetyl groups:

Tetrasaccharide starting materials were dissolved in acetic acid (1.5 mL for 0.03 mmol) and heated at 50°C under Ar. A large excess of Zn-Cu couple was then added in five portions at 1 h intervals and then stirred for an additional 19 h. The mixture was then cooled, filtered through a pad of Celite and concentrated. The filtrate was concentrated, and the residue was purified by silica gel column chromatography using a gradient of hexanes or toluene and EtOAc to afford the product.

General procedure for cleavage of Lev esters:

Anhydrous hydrazine acetate (5 equiv per Lev group) was added to a solution of the starting material in a mixture of ethanol and toluene (2/1, v/v, 5 mL for 150 mg).

Stirring was continued until TLC analysis indicated the disappearance of starting material (~2 h). The reaction mixture was diluted with DCM (30 mL), washed with water and brine, dried (MgSO4), and filtered. The filtrate was concentrated, and the residue was purified by silica gel column chromatography using a gradient of hexanes or toluene and EtOAc to afford the product.

General procedure for O-sulfation:

Sulfur trioxide pyridine complex (10 equiv per OH) was added to a solution of the starting material in DMF (1.0 mL for 0.02 mmol). The mixture was stirred at 65^{O} C for 24 h-72 until TLC ($CHCl_3$, CH_3OH 90/10, v/v) indicated completion of the reaction. After the addition of pyridine (0.2 mL) and CH_3OH (0.5 mL) stirring was continued for 30 min. The mixture was concentrated in vacuo (bath temperature 20 °C), and the residue was applied to a column of Iatrobeads (1.5 g), which was eluted with a gradient of CH_3OH in $CHCl_3$ (96/4→88/12 v/v, containing 0.2% pyridine). The fractions containing product were concentrated *in vacuo* (bath temperature 20 °C), and the residue was immediately passed through a column of Biorad 50 x 8 Na+ resin (0.6 x 5cm) using CH_3OH as eluent, providing the product as the sodium salt.

General procedure for saponification of methyl esters and de-O-acetylation:

A premixed solution of 30% solution of H_2O_2 in water (100 equiv per CO_2Me) and 1 M LiOH (50 equiv per CO_2Me) were added to a solution of the starting material in THF (0.02 M). The reaction mixture was stirred at room temperature for 8 h. A 4 N solution of NaOH (1.0 mL) was added until pH 14. The reaction mixture was

left stirring for 18 h at room temperature. In the case that the reaction had not gone to completion, stirring was continued at 35°C for an additional 12 h. The mixture was then brought to pH 8-8.5 by addition of AcOH, and the mixture was concentrated in vacuo (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (10 times the weight of starting material), which was eluted with a stepwise gradient of H_2O and CH_3OH (from 90/10→70/30, v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad 50 x 8 Na+ resin (0.6 x 5 cm) using CH_3OH as eluent providing product.

General procedure for selective N-acetylation:

Acetic anhydride (10 equiv per NH_2) was added to a solution of the starting material in a mixture of anhydrous CH_3OH (500 µL for 0.011 mmol) and Et_3N (20 equiv per NH_2) at 0 °C. The progress of the reaction was monitored by TLC (silica gel, $CHCl_3/CH_3OH/H_2O$, 60/30/3, v/v/v; RP18 silica gel, H_2O/CH_3OH, 40/60, v/v). After 5 h, another portion of Et_3N and Ac_2O was added at 0 °C. After stirring for 1 h at room temperature, the mixture was co-evaporated with toluene *in vacuo* (bath temperature 20°C) and the residue passed through a short column of Biorad 50 x 8 Na+ resin (0.6 x 5cm) using a mixture of CH_3OH and H_2O (90/10, v/v) as eluent, and appropriate fractions were concentrated in vacuo. The residue was vortexed with water and applied to a small RP-18 column (20 times the weight of starting material), which was eluted with a stepwise gradient of H_2O and CH_3OH (from 90/10 to 40/60, v/v). The appropriate fractions were concentrated *in vacuo* to obtain N- acetylated product.

General procedure for global debenzylation:

Pd/C (10%, 1.5 times the weight of starting material) was added to a solution of the starting material in CH_3OH and H_2O (1/1, v/v, 1 mL for 5 mg). The mixture was placed under an atmosphere of hydrogen, and progress of the reaction was monitored by TLC (silica gel, $CHCl_3/CH_3OH/H_2O$ 60/40/10, v/v/v; EtOAc/pyridine/water/CH_3CO_2H, 3/5/3/1,v/v/v). The hydrogenation was stopped when TLC indicated the disappearance of the starting material and the presence of a ninhydrin-positive main spot (2 h). The mixture was filtered through a PTFE syringe filter (0.2 mm, 13 mm) and washed with a mixture of CH_3OH and H_2O (1/1, v/v, 2 mL), and the solvents were concentrated in vacuo. The residue was dissolved in distilled water (1.5 mL), and palladium hydroxide on carbon (Degussa type, 20%, 1.5 times the weight of starting material) was added. The resulting mixture was placed under an atmosphere of hydrogen, and after 12 h, TLC (EtOAc/pyridine/water/CH_3CO_2H 4/5/3/1, v/v/v/v) indicated the completion of the reaction. The mixture was filtered through a PTFE syringe filter, and the residue was washed with H_2O (2mL). The filtrate was freeze dried; the residue was passed through a short column of Biorad 50 x 8 Na+ resin (0.6 x 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze-dried to provide the final product.

_N_-benzyloxycarbonyl-5-aminopentyl _O_-((methyl-(2-methylnaphthyl)-3-_O_-benzyl-2-_O_-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-6-_O_-benzyl-2-deoxy4-O-levulinyl-2-trichloroacetamido-β-D-galactopyranoside (6)

Disaccharide **1** (.728g , .599 mmol), was de-silylated and transformed into a trichloroacetimidate by general procedures respectively to give compound **3.** The imidate was used directly after a short column. TMSOTf (4 µl, 2.95 µmol) was added to a solution of **3** (247 mg, .202 mmol) and the monosubstituted linker acceptor $HO(CH_2)_5NHCbz$ (97.7 mg, .304 mmol) in anhydrous DCM (5 ml). The mixture was stirred at -20^{0}C for 1hr. Upon completion the reaction was quenched with TEA and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 7:3) providing glycoside **6** (182 mg, 73%) . ^{1}H NMR (500 MHz, Chloroform-d) δ 7.88 – 7.04 (m, 25H, CH Aromatic), 6.80 (d, *J* = 7.3 Hz, 1H, N*H*TCA), 5.53 (d, J = 3.4 Hz, 1H, H-4^a), 5.21 (dd, J = 8.8, 7.4 Hz, 1H, H-2^b), 5.08 (s, 2H, PhC*H*$_2$), 4.89 (dd, J = 18.9, 9.7 Hz, 2H, NapC*H*H, H-1^a), 4.83 – 4.73 (m, 4H, PhC*H*$_2$, , H-1^b), 4.67 (d, J = 11.4 Hz, 1H, NapC*H*H), 4.60 (dd, J = 10.6, 3.4 Hz, 1H, H-3^a), 4.51 (d, J = 1.3 Hz, 2H, PhC*H*$_2$), 4.07 (t, J = 9.2 Hz, 1H, H-4^b), 3.99 (d, J = 9.7 Hz, 1H, H-5^b), 3.87 (dt, J = 9.5, 6.1 Hz, 1H, C*H*H-linker), 3.82 (t, J = 6.2 Hz, 1H, H-4^a), 3.76 (t, J = 8.8 Hz, 1H,H-3^b), 3.71 (s, 3H, OC*H*$_3$), 3.62 – 3.50 (m, 3H, C*H*$_2$-linker, H-2^a), 3.45 (q, J = 7.3 Hz, 1H, C*H*H-linker), 3.15 (q, J = 6.8 Hz, 2H, C*H*$_2$-linker), 2.87 (dt, J = 19.2, 7.5 Hz, 1H), 2.76- 2.55(m, 4H, C*H*$_2$-Lev), 2.19 (s, 3H, C*H*$_3$-Lev), 1.58 - 1.22 (m, 6H, C*H*$_2$-linker). ^{13}C NMR (126 MHz, cdcl3) 128.00, 127.96, 126.73, 126.07, 121.42, 118.48, 118.41, 100.12, 98.68, 81.7, 79.16, 75.18, 75.08, 75.07, 73.69. 74.36, 73.62, 72.78, 72.96, 69.94, 69.87, 69.36, 68.7, 66.61, 56.5, 52.6, 40.93, 38.36, 38.19, 38.1, 29.82, 29.69, 29.66, 29.01, 28.13, 27.91, 23.24 HR MALDI-TOF MS: m/z: calcd for $C_{65}H_{67}Cl_3F_2N_2O_{17}$ [M+Na]+ : 1315.3473; found 1315.4081.

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-((methyl-(2-methylnaphthyl)-3-_O_-benzyl-2-_O_-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-6-_O_-benzyl-2-deoxy4-O-levulinyl-2-trichloroacetamido-β-D-galactopyranoside (7)

Disaccharide **1** was de-silylated and transformed into the trifluoroacetimidate by general procedures respectively to give compound **4.** The imidate was used directly after a short column. Then TMSOTf (10 µl, 54.3 µmol) was added to a solution of the above **4** (661 mg, .543 mmol) and the disubstituted linker acceptor $HO(CH_2)_5NBnCbz$ (403 mg, 1.23 mmol) in anhydrous DCM (1 ml) and was stirred at -20^0C for 1hr. Upon completion the reaction was quenched with TEA and concentrated in vacuo. Column chromatography (hexane/EtOAc 7:3) providing glycoside (**7**) (680 mg, 98%) ^1H NMR (500 MHz, Chloroform-d) δ 7.87 – 7.00 (m, 30 H, CH Aromatic), 6.94 – 6.65 (m, 1H, N*H*TCA), 5.53 (d, J = 3.4 Hz, 1H, H-4^a), 5.18 (dd, J = 8.8, 7.4 Hz, 1H, H-2^b), 5.08 (s, 2H, PhCH_2), 4.89 (dd, J = 18.9, 9.7 Hz, 2H, NapC*H*H, H-1^a), 4.83 – 4.73 (m, 4H, PhCH_2, , H-1^b), 4.67 (d, J = 11.4 Hz, 1H, NapC*H*H), 4.60 (dd, J = 10.6, 3.4 Hz, 1H, H-3^a), 4.51 (d, J = 1.3 Hz, 4H, PhCH_2, PhCH_2), 4.06 (t, J = 9.2 Hz, 1H, H-4^b), 3.99 (d, J = 9.7 Hz, 1H, H-5^b), 3.87 (dt, J = 9.5, 6.1 Hz, 1H, C*H*H-linker), 3.82 (t, J = 6.2 Hz, 1H, H-4^a), 3.72 (t, J = 8.8 Hz, 1H,H-3^b), 3.71 (s, 3H, , OCH_3), 3.62 – 3.52 (m, 3H, CH_2-linker, H-2^a), 3.45 (q, J = 7.3 Hz, 1H, C*H*H-linker), 3.15 (q, J = 6.8 Hz, 2H, CH_2-linker), 2.87 (dt, J = 19.2, 7.5 Hz, 1H), 2.79- 2.54(m, 4H, CH_2-Lev), 2.17 (s, 3H, CH_3-Lev),1.77-1.07(m, 6H, CH_2-linker). ^{13}C NMR (126 MHz, $cdcl_3$) 128.15., 127.91, 127.89, 126.78, 126.06, 121.54, 118.4, 118.3, 100.08, 98.72, 81.65,79.17, 75.16 , 75.03, 74.94, 74.3,

73.65, 73.65, 72.75, 72.71, 70.04, 69.91, 69.27, 68.61, 67.11, 56.36, 52.55, 50.3, 46.73, 38.08, 38.00, 29.7, 28.27, 27.86, 23.08 HR MALDI-TOF MS: m/z: calcd for $C_{72}H_{73}Cl_3F_2N_2O_{17}$ [M+Na]+ : 1403.3943; found 1403.2050

_N_-benzyloxycarbonyl-5-aminopentyl _O_-((methyl-(2-methylnaphthyl)-3-_O_-benzyl-2-_O_-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-6-_O_-benzyl-2-deoxy4-O-levulinyl-2-trichloroacetamido-β-D-galactopyranoside (9)

The Nap ether was removed from disaccharide **6** (182 mg, .141 mmol) by the general procedure of cleavage of Nap ethers in 2:.5 (DCM:MeOH) providing alcohol **9** (91 mg, 56 %). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.46 – 7.06 (m, 18 H, CH Aromatic), 6.92 (d, *J* = 7.3 Hz, 1H, N*H*TCA), 5.59 (d, J = 3.4 Hz, 1H, H-4^{a}), 5.18 – 5.02 (m, 3H, PhC*H*$_2$, H-2^{b}), 4.92 – 4.84 (m, 2H, H-1^{a}, PhC*H*H), 4.79 – 4.64 (m, 3H, PhC*H*H, H-1^{a} , H-3^{a}), 4.51 (m, 2H, PhC*H*$_2$), 4.12 – 3.97 (m, 1H, C-5^{b}), 3.93 – 3.76 (m, 6H, H-4^{b}, H-5^{a} , C*H*H-linker,OC*H*$_3$), 3.68 – 3.39 (m, 3H,H-3^{b} H-6ab,), 3.19 (d, J = 2.6 Hz, 5H, H-2^{a}, C*H*H-linker, O*H*, C*H*$_2$-linker), 2.95 – 2.46 (m, 4H, C*H*$_2$-Lev), 2.20 (s, 3H, CH_3-Lev), 1.67-1.21 (m, 6H, C*H*$_2$-linker). ^{13}C NMR (126 MHz, $cdcl_3$) δ 118.35, 125.44, 128.10, 121.46, 127.98, 118.39, 69.63, 73.17, 66.63, 98.58, 74.67, 74.60, 74.64, 100.40, 72.69, 71.10, 73.69, 72.10, 70.11, 73.15, 50.23, 52.87, 70.60, 80.57, 68.65, 66.04, 56.61, 70.04, 40.97, 38.15, 38.16, 38.19, 27.99, 28.08, 29.86, 27.20, 25.63, 29.05, 26.67, 29.65, 26.23, 23.26, 29.77 MS: m/z: calcd for $C_{54}H_{59}Cl_3F_2N_2O_{17}$ [M+Na]+ : 1175.4098; found 1175.3824

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-((methyl-(2-methylnaphthyl)-3-_O_-benzyl-2-_O_-difluorobenzoyl-β-D-glucopyranosyluronate)-(1→3)-6-_O_-benzyl-2-deoxy4-O-levulinyl-2-trichloroacetamido-β-D-galactopyranoside (10)

The Nap ether was removed from disaccharide **7** (680 mg, .521mmol) by the general procedure of cleavage of Nap ethers in 8:2 (DCM:MeOH) providing alcohol **10** (424 mg, 63%). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.46 – 7.02 (m, 23H, CH Aromatic), 6.89-6.69 (m, 1H, N*H*TCA), 5.57 (d, J = 3.4 Hz, 1H, H-4^{a}), 5.23 – 5.05 (m, 3H, PhC*H*$_2$, H-2^{b}), 4.95 – 4.77 (m, 2H, H-1^{a}, PhC*H*H), 4.74 – 4.58 (m, 3H, PhC*H*H, H-1^{a} , H-3^{a}), 4.55 – 4.37 (m, 4H, PhC*H*$_2$, PhC*H*$_2$), 4.22 – 3.96 (m, 1H, C-5^{b}), 3.93 – 3.67 (m, 6H, H-4^{b}, H-5^{a} , C*H*H-linker,OC*H*$_3$), 3.67 – 3.52 (m, 3H,H-3^{b} H-6ab,), 3.19 (d, J = 2.6 Hz, 5H, H-2^{a}, C*H*H-linker, O*H*, C*H*$_2$-linker), 2.98 – 2.42 (m, 4H,CH$_2$-Lev), 2.18 (s, 3H, CH$_3$-Lev), 1.70-1.04(m, 6H, C*H*$_2$-linker) ^{13}C NMR (75 MHz, cdcl3) 125.38. , 121.50, 118.41, 118.39, 100.43, 98.59, 96.05, 80.56, 74.6, 74.53, 73.68, 73.2, 73.19, 72.65, 72.93, 72.09, 71.08, 68.57, 67.13, 60.33, 56.58, 52.75, 50.35, 50.3, 50.1, 46.74, 38.06, 38.06, 29.73, 28.31, 27.89, 23.05, 20.92,14.17. HR MALDI-TOF MS: m/z: calcd for $C_{61}H_{65}Cl_3F_2N_2O_{17}$ [M+Na]+ : 1265.5348; found 1265.2428

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-(Methyl-2-O-2,5difluorobenzoyl,3-O-benzyl,4-_O_- naphthyl-β-Dglucopyranosyluronate)-(1_3)-(4,6-di-_O_-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1_4)-(methyl-2-O-2,5difluorobenzoyl,3-O-benzyl-β-D-

glucopyranosyluronate)-(1_3)-6-*O*-benzyl-4-*O*-levulinoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (17)

Donor **3** (91 mg, 78.9 μmol) and acceptor **9** (144 mg, 118 μmol) were combined in a flask, and coevaporated with toluene (3 × 3 mL). Freshly activated 4 Å molecular sieves were added, and the mixture was stirred for 30 min at ambient temperature and then cooled to 0 °C. TfOH (2 μl, 19.7 μmol) was added to the mixture, and stirring was continued until TLC indicated disappearance of the glycosyl donor (~30 min). The reaction mixture was quenched by addition of TEA and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/EtOAc 7:3) to afford **17** (88mg, 48%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.87 – 7.79 (m, 41H, CH Aromatic), 6.85-6.77 (m, 2H, N*H*TCA^{a}, N*H*TCA^{c}), 5.55-5.44 (m, 1H, H-4^{a},H-4^{c}), 5.11 (dd, J = 8.2, 7.1 Hz, 1H,H-2^{d}), 5.19 – 5.02 (m, 3H, PhC*H*$_{2,}$ H-2^{d}), 4.96 (d, J = 8.5 Hz, 1H, H-1^{d}), 4.92 – 4.83 (m, 3H, NapC*H*H, PhC*H*H, H-1^{a}), 4.82 – 4.73 (m, 3H PhC*H*$_{2}$,H-1^{c}), 4.72 – 4.66 (m, 2H, NapC*H*H, H-1^{b}), 4.59 (s, 1H. H-3^{a}), 4.53 – 4.44 (m, 4H, PhC*H*$_{2,}$ CH_2-linker), 4.25 – 4.15 (m, 2H, H-3^{c}, H-4^{b}), 4.12 – 3.92 (m, 4H,H-4^{b}, H-5^{d}, H-2^{c}, H-6a), 3.90 (d, J = 9.1 Hz, 1H, H-5^{b}), 3.88 – 3.76 (m, 5H, H-5^{c}, H-6b,H-5^{a}, H-3^{d}, C*H*H), 3.73 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.64 (t, J = 8.3 Hz, 1H, H-3^{b}), 3.53 (qd, J = 10.0, 6.2 Hz, 2H, H-6ab^{a}), 3.46 (s, 1H, H-2^{a}), 3.16 (d, J = 33.0 Hz, 2H, N-CH_2-linker), 2.80 - 2.52 (m,4H, CH2-Lev 2.17 (d, J = 8.2, 7.1 Hz ,6H,CH_3-Lev), 1.26 (m, 6H, CH_2-linker). HR MALDI-TOF MS: m/z: calcd $C_{107}H_{111}Cl_6F_4N_3O_{31}$ [M+Na]+ : 2223.7486; found : 2223.6865

Tetrasaccharide Deprotection:

5-Aminopentyl(β-D-glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranosyl)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranoside (22)

Cleavage of Lev esters: Upon N-trichloroacetyl reduction, the compound was directly taken to the next step. The acetamido (70 mg, 34.9 μmol) was de-levulinoylated according to general procedure providing **19** (30 mg, 47 %). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.93 – 6.94 (m, 37H, CH Aromatic), 5.92 (d, *J* = 7.2 Hz, 1H, N*H*C=OCH$_3$), 5.67 (d, *J* = 6.6 Hz, 1H, N*H*C=OCH$_3$), 5.27 (t, *J* = 7.0 Hz, 1H,H-2^{b}), 5.17 (dd, *J* = 12.9, 5.9 Hz, 1H, H-2^{d}), 5.04 (s, 2H, PhCH$_2$-linker), 4.96 (d, *J* = 8.0 Hz, 1H, H-1^{d}), 4.91 – 4.88 (m, 2H, H-1^{a}, NapC*H*H), 4.84 (d, *J* = 8.0 Hz, 1H, H-1^{c}) 4.82 – 4.71 (m, 3H, PhCH$_2$, H-1^{b}), 4.67 (d, *J* = 11.4 Hz, 1H, NapC*H*H), 4.60 – 4.34 (m, 6H, H-4^{a,c}, 2PhCH$_2$), 4.25 (t, *J* = 7.0 Hz, 1H, H-3^{c}), 4.07- 4.04 (m, Hz, 3H, H-4^{d}, H-5^{d}, H-3^{a}), 3.88 – 3.57 (m, 7H, H-4^{b}, H-5^{a-c}, 2H-6ab^{a,c}, 2OCH$_3$, H-3^{b,d}), 3.52 (dd, *J* = 8.7, 4.8 Hz, 1H, C*H*H-linker), 3.41 (d, *J* = 9.2 Hz, 1H, C*H*H-linker), 3.28 (s, 1H, H-2^{a}), 3.16 (d, *J* = 41.4 Hz, 3H, H-2^{c}, CH$_2$-linker), 1.66-1.53 (m, 6H, NHC=OCH$_3$), 1.45-1.12 (m, 6H, C*H*$_2$-linker). ^{13}C NMR (151 MHz, cdcl$_3$) δ 127.93, 127.96, 126.61, 118.60, 126.25, 126.26, 128.14, 128.00, 77.23, 81.29, 73.17, 127.56, 121.57, 125.75, 120.03, 127.82, 131.51, 127.76, 118.31, 73.28, 67.10, 66.55, 98.32, 74.97, 98.70, 98.38, 75.04, 99.91, 75.00, 74.63, 101.26, 74.80, 73.90, 73.63, 73.46, 78.05, 50.34, 73.45, 73.66, 76.56, 79.07, 68.01, 74.11, 81.44, 69.34, 79.64, 69.27, 52.82, 72.96, 52.67, 62.70, 54.63, 40.97, 23.12, 22.85, 29.17,

22.88, 29.62, 13.96, 18.99, 19.99. HR MALDI-TOF MS: m/z: calcd $C_{97}H_{105}F_4N_3O_{27}$ [M+Na]+ : 1843.8946; found : 1843.9586

O-sulfation: Alcohol **19** (28 mg, 18.7 15.5 mmol) was dissolved in 1.5 ml of DMF and O-sulfated according to the general procedure providing **20** (21 mg, 67 %) as a sodium salt. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.92 – 7.01 (m, 37H, CH Aromatic), 5.38 (t, *J* = 8.1 Hz, 1H, H-2^b), 5.21 (t, *J* = 7.0 Hz, 1H, H-2^d), 5.05 (s, 2H,PhCH_2-linker), 5.00 – 4.80 (m, 6H, H-1^d, H-1^a, H-4^{a,c}, NapC*H*H, PhC*H*H), 4.68 (d, *J* = 11.5 Hz, 1H, PhC*H*H), 4.63 (d, *J* = 7.7 Hz, 1H, H-1^c), 4.60 – 4.52 (m, 3H, NapC*H*H, PhCH_2), 4.40 (dd, *J* = 16.4, 7.6 Hz, 2H, H-1^b, H-3^c), 4.35 (s, 2H, PhCH_2), 4.20 – 4.09 (m, 2H, H-4^d, H3-^{a}), 4.04 (d, *J* = 9.0 Hz, 1H, H-5^d), 4.00 – 3.76 (m, 10H,H-2^{ac} ,H-4^b, H-5^{a,b,c} 2H-6ab), 3.76 – 3.62 (m, 9H, 2OCH_3, H-3^d, H-3^b, C*H*H-linker), 3.48 – 3.40 (m, 1H, C*H*H-linker), 3.07 (t, *J* = 7.0 Hz, 2H, CH_2-linker), 1.66 (d, *J* = 6.3 Hz, 6H, NHC=OCH_3), 1.56 – 1.18 (m, 6H, CH_2-linker). ^{13}C NMR (126 MHz, cd$_3$od) δ 127.65, 126.36, 118.60, 118.16, 125.66, 121.61, 127.60, 126.60, 127.73, 118.43, 127.88, 126.31, 127.49, 74.16, 73.80, 65.92, 101.36, 74.41, 100.65, 75.56, 74.54, 74.67, 101.09, 72.90, 74.44, 74.33, 100.85, 76.89, 73.02, 74.18, 79.04, 74.27, 52.00, 76.89, 82.04, 77.36, 51.75, 70.03, 80.65, 68.78, 51.96, 53.08, 73.82, 51.77, 52.89, 73.89, 69.95, 68.81, 47.90, 40.39, 35.60, 20.70, 21.69, 28.73, 29.14,22.76. ESI-MS: m/z: calcd. for $C_{97}H_{103}F_4N_3O_{33}S_2$: 980.7774; found : 980.6965 $[M-2H]^{2-}$

Saponification of methyl esters and de-O-acetylation: The compound **20** (21.0 mg, 10.4 µmol) was dissolved in THF (1 mL) and subjected to saponification and de-O-acetylation according to the general procedure of saponification of methyl ester

and de-Oacetylation to give **21** (7 mg, 40%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.85 – 7.13 (m, 32H, CH Aromatic), 5.07 (s, 2H, PhCH_2-linker), 5.00 – 4.89 (m, 2H, NapC*H*H, PhC*H*H), 4.84 – 4.68 (m, 4H, H-4^{a,c}, NapC*H*H, PhC*H*), 4.64 – 4.61 (m, 1H, H-1^{d}), 4.58 (d, *J* = 4.0 Hz, 2H, PhCH_2), 4.51 (d, *J* = 7.8 Hz, 1H, H-1^{a}), 4.48 – 4.41 (m, 3H, H-1^{c}, H-1^{b}, PhC*H*), 4.37 (d, *J* = 11.9 Hz, 1H, PhC*H*), 4.2 (t, J=7.5 Hz, 1H, H-2^{a}) , 4.03 – 3.93 (m, 3H, H-2^{c}, H-4^{d}, H-3^{a}), 3.93 – 3.71 (m, 9H, H-5 $^{a-d}$,H-4^{b}, 2H-6ab), 3.70 – 3.60 (m, 2H, C*H*H-linker, H-3^{d}), 3.58 – 3.48 (m, 3H, H-2^{b}, H-3^{b}, C*H*H-linker), 3.45 (t, *J* = 8.7 Hz, 1H, H-2^{d}), 3.11 (t, *J* = 7.0 Hz, 2H, CH_2-linker), 2.08 (s, 3H, NHC=OCH_3), 1.97 (s, 3H, NHC=OCH_3), 1.63 – 1.27 (m, 8H, m, 6H, CH_2-linker).^{13}C NMR (126 MHz, cd$_3$od) δ 126.59, 127.38, 128.65, 126.22, 126.18, 125.50, 127.68, 127.63, 127.69, 65.70, 74.04, 74.10, 75.66, 74.08, 75.45, 74.07, 100.23, 72.78, 104.83, 101.28, 104.02, 73.00, 72.96, 51.90, 51.81, 76.50, 78.16, 79.17, 69.57, 80.40, 77.44, 69.78, 73.20, 73.59, 77.27, 69.37, 83.39, 71.96, 68.92, 82.08, 48.18, 49.10, 47.55, 40.12, 21.82, 21.84, 28.46, 28.96, 22.20. ESI-MS: m/z: calcd. for $C_{80}H_{89}N_3O_{31}S_2$: 834.7466 ; found : 834.3659 $[M-2H]^{2-}$

Global debenzylation: A solution of tetrasaccharide **21** (7 mg, 4.32 µmol) in MeOH (2 mL) was subjected to debenzylation according to the general procedure for global debenzylation to render tetrasaccharide **22** (2.5 mg, 63%)^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.89 (d, J = 2.2 Hz, H, H-4^{a}) 4.63 (d, J = 2.0 Hz, H, H-4^{c}), 4.49 – 4.45 (m, 1H, H-1^{a}), 4.44 – 4.40 (m, 1H, H-1^{c}), 4.37 (dd, J = 10.5, 7.8 Hz, 2H, H-1^{b}, H-1^{d}), 4.00 – 3.88 (m, 4H, H-2^{a,c}, H-3^{a,c}), 3.81 (dt, J = 10.2, 6.0 Hz, 1H,O-C*H*H-linker), 3.77 – 3.62 (m, 7H, H-4^{b,} H-5^{a,c}, H-6ab^{a,c}), 3.54 (ddd, J = 13.0, 10.0, 5.3 Hz, 3H, H-5^{b}, H-5^{d}, O-C*H*H-linker), 3.51 – 3.45 (m, 1H, H-3^{b}), 3.42 (t, J

= 9.4 Hz, 1H, H-4^{d}), 3.36 (t, J = 9.2 Hz, 1H, H-3^{d}), 3.30 (dd, J = 9.4, 7.9 Hz, 1H, H-2^{b}), 3.24 (dd, J = 9.2, 7.8 Hz, 1H, H-2^{d}), 2.94 – 2.83 (m, 2H, N-CH_2-linker), 1.92 (d, J = 11.9 Hz, 6H, CH_3), 1.66 – 1.45 (m, 4H, CH_2-linker), 1.37 – 1.25 (m, 2H, CH_2-linker). ^{13}C NMR (126 MHz, cdcl$_3$) 103.36, 101.2, 101.05, 80.57, 80.57, 76.58, 76.48, 76.36, 75.06, 74.88, 74.44, 73.63, 72.55, 72.07, 71.83, 70.00, 70.04, 61.03, 61.04, 51.7, 39.38, 28.02, 26.26, 22.44, 22.2,22.04. ESI-MS: m/z: calcd. for $C_{33}H_{51}N_3O_{29}S_2$: 579.1375 ; found : 579.1658 $[M-2H]^{2-}$

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-(Methyl 2-O-2,5difluorobenzoyl,3-O-benzyl,4-_O_- naphthyl-β-Dglucopyranosyluronate)-(1→3)-(4,6-di-_O_-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl2-O-2,5difluorobenzoyl,3-O-benzyl-β-D-glucopyranosyluronate)-(1→3)-6-_O_-benzyl-4-_O_-levulinoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside(23)

After the desilylation of disaccharide **11,** NaH (6.7 mg, .282 mmol) was added to a mixture of the subsequent lactol (273 mg, .282 mmol) and $CF_3CNPhCl$ (.281 mL, 1.41 mmol) in DCM. After 1 h, the mixture was dried *in vacuo* and the residue was purified by a short silica gel column. The resulting imidate was used directly. Acceptor **4** (50mg, 40.7 µmol) and donor **10** (55mg, 48.2 µmol)) were combined in a flask, and coevaporated with toluene (3 × 3 mL). Freshly activated 4 Å molecular sieves were added, and the mixture was stirred for 30 min at ambient temperature and then cooled to -35 °C. TfOH (3µl, 40.7 µmol) was added to the mixture, and stirring was continued until TLC indicated disappearance of the glycosyl donor

(~30 min). The reaction mixture was quenched by addition of TEA and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/EtOAc 7:3) to afford **23** (80mg, 71%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.87 – 7.01 (m, 41H, CH Aromatic), 6.95-6.70 (m, 2H, N*H*TCA^{a}, N*H*TCA^{c}), 5.53 (d, J = 3.6 Hz, 1H, H-4^{a}), 5.41 (d, J = 3.4 Hz, H-4^{c}), 5.21 (dd, J = 8.2, 7.1 Hz, 1H,H-2^{d}), 5.18 – 5.05 (m, 3H, PhC*H*$_{2,}$ H-2^{d}), 4.94 (d, J = 8.5 Hz, 1H, H-1^{d}), 4.92 – 4.83 (m, 3H, NapC*H*H, PhC*H*H, H-1^{a}), 4.82 – 4.73 (m, 3H PhC*H*$_{2}$,H-1^{c}), 4.72 – 4.66 (m, 2H, NapC*H*H, H-1^{b}), 4.59 (s, 1H. H-3^{a}), 4.56 – 4.42 (m, 4H, PhC*H*$_{2,}$ CH_2-linker), 4.25 – 4.15 (m, 2H, H-3^{c}, H-4^{b}), 4.12 – 3.92 (m, 4H,H-4^{b}, H-5^{d}, H-2^{c}, H-6a), 3.88 (d, J = 9.1 Hz, 1H, H-5^{b}), 3.85 – 3.74 (m, 5H, H-5^{c}, H-6b,H-5^{a}, H-3^{d}, C*H*H), 3.73 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.64 (t, J = 8.3 Hz, 1H, H-3^{b}), 3.53 (qd, J = 10.0, 6.2 Hz, 2H, H-6ab^{a}), 3.46 (s, 1H, H-2^{a}), 3.16 (d, J = 33.0 Hz, 2H, N-CH_2-linker), 2.80 - 2.52 (m,4H, CH2-Lev 2.17 (s, 3H,CH_3-Lev), 2.03 (d, J = 14.3 Hz, 6H, *H*$_{3}$CC=O), 1.26 (m, 6H, CH_2-linker). ^{13}C NMR (126 MHz, cdcl3) 128.09, 127.95, 127.83, 127.64, 126.72, 126.03, 124.07, 121.35, 118.54, 118.19, 118.13, 100.09, 99.72, 99.68, 98.17,81.82, 79.74,78.85, 77.38, 75.08,75.03, 74.91, 74.77, 74.7, 74.69, 74.59, 74.35, 73.7, 73.62, 73.6, 73.45, 72.98, 72.85, 71.75, 69.94, 69.58, 68.56, 68.3, 67.16, 61.66, 61.61, 56.44, 52.94, 52.59, 50.37, 46.84, 38.15, 38.08, 29.89, 29.76, 28.55, 28, 27.9, 23.34, 20.73,20.54. HR MALDI-TOF MS: m/z: calcd $C_{105}H_{105}Cl_6F_4N_3O_{31}$ [M+Na]+ : 2216.6786; found 2216.5654

Tetrasaccharide Deprotection:

5-Aminopentyl(β-D-glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranoside (28)

N-trichloroacetyl reduction: Compound **23** (70 mg, 35 μmol) was subjected to N-trichloroacetyl reduction conditions to provide acetamido **24** (30 mg, 72%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87 –7.02 (m, 39H, CH Aromatic), 5.76 (s, 1H, H-4^{a}), 5.48 (s, 1H, H-4^{c}), 5.37 (d, *J* = 3.5 Hz, 1H, H-2^{d}), 5.21 (dd, *J* = 8.4, 7.2 Hz, 1H,), 5.14 (d, *J* = 24.3 Hz, 3H, PhCH$_2$, H-2^{b}), 4.93 – 4.83 (m, 3H, H-1^{d}, H-1^{a}, Nap*H*H), 4.82 – 4.69 (m, 4H, Nap*H*H, Ph*H*H, H-1^{c}, H-1^{b}), 4.47 (d, *J* = 4.1 Hz, 4H, PhCH$_2$, CH$_2$-linker), 4.23 (s, 1H,H-3^{a}), 4.10 – 3.92 (m, 4H, H-4b, H-5d, H-2c, H-6a), 3.90 – 3.64 (m, 11H, H-5^{c}, H-6b,H-5^{a}, H-3^{d}, C*H*H, OCH$_3$, OCH$_3$), 3.50 (d, *J* = 6.0 Hz, 2H,CH2-linker), 3.18 (d, *J* = 27.4 Hz, 3H), 2.71– 2.40 (m, 4H, CH$_2$Lev), 2.14 (s, 3H, CH$_3$ Lev), 1.98 (d, *J* = 5.6 Hz, 6H, CH$_3$), 1.64 (s, 6H,NHC=OCH$_3$) 1.55 -1.26 (m, 6H, CH$_2$-linker). ^{13}C NMR (126 MHz, cdcl$_3$) δ 128.09, 127.95, 127.83, 127.64, 126.72, 126.03, 124.07, 121.35, 118.54, 118.19, 118.13, 100.09, 99.72, 99.68, 98.17, 81.82, 79.74, 78.85, 77.38, 75.08, 75.03, 74.91, 74.77, 74.7, 74.69, 74.59, 74.35, 73.7, 73.62, 73.6, 73.45, 72.98, 72.85, 71.75, 69.94, 69.58, 68.56, 68.3, 67.16, 61.66, 61.61, 56.44, 52.94, 52.59, 50.37, 46.84, 38.15, 38.08, 29.89, 29.76, 28.55, 28, 27.9, 23.34, 20.73,20.54. HR MALDI-TOF MS: m/z: calcd for $C_{105}H_{111}F4N_3O_{31}$ [M+Na]+ : 2008.7138; found 2008.6571

O-sulfation: After de-levulinoylated according to the general procedure for cleavage of levulinoyl esters, tetrasaccharide **25** (26 mg, 18.7 µmol) was dissolved in 1.5 ml of DMF and O-sulfated according to the general procedure providing **26** (12 mg, 35 %) as sodium salt. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.06 – 6.54 (m, 31H, CH Aromatic), 5.30 (m, 1H, d, J = 3.6 Hz, 1H, H-4^{a}), 5.17 (t, *J* = 6.9 Hz, 1H, H-2^{b}), 5.14 – 5.05 (m, 3H, CH-2-linker, H-2^{d}), 4.92 – 4.80 (m, 4H, Nap*H*H, C-4^{c}, H-1^{d}, H-1^{a}), 4.75 (t, *J* = 12.0 Hz, 1H, Ph*H*H), 4.60 (d, *J* = 11.6 Hz, 1H, Ph*H*H), 4.53 (dd, *J* = 22.6, 7.8 Hz, 2H, H-1^{c}, Nap*H*H), 4.44 (s, 2H, CH_2-linker), 4.36 (s, 1H, H-1^{d}), 4.25 (t, *J* = 7.9 Hz, 1H, H-3^{c}), 4.07 (dd, *J* = 26.3, 8.7 Hz, 2H, H-4^{d}, H-5^{d}), 4.01 – 3.74 (m, 9H, C*H*H-linker, H-2^{a,c}, H-4^{b}, H-5^{b}, H-5^{c}, H-5, H-6ab, H-3^{d}), 3.70 (d, *J* = 14.5 Hz, 8H, 2OCH_3, H-3^{b}, C*H*H-linker), 3.67 – 3.62 (m, 1H, C*H*H-linker), 3.16 (d, J = 33.0 Hz, 2H, N-CH_2-linker) 1.94 (d, *J* = 13.3 Hz, 6H, 2H_3CC=O), 1.57 (s, 6H, NHC=OCH_3), 1.46– 1.14 (m, 6H, 2CH_2-linker). ^{13}C NMR (151 MHz, cd$_3$od) δ 127.84., 127.57, 127.49, 127.43, 127.4, 127.29, 126.54, 126.28, 125.66, 125.51, 121.6, 118.29, 117.97, 101.29, 100.81, 100.63, 81.62, 79.82, 79.52, 77.19, 76.22, 75.57, 74.67, 74.49, 74.46, 74.19, 73.92, 73.82, 73.71, 73.36, 72.86, 70.84, 70.2, 70.13, 68.67, 68.66, 66.89, 62.18, 62.1, 52.01, 51.81, 51.68, 49.95, 47.89, 46.65, 30.2, 29.26, 27.38, 27.21, 26.35, 21.5. ESI-MS: m/z: calcd. for $C_{93}H_{98}F_4N_3O_{32}S$: 1967.5796 ; found : 1967.4878

Saponification of methyl esters and de-O-acetylation: The compound **26** (12.0 mg, 6.03 µmol) was dissolved in THF (1 mL) and subjected to saponification and de-O-acetylation according to the general procedure of saponification of methyl ester and de-Oacetylation to give **27** as sodium salt (7 mg, 72%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.87 – 7.04 (m, 19H, CH Aromatic), 5.13 (d, *J* = 17.5 Hz, 2H, CH_2-linker), 5.00 – 4.85 (m, 3H, Nap*H*H, PhCH_2, Ph*H*H), 4.74 (dd, *J* = 33.3, 10.9 Hz, 3H, Ph*H*H,

Nap*H*H, H-4[a]), 4.55 (dd, *J* = 12.9, 8.4 Hz, 3H, $PhCH_2$, H-1[d]), 4.48 (s, 2H, $PhCH_2$-linker), 4.46 – 4.37 (m, 3H, H-1[a], H-1[b], H-1[C]), 4.12 (t, *J* = 6.9 Hz, 1H, H-2[a]) 4.06 (d, *J* = 3.3 Hz, 1H, H-4[c]), 3.99 – 3.90 (m, 2H, H-3[c], H-2[a]), 3.88 – 3.84 (m, 1H, C*H*H-linker), 3.83 – 3.68 (m, 5H, H-6ab, H-4[d], H-5[d], H-4[b]), 3.64 (dd, *J* = 11.8, 4.4 Hz, 1H, C*H*H-linker), 3.59 (dd, *J* = 10.6, 3.3 Hz, 1H, H-5[b]), 3.54 – 3.44 (m, 4H, H-5[c,d], H-3[d,b]), 3.21 (d, *J* = 24.9 Hz, 2H.), 2.04 (s, 2H), 1.95 – 1.82 (m, 3H), 1.57 – 1.11 (m, 15H), 1.08 (d, *J* = 6.5 Hz, 2H), 0.97 – 0.75 (m, 4H). ^{13}C NMR (151 MHz, cd_3od) δ 127.10, 126.40, 127.29, 128.63, 129.94, 125.87, 126.11, 127.39, 123.28, 125.28, 123.97, 127.91, 127.63, 126.40, 127.69, 127.23, 117.73, 66.96, 66.92, 74.72, 74.27, 74.34, 74.35, 75.72, 73.40, 74.65, 100.06, 72.89, 49.90, 47.96, 49.96, 51.30, 101.09, 104.51, 51.57, 68.00, 52.04, 77.09, 77.43, 69.83, 61.50, 80.53, 76.95, 69.80, 71.19, 73.24, 68.94, 77.14, 80.47, 61.50, 82.82, 82.31, 83.69, 73.92, 72.21, 82.23, 75.79, 77.13, 68.94, 48.43, 47.87, 45.88, 49.21, 46.83, 46.19, 39.48, 31.33, 28.33, 37.87, 22.08, 20.04, 20.79, 23.39, 21.89, 22.64, 28.77, 27.19, 28.76, 22.57, 29.26, 30.57, 27.94, 31.37, 28.36, 22.08, 13.03, 21.64,18.84. ESI-MS: m/z: calcd. for $C_{75}H_{90}N_3O_{28}S$: 1575.5437; found : 1575.6952

Global debenzylation: A solution of the tetrasaccharide **27** (7 mg, 4.32 µmol) in MeOH (2 mL) was subjected to debenzylation according to the general procedure for global debenzylation to give tetrasaccharide **28** (2.7 mg, 63%) ^{1}H NMR (800 MHz, Deuterium Oxide) δ 4.57 (d, J = 2.3 Hz, 1H, H-4[a]), 4.40 – 4.28 (m, 4H, H-1[a] and H-1[c], H-1[b]and H-1[d]), 4.01 (d, J = 3.0 Hz, H1,H-4[c]), 3.91 – 3.82 (m, 3H, H-3[a], H-2[a], H-2[c]), 3.76 (ddt, J = 11.9, 9.2, 4.2 Hz, 2H,O-C*H*H), 3.68 – 3.46 (m,11H, H-3[c], H-5[a], H-5[c], H-6ab[a,c],H-4[b], H-5[b,d], O-CH_2-linker), 3.41 (td, J = 9.1, 2.7 Hz, 1H,H-3[b]), 3.31 (dd, J = 8.2, 3.5 Hz, 2H, H-3[d], H-4[d]), 3.24 (td, J = 8.8, 2.9 Hz, 1H, H-2[b]), 3.15 (td, J = 8.1, 3.1 Hz, 1H, H-2[d]), 2.83 (t, J = 7.6 Hz, 2H, N-CH_2-linker), 1.86

(dd, J = 8.3, 2.6 Hz, 6H, CH_3, NHC=OCH_3), 1.51 (t, J = 7.8 Hz, 2H, CH_2-linker), 1.45 (t, J = 6.7 Hz, 2H, CH_2-linker), 1.29 – 1.22 (m, 2H, CH_2-linker). ^{13}C NMR (201 MHz, d_2o) δ 103.64 , 100.94, 80.09, 76.23, 76.09, 75.08, 74.71, 74.69, 74.21, 73.4, 73.25, 72.55, 71.96, 71.57, 69.86, 69.85, 67.4, 60.85, 60.82, 51.37, 51.3, 50.92, 39.16, 27.86, 26.15, 22.04,21.94. ESI-MS: m/z: calcd for $C_{33}H_{54}N_3O_{26}S$: 469.6153, found: 469.5938 $[M-2H]^{2-}$.

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-[(Methyl 2-O-2,5difluorobenzoyl,3-O-benzyl,-β-Dglucopyranosyluronate)-(1→3)-(4,6-di-_O_-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2-O-2,5difluorobenzoyl,3-O-benzyl - β-D-glucopyranosyluronate)]-(1→3)-6-_O_-benzyl-4-_O_-levulinoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (29)

The Nap ether was removed from tetrasaccharide **23** (126 mg, 57.4 μmol) in 3:.3 (DCM: PBS buffer) by the general procedure of cleavage of Nap ethers to provide **28** (56 mg, 47%). 1H NMR (500 MHz, Chloroform-d) 7.77 – 7.03 (m, 35H, CH Aromatic), 6.95-6.72 (m, 2H, N*H*TCA^a, N*H*TCA^c), 5.53 (d, J = 3.6 Hz, 1H, H-4^a), 5.41 (d, J = 3.4 Hz, H-4^c), 5.21 (dd, J = 8.2, 7.1 Hz, 1H,H-2^d), 5.19 – 5.05 (m, 3H, PhC*H*$_2$, H-2^d), 4.94 (d, J = 8.5 Hz, 1H, H-1^d), 4.92 – 4.83 (m, 3H, NapC*H*H, PhC*H*H, H-1^a), 4.82 – 4.74 (m, 3H PhC*H*$_2$,H-1^c), 4.72 – 4.66 (m, 2H, NapC*H*H, H-1^b), 4.60 (s, 1H. H-3^a), 4.56 – 4.42 (m, 4H, PhC*H*$_2$, CH_2-linker), 4.25 – 4.15 (m, 2H, H-3^c, H-4^b), 4.09 – 3.92 (m, 4H,H-4^b, H-5^d, H-2^c, H-6a), 3.88 (d, J = 9.1 Hz, 1H, H-5^b), 3.85 – 3.74 (m, 5H, H-5^c, H-6b,H-5^a, H-3^d, C*H*H), 3.73 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.64 (t, J = 8.3 Hz, 1H, H-3^b), 3.53 (qd, J = 10.0, 6.2 Hz, 2H, H-6ab^a), 3.46

(s, 1H, H-2[a]), 3.16 (d, J = 33.0 Hz, 2H, N-CH_2-linker), 2.80 - 2.52 (m,4H, CH2-Lev 2.17 (s, 3H,CH_3 - Lev), 2.03 (d, J = 14.3 Hz, 6H, H_3CC=O), 1.26 (s, 6H, CH_2-linker). ^{13}C NMR (126 MHz, cdcl$_3$) δ 128.21, 128.00, 127.73, 121.41, 118.62, 118.37, 118.28, 100.29, 100.21, 99.38, 98.63, 80.68, 80.16, 77.27, 74.6, 74.58, 74.57, 73.84, 73.79, 73.67, 73.19, 73.05, 72.81, 71.86, 71.49, 69.8, 69.52, 68.51, 68.49, 67.13, 61.68, 61.66, 56.39, 52.9, 52.78, 50.31, 46.62, 38.09, 37.88, 29.71, 29.1, 28.04, 27.8, 23.17,20.57. $C_{94}H_{97}Cl_6F_4N_3O_{31}$: 2076.4936; found 2076.5986

_N_-(Benzyl)-benzyloxycarbonyl-5-aminopentyl _O_-[(methyl - 2-O-2,5difluorobenzoyl,3-O-benzyl,-β-Dglucopyranosyluronate)-(1→3)-O-(6-_O_-benzyl-4-_O_-levulinoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside)-(1→4)-O-(methyl -2-O-2,5difluorobenzoyl,3-O-benzyl,4-_O_- naphthyl-β-Dglucopyranosyluronate)-(1→3)-O-(4,6-di-_O_-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-O-(methyl -2-O-2,5difluorobenzoyl,3-O-benzyl,-β-Dglucopyranosyluronate)]-(1→3)-6-_O_-benzyl-4-_O_-levulinoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside(29)

Disaccharide N-phenylimidate donor **4** (50 mg, 40.8 μmol) and tetrasaccharide acceptor **28** (56 mg, 27.2 μmol) were combined in a flask, coevaporated with toluene (3 × 3 mL), and dissolved in anhydrous DCM (500 μL) . Powdered freshly activated 4 Å molecular sieves were added, and the mixture was stirred for 30 min at ambient temperature and then cooled to -35 °C. TfOH (2.5 μL, 27.2 μmol) stirring was continued until TLC indicated the disappearance of donor (~30 min). The reaction was then quenched by the addition of TEA. The mixture was filtered and

the filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography using a gradient of toluene and EtOAc to give pure Hexasaccharide (68 mg, 80%).^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.93 – 6.96 (m, 51 H, CH Aromatic), 6.82 (dd, *J* = 20.9, 8.2 Hz, 3H, NHTCA^{a,b,c}), 5.50 (s, 2H, H-4^{a,e}),5.39 (s, 1H, H-4^{c}) 5.23 – 5.03 (m, 5H, H-2^{b,d,f}, PhC*H*$_2$), 4.95 (d, J=8.5, 1H, H-1^{d}), 4.93 – 4.64 (m, 10H, NapC*H*H, PhC*H*H, H-1^{a} , PhC*H*$_2$,H-1^{c},H-1^{e}, H-1^{f} NapC*H*H, H-1^{b}), 4.62 – 4.37 (m, 6H, H-3^{a,e}, PhC*H*$_2$, CH_2-linker), 4.28 – 4.04 (m, 5H, $PhCH_2$, H-3^{c}, H-4^{b}, H-4^{f}), 4.04 – 3.92 (m, 3H, H-4^{b}, H-2^{a}, H-6a^{c}), 3.92 – 3.83 (m, 2H, H-5^{b}, H-5^{f},H-2^{b}), 3.80 (s, 3H, OCH_3), 3.78 – 3.59 (m,14H, H-5^{c}, H-6b^{c},H-5^{a},H-5^{e} H-3^{d}, C*H*H, 2OCH_3, H-3^{b,} H-3^{e}), 3.60 – 3.47 (m, 2H, H-6ab^{a}), 3.44 (s, 1H, H-2^{e}), 3.30 (d, *J* = 6.3 Hz, 2H, , H-6ab^{e}), 3.19 (d, *J* = 8.7 Hz, 1H), 3.12 (s, 1H), 2.73 – 2.40 (m,8H, CH_2-Lev), 2.14 (d, *J* = 12.5 Hz, 6H,CH_3-Lev), 2.05 – 1.95 (m, 6H, *H*$_3$CC=O), 1.49-1.1 (m, 6H, CH_2-linker). ^{13}C NMR (151 MHz, cdcl3) δ 128.02, 128, 127.82, 126.96, 126.87, 126.74, 126.07, 125.83, 125.28, 121.31, 118.53, 118.27, 118.24, 100.16, 99.96, 99.85, 99.79, 99.18, 98.49, 81.69, 79.8, 78.88, 77.27, 77.27, 77.14, 75.15, 75.09, 75.05, 74.87, 74.76, 74.64, 74.59, 74.55, 74.42, 74.3, 74.12, 73.84, 73.65, 73.65, 73.64, 73.63, 73.58, 73.39, 73.04, 72.93, 72.76, 72.66, 71.45, 70.34, 69.98, 69.97, 69.18, 68.54, 68.49, 67.95, 67.13, 67.13, 65.54, 61.97, 61.95, 56.39, 54.87, 54.86, 54.58, 53.03, 52.92, 52.61, 50.3, 49.96, 47, 45.97, 38.06, 38.06, 38.06, 34.94, 34.16, 29.76, 29.66, 29.03, 27.9, 27.48, 26.22, 26.02, 25.4, 25.33, 25.26, 24.54, 23.24, 21.45, 20.75, 20.66, 17.63, 16.85, 15.93,15.09. HR MALDI-TOF MS: m/z: calcd for $C_{146}H_{145}Cl_9F_6N_4O_{45}$ [M+Na]+ : 3131.7894; found 3131.6037

Hexasaccharide Deprotection:

5-Aminopentyl(β-D-glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4-O-sulfonato-β-D-galactopyranoside)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4-O-sulfonato-β-D-galactopyranoside (31)

O-sulfation: After *cleavage of Lev esters,* hexasaccharide **29** (33 mg, 20.6 μmol) was dissolved in a 6M solution of SO_3·TMA and DMF and O-sulfated according to the general procedure providing **30** (35 mg, 98 %) as a sodium salt. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.02 – 6.98 (m, 51H, CH Aromatic), 5.40 (t, *J* = 7.4 Hz, 1H, H-2^b), 5.27 (m, 1H, d, J = 3.6 Hz, 1H, H-4^c), 5.19 (t, J = 7.4 Hz, 1H, H-2^d) , 5.11 (d, J = 16.4 Hz, 2H, CH_s-linker), 5.01 – 4.93 (m, 3H,PhCH_2, H-2^f), 4.92 – 4.80 (m, 6H, Nap*H*H, H-4^{a,e}, H-1^d, H-1^a, H-1^e), 4.75 (t, *J* = 12.0 Hz, 1H, Ph*H*H), 4.60 (d, *J* = 11.6 Hz, 1H, Ph*H*H), 4.53 (dd, *J* = 22.6, 7.8 Hz, 3H, H-1^{c,f}, Nap*H*H), 4.44 (s, 2H, CH_2-linker), 4.36 (s, 1H, H-1^d), 4.25 (t, *J* = 7.9 Hz, 1H, H-3^c), 4.07 (dd, *J* = 26.3, 8.7 Hz, 2H, H-4^d, H-5^d), 4.01 – 3.74 (m, 9H, C*H*H-linker, H-2^{a,c,e}, H-4^{b,f}, H-5^b, H-5^c, H-5, H-6ab, H-3^d), 3.70 (d, *J* = 14.5 Hz, 9H, 2OCH_3, H-3^{b,f}, C*H*H-linker), 3.67 – 3.62 (m, 1H, C*H*H-linker), 3.16 (d, J = 33.0 Hz, 2H, N-CH_2-linker) 1.94 (d, *J* = 13.3 Hz, 6H, 2H_3CC=O) 1.46– 1.14 (m, 6H, 2CH_2-linker). ^{13}C NMR (151 MHz, cd$_3$od) δ 128.51, 128.32, 127.72, 127.62, 127.51, 127.44, 126.3,

125.62, 125.59, 121.12, 118.09,100.7, 100.67, 100.56, 100.45, 100.18, 99.47, 81.93, 79.61, 79.48, 79.03, 76.59, 76.1, 75.37, 74.75, 74.73, 74.72, 74.61, 74.6, 74.49, 74.47, 74.26, 74.14, 73.81, 73.79, 73.74, 73.7, 73.64, 73.3, 73.15, 72.98, 72.98, 70.34, 70.1, 70.09, 68.78, 66.96, 54.31, 54.04, 53.78, 51.34, 50.63, 50.02, 49.96, 48.77, 48.42, 47.87, 41.32, 35.56, 29.31, 28.86, 27.43, 22.87,19.32. ESI-MS: m/z: calcd. for $C_{136}H_{131}Cl_9F_6N_4O_{47}S_2$: 1535.2265 found: 1535.7038 $[M-2H]^{2-}$.

Global debenzylation: After the saponification of methyl esters and de-O-acetylation followed by subsequent selective N-acetylation, the final target was produced by utilizing the general procedure for global debenzylation. .500 mg of **31** was rendered. ^{1}H NMR (800 MHz, Deuterium Oxide) δ 4.58 (d, J = 2.3 Hz, 1H, H-4^{a}, H-4^{e}), 4.50 – 4.28 (m, 6H, H-1^{a}, H-1^{c}, H-1^{e} H-1^{b} H-1^{d}, H-1^{f}), 3.99 (d, J = 3.0 Hz, H1,H-4^{c}), 3.96 – 3.75 (m, 5H, H-3^{a},H-3^{e} H-2^{a},H-2^{e} H-2^{c}), 3.76 (ddt, J = 11.9, 9.2, 4.2 Hz, 2H,O-C*H*H), 3.68 – 3.46 (m,11H, H-3^{c}, H-$5^{a,e}$, H-5^{c}, H-6ab^{a,c},H-$4^{b,}$, H-$5^{b,d,f}$, O-CH_2-linker), 3.41 (td, J = 9.1, 2.7 Hz, 1H,H-3^{b}), 3.33 (dd, J = 8.2, 3.5 Hz, 2H, H-3^{d}, H-4^{d}), 3.24 (td, J = 8.8, 2.9 Hz, 1H, H-$2^{b,}$ H-2), 3.15 (td, J = 8.1, 3.1 Hz, 1H, H-2^{d}), 2.83 (t, J = 7.6 Hz, 2H, N-CH_2-linker), 1.83 (dd, J = 8.3, 2.6 Hz, 9H, NHC=OCH_3), 1.51 (t, J = 7.8 Hz, 2H, CH_2-linker), 1.45 (t, J = 6.7 Hz, 2H, CH_2-linker), 1.29 – 1.22 (m, 2H, CH_2-linker). ^{13}C NMR (201 MHz, d2o) δ 104.27, 103.28, 101.06, 100.83, 80.14, 76.6, 76.51, 76.44, 76.33, 75.03, 74.89, 74.81, 74.42, 73.59, 72.43, 72.1, 71.78, 69.99, 69.98, 67.62, 61, 51.65, 51.61, 50.93, 39.3, 27.96, 26.33, 22.49, 22.26, 22.05. ESI-MS: m/z: calcd. for $C_{47}H_{71}N_4O_{40}S_2$: 710.2565, found: 710.1383$[M-2H]^{2-}$

3.4 REFERENCES

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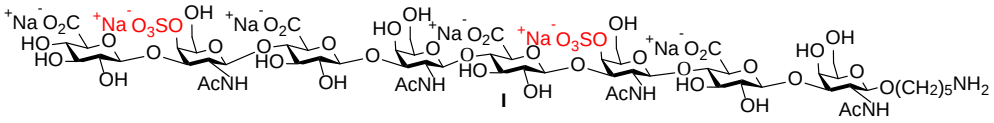
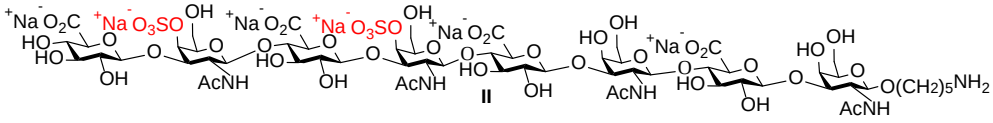
CHAPTER IV

CONCLUSION

4.1 CONCLUSION AND FUTURE OUTLOOK

Since the deprotection and sulfation of the hexasaccharide proved to be very difficult, we are currently investigating ways to optimize these steps. The synthetic strategy utilized must apply to substantial molecules such as octassacharides and possibly dodecasaccharides. After a thorough examination of our previous route, we have concluded that the TCA protection of the 2-amino-2-deoxy sugar GalNac was the feature creating the most difficulty.We surmised that this was due to it is stability to basic conditions. Therefore, we decided to switch to the more base labile ethoxy carbonyl Troc. Though it is less stable in basic conditions, it also acts as a neighboring group auxillary assuring 1,2-trans-glycosides.





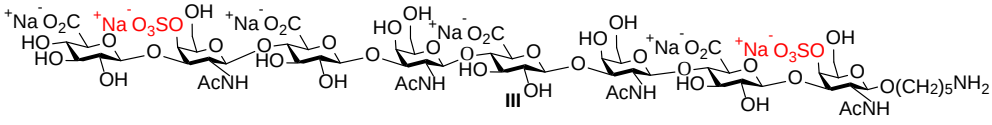
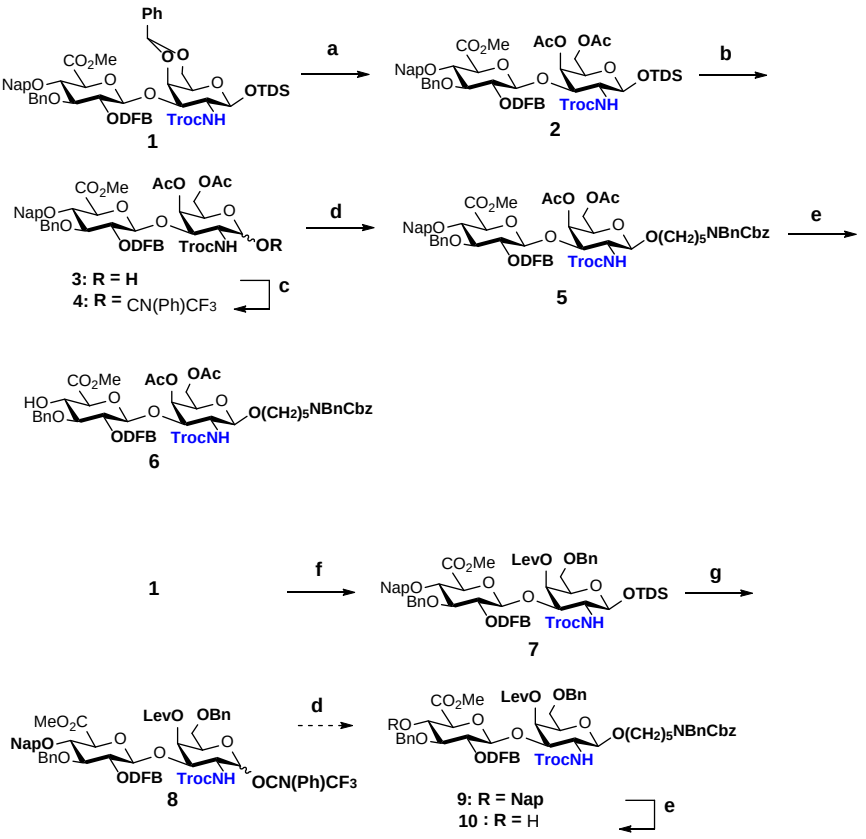


Figure 4.1: Low sulfated CSA Octasaccharide Targets

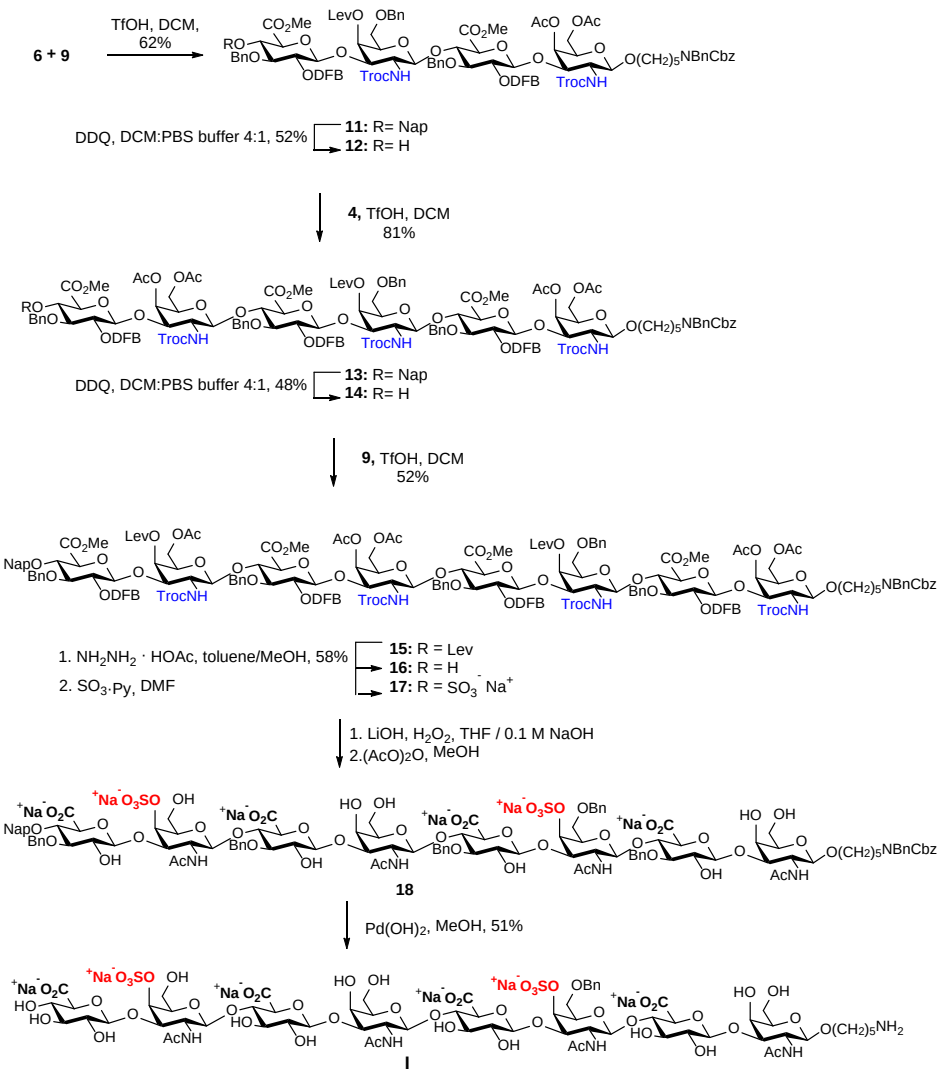
Troc bearing disaccharide **1** has been modified into glycosyl donors (**4** , **8**) and acceptors (**6 , 10**) according to established protocols (Scheme 4.1). These disaccharides are now being utilized to build octasaccharide targets **I**, **II**, and **III**. The present targets are based on low sulfated CS fragments observed as responsible for the sequestering of the parasite *Plasmodium falciparum*-infected red blood cells in the placenta. These compounds will be used to elucidate the definite structures responsible for this pathogenic activity.



Scheme 4.1: a) i.TFA: DCM:H_2O 64% ii. Ac_2O, DMAP, Pyr 84% b) HF, Pyr, 0º C, 76% c) $F_3C(NPh)Cl$, NaH d) DCM, $BnCbzN(CH_2)_5OH$, TMSOtf,DCM, 77%, 0 ^{0}C e)

DDQ, 4:1 DCM: H_2O f) i. TfOH, Et_3SiH, DCM,-78^{0}C ,86% ii. Lev_2O,DMAP,72% g) $F_3C(NPh)Cl$, NaH,DCM

Iterative cycles of Nap removal and glycoslations yielded the fully protected octasaccharide **15** in 52% yield (Scheme 4.1). After Lev removal and sulfation, molecule **17** was saponified, and all esters, as well as Troc groups, were removed overnight. This was a significant improvement because with the previous route this reaction took several days and had a very poor yield. The acetylation also only took 30 min which was a marked improvement from the previous synthesis. Finally, hydrogenation gave the fully the deprotected CSA-CSO-CSA-CSO octasaccharide **I** target in a 51% yield.



Scheme 4.2: Synthesis of Heterogeneous CS-A-CS-O-CS-A-CS-O Octasaccharide

The synthesis of additional targets **II** and **III** are currently ongoing in our lab. Upon completion, these compounds will undergo binding affinity studies by the Gowda lab. This research group specializes in molecular interactions and signaling mechanisms in malaria pathogenesis and protective immunity development.