NEW METHODS FOR THE CHEMICAL SYNTHESIS OF GLYCOSAMINOGLYCANS

by

OMKAR PRABHAKAR DHAMALE

(Under the Direction of Geert-Jan Boons)

ABSTRACT

Glycosaminoglycans (GAGs) represent a major portion of proteoglycans which in turn are an integral part of the extracellular matrix surrounding mammalian cells. GAGs promote important biological functions as they participate in a wide range of physiological processes like metabolism, transport and regulation. Many of these interactions have been extensively studied and characterized, but the GAG motifs that mediate these important interactions have been seldom understood. The incapability to perform necessary structure-activity-relationship studies is due to the lack of novel synthetic technologies to access a library of well-defined GAG oligosaccharides. This difficulty can be attributed to the structural complexity associated with GAG which arises from an equally complex biosynthetic pathway. Significant members of the GAG family are heparin and heparan sulfate (HS) and dermatan sulfate (DS).

Therefore, we have designed new techniques for the stereoselective synthesis of HS and DS oligosaccharides that employ a fairly small number of orthogonally protected building blocks, which can easily be converted into glycosyl donors and acceptors and utilized to construct respective GAG fragments. We have used a preglycosylation

oxidation approach to address the issue of installation of uronic acids. A novel C2 ester participating functionality (pivaloyl acetate) was utilized for stereoselective 1,2-trans glycosidic linkages without compromising coupling efficiency. In the modular synthesis of DS, our strategic design allowed us to incorporate sulfates at all the required positions with absolute control and precision. We utilized orthogonal protecting groups for the construction of iduronic acid and galactosamine building blocks which allowed us to rapidly disassemble the derivatives to their final deprotected forms. We also attempted to construct large HS oligosaccharides fragments using a modular strategy by performing a rigourous study to achieve efficient stereoselective α-1,2 cis glycosidic coupling between glucosamine donor and GlcA acceptor using a non-participating azido functionality at the C2 position of the glucosamine donor.

Thus, we have reported new chemical strategies for the synthetic construction of GAG motifs which will be helpful in addressing the problems associated with the poor reactivity profile of uronic acids. Using the discussed methodologies we set a premise for generating newer synthetic strategies for the construction of complex GAG oligosaccharides.

INDEX WORDS: Glycosaminoglycans, heparan sulfate, dermatan sulfate, uronic acids, modular synthesis, stereoselective synthesis, pre/post-glycosylation oxidation.

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DEDICATION

I would like to dedicate my thesis to my parents Deepa Dhamale and Prabhakar Dhamale for their undying support, love and encouragement. Your confidence and belief in me and my abilities has given me immense motivation.

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LIST OF ABBREVIATIONS

Ac .....Acetyl

AcCl…….....Acetyl chloride

AcOH.....Acetic acid

Ac_2O.....Acetic anhydride

AgOTf.....Silver triflate

Ar .....Aryl

AT III.....Antithrombin

Bn .....Benzyl

BuOH.....Butanol

Bz .....Benzoyl

C.....Carbon

Cbz .....Benzyloxycarbonyl

$C_6H_5CH(OMe)_2$.....Benzaldehyde dimethylacetal

$CHCl_3$ .....Chloroform

CH_3CN.....Acetonitrile

CSA.....Camphorsulfonic acid

CO_2.....Carbon dioxide

$CuSO_4$.....Copper sulfate

CuO.....Copper (II) oxide

$CuCl_2$ .....Copper (II) chloride

COSY .....Correlation spectroscopy

DBU.....1,8-Diazabicylco[5.4.0]undec-7-ene

DCC.....Dicyclohexyl carbodiimide

DCE.....1,2 Dichloroethane

DCM .....Dichloromethane/Methylene chloride

DMAP .....N, N-Dimethylaminopyridine

DMF.....N, Ndimethylamineformamide

E. coli .....Escherichia coli

ESI-MS .....Electrospray ionization mass spectrometry

Et_3SiH .....Triethylsilane

EtSH .....Ethanethiol

EtOH..... Ethanol

Et_2O.....Diethyl ether

GlcA.....Glucuronic acid

GlcNAc .....N-Acetyl glucosamine

GlcNS .....N-sulfonato glucosamine

H_2.....Hydrogen

HEPES .....Hydrogen Bromide

HF.....Hydrogen fluoride

HMBC.....Heteronuclear multiple bond correlation

NAN_3.....Sodium azide

NaOH.....Sodium hydroxide

NaOMe.....Sodium Methoxide

$Na_2S_2O_3$ .....Sodium thiosulfate

NH_3 .....Ammonia

NH_2NH_2 .....Hydrazine

o.....Ortho

OD.....Optical density

p .....Para

Pd .....Palladium

$Pd(OH)_2$ .....Palladium hydroxide

PBS .....Phosphate buffered saline

Ph_2SO.....Diphenyl sulfoxide

PivOAc.....Pivaloyl acetate

PMe_3 .....Trimethyl phosphine

ppm .....parts per million

PTFE .....Polytetrafluoroethylene

TBDMS.....Tert-butyl dimethylsilyl

TFA.....Trifluoroacetic acid

Tf_2O .....Trifluoromethane sulfonic anhydride

TfOH.....Trifluoromethane sulfonic acid

TLC.....Thin layer chromatography

THF.....Tetrahydrofuran

TMS.....Trimethylsilyl

TMSOTf .....Trimethylsilyl trifluoromethanesulfonate

TNF-α.....Tumor necrosis factor alpha

TOCSY .....Total correlation spectroscopy

TsOH.....p-Toluenesulfonic acid

UV .....Ultraviolet

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Glycosaminoglycans

Proteoglycans are an integral part of the extracellular matrix that surrounds all mammalian cells. Different core proteins are used to anchor the glycosaminoglycans (GAGs) polysaccharides outside the lipid bilayer. Protoeglycans consist of a core protein and one or more covalently attached GAG chains. GAGs are linear polysaccharides whose disaccharide building blocks consist of an amino sugar (*N*-acetylglucosamine, glucosamine that is variously *N*-substituted or *N*-acetylgalactosamine) and uronic acid (glucuronic acid or iduronic acid) or galactose. Heparin and heparin sulfate are the most complex GAGs, a family of molecules that comprises chrondroitin sulfate, keratin sulfate and dermatan sulfate (Figure 1.1). GAGs promote important biological functions by binding to different growth factors, enzymes, morphogens, cell adhesion molecules, and cytokines. [1-6]

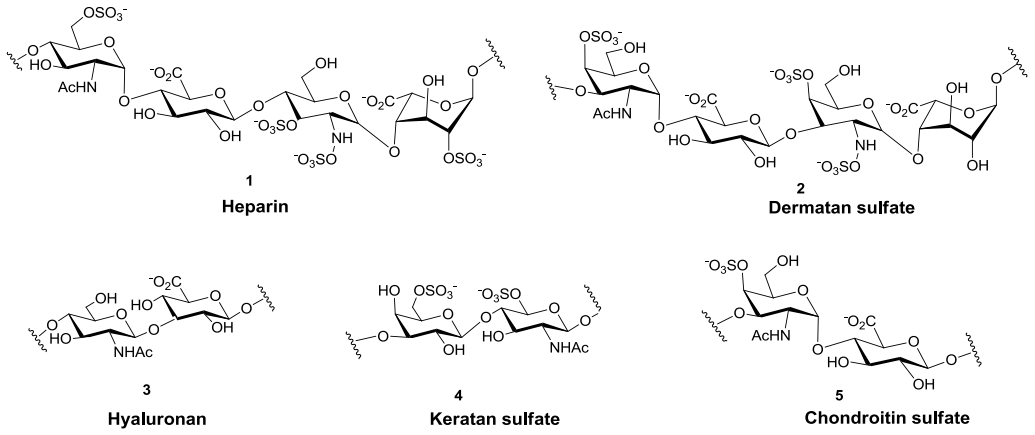

Figure 1.1: Examples of glycosaminoglycans

GAGs are associated with a high degree of heterogeneity with regards to disaccharide construction, molecular weight and sulfation pattern which can be attributed to the fact that GAG synthesis, unlike proteins or nucleic acids, is not template driven, and dynamically controlled by processing enzymes.[7] Based on the core disaccharide structures, GAGs can be classified into five groups.[8] (Figure 1.2)

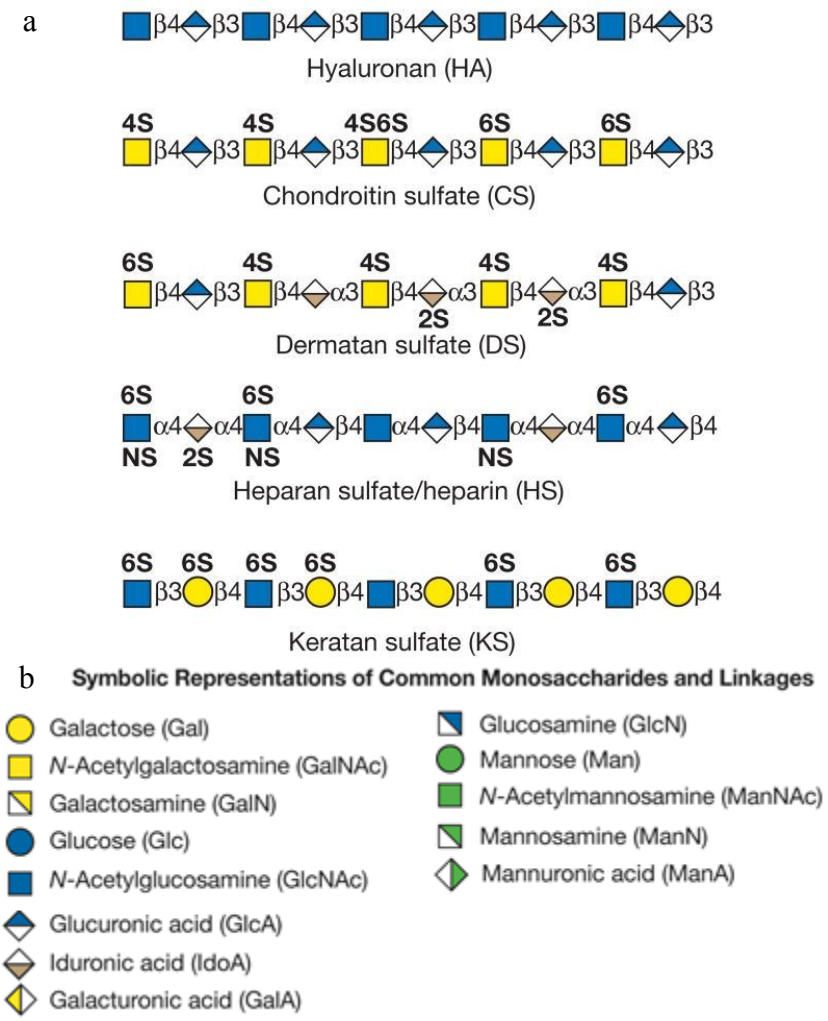

Figure 1.2: a) Carbohydrate sequences of the five types of glycosaminoglycans using the monosaccharide symbols Hyaluronan, Chondroitin sulfate, Dermatan sulfate, Heparin/Heparan sulfat and Keratan sulfate. Possible sulfation presence and location (2S, 4S or 6S) is indicated. b) Key to monosaccharide symbols.

Heparin/Heparan sulfate[9] and chrondroitin[10] /dermatan sulfate[11] are synthesized in the golgi apparatus, where core proteins are posttranslationally modified with *O*-linked glycosylations by glycosyltransferases. Heparan and heparin glycosaminoglycans are complex heterogeneous mixtures of repeating disaccharide units consisting of an uronic acid (D-glucuronic or L-iduronic acid) and D-glucosamine. Various degrees of sulfation occur (at the oxygen and/or nitrogen containing groups) on each monosaccharide unit, ranging from zero to tri-sulfation. Heparan sulfate is less sulfated than heparin.[9] (Figure 1.3)

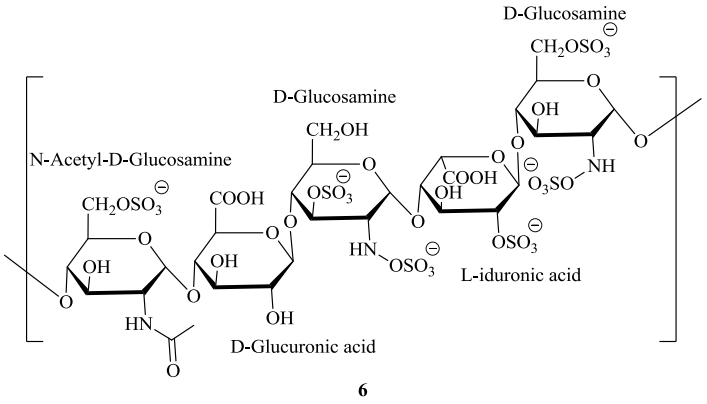

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Figure 1.3: Structures of Heparan and heparin glycosaminoglycans

Chondroitin sulfate and dermatan sulfate (chondroitin sulfate B) (Figure 1.4) are composed of disaccharide units containing *N*-acetylgalactosamine (GalNAc) and an uronic acid joined by β (1→3) linkages, respectively. Chondroitin sulfate contain glucuronic acid (GlcA) and are 4-*O*-sulfated (chondroitin sulfate A) or 6-*O*-sulfated (chondroitin sulfate C). Dermatan sulfate also contains *N*-acetylgalactosamine (GalNAc), but the uronic acid present in dermatan is L-iduronic acid (IdoA).[10,11]

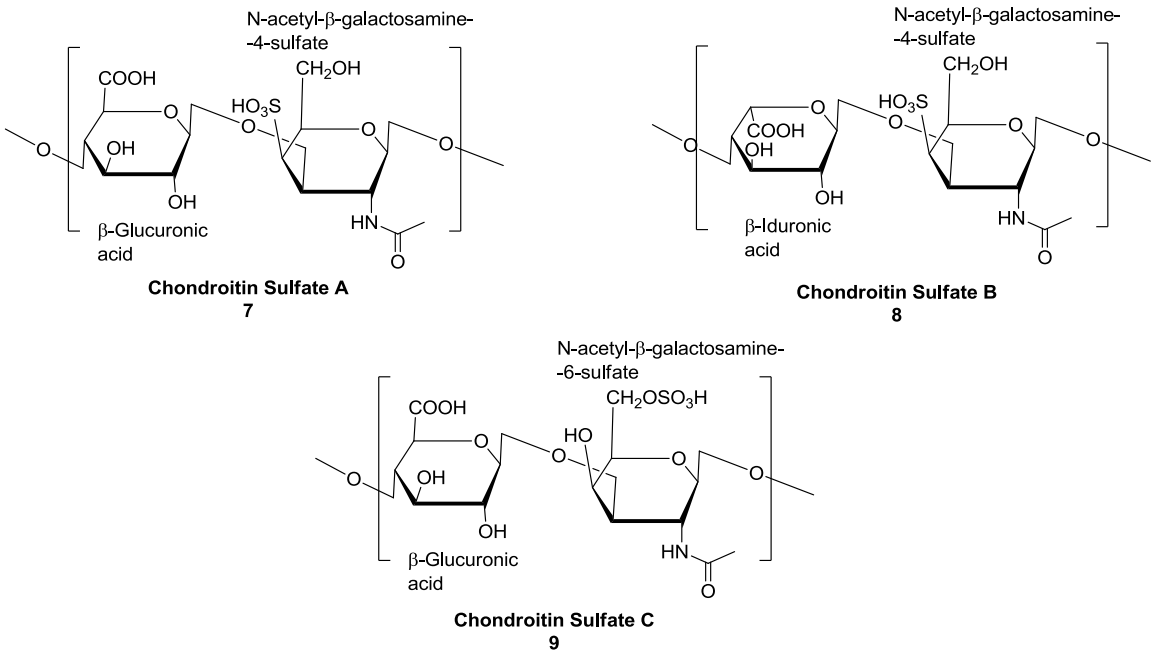
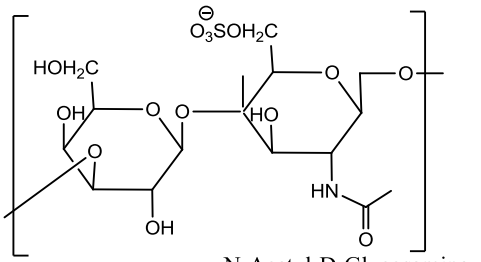


Figure 1.4: Structures of a) Chondroitin Sulfate A consisting of an alternating copolymer β-glucuronic acid-(1-3)-*N*-acetyl-β-galactosamine-4-sulfate; b) Chondroitin sulfate B (dermatan sulfate) consists of an alternating copolymer β-iduronic acid-(1-3)-*N*-acetyl-β-galactosamine-4-sulfate, c) Chondroitin sulfate C consists of an alternating copolymer β-glucuronic acid-(1-3)-*N*-acetyl-β-galactosamine-6-sulfate.

The third class of GAGSs, keratan sulfate, may modify core proteins through *N*-linked glycosylation or *O*-linked glycosylation of the proteoglycan. Keratan sulfate differs from the other glycosaminoglycan chains in that it does not contain uronic acid residues. Keratan is made up of *N*-acetyllactosamine (βGal-β (1→4)-GlcNAc) subunits.[8, 12] (Figure 1.5)



Keratan Sulfate
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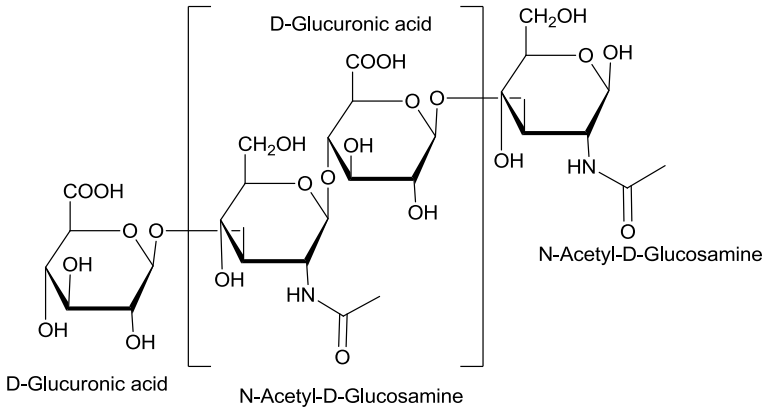
Figure 1.5: Structure of Keratan Sulfate consisting of an alternating polymer of β-D-Gal and GlcNAc

The fourth class of GAGs, hyaluronic acid is not synthesized by the golgi but by the integral membrane synthases. Unlike the other GAGs, Hyaluronan is not sulfated. Hyaluronan (HA; hyaluronic acid) is composed of alternating residues of β-D-(1→3) glucuronic acid (GlcA) and β-D-(1→4)-*N*-acetylglucosamine (GlcNAc).(Figure 1.6) Unlike the other glycosaminoglycans, hyaluronan does not attach to proteins to form proteoglycans.[13]



Hyaluronic acid
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Figure 1.6: Hyaluronic acid is composed of alternating residues of β-D-(1-3) glucuronic acid and β-D-(1-4)-N-acetylglucosamine.

Heparin and Heparan sulfate

Heparin has served as an anticoagulant in heart disease for more than 60 years based on its ability to accelerate the rate at which antithrombin inhibits serine proteases in the blood coagulation cascade. Heparin is second only to insulin as what could be conceived as a highly successful natural therapeutic agent.[5] Heparin is a linear polymer consisting of repeating units of 1→4- linked pyranosyluronic acid and 2-amino-2-deoxyglucopyranose residues. The uronic acid residues typically consist of 90% L-idopyranosyluronic acid (L-iduronic acid) and 10% D-glucuronic acid). Heparin has the highest negative charge density among all the biological macromolecules as a result of its high content of negatively charged sulfate and carboxyl groups.[14] A prototypical heparin disaccharide contains three sulfate groups with the *O*-sulfation normally occuring at the 2-position of the uronic acids and the 3- and /or 6-position of the glucosamine. In addition, the glucosamine nitrogen may be sulfated, acetylated or, less frequently may remain unmodified, thus resulting in 48 possible disaccharides that make up heparin.[1,15]

Heparan sulfate is structurally related to heparin but is much less substituted with sulfate groups than heparin and has a more varied structure. Like heparin, heparan sulfate, is a repeating linear copolymer of an uronic acid 1→4- linked to glucosamine. While D-glucuronic acid predominates in heparan sulfate, it can contain substantial amounts of L-iduronic acid.[16] Heparan sulfates generally contain only about one sulfate group per disaccharide.[17,18] While heparan sulfate contains all of the structural variations found in heparin, heparin is known to have greater structural variability, thus making the latter molecule much more complex. In theory, Heparan sulfate can contain 48 possible disaccharide structures, which can combine to form 110,592 hexasaccharides and 5

million octasaccharides. Of these only 23 disaccharides are known from structural studies.[6,15]

Structure of Heparin/ Heparan Sulfate

Heparin does not fold into tertiary structures like proteins but exists primarily as a helical structure.[19] The conformational flexibility of L-iduronic acid residue within heparin is believed to be responsible for the wide range of specific protein interactions.[20] These features are contrary to the poor binding properties and fewer biological interactions associated with the other GAGs having approximately the same degree of sulfation and molecular weight. Even though the IdoA residue is quite flexible, the glycosidic linkage in heparin is very rigid and therefore, the IdoA conformers do not alter the shape of the heparin polysaccharide. The sulfate and carboxyl groups of heparin also promote specific interactions with biologically important proteins.[21-23]

The pyranose rings of the monosaccharide residues adopt the $^{1}C_{4}$ and $^{4}C_{1}$ chair conformations as well as the $^{2}S_{0}$ skew boat conformation (Figure 1.7).[24-26] The conformation of the flexible L-iduronic acid (Figure 1.8) differs according to the substitution pattern and also its relative position in the chain. If the IdoA residue is present at the reducing end of the oligosaccharide, it prefers three conformations, the $^{4}C_{1}$ and $^{1}C_{4}$ chair and the $^{2}S_{0}$ skew boat.[29] When the IdoA bears a sulfate group at *C*2, the $^{1}C_{4}$ chair and the $^{2}S_{0}$ skew boat conformations are preferred. NMR studies have shown that in the latter case, the IdoA residues exist in an equilibrium between the chair and skew boat conformations.[30] The skew boat form is slightly favored as it affords conformational stability by avoiding the unfavorable 1,3-diaxial nonbonded interactions observed in the chair form. However, the energy barrier to the chair form is not high and therefore it is

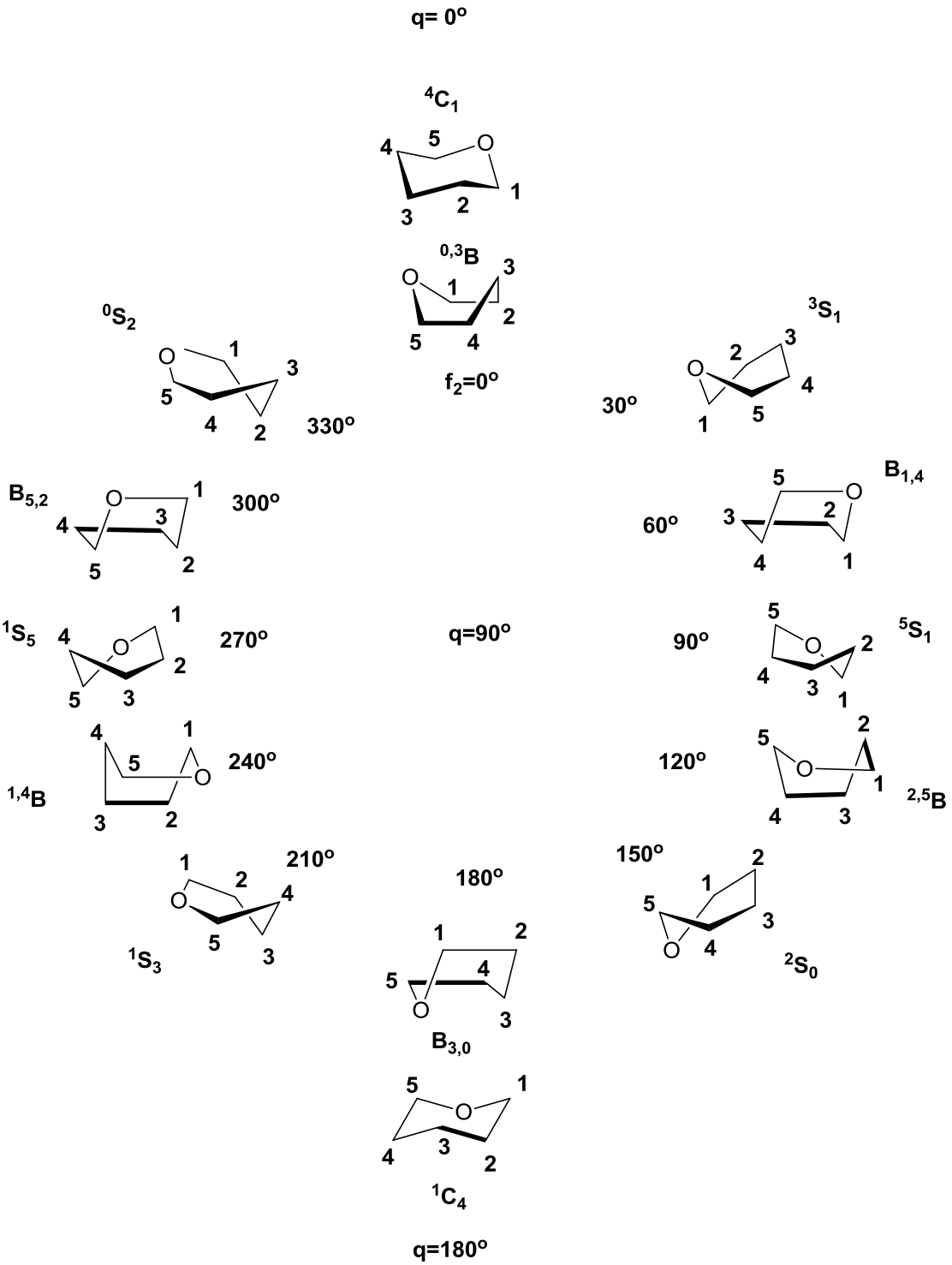


Figure 1.7: Schematic representation[25] of the conformational itinerary of the pyranose ring as defined by Jeffrey and Yates[27] and according to Cremer and Pople.[28]

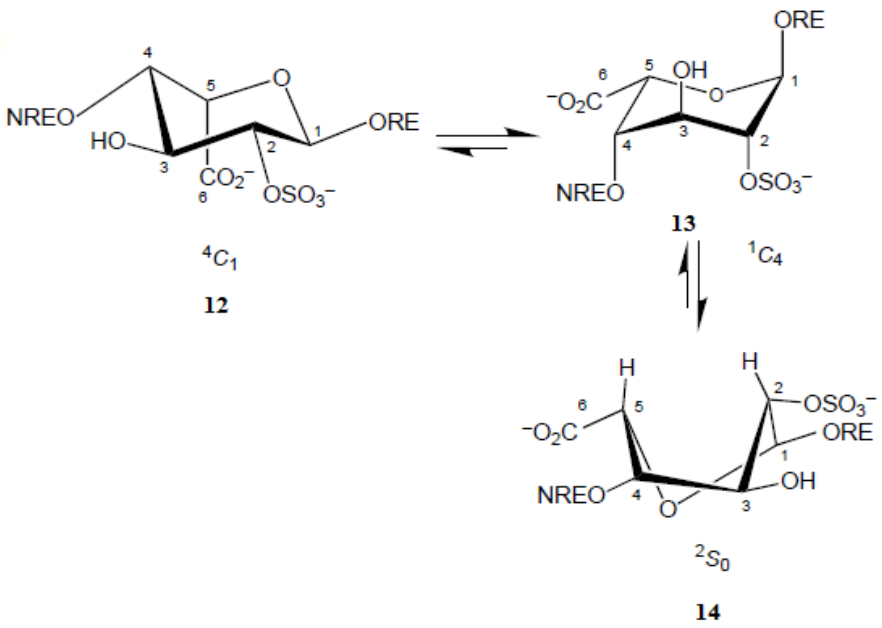
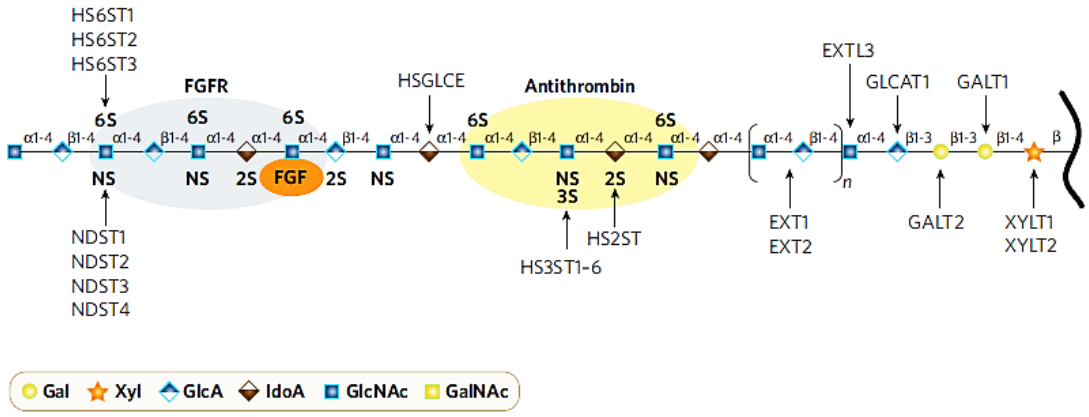


Figure 1.8: Conformational flexibility of IdoA residues.[5]

possible for the IdoA ring to flip between the two conformations. Unsunbstituted IdoA however resides predominantly in the $^1C_4$ chair form.[31]Different attractive forces come into play when heparin interacts with proteins. The negatively charged sulfate and carboxyl groups form ionic pairs with the positively charged amino acids on the proteins.[32] Nonionic interactions for example hydrogen bonding and hydrophobic forces are also involved in the binding. The binding affinity of the interaction depends on the ability of the oligosaccharide to provide optimal orientation of the sulfate groups complimentary with the protein. The complexity associated with the GAGs has further complicated the study of the structure-activity relationships. However, it has been shown that defined length and sequences of GAGs are responsible for the binding to proteins and hence play a role in modulating the biological activity of different proteins.[33]

Biosynthesis of Heparin

There have been several reports on the biosynthesis of heparin and heparan sulfate. However, the biosynthesis and the mechanism for the incorporation of different saccharide moieties along the backbone are still poorly understood. (Scheme 1.1)



Scheme 1.1: Biosynthesis of HS and formation of binding sites for ligands[9d]

The biosynthesis of GAG is known to take place primarily in the golgi apparatus. It begins with the attachment of a tetrasaccharide primer sequence to a serine residue in the backbone of the core protein.[34] The sequence of this linkage-region tetrasaccharide is *β*-GlcAp(1→3)-*β*-Gal(1→3)-*β*-Gal(1→4)-*β*-Xyl-1→Ser.Four different glycosyltransferases are responsible for the synthesis of each of the four glycosidic linkages within the primer sequence.[35] The glucuronyltransferase that attaches the second Gal residue and the galactosyltransferase that attaches the terminal GlcA[36] have been identified and characterized. This tetrasaccharide primer fragment will then act as an initiator sequence to further lengthen the glycosaminoglycan chain. The addition of the first *N*-acetylglucosamine (GlcNAc) or *N*-acetyl galactosamine (GalNAc) residue to the tetrasaccharide primer sequence determines whether the chain will be a glucosaminoglycan (heparin or heparan sulfate) or a galactosaminoglycan (chondroitin

sulfate or dermatan sulfate). The addition of the first hexosamine residue onto the tetrasaccharide primer is influenced by the adjacent peptide chain. It has been suggested that the amino acid linkage close to the tetrasaccharide primer region may be encoded with a signal for the addition of a GlcNAc residue, thereby intitiating heparin/heparan sulfate formation. The GlcNAc transferase which is believed to bring about this reaction has been identified.[37] The next step in the biosynthesis involves the transfer of GlcA and GlcNAc residues in an alternate fashion from their corresponding UDP-sugar nucleotides to the non-reducing end of the propagating chain to thus produce the remainder of the GAG chain. One unique enzyme, formed by a hetero-oligomeric complex of two proteins (EXT1 and EXT2) is believed to have both GlcA and GlcNAc transferase activity.[38] The synthesis of the linear polysaccharide chain is terminated after addition of almost 300 sugar residues.[39] Along with the elongation of the chain, other modification reactions also occur. The modification along the backbone of the biopolymer begins with the *N*-deacetylation and *N*-sulfation of the GlcNAc residues by enzymes, *N*-deacetylase and *N*-sulfotransferase respectively. Next, a C5 epimerase catalyzes the transformation of the D-glucuronic acid residues to the L-iduronic acid residues, followed by *O*-sulfation of the iduronic acid residues at the C2 position by an iduronosyl 2-*O*-sulfotransferase.[40] In order to introduce a sulfate group on the C2 position of iduronic acid residue in the disaccharide sequence IdoA-GlcNS, the enzyme iduronosyl 2-*O*-sulfotransferase has a functional requirement to have a free C6 hydroxyl group in the GlcNS residue.[41] The next step is the installation of the an-*O*-sulfate group at the C-6 position of GlcNAc and GlcNS with the aid of glucosamine 6-*O*-sulfotransferase.[41] The final step involves the action of 3-*O*-sulfotransferase to modify certain GlcNS6S residues.[42] 3-*O*- sulfation along

with a pentasaccharide sequence is minimally required to retain the anticoagulant activity of heparin. All the sulfotransferase reactions described above require 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a sulfate donor.

The structural heterogeneity of the polymer is thought to be brought about by the random and incomplete nature of the initial *N*-deacetylation. The specificity of this enzyme after its initial modification is also responsible for the block structures seen in heparan sulfate, where highly sulfated sequences are separated by nonsulfated disaccharides. The overall structural variability of the heparin/heparan sulfate polymer is the result of the incomplete nature of modifications by the enzymes and the factors which may serve as the regulating elements for these enzymes in the biosynthetic pathway are still unclear.[17,34]

Heparan sulfate's role in mammalian physiology

Owing to its highly sulfated nature, heparan sulfate interacts with a wide variety of proteins. These include growth factors, enzymes, extracellular matrix proteins and proteins found on the surface of pathogens. The overwhelming number of interactions led to the misconception that these interactions of heparin are nonspecific. However, this idea was quickly dismissed based on the mounting evidence for interactions between specific consensus structural motifs in HS and many HS-binding proteins and for the regulatory roles of these interactions.[3,43-45]

Heparan sulfate proteoglycans modulate nutritional metabolism:

In the circulatory system, HSPGs are known to influence lipid metabolism by acting as receptors for lipases, attaching them to endothelial cell surfaces and by acting as clearance receptors in the liver.[9d,46] It is known that dietary triglycerides and triglycerides

synthesized in the liver enter the circulation where they encounter lipases that are responsible for hyddrolysing triglycerides from the core, thereby generating fatty acids

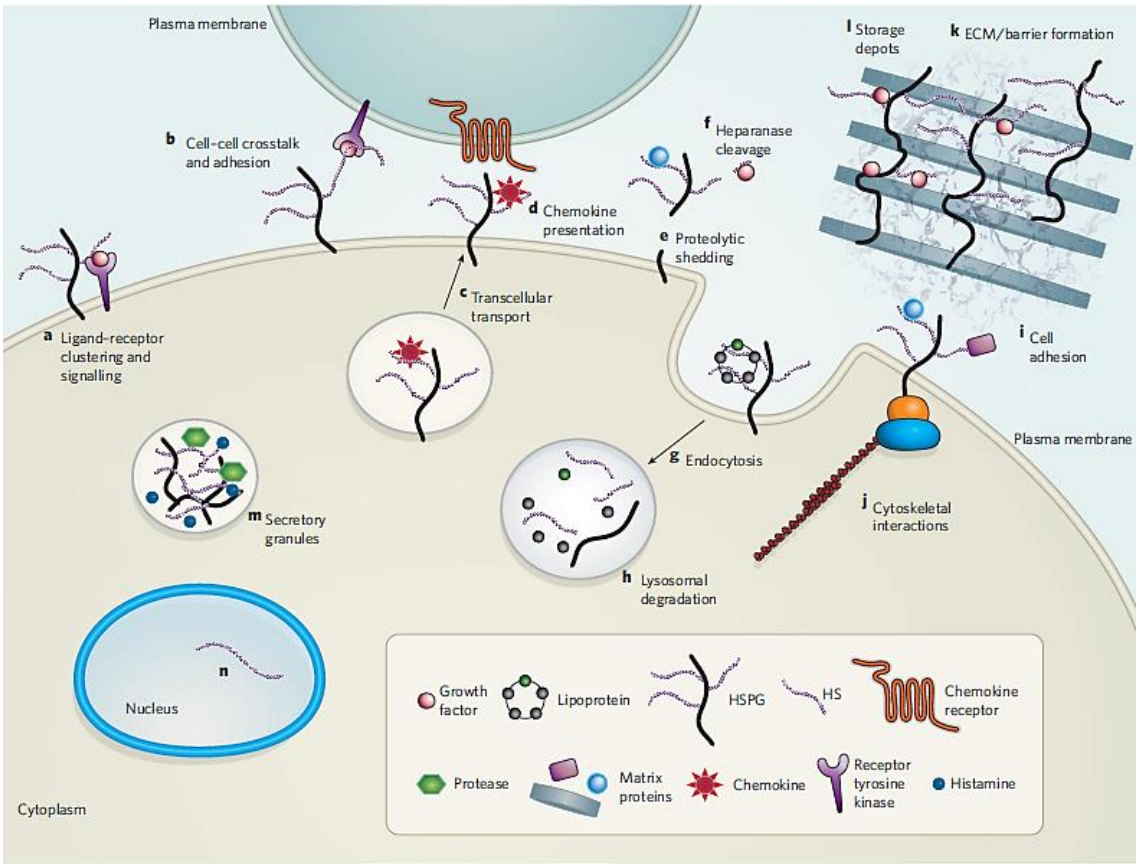


Figure 1.9: Role of HS proteoglycans in cellular physiology[9d]

useful for energy metabolism in peripheral tissues and lipid storage in adipose tissue. These lipases are usually not present in the plasma. They are instead found attached to the cells and are modulated by HSPGs. After hydrolyzing of the triglyceride core, (Figure 1.9) the remaining particles enter the liver sinusoids where the HSPGs facilitate their sequesteration and receptor mediated clearance, thereby allowing degradation of the particles in the lysosomes.[46]

Heparan sulfate proteoglycans in cell signaling and morphogenesis:

HSPGs play an important in the cellular machinery. They act as co-receptors for growth receptors as well as receptor tyrosine kinases. They also assist in the transport of chemokines across cells and their presentation on the cell surface. HSPGs are also known to facilitate cell adhesion to the extracellular matrix and form linkages to the cytoskeleton.[9d]

Heparan sulfate proteoglycans and their interactions with Antithrombin III:

The most well studied heparin binding protein is the serine protease inhibitor antithrombin III (AT III) that interacts with thrombin and factor Xa in the blood coagulation cascade.[47] The heparin-AT III interaction is responsible for the anticoagulant activity of heparin. A characteristic heparin pentasaccharide sequence, termed DEFGH, is required for binding to AT III.[48,49] The antithrombotic activity of heparin results from a ternary complex formed between heparin, AT III, and thrombin.[50,51] The protein undergoes a conformational change due to the interaction of AT III with the pentasaccharide sequence. AT III-pentasaccharide binding directly accelerates the inhibition of factor Xa but not that of thrombin. Although the pentasaccharide sequence is essential for binding for binding to AT III, a heparin chain containing 14-20 saccharide units is required to accelerate the AT III/thrombin interaction.(Figure 1.10) [52-54]

Involvement of heparan sulfate in various diseases:

The examples discussed above show how HSPGs have crucial roles in physiology. However under certain conditions, HSPGs are also known to contribute to pathophysiology. As an example, growth factor dependent signaling mediated by HSPGs is known to bring about primary tumor growth and angiogenesis in cancer.[9d,55] HSPGs

also promote amyloid deposition by facilitating formation of insoluble fibrils. The amyloid plaques seen in disorders such as

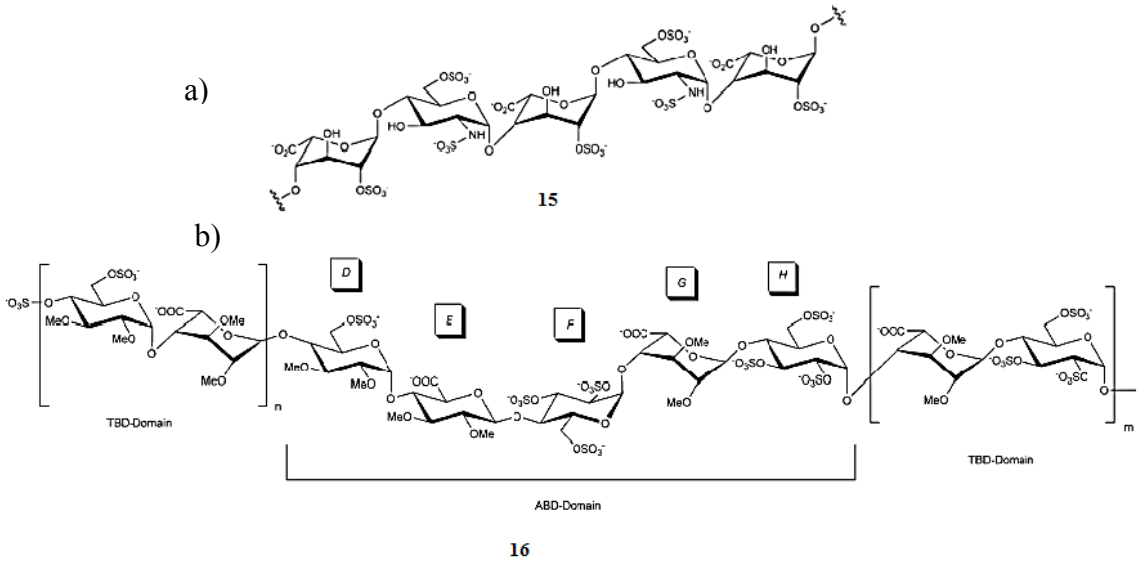


Figure 1.10: Structure of Heparin Oligosaccharides[1] a) the FGF Binding domain, b) the AT III binding domain

Alzeheimer's disease contain HSPGs supporting the fact that several amyloidogenic polypeptides bind to HS.[56-58] Literature has shown that there are a great number of proteins known to bind to heparin and therefore there is considerable interest in determining the HS sequences that make up the binding motifs for these proteins. This information could lead to the development of HS-based protein inhibitors [5,6,56]

Heparin/Heparan Sulfate based Drugs

Glycans recently have gained impetus as novel targets for drug targets owing to the important role they play in cellular interactions with drugs already being developed for the treatment of metabolic disorders, cancer and infection.[56] Heparin/Heparan sulfate and their mimetics are extremely important in this owing to their ability to bind and bring

about immobilization and/ or activation of a wide range of proteins which include growth factors, chemokinases and metalloproteinases.[59-61]

Heparin/Heparan sulfate mimetics as anticoagulants:

Anticoagulants based on heparin/HS are drugs of choice in the therapy and prophylaxis of thromboembolic diseases.[59] Structural studies have shown a unique pentasaccharide known as DEFGH GlcNAc/NS6S→GlcA→ GlcNS3S6S → IdoA2S → GlcNS6S (where Glc is glucosamine, IdoA is iduronic acid and GlcA is glucoronic acid, which are either sulphated or acetylated), comprises the AT-binding domain and is responsible for the anticoagulant activity of heparin. The 3-*O* sulfate group at position F is responsible for specific interactions with AT. The pentasacccharide sequence only inhibits the activity of FXa mediated by AT. A larger oligosaccharide sequence is required for the AT-mediated inhibition of thrombin. The structure activity relationship studies along with the crystal studies showed that the charged sulfate and carboxylate groups play an important role in the activation of AT and also increase the biological activity. Further, the hydrophobic interactions between the heparin pentasaccharide and AT also contribute to increasing the binding affinity.[62]

Since the successful clinical development programs of the 1990s, research on the heparin mimetic anticoagulants has gained tremendous impetus. GlaxoSmithKline registered Fondaparinux (**18**) (SR90107, Org31540) as a new anti-thrombotic drug under the name Arixtra1 after being granted approval from the US FDA and the European Committee for Proprietary Medical Products.[63] SR123781 is a short-acting hexadecasaccharide analogue of heparin with *N*-sulfate groups replaced by *O*-sulfates and alkylated hydroxyl groups in the AT-binding domain. It has tailor-made FXa- and thrombin-inhibitory activities

combined with more selectivity in its mode of action. Sanofi-Aventis discontinued the development of SR123781, after the success of heparin mimetic AVE 5026.[64] Idraparinux (**19**) is a synthetic pentasaccharide analogue of Fondaparinux, in which the hydroxyl groups are methylated and the *N*-sulfate groups are replaced by *O*-sulfates. Idraparinux (Kd of 1nM) is known to interact more strongly than Fondaparinux (Kd of 50nM) with AT through non-ionic interactions and also requires less rigorous synthetic methodology.[65] Idrabiotaparinux (**20**) (SSR126517) is a novel synthetic anticoagulant linked to biotin at position 2 of the non-reducing end of the glucose in Idraparinux. (Figure 1.11) Presence of biotin at this position in the pentasaccharide prevents interaction of the pentasaccharide with AT or FXa in vitro. Sanofi-Aventis has halted the development of Idrabiotaparinux in AF in phase III trials, owing to its lack of potential benefit over oral anticoagulants, such as vitamin K antagonists, which are currently in clinical trials.[59]

AVE5026 was developed by Sanofi Aventis for the prevention of venous thromboembolism. It is known to primarily target FXa while having little effect on

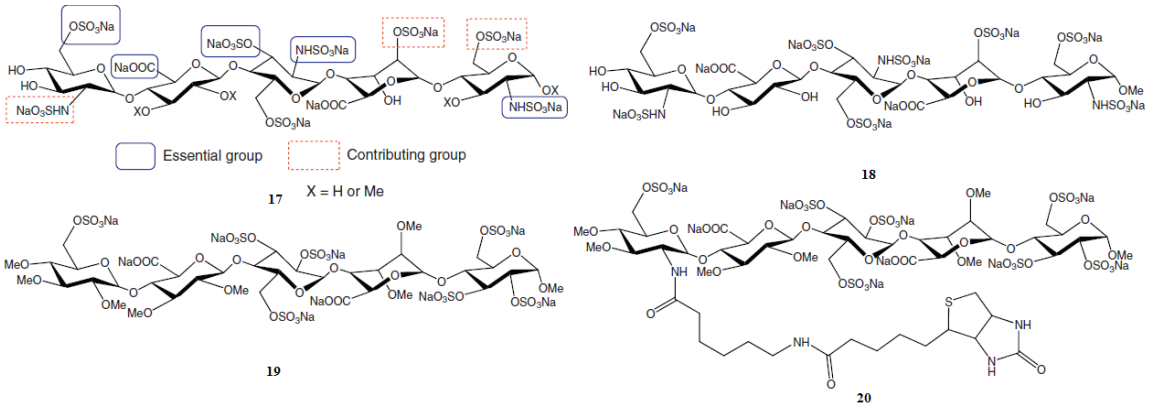


Figure 1.11: Chemical structures of heparin pentasaccharide derivatives. (**17**) The AT-binding motif. Highlighted functional groups are necessary for AT activation.(**18**)

Structure of Fondaparinux, (**19**) Structure of Idraparinux, (**20**) Structure of Idrabiotaparinux.

thrombin. M118, developed by Momenta Pharmaceuticals, is a novel antibiotic used for the treatment of acute coronary syndrome.[66]

Non-anticoagulant heparin/HS analogues

Heparin has also known to play a role in the inhibition of synthesis, expression and function of adhesion molecules, cytokines, chemokines, proteases and viral proteins. Therefore, it is not surprising that recently attention has been focused on the non-coagulant properties of heparin, which are known to inhibit inflammation and metastatic spread of tumor cells.[67] For example; heparanase is an endoglycosidase enzyme that is known to play vital roles in inflammation, tumor cell invasion, metastatis and angiogensis. It is also known to be responsible for processing HS. Several sulfated molecules such as cyclitols and glycol split derivatives have been discovered as selective inhibitors of heparanase-heparin interactions. Progen Pharmaceuticals developed one such molecule, PI-88 (**21**) which is known to inhibit heparanase and the cleavage of HS by binding competitively with HS, thus preventing the release of growth factors involved in angiogenesis.[68] (Figure 1.12)

HS/Heparin is known to be associated with neuritic plaques in Alzheimer's disease. HS has been shown to promote the aggregation of amyloid β-peptides and have an important role in plaque formation.[69] Tramiprosate (**22**)[70] (also referred to as 3-amino-1-propanesulfonic acid, 3-aminopropylsulfonic acid), a GAG mimetic and Ephrodisate sodium[71] (1,3-propanedisulfonic acid disodium salt) (**23**), a low molecular weight,

negatively charged sulfonated molecule that shares certain structural similarities with HS are examples of molecules being developed to prevent HS-induced aggregation of Aβ.

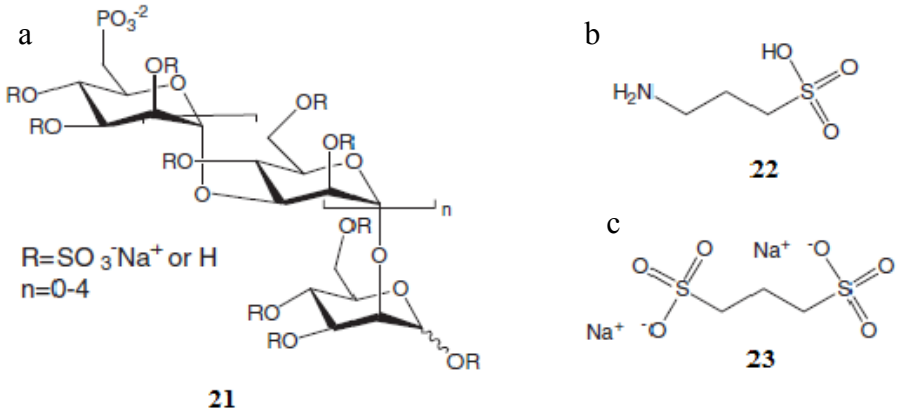
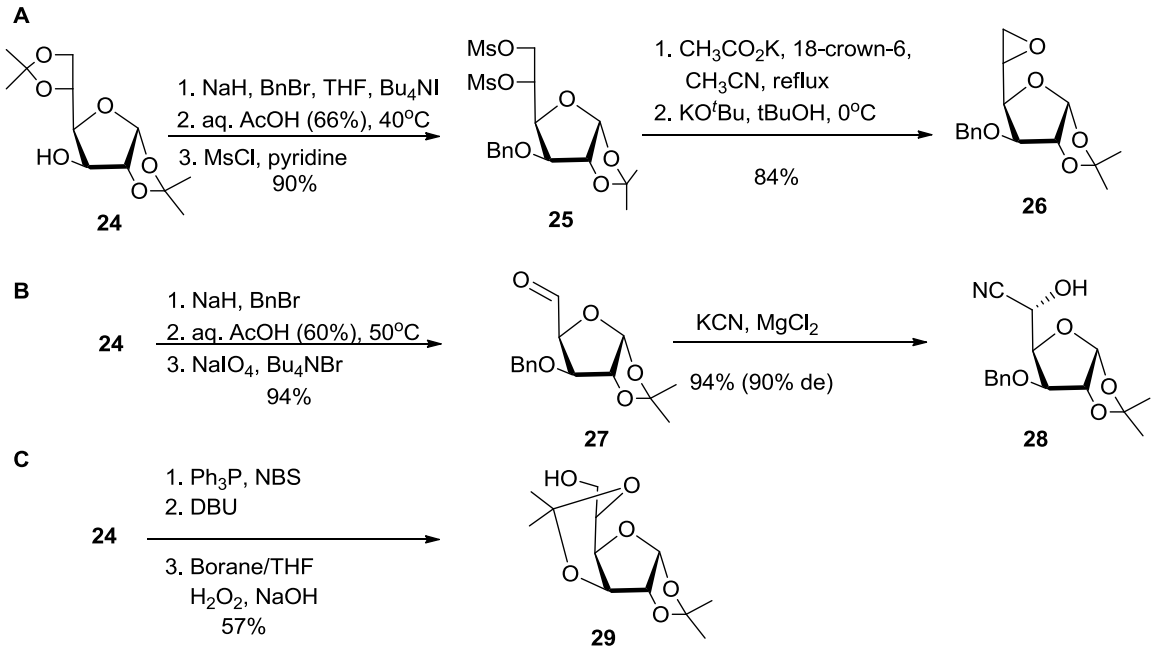


Figure 1.12: Chemical Structures of a) PI-188, b) Tramiprosate and c) Ephrodisate sodium

Chemical and Enzymatic Approaches to Synthesis of HS

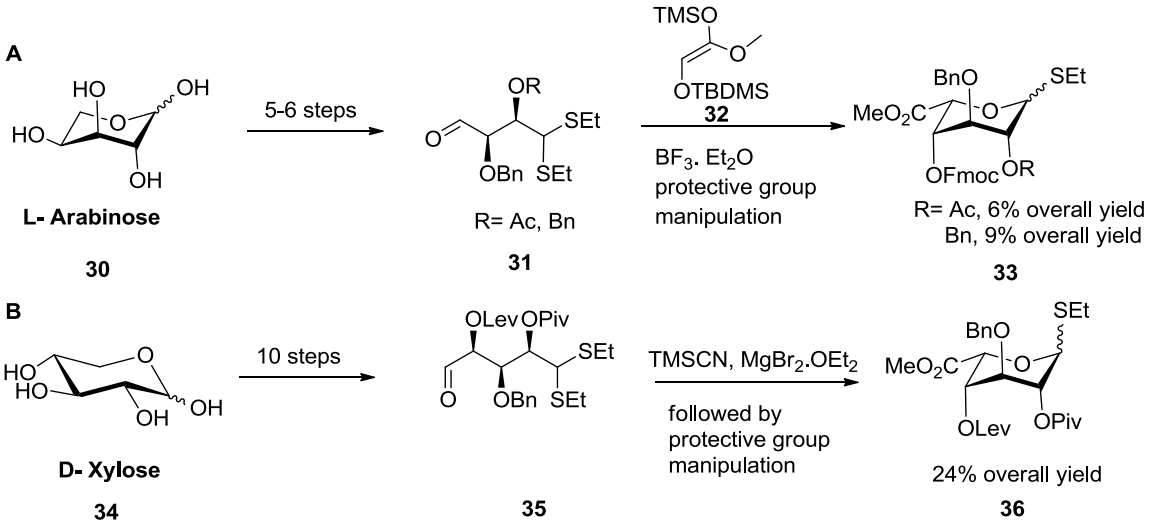
Despite the successful establishment of the AT III-binding site, the heterogeneous nature of heparin and heparan sulfate obtained from natural sources poses a challenge for obtaining pure materials in sufficient quantities for undertaking structure-activity relationship studies. To circumvent this issue, a commonly employed strategy has involved the chemical modification of natural heparin and HS.[72] However; this can lead to a mixture of partially formed products from incomplete reactions. Therefore, the synthesis of pure and homogeneous oligosaccharide sequences of parent oligosaccharides has gained tremendous importance for studying this biologically relevant molecule. The synthetic strategy entails the synthesis of disaccharide building blocks composed of hexuronic acid and D-glucosamine units with the aid of a protecting group strategy that will allow for selective *O*- sulfation and the easy assembly of larger oligosaccharides by deblocking their reducing or non-reducing ends.[73] The major roadblock in the gram scale

synthesis of extremely pure and homogeneous heparan sulfate is the synthesis of L-idose or L-iduronic acid as it is not readily accessible from natural sources and it is quite expensive commercially. Therefore, since the early 1980s, a lot of research has been conducted to synthesize L-idose and its derivatives efficiently.[73] The most commonly employed strategy begins with the commercially available 1,2:5,6-di-O-isopropylidene-a-D-glucofuranose (**24**), followed by the inversion of the configuration at C-5 through formation of an L-ido epoxide as in **26** (Scheme 1.2A).[73] Another commonly employed strategy using **24** involves oxidation of the 5-hydroxyl group to aldehyde **27** through a three-step process, followed by the stereoselective addition of the cyano group or elimination of the primary hydroxyl group with subsequent hydroboration to invert the stereochemistry at C-5 (**29**).



Scheme 1.2: Various routes for the synthesis of L-idose derivatives starting from D-glucose.

Seeberger and coworkers have utilized a *de novo* approach for the synthesis of L-idose beginning with L-arabinose.(Scheme 1.3A) However, the low selectivity in the Mukaiyama aldol reaction with aldehyde **31** resulted in a low overall yield (6%).[74a] Starting instead with *D*-xylose (Scheme 1.3B) and using the more selective cyanation furnished the idose building block in 24% overall yield.[74b] However, inspite of the different routes being developed for the synthesis of idose building blocks, the synthesis of heparin/HS building blocks remains tedious involving the use of 8-12 lengthy steps to prepare a single monosaccharide building block.

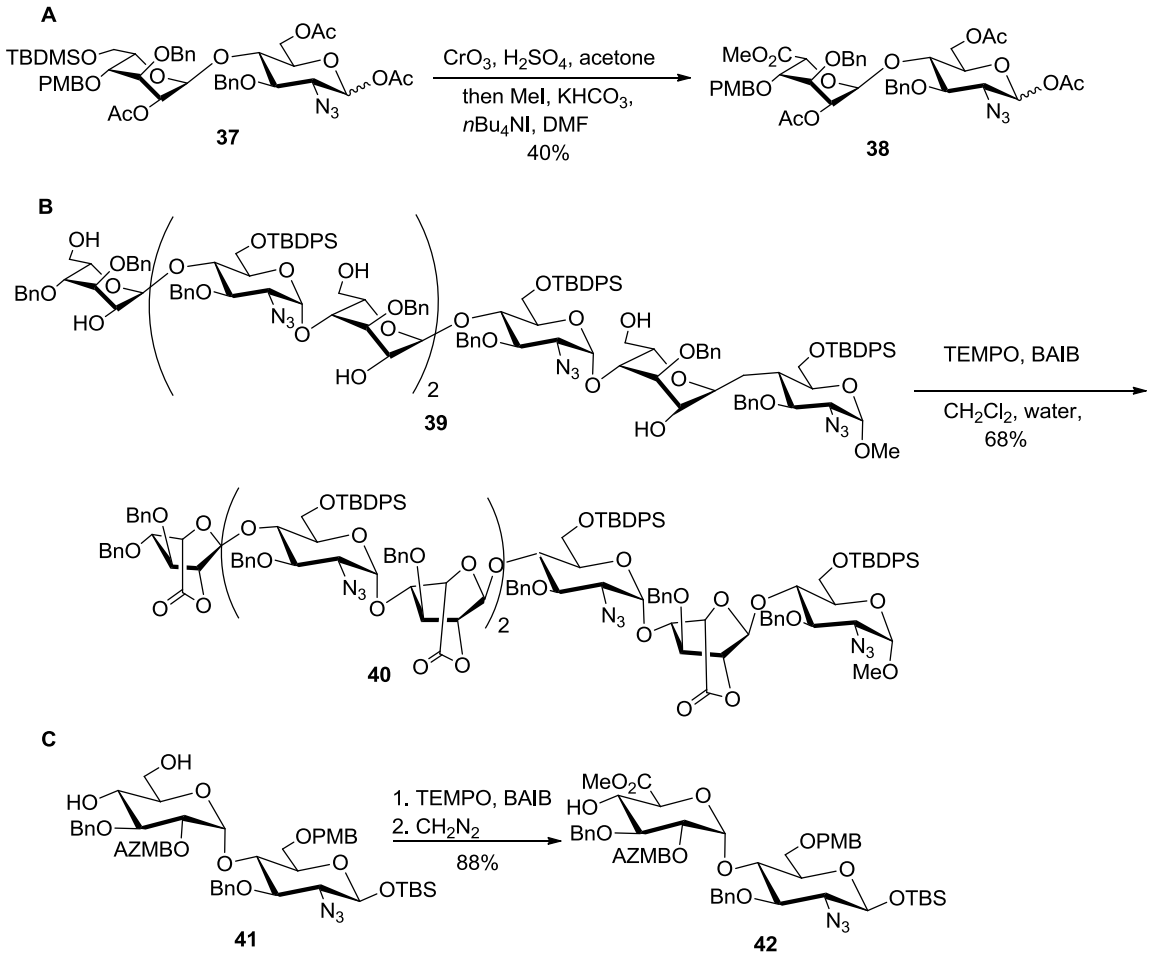


Scheme 1.3: *De-novo* synthesis of L-IdoA derivatives.

Another key issue in the synthesis of complex and polyfunctional molecules is the proper and careful choice of the protecting groups. Presence of a participating group at the C-2 position of the uronic acids is necessary to ensure the installation of 1,2-*trans* glycosidic linkage, while the C-2 amino group on the D-GlcN should be either masked or protected to allow for the difficult introduction of the 1,2-*cis*-linked D-glucosamine units. Normally the nonparticipating azido group is used to prevent the formation of β-glycoside. Another factor to be considered is the differentiation between the hydroxyl groups destined to be

sulfated and those that do not require sulfation. This can be brought about by the use of two orthogonal sets of protecting groups. The protecting groups chosen should also remain unaffected under the reaction conditions selected for oxidation of the *C*-6 hydroxyl groups. The removal of the permanent protecting groups should also be compatible with the presence of *O*- and *N*-sulfate functionalities.[73a]

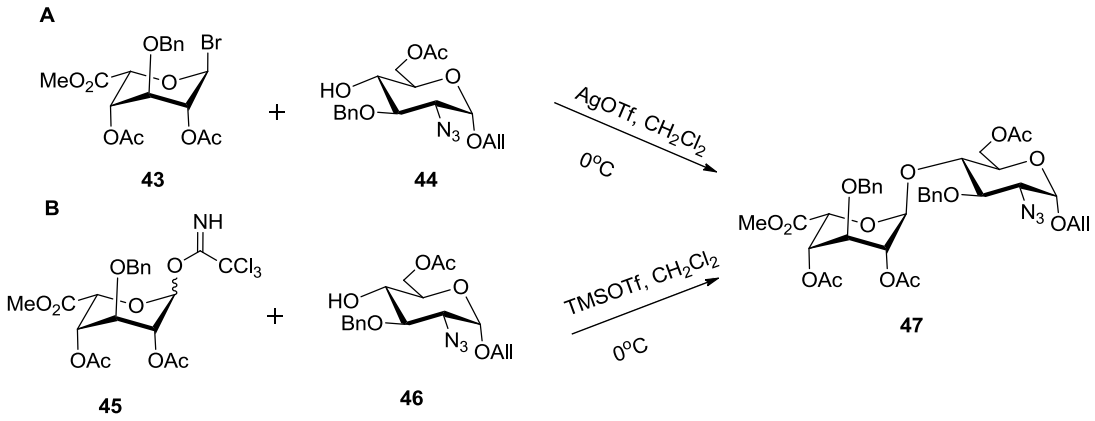
Use of glycosyl donors based on uronic acids presents another challenge as these molecules are susceptible to epimerization during their preparation and are known to be less reactive than the corresponding glycopyranosides. This approach, although attractive, presents a problem of performing oxidation on the oligosaccharide at the late stage of its assembly. As the size of the oligosaccharide increases, high yielding oxidation reactions can become even more difficult.[75] Early syntheses employed Jones oxidation or the use of similar chromium reagents to afford the uronic acid oligosaccharides.(Scheme 1.4A) However, chromium reagents are known to be toxic and the reactions were low yielding.[76] The use of milder oxidizing reagent like the (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) in the presence of a co-oxidant like sodium hypochlorite (NaOCl)[77] or bis(acetoxy)iodobenzene (BAIB)[78] (Scheme 1.4B) is known to furnish the oxidized oligosaccharide in fairly high yields. Alternatively, the high anomeric reactivity of the glycopyranosides can be utilized to assemble disaccharides as intermediates for longer oligosaccharides. The oxidation state of the disaccharides can then be adjusted by performing oxidation on the *C*6 hydroxyl at the nonreducing end of the uronic acid, thereby avoiding a late stage oxidation of the more expensive oligosaccharides. (Scheme 1.4C)



Scheme 1.4: Conversion of glycopyranosides into uronic acids a) Synthesis using Jones oxidation; b) Synthesis using milder oxidizing agents like TEMPO and c) Late stage oxidation at the C-6 of the non-reducing end.[79]

The uronic acid monosaccharides after further modification can be used directly as donors for glycosylation.[80] Use of uronic acid based glycosyl bromides was demonstrated by Sinay and co-workers for the synthesis of the AT III binding pentasaccharide.[73e,81] They were able to achieve the glycosylation in fairly good yields.(Scheme 1.5A) Bonaffe and co-workers used the trichloroacetamidate donor **45** for the synthesis of disaccharide building block **47** shown in Scheme 1.5B[16,82]. The disaccharide was furnished in 91% yield as opposed to the 75% yield that was observed when they employed the bromide

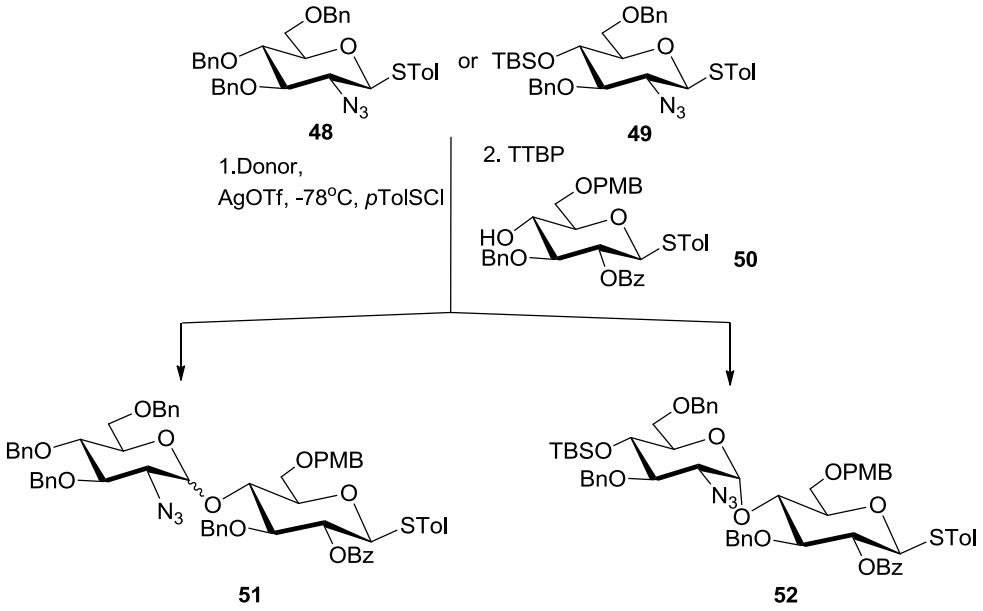
donor. The resultant disaccharide was then used in a highly convergent fashion to synthesize a dodecasaccharidederivaive that was used for the synthesis of HS proteoglycan analogue.



Scheme 1.5: Glycosyl bromide *vs.* glycosyl trichloroacetimidates as donors for glycosylation.

An essential point concerns the glycosylation strategies adopted for the assembly of heparan sulfate oligosaccharides. Need for stereochemical control during the glycosylations often dictates the choice of the protecting groups. The formation of 1,2-*trans* glycosidic linkage from the uronic acid to glucosamine is usually achieved through use of a participating group at the 2-position of the uronic acid. However, the formation of the 1,2-*cis* linkage is quite difficult. The azido group is commonly used as the precursor for the nitrogen atom at the C2 of glucosamine. However, being a non-participating group, the presence of the azido group leads to the preferential formation of the thermodynamically more stable α-glycosides.[73a,83] In case of the L-idosyl acceptors, the reaction proceeds with high stereoselectivities. For the D-glucuronic acid based acceptors, on the other hand, use of the azido group leads to the formation of anomeric mixtures.[84] However, careful selection of the protecting groups can help achieve high

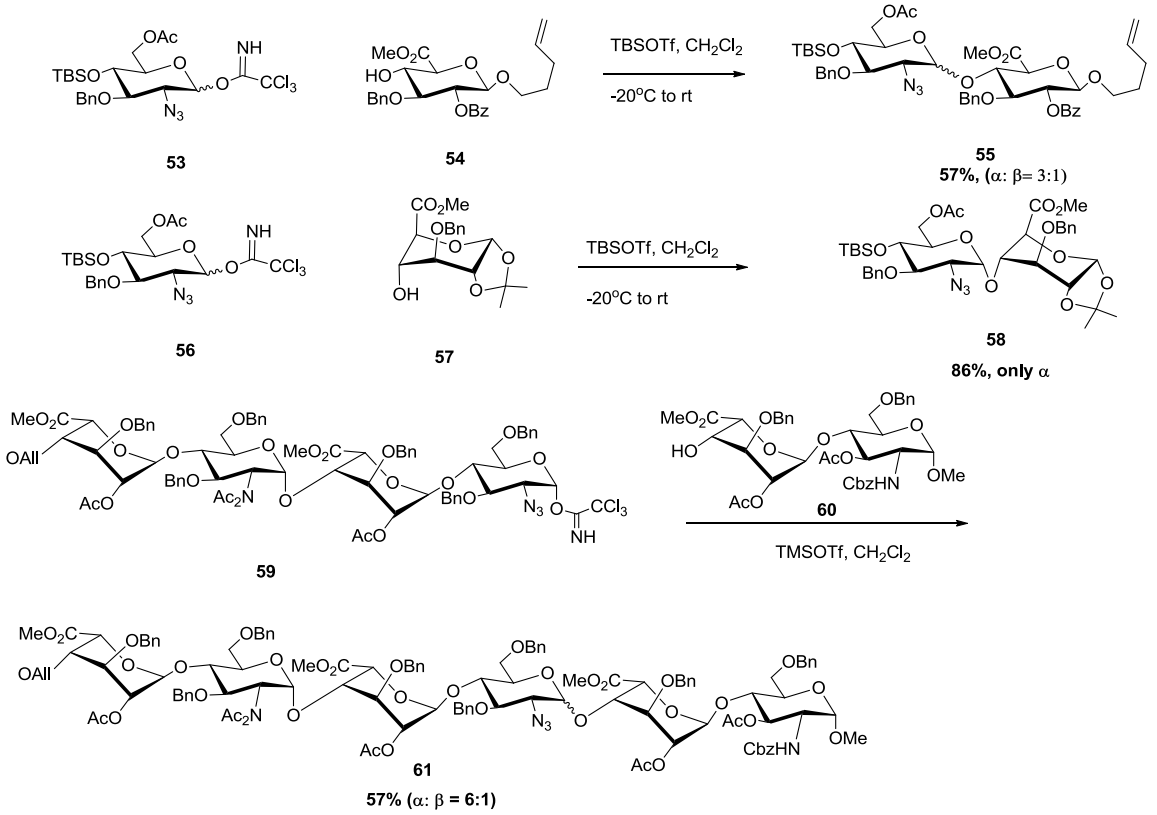
stereoselectivities. Huang and co-workers replaced the 4-benzyl ether in donor **48** by a 4-*t*-butldimethylsilyl ether donor **49**, resulting in the exclusive formation of the α-linked disaccharide.[84b] (Scheme 1.6)



Scheme 1.6: Strategy employed to improve the stereoselectivity of glycosylation.

Use of bulky protecting groups at *O*-6 of the glucosamine sugar has also been shown to decrease the tendency of the formation of β-anomer.[85] The formation of the desirable stereoisomer can also be dictated by the conformation of the acceptor. Seeberger and coworkers have shown that glycosylation of the pentenyl glycoside **54** with the trichloroacetimidate **53** resulted in α:β mixture in the ratio 3:1 (Scheme 1.7A). However, locking the glucuronic acid in the $^1C_4$ conformation led to the formation of the α anomer exclusively (Scheme 1.7B).[86a,b] However, similar results were not obtained in the assembly of larger oligosaccharides. Therefore, when the L-idosyl configured disaccharide derivative **36** was treated with the tetrasaccharide donor **35**, the resulting

hexasaccharide product was obtained as an inseperable anomeric mixture (Scheme 1.7C).[86c]



Scheme 1.7: Strategies to enhance the stereoselectivities of glycosylation.

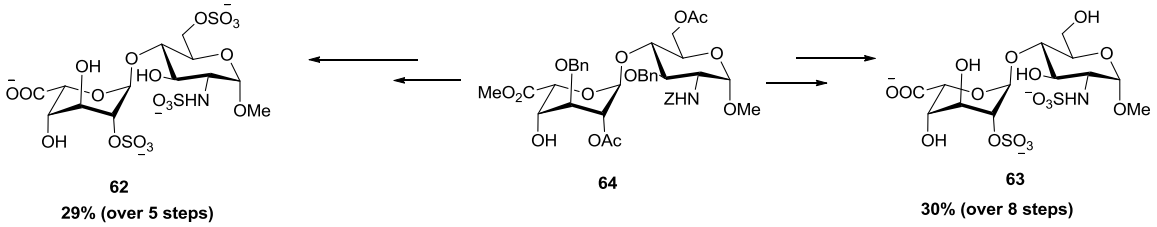
In the early years of heparin/heparan sulfate synthesis, focus was given to a single heparin oligosaccharide that gave several valuable insights. However, in the recent years, the attention has been shifted towards the modular synthesis of a broad range of heparin/heparan sulfate oligosaccharides.

Modular Synthesis of Heparin/Heparan Sulfate Oligosaccharides:

Recently, the attention has shifted to the modular strategies for the assembly of oligosaccharides that differ in protecting group patterns for the convenient synthesis of a variety of the structurally related HS oligosaccharides. The reported approaches differ in protecting groups, glycosylation strategies and the timing of the oxidation step to

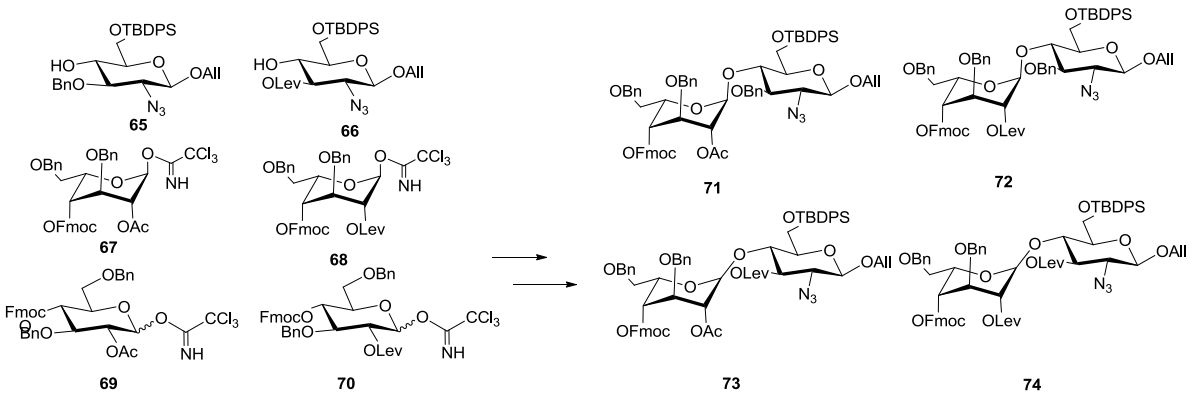
introduce the carboxylic acids of the uronic acids. However, till date, there is no one general strategy that has been reported to accommodate for all the unique characteristics displayed by the naturally occurring HS.[16,76c, 86a,b, 87.]

The first modular approach targeted the common heparin disaccharide repeating unit **62** and its 6-O sulfated counterpart **63** (Scheme 1.8). Disaccharide **62** was prepared in five steps from disaccharide **64** in five steps (29% overall yield), whereas the assembly of **63** required eight steps (30% overall yield).[88]



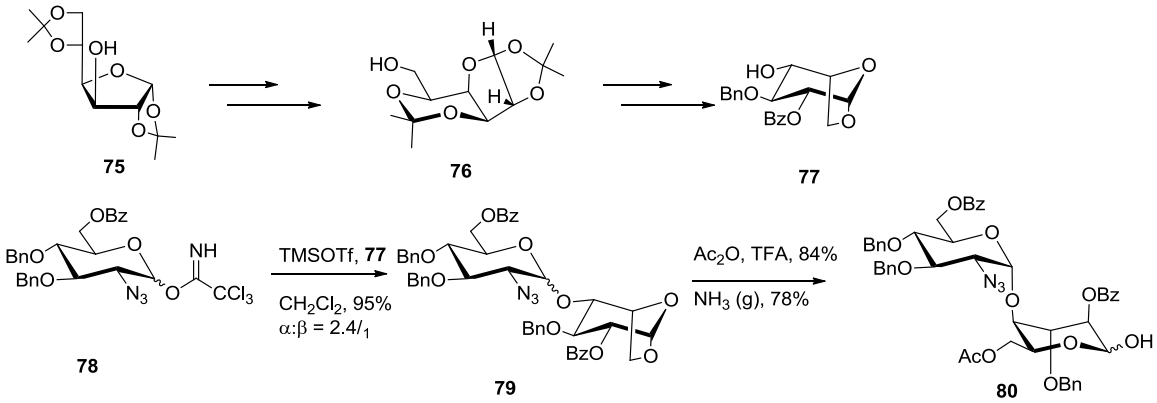
Scheme 1.8: Modular assembly approach for the synthesis of the regular disaccharides of heparin.

Boons and coworkers synthesized six different monosaccharide building blocks that can then be combined to form 20 disaccharides. Four different protecting groups' fluorenylmethyloxycarbonyl (Fmoc), levulinoyl (Lev), *t*-butyldiphenylsilyl (TBDPS) and allyl (All) were employed in the synthesis. The Fmoc group was chosen to mark the C4 hydroxyls which served as points of elongation, while the anomeric hydroxyls were protected as allyl glycosides. The primary hydroxyls that were to be further oxidized to carboxylic acids and the secondary hydroxyls that remain sulfated were protected as benzyl ethers. Lev esters were used to mask the C2 position to afford stereoselectivity during glycosylation. The C6 hydroxyl of the glucosamine was protected as TBDPS ether (Scheme 1.9)[87d]



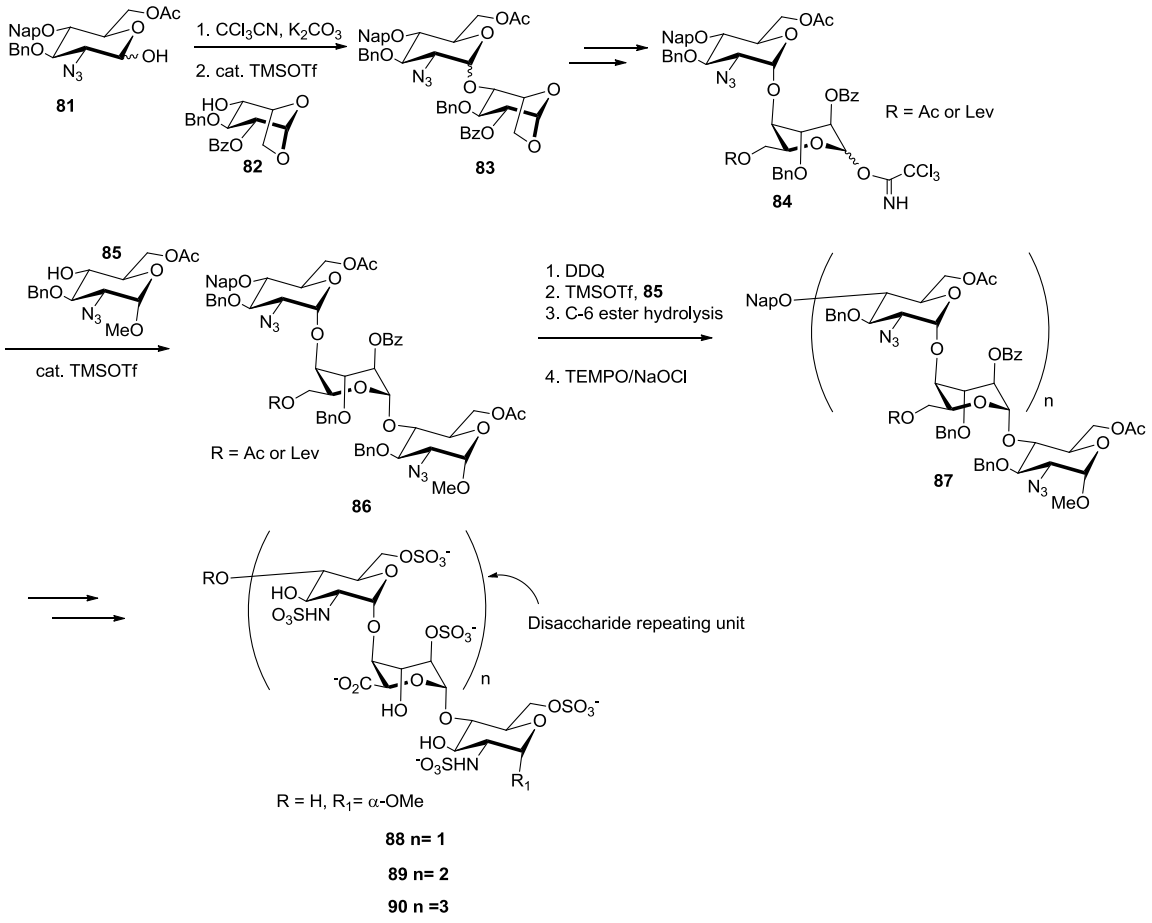
Scheme 1.9: Modular approach designed by Boons and co-workers for the synthesis of 20 disaccharides.

Hung and co-workers reported a synthetic strategy that begins with diacetone glucose **75** that was converted to the desired 1, 6-anhydro-β-L-hexopyranose **77** via the 1,2:3,5-di-*O*-isopropylidene-β-L-iodofuranose **76** (Scheme 1.10). Glycosylation of **77** and **78** was performed in the presence of trimethylsilyltrifluoromethanesulfonate (TMSOTf) to furnish disaccharide **79**. The α-isomer was isolated and then acetylated and the 1, 6-anhdyro-β-L-idopyransosyl ring was opened. Deprotection of the anomeric acetal provided the lactol **80** that could be used for further glycosylations.[89]



Scheme 1.10: Modular approach for the synthesis of 1, 6-anhydro-β-L-hexopyranose.

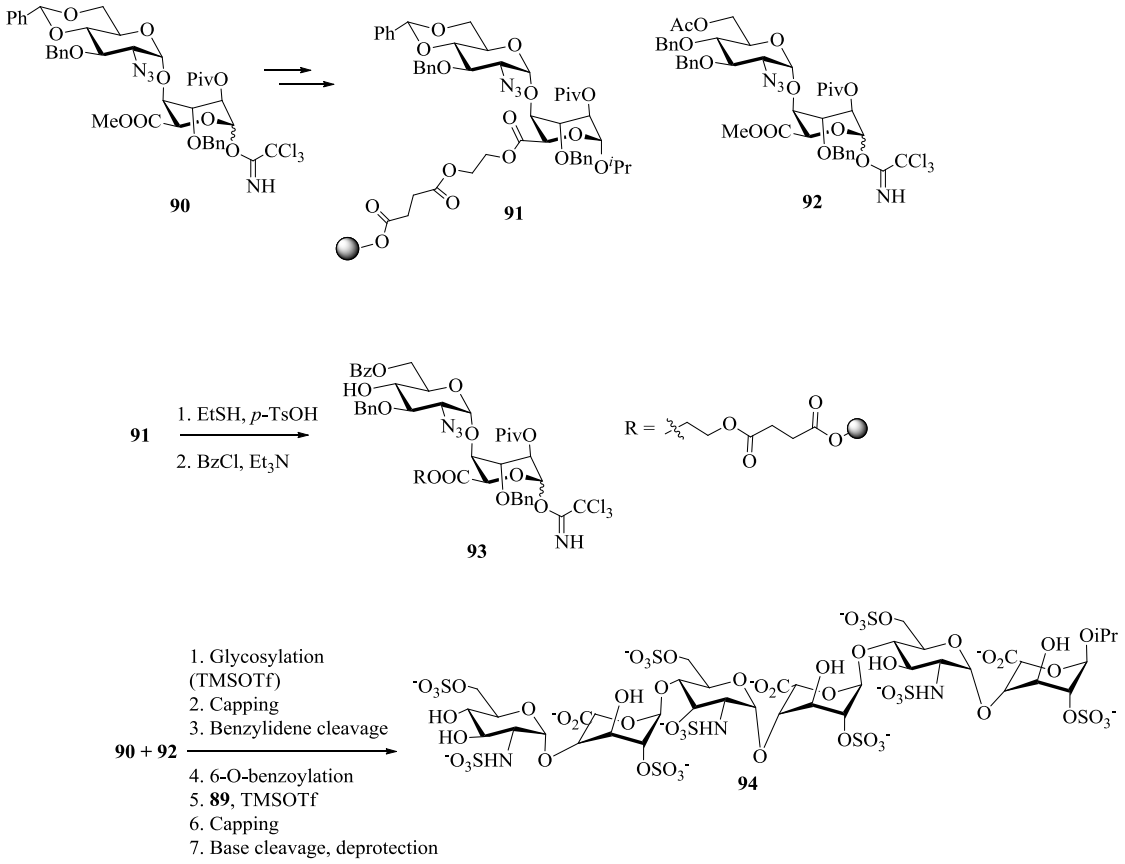
Hung and co-workers also utilized the 1, 6-anhydroidose **82** as a glycosyl acceptor and the 2-deoxy-2-azido-a-D-glucopyranose as the donor to furnish disaccharides which were then converted to the tricholoroacetimidate donor **84** (Scheme 1.11). The donor was coupled with monosaccharide **85**. Using **83** as the elongating block oligosaccharides **88-90,** were synthesized.[87b,90]



Scheme 1.11: Hung's modular approach using 1,6- anhydroidose.

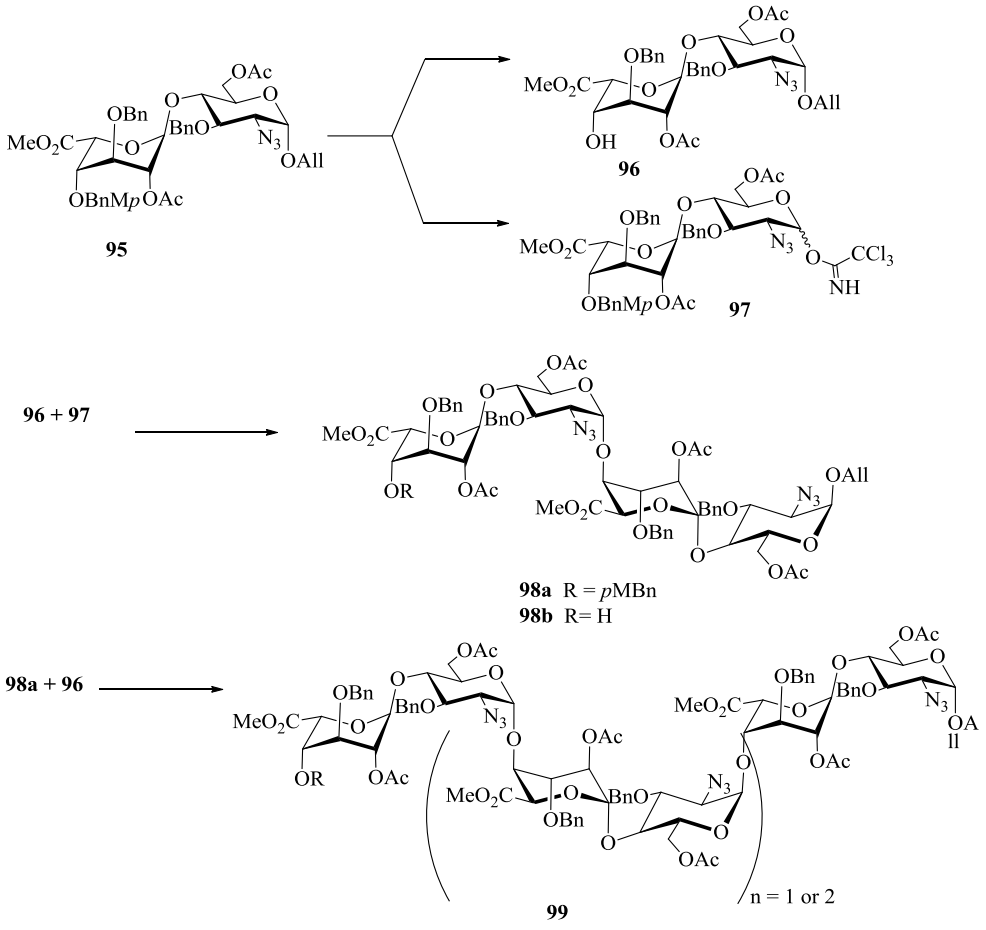
Automated solid phase synthesis has been employed for assembling oligonucleotides and peptides. Therefore, ideally it could be utilized for the synthesis of well defined heparin sequences. Although, the solid-phase synthesis of oligosaccharides is well reported, [91] only a few polymer-supported syntheses of heparin oligosaccharides have been

disclosed.[82,92] Martin-Lomas and co-workers utilized two building blocks **90** and **92** having an α-glucosamine at the non-reducing end. Use of participating groups like pivaloyl (Piv) and benzoyl (Bz) helped achieve stereocontrol during glycosylation. The benzylidene acetal cleavage followed by selective C6 *O*-benzylation after each coupling provided the free hydroxyl at C-4 (Scheme 1.12). The terminating block **91** contained an isopropyl (*i*-Pr) ether group at the reducing terminus and a soluble polymer support of polyethylene glycol ω-monomethyl ether (MPEG) for polymer supported synthesis. The unreacted acceptor was capped with an acid functionalized solid support. The low reactivity of the coupling partners necessitated this rather tedious synthetic strategy.



Scheme 1.12: Synthesis of polymer bound oligosaccharides

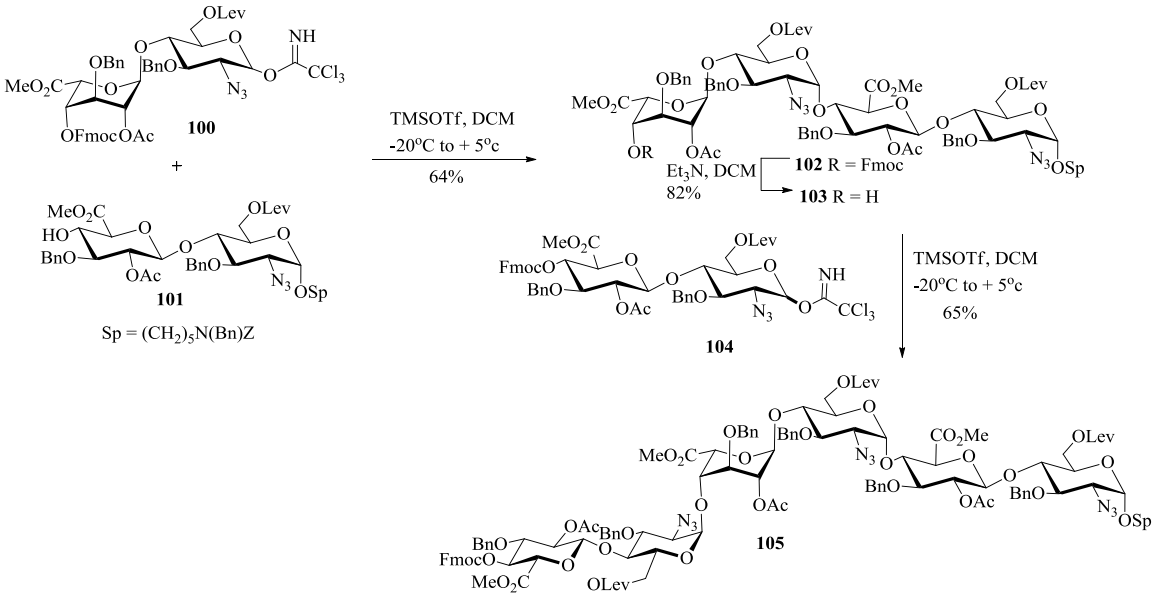
The final hexasaccharide was obtained in 37% yield.[92] Using a 2+2, 2+4 and 4+4 glycosylation strategy, Bonaffe and coworkers[16, 93] reported the synthesis of tetra-, hexa- and octasaccharides which were further used to examine the interaction between heparin and interferon γ (IFN-γ). The key disaccharide **95** had a uronic acid at the non-reducing end which can be converted into the trichloroacetimidate donors by removal of the anomeric *O*-allyl group and into acceptors by removal of the *O*-paramethoxybenzyl (pMBn) ether at the C-4 position (Scheme 1.13). Allyl glycosides are commonly



Scheme 1.13: Bonnaffe's modular approach for the synthesis of glycoconjugate mimetics.

employed for the active-latent glycosylation strategy. They offer several advantages. Ally glycosides are inert to donor activation conditions, they can be readily transformed to vinyl glycosides which can then act as active glycosyl donors in Lewis-acid catalyzed glycosylations.[78]

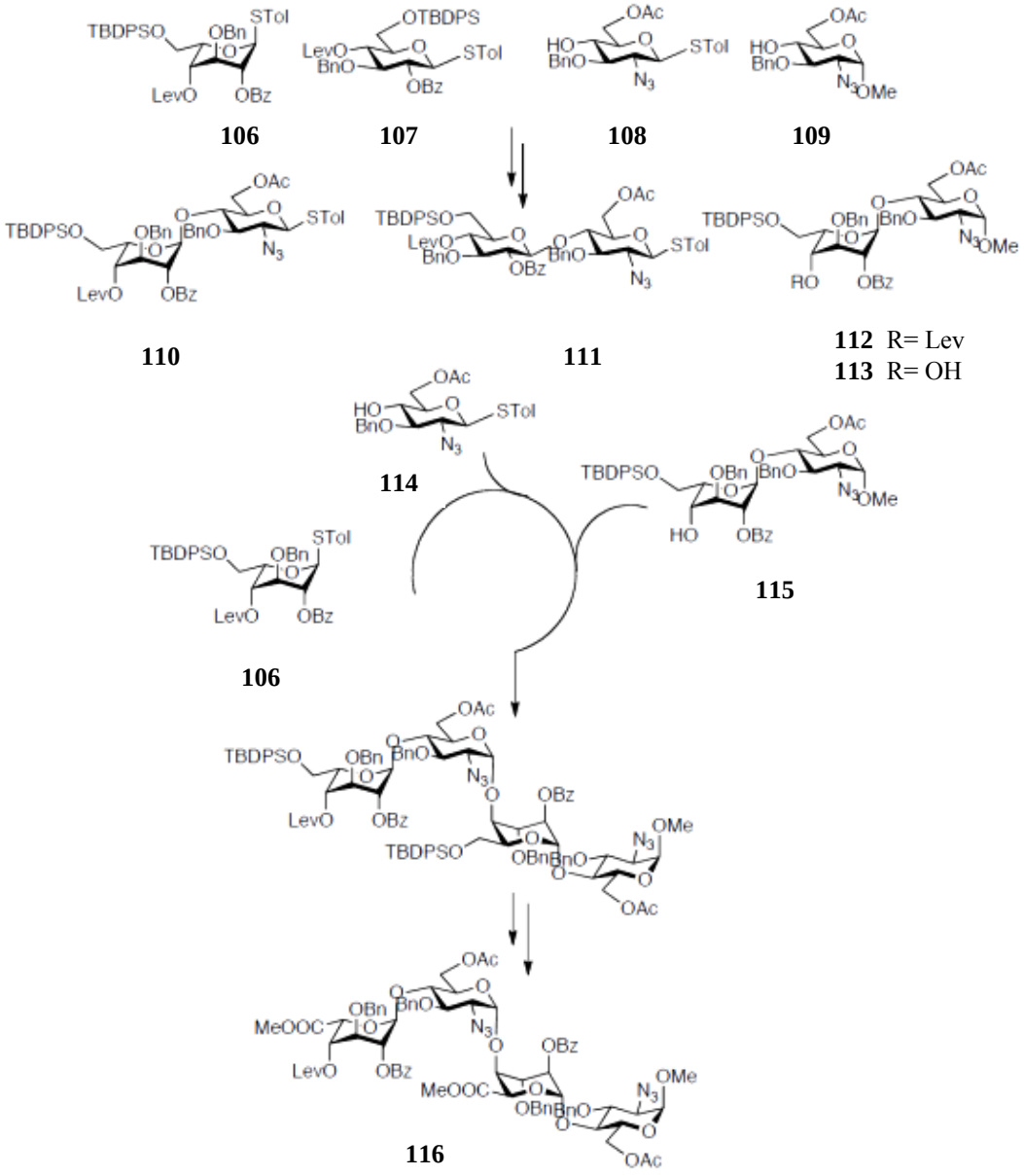
The deprotection of allyl groups however is quite tedious. It requires the use of expensive transition-metal reagents and toxic mercury salts. Therefore silyl protecting groups serve as attractive alternatives for anomeric protecting groups in the active-latent strategy. They are known to be compatible with the trichloroacetimidate donors and quite robust and have been applied with thioglycosides. Boons and coworkers used this strategy to prepare disaccharide building blocks for their heparin/HS oligosaccharide synthesis. All couplings resulted in similar yields (51-65%) and with excellent stereoselectivity.Eight disaccharide building blocks were synthesized which were then utilized to prepare a library of 11 heparin/HS tetrasaccharides and 1 hexasaccharide having different backbone



Scheme 1.14: Modular synthesis of Heparin oligosaccharides

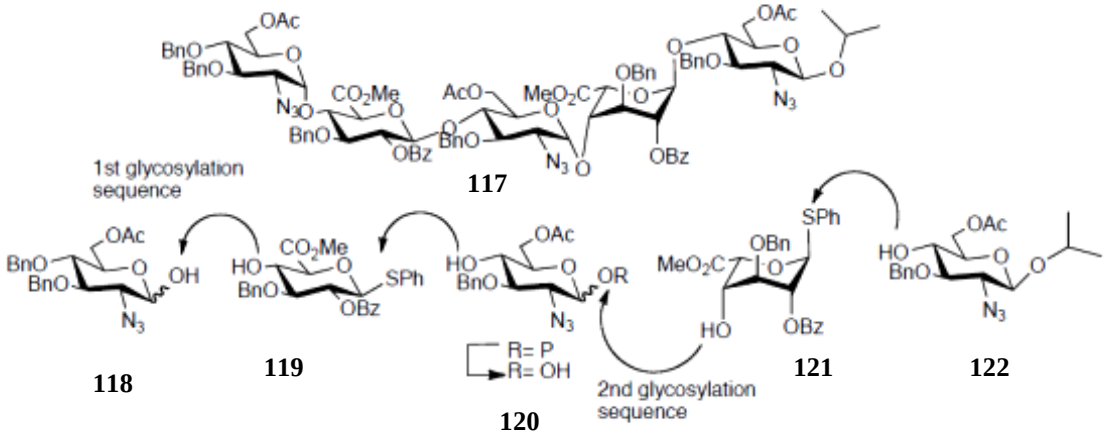
structures and sulfation patterns (Scheme 1.14).[79a]

One-pot method for the synthesis of HS oligosaccharides based on the anomeric reactivity of thioglycoside donors was reported by Wong and Polat.[94] The thioidosyl donor **106** and the thioglucosyl donor **107** were coupled with the 2-deoxy-2-azido-a-D-glucopyranoside acceptors **108/109** to obtain the disaccharides **110-112.** The one-pot



Scheme 1.15: One- pot method for the synthesis of oligosaccharides.

procedure was further used to assemble the tetrasaccharide by first reacting the idosyl donor **106** and acceptor **108** followed by glycosylation with disaccharide acceptor **110**. The desired yield and stereoselectivity was obtained primarily because of the higher reactivity of the idosyl donor or glucosyl donor as compared to the 2-azido-thioglucosyl acceptor (Scheme 1.15).The van der Marel[84b] group used a sequential glycosylation strategy to assemble pentasaccharide using monomeric building blocks that allowed for flexibility. Using the 1-hydroxyl 2-deoxy-2-azido-α-D-glucosyl donors (**118** and **120**), 1-thioglucuronyl donors and the 1-thioioduronyl donors (**119** and **121**) instead of traditional trichloroacetimidates, they were able to synthesize a model pentasaccharide in two consecutive glycosylation reactions (Scheme 1.16).



Scheme 1.16: Sequential glycosylation strategy for the synthesis of pentasaccharide.

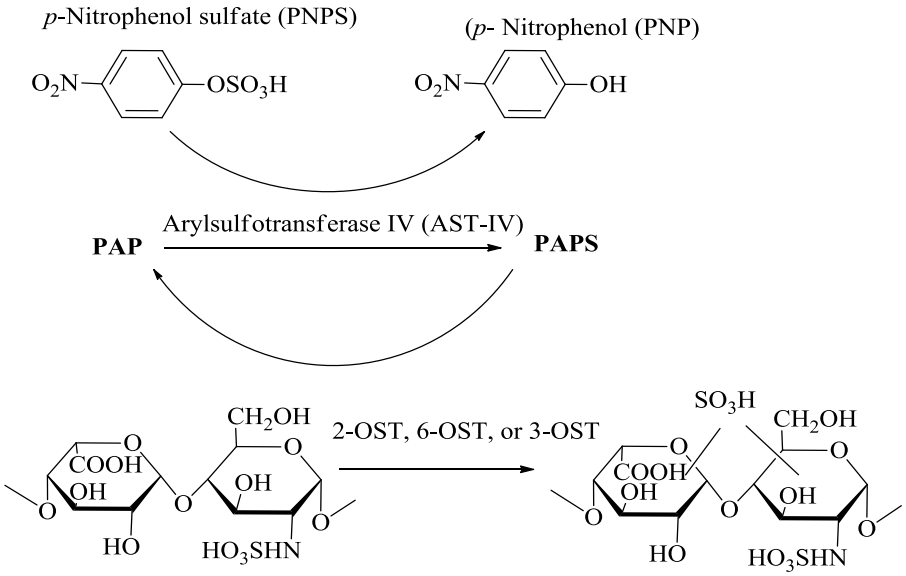
Chemoenzymatic Synthesis of Heparan Sulfate:

The complexity associated with the size and structure of HS renders its chemical synthesis quite challenging. It entails long synthetic routes with several protection/deprotection steps, making the process quite cumbersome and expensive. For example, the chemical synthesis of Arixtra requires about 50 steps with a yield of only ~

0.1%, thereby making it an impractical method.[95] An attractive alternative to the chemical synthesis is the enzymatic method that employs biosynthetic enzymes.[96] Rosenberg and coworkers in 2003 reported the synthesis of AT-binding HS polysaccharide using cloned enzymes that mimicked the ones present in cells.[97] They employed a K5 polysaccharide from *E.coli* as starting material and were able to achieve the synthesis of the pentasaccharide in just six steps with a yield that was much better than the one seen with chemical synthesis. This work paved the way for the use of enzymatic approaches to synthesize HS. However, enzymatic approach had an inherent disadvantage- the synthesis could be completed in microgram scale which is not nearly adequate for satisfying the needs of biological studies.[97]

Focus then shifted to large-scale enzymatic synthesis which was made possible because of three reasons:

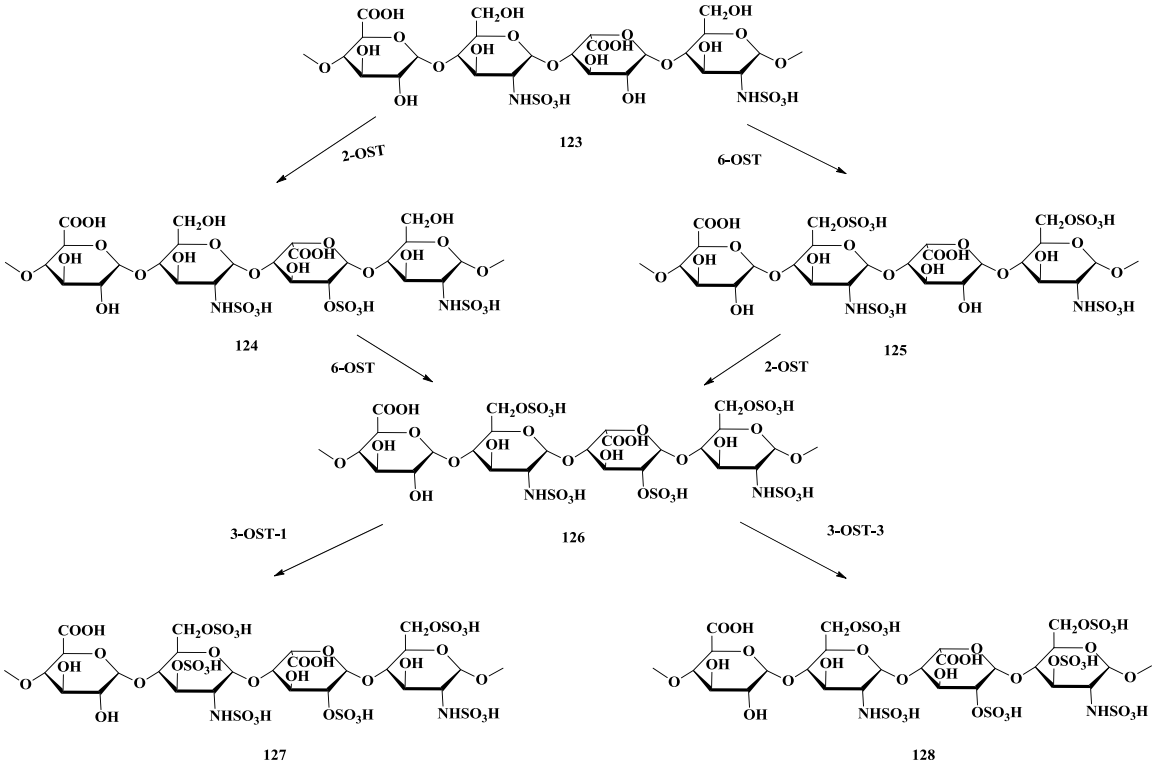
1. Recombinant enzymes can be expressed in bacteria thus providing for the enzymes in a large scale.



Scheme 1.17: PAPS regeneration scheme

2. Wong and coworkers developed an inexpensive sulfate donor, *p*-nitrophenol sulfate (PNPS) that can regenerate the PAPS, thereby preventing the accumulation of 3'-phosphoadenosine 5'-phosphate (PAP), a precursor of PAPS. Accumulation of PAP can potentially inhibit the reaction (Scheme 1.17).
3. Heparosan, an *E.coli* K5 bacterial capsular polysaccharide, can be obtained in gram scale, thus acting as a convenient starting material for enzymatic reactions.[96]

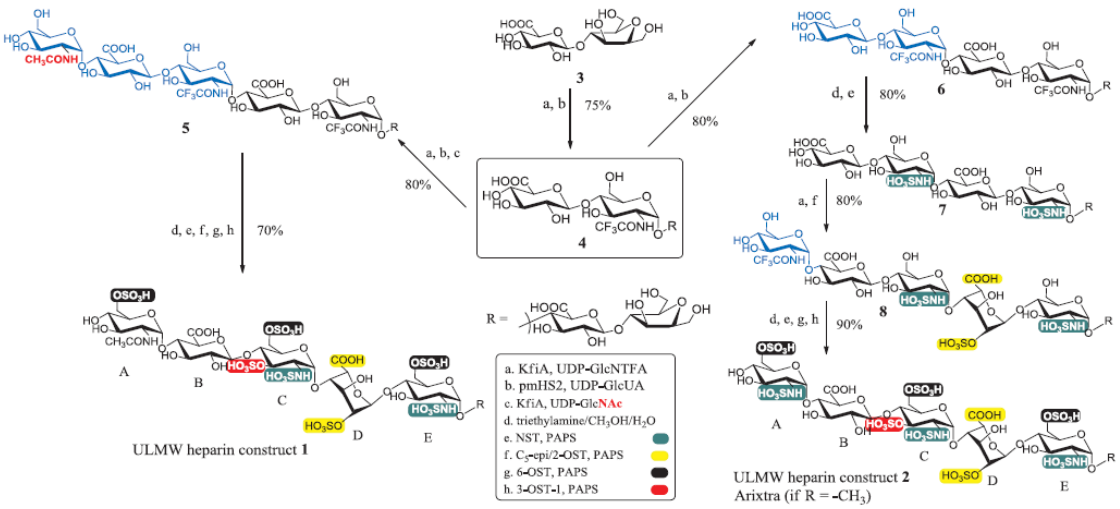
Pioneering work in the field of enzymatic synthesis has been done by the groups of Liu and Linhardt, whereby they were able to synthesize HS polysaccharides (Scheme 1.18) capable of binding to the AT III (**127**), FGF (**126**) and Herpes simplex virus -1 (HSV-1) (**128**).[98]



Scheme 1.18: Enzymatic synthesis of HS oligosaccharides.

Enzymatic synthesis of HS polysaccharides has certain drawbacks. For example, the major drawback of the reaction is the tendency to obtain a mixture of polymers having

different sizes and sulfation patterns. Incomplete nature of enzymatic reactions and the heterogeneity of the starting materials often lead to unnatural sulfation patterns. In order to overcome these problems, one needs to ensure that the starting material is homogeneous and well-defined. Such structures can only be obtained via chemical synthesis.[96] This created the need for the development of chemoenzymatic methods for the synthesis of HS polysaccharides. The groups of Linhardt and Liu were able to successfully synthesize a library of homogeneous ultralow molecular weight heparins in 10-12 steps using a simple disaccharide (Scheme 1.19). These molecules further displayed excellent *in vivo* anticoagulant activity and comparable pharmacokinetic properties to Arixtra.[99]



Scheme 1.19: Chemoenzymatic synthesis of ultralow molecular weight heparin constructs.[99]

By careful selection of the substrate size, sulfation sequence and the target structure, they were able to avoid by-product formation. Thus chemoenzymatic synthesis has provided researchers with a tool to probe the bioactivity of structurally defined HS

oligosaccharides. A thorough understanding of the HS-modifying enzymes has allowed for the strategic installation of the IdoA residues and sulfate groups in substrates. This technology encompasses the advantages of both the synthetic methods as well as the enzymzatic methods, thus enabling the generation of well-defined HS structures that can have potential therapeutic applications (for example new anticoagulant drugs) as well as providing help elucidating the role of HS in various physiological processes.[99]

Dermatan Sulfate

Dermatan sulfate sub-family of GAGs consist of *N*-acetylgalactosamine (GalNAc) residues that alternate with iduronic acid residues. The GalNAc residues are substituted to varying degrees with sulfates that are linked to the 4- and/ or 6-hydroxyl positions. The uronic acid residues may be substituted to a lesser extent with the sulfate at the 2- and rarely at the 3-hydroxyl position, thus imparting sufficient anionic character to these molecules. Dermatan sulfate chains can vary in size up to a hundred or more disaccharide repeating units. These GAGs are attached to the core protein via serine using the same glucuronic acid-galactose-galactose-xylose tetrasaccharide as heparin/heparan sulfate.[100]

Biosynthesis of Dermatan Sulfate

The nascent core protein biosynthesis is initiated by translation in the cytosol followed by the translocation of the polypeptide into the lumen of the ER. This is followed by the addition of the *N*-linked oligosaccharides to asparagine (Asn) residues from the dolichol phosphate intermediates. The next steps entail chaperone mediated folding, formation of the disulfide bonds, and the initial trimming of the *N*-linked oligosaccharides which occur before the modified core protein exits the ER. Misfolded proteins are degraded and never leave the ER. [101]

Glucose is the main precursor for all the sugars of the dermatan sulfate, although galactose, mainly derived from lactose, can be the precursor for the galactose, glucuronic acid and xylose residues through the formation of UDP-Gal, UDP-GlcA and UDP-Xyl. Glucosamine can act as a substrate for the *N*-acetylgalactosamine residues via the formation of the UDP-GlcNAc.[101] Sulfation of the sugars is brought about by 3'-phosphoadenosine 5'- phosphosulfate (PAPS) as shown in the Figure 1.13. Cells normally capable of synthesizing GAGs also contain all the necessary enzymes for the formation of the precursors, as the sugar nucleotides and PAPS normally do not enter the cells. Next, the sugar nucleotides and PAPS are transported from the cytosol across the lumen into the ER.[102]

Direct addition of the single sugars onto the appropriate receptors without the aid of intermediates such as the dolichol pyrophosphate oligosaccharides normally involved in the glycoprotein formation results in the formation of the oligosaccharide linkage region and the attachment of the chrondroitin polymer to the core proteins. All the steps of the synthesis are carried out within the intracellular membrane system composed of the endoplasmic reticulum (ER), transfer vesicles, and Golgi apparatus. [101]

Dermatan sulfate and heparin/heparan sulfate chains are linked to serine residues in the core proteins by the same tetrasaccharide sequence.[103] The transfer of the GalNAc to the linkage oligosaccharide is the first step that provides the specificity for chondroitin/dermatan sulfate. This transfer is brought about by the enzyme GalNAc transferase. Once the addition is complete, the polymerization of the chondroitin sulfate chain continues by the alternate addition of GlcA and GalNAc residues from activated precursors. The polymerization occurs in a highly ordered fashion, with the concerted

action of both GalNAc and GlcA transferase adding the respective sugars to the nonreducing end of a growing proteoglycan, thereby resulting in the formation of a chondroitin sulfate chain as large as 70kDa.[101]

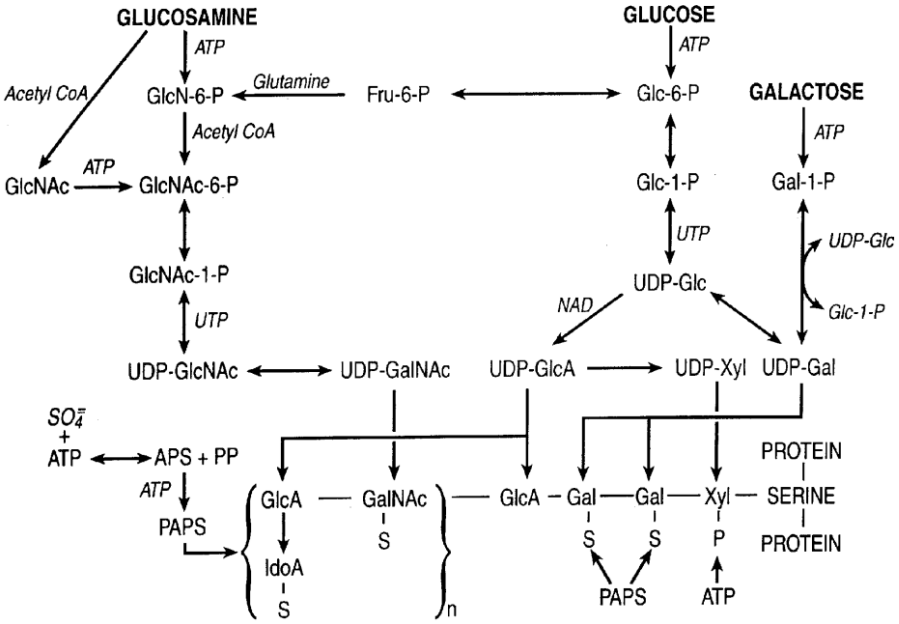


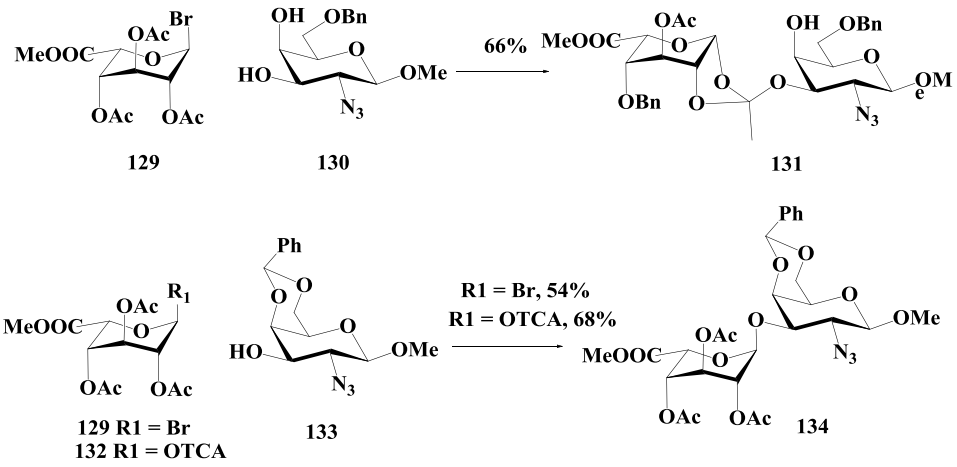
Figure 1.13: Biosynthesis of DS[101]

Chemical Synthesis of Dermatan Sulfate

Dermatan sulfate unlike chondrotin sulfate has L-IdoA in its backbone. Since L-IdoA is a rare sugar that is not available commercially, the efficient preparation of L-IdoA derivatives is a major roadblock in the synthesis of DS. The past decade has seen several advances in the preparation of L-IdoA[104] however the most commonly techniques for the preparation remains the intramolecular nucleophilic substitution at C-5 starting from 3-*O*-benzyl-1, 2-isopropylidene-α-D-glucofuranose.[105]

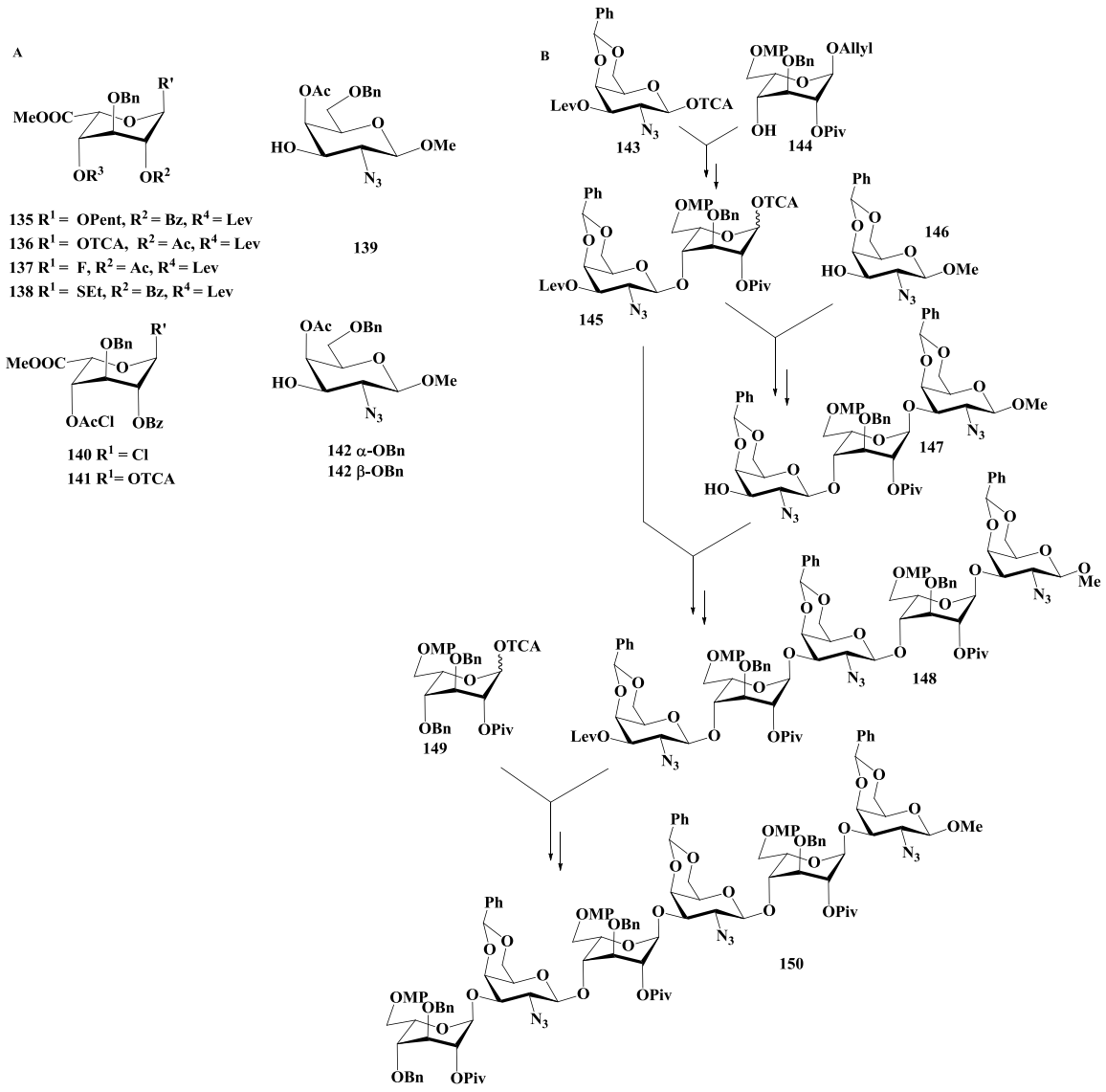
There have been a few reports on the syntheses of DS oligosaccharides. The first synthesis of IdoA-GalN(4S)-OMe disaccharide was reported by Marra and coworkers.[106]

They utilized D-GalN 3,4-diol **130** as an acceptor, which however led to the formation of 1,2-orthoacetate **131** instead of forming the desired product. On switching to the IdoA derivatives **129** and **132** as donors, donor **132** was found to give higher yield than the corresponding bromide donor **129** when treated with the acceptor **133**.



Scheme 1.20: Glycosylation of 3,4-diol *vs* 3-OH D-GalN with L-IdoA donors.[106]

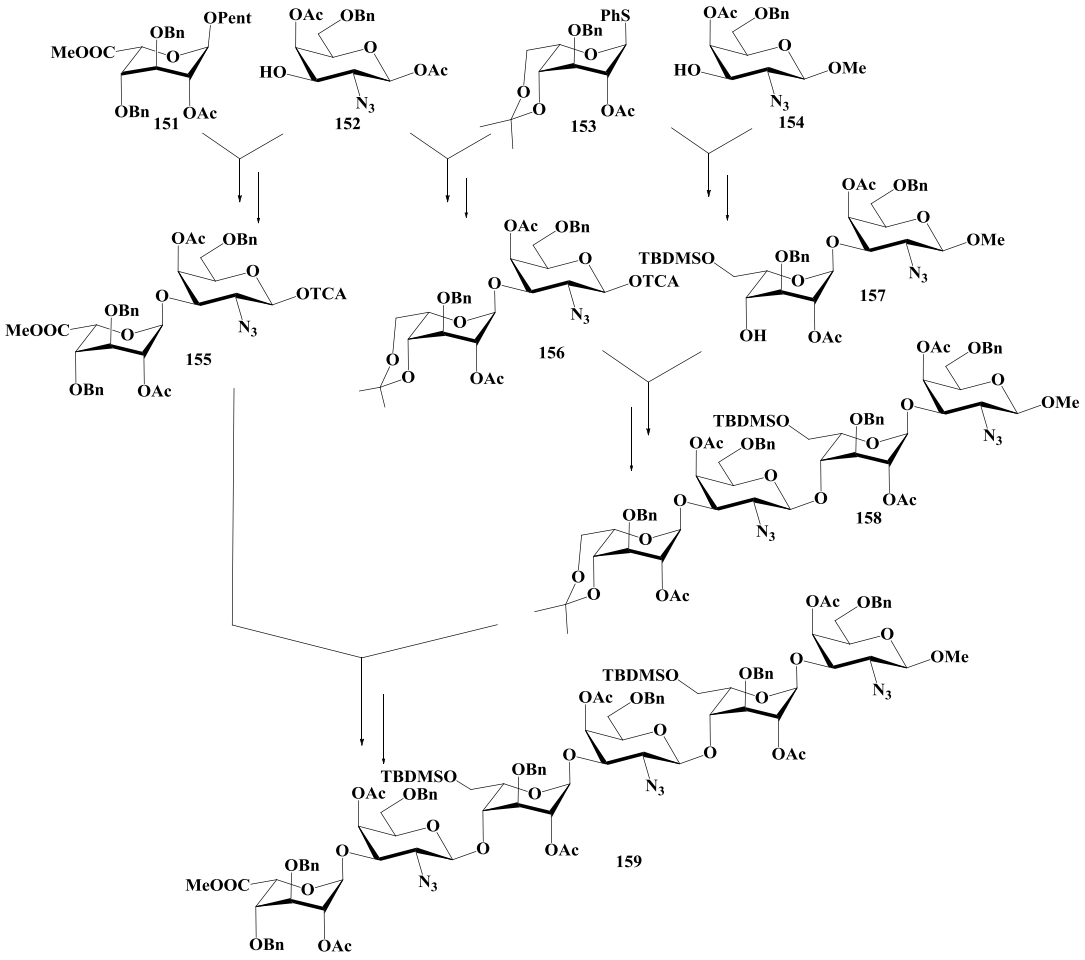
Sinay and coworkers[107] conducted an investigation into the reactivity of various L-IdoA donors for example thioglycosides, trichloroacetimidates, *n*-pentenyl and fluoride. They found that trichloroacetimidate and *n*-pentenyl proved to be more efficient donors than the thioglycosides and fluorides (Scheme 1.21 A). Similar data was reported also by Rochepeau-Jobron and Jacquinet[108] for the synthesis of a disaccharide unit of DS. They observed the exclusive formation of β-linked disaccharides when the L-IdoA chloride donors were used in the presence of a participating group at C-2 position. Use of IdoA derivatives as donors in the synthesis of DS oligosaccharides is quite popular. However, they behave as poor acceptors when glycosylated with D-GalN donors. Therefore, for the



Scheme 1.21: A) L-IdoA as donors in glycosylation; B) Synthesis of DS hexasaccharide using L-Ido donors

synthesis of longer oligosaccharides, L-Ido derivatives are often used as acceptors which are then subsequently oxidized to the corresponding L-IdoA derivatives. This strategy was employed by Goto and Ogawa[109] in 1994 and Sinay and coworkers[110] in 1997 when assembling the hexasacchrides containing IdoA(2S)-GalN(4S). The synthesis consisted of successive addition of a common disaccharide **145**, which was obtained in 83% yield

from the reaction of L-Idose **143** with the trichloroacetimidate **144.** The hexasaccharide obtained was subjected to Swern oxidation followed by treatment with sodium hypochlorite and after esterification to yield the desired urinate in 39% overall yield.

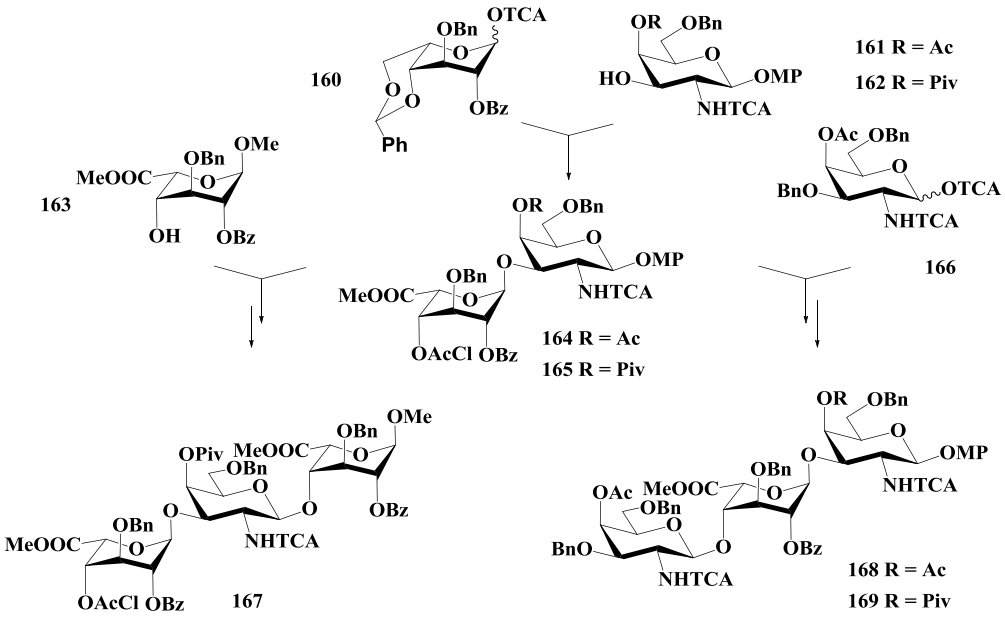


Scheme 1.22: Synthesis of DS hexasaccharide methyl glycoside.

In 1997, Sinay and coworkers[110] reported a new method for the synthesis of hexasaccharide. They synthesized three disaccharides **155, 156** and **157** that were then subjected to 2+2 and 2+4 reactions. Despite the presence of a non-participating azido group at the C-2 position, the 2+2 coupling reaction of **156** and **157** gave β-linked glycoside exclusively. This was attributed to the formation of the α-nitrilium ion in the

acetonitrile solvent. The same stereoselectivity was observed in case of the hexasaccharide. Oxidations were performed on the tetra-and hexasaccharides using the Swern and Dess Martin reagents respectively.

In 2000, Barroca and Jacquinet[105b,111] reported the synthesis of DS disaccharide GalN(4S)-IdoA(2S) using the relatively unreactive 4-OH L-IdoA acceptors with the 2-deoxy-2-trichloroacetimido-D-GalN trichloroacetimidate donors. They were also



Scheme 1.23: Synthesis of various sulfoforms of DS trisaccharide methyl glycosides

successful in applying this method for the synthesis of larger oligosaccharides. The interesting point of their synthesis was the regioselective oxidation of the disaccharides containing the 4,6-Ido diol residue using TEMPO followed by esterification. The L-IdoA acceptor **163** was then glycosylated with the disaccharide donor **165** to five the 1,2-*trans* trisaccharide in 61% yield. By using two different esters, the pivalate and the acetate, they were able to differentially sulfonate the trisaccharide.

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CHAPTER 2

EXPLORING THE REACTIVITY OF GLUCURONIC ACID DONORS IN HEPARAN SULFATE SYNTHESIS[1]

Abstract

Glucuronic acid (GlcA) is a C5 carboxylic acid derivative of glucose found in glycosaminoglycans (GAGs). In most GAGs, GlcA occur in a disaccharide repeat sequences coupled to hexosamines at both the reducing and non-reducing ends. Being an integral part of GAGs, GlcA is involved in modulating various important protein interaction events at the cellular level. An access to synthetic fragments of GAGs with defined composition and functionality is essential to perform structure activity relationship studies between GAGs and proteins. Heparan sulfate (HS) is one of the most important members of the GAG family. GlcA is major component of HS, which is β (1-4) linked to glucosamine. Due to the presence of electron withdrawing carboxylic acid functionality the stereoselective incorporation of GlcA into synthetic HS fragments is quite challenging. This synthetic inefficiency is often circumvented by use of masked non-oxidized precursors of GlcA. The delayed introduction of the acid functionality occurs at the cost of late stage manipulations which tend to increase the length of the synthesis while decreasing the overall efficiency. The poor reactivity of the GlcA moiety is attributed to the disarming effect of carboxylic acid functionality which may be counter balanced by the presence of armed substituents. We used the oxidized derivatives of differentially substituted GlcA donors to perform coupling experiments with glucosamine

[1]O.P Dhamale, C Zhong, K.A Mafraji, G-J Boons. *To be submitted to Organic Letters*

derivatives. We employed a novel C2 protecting group, Pivaloyl acetate (PivOAc) and compared its stereoselectivity, efficiency and orthogonality with classic groups like Ac and Lev. We also studied the effect of C4 substituents on the glycosylation efficiency of GlcA donors. In the underlying study we propose a new synthetic strategy which employs pre-oxidized GlcA derivatives which efficiently coupled with glucosamine acceptors with excellent stereoselectivity utilized for the construction of heparan sulfate disaccharide fragments.

Introduction

Glucuronic acid (GlcA) is the C5 carboxylic acid derivative of glucose. GlcA is a major component of uronic acids found in glycosaminoglycans (GAGs). GlcA is coupled to glucosamines or galactosamines at the reducing and non-reducing ends within the different members of the family of GAGs like heparin, heparan sulfate, dermatan sulfate, chondroitin sulfate among others. Being a part of complex carbohydrates GlcA plays important roles in various biological processes at the cellular level.[1] Therefore, ever since their discovery the synthesis of uronic acid based oligosaccharides and glycoconjugates has received significant attention from the synthetic organic chemistry community.[2,3,4,5] The incorporation of GlcA in GAGs is performed in two ways; (i) by using a pre-oxidized derivative and (ii) via the non-oxidized glucose based derivative. The latter method has been a favored choice due to the poor reactivity of GlcA both as a donor and acceptor.[3] The post glycosylation incorporation of GlcA utilizes a more reactive non-oxidized precursor of GlcA wherein the electron withdrawing C5 carboxylic acid functionality is masked with a temporary protecting group. Although the post glycosylation oxidation method is associated with efficient coupling reaction, it suffers

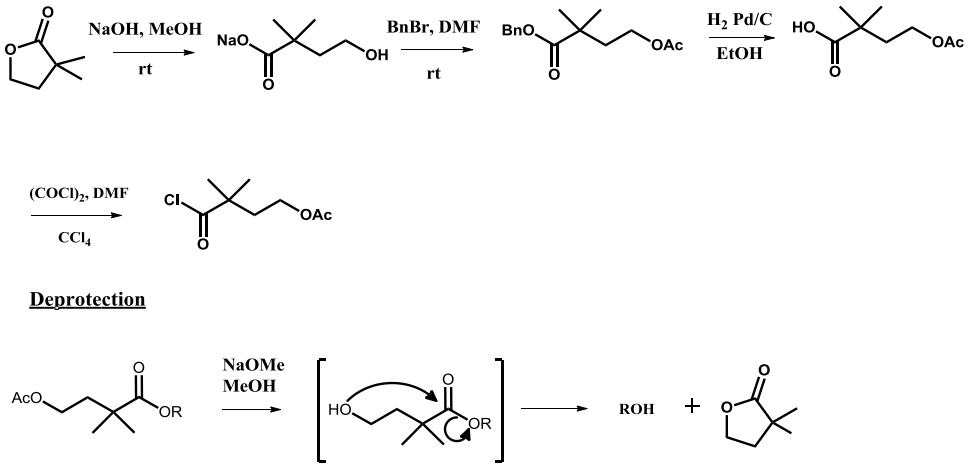
from the drawback of requiring further manipulations for introducing the carboxylic acid functionality on the coupled product. The presence of the electron withdrawing carboxylic acid functionality has a disarming effect on GlcA which hampers its reactivity profile as donors thus reducing its popularity in coupling reactions. The carboxylic acid functionality in GlcA tends to increase the electronegativity of the endocyclic oxygen which translates to increased resistance towards the formation of the oxonium ion. Thus the departure of the leaving group is detained and the overall reactivity of the donor is reduced. On the contrary, in case of a non-oxidized GlcA donor the capacity of the endocyclic oxygen to participate in forming the oxonium ion is unaffected and the innate tendency of the leaving group to depart is retained. Although the pre-oxidized GlcA derivatives suffer from poor reactivity, its installation prior to the coupling reaction can reduce number of steps required for introducing the acid functionality in comparison to the post-glycosylation oxidation approach. There have been reports of use of pre-oxidised GlcA derivatives in the construction of GAGs but their coupling efficiency has been found to be poor to mediocre.[4] Thus technically neither of the methods is truly efficient. A truly efficient approach would utilize and employ the best of both the methods described above, wherein a pre-oxidized GlcA derivative would be used in a highly efficient coupling reaction which require minimum further manipulations on the coupled product. Unlike GlcA, the pre-oxidized form iduronic acid (idoA) has been often used during glycosylation reactions for construction of several GAGs.[5] IdoA, both as a donor and an acceptor yields efficient coupling reactions with high stereoselectivity attributed to the double stereodifferentiation during their glycosylation transition state.[5] Currently reported synthetic strategies lack an efficient synthetic methodology for the stereoselctive

incorporation of GlcA motif in the construction of different GAGs systems.[4] We report a new synthetic methodology which employs pre-oxidized GlcA derivatives to couple with glucoamine acceptors with high yield and excellent stereoselectivity utilized for the construction of heparan sulfate disaccharide fragments.

Results and discussion

In the past, our group relied on introduction of the uronic acids at the disaccharide level.[2] However, here we have introduced the acid functionality on monosaccharide sugars of glucose using the regioselective C-6 oxidation reaction with help of 2,2,6,6-tetramethyl-1-piperdinyloxy (TEMPO) and bis acetoxy iodo benzene (BAIB).[6] The C2 position of the GlcA donor is a critical position which influences the stereochemical outcome of the glycosidic linkage. We have utilized PivOAc, a novel ester functionality to ensure the formation of 1,2-trans-glycosidic linkage.[7] We have performed a profiling study for reactivity and coupling efficiency of pre-oxidized GlcA donors. We constructed differentially substituted GlcA donors and tested their coupling efficiency with protected glucosamine derivatives which are summarized in scheme 2.2. For our synthetic methodology we employed GlcA donors with C2 acetyl, lev and PivOAc esters along with C4 Fmoc, Bn and NAP groups. Trichloroacetimidate was used as leaving group on all the GlcA donors. These donors were then coupled with glucosamine derivatives protected with C6 Ac or Lev esters and the anomeric position was either protected with a TDS ether or a masked amino pentyl linker. The construction of the glucosamine was based on our previous work.[2] Based on our previous results and some model studies, we have observed that the use of acetyl groups as C-2 protecting groups of hexuronyl donors may lead to formation of ortho esters. The problem of ortho-ester formation can be

circumvented or minimized with the use of benzoyl or pivaloyl esters.[7] However their deprotection employs strong basic conditions which may prove to be detrimental to the synthetic design of HS as sulfate groups can be incompatible with rigorous basic conditions. The above discussed issues presented us with an opportunity to study the application of the new found auxiliary PivOAc, in our synthesis which has not been reported in the synthesis of glycosaminoglycans to the best our knowledge. This group offers a twofold advantage as it performs neighboring group participation as effectively as a traditional pivaloyl or benzoyl group but can be removed under milder conditions by a relay mechanism, where the extended acetyl group is cleaved under normal deprotection condition followed by intermolecular lactone formation (scheme 2.1).[7] The PivOAc protecting group was stable under



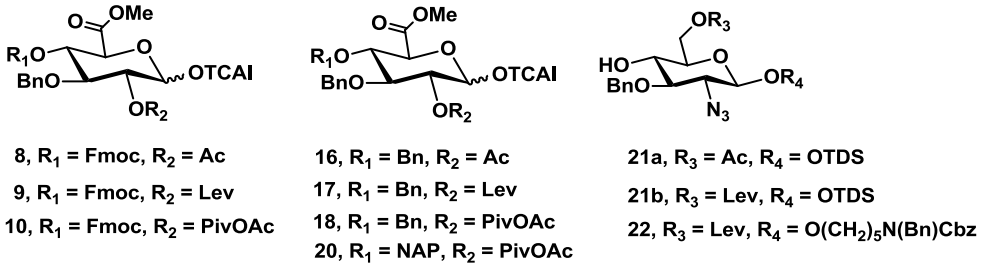
Scheme 2.1: Synthesis and application of PivOAc ester.[7]

deprotection conditions for Lev, Fmoc and TDS removal, and this orthogonality feature was helpful in facilitating the late stage deprotection and sulfation at required positions. Use of PivOAc ester group as a C2 auxiliary during the installation of 1,2 trans

stereoselective glycosylations coupled with its orthogonal stability towards required deprotections made it an ideal protecting group for our proposed synthetic strategy.

Synthesis of monosaccharide building blocks

The strategy uses 10 monosaccharides building blocks which were differentially protected with orthogonal protecting groups (scheme 2.2). These monosaccharides were divided comprised of two batches viz., glucuronic acid donors **8-10** & **16-20** and 2-deoxy-2-azido-D-glucopyransides acceptors **21a, 21b** & **22**. Further these donors and acceptors can be strategically coupled to generate a library of disaccharides compounds. The synthesis of glucosazide acceptor was based on our previous work.[2]

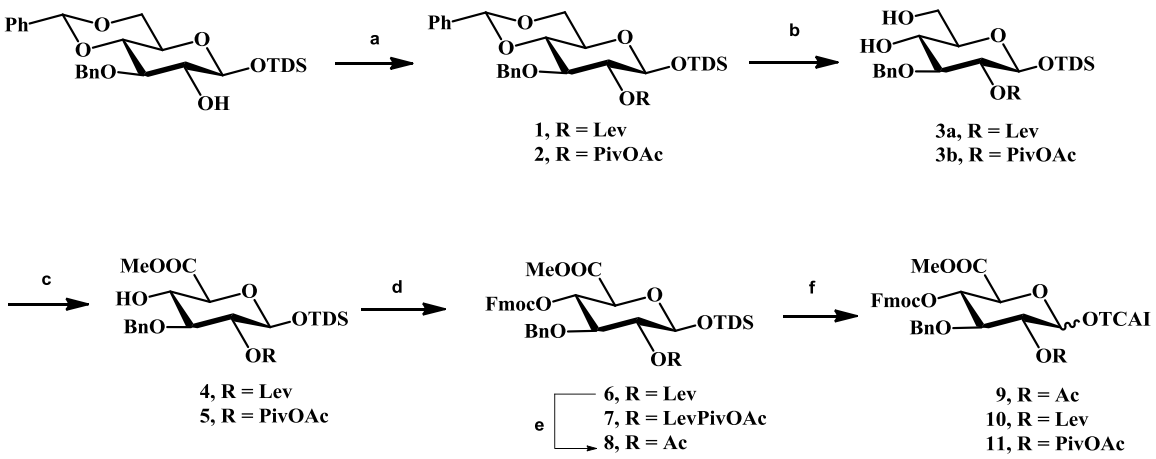


Scheme 2.2: Building blocks for HS synthesis

Synthesis of Glucuronic acid donor

The synthesis of the glucuronic acceptor began with benzylidene protected glucose derivative[8] with anomeric TDS ether (scheme 2.3). This compound was separately treated with levulinic acid and PivOAc-Cl to introduce Lev ester and PivOAc ester respectively on the C2 position to give derivatives **1** and **2** with 90% and 86% yields respectively. Both the compounds were subjected to acidic hydrolysis to cleave the benzylidine acetal to give the 4,6 diol compounds **3a** and **3b** respectively with good yields. The diol compounds with C2 lev and PivOAc were separately treated with TEMPO and BIAB to regioselectively oxidize C-6 hydroxy to carboxylic acid followed by methylation in

presence of diazomethane to give compounds **4** and **5** respectively with 68% and 88% yields respectively. Next, both these compounds **4** and **5** were separately treated with Fmoc-Cl in pyridine to introduce the the carbamate functionality at the C4 positions of compounds **6** and **7** respectively. Further to get the C2 acetyl derivative of GlcA, compound **6** was subjected to de-levulination conditions to give C2 hydroxy derivative followed by treatment with Ac_2O in pyridine to give compound **8** with C2 Ac with 81% yield. Next, compounds **6, 7** and **8** were separately treated with HF in pyridine to cleave the anomeric silyl ether to give respective lactol derivatives which were then treated with



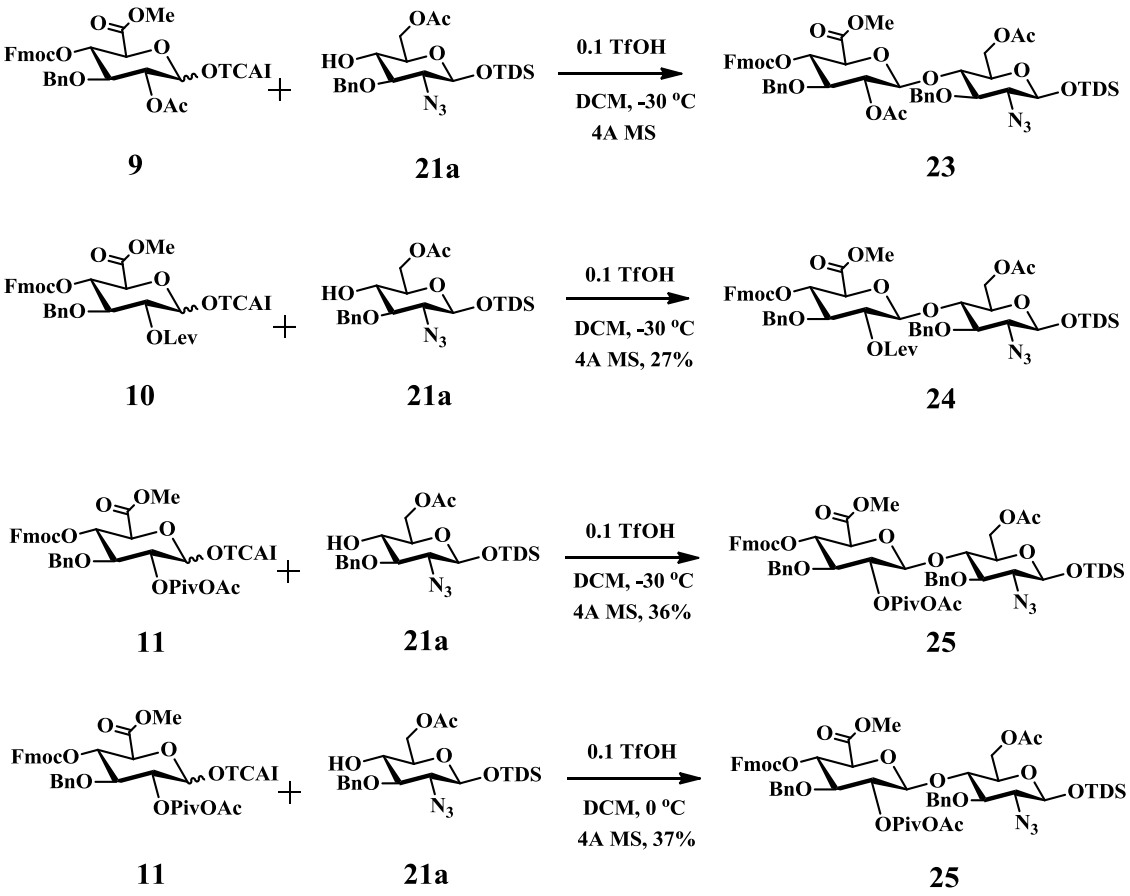
Scheme 2.3 Synthesis of Glucuronic acid donors with C4 Fmoc ester.

Reagents and conditions: (a) Levulinic acid, DCC, DMAP, rt, DCM, (90%, **1**); PivOAc-Cl, DMAP, Pyr., (86%, **2**); (b) DCM:TFA:H_2O (**3a**) ; EtSH, TsOH, DCM, (77%, **3b**); (c) (i) TEMPO, BAIB, rt, DCM, H_2O; (ii) CH_2N_2, Et_2O, (68%, **4**); (88%, **5**); (d) Fmoc-Cl, DMAP, Pyr. (87%, **6**); (76%, **7**) (e) (i) NH_2NH_3:OAc, Tol:EtOH (9:1); (ii)Ac_2O, Pyr. (81%, **8**); (f) (i) HF:Pyr. THF; (ii) Cl_3CCN, NaH, DCM.

trichloroacetonitrile in dichloromethane (DCM) to give trichloroacetimidate compounds **9, 10** and **11** respectively which were directly used in performing glycosylation reactions. With the preparation of monosaccharide blocks viz., glucuronyl donors and 2-azido-glycosyl acceptor, the next step was to study the reactivity profile of GlcA donors by performing their glycosylations under identical conditions to prepare the modular disaccharide motifs (scheme 2.4). The TfOH mediated coupling of GlcA donor with C4 Fmoc and C2 acetate **8** and glucosazide acceptor with C6 Ac **21a** at -30 ^{o}C in DCM gave hydrolysis of donor along with trihalose formation. Similarly, the coupling between GlcA donor with C2 Lev **9** and the same acceptor **21a** gave disaccharide product **24** in 27% yield along with hydrolysed donor. Next the coupling with C2 PivOAc substituted GlcA donor, **10** and acceptor **8** gave 36% disaccharide **25** formation. As expected the GlcA donors succumbed to its nature and exhibited poor reactivity leading to the hydrolysis of its trichloroacetimidate functionality with no or little disaccharide formation. But, in spite of the poor performance of the GlcA donors, we saw steady increment in the yields in the coupling reactions. Clearly the C2 substitution had evident effect on the reactivity of the donors. The GlcA donor with C2 Acetyl showed the poorest reactivity while the substitution with PivOAc exhibited comparative improvement. The GlcA donor with C2 Lev showed intermittent reactivity. The glycosylation of PivOAc substituted GlcA with the acceptor at higher temperature (0 ^{o}C) did not show improvement in the yield of the product formation (0 ^{o}C, **25**).

In order to improve the reactibity of GlcA donor we decided to revisit our monosaccharide protecting group design. In order to reduce the disarming effect caused by the substitution at C4, we decided to replace the Fmoc ester with benzyl ether. We

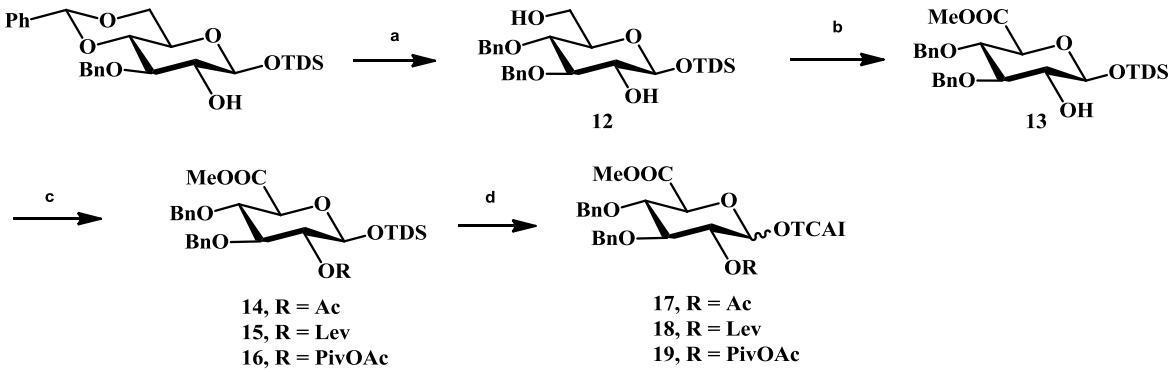
reconstructed all the GlcA donors with C4 benzyl ether. New donors **17** and **19** wrer synthesized with C2 acetyl, levulinoyl and pivaloyl acetate respectively (Scheme 2.5).



Scheme 2.4: Synthesis of disaccharides with C4 Fmoc esters

The benzylidene group was regioselectively opened on the C4 position in presence of Et_3SiH and $PhBCl_2$ at -78 ^oC to give diol **12**. The resulting free C6 primary alcohol was chemoselectivily oxidized to carboxylic acid in presence of TEMPO and BAIB. The free carboxylic acid was then methylated to give the methyl ester of the carboxylic acid in presence of CH_2N_2 to give derivative **13**. Further the modular design permitted us to use this common precursor to introduce Ac, Lev and PivOAc to give compounds **17-19** respectively with good yields. Next these compounds were subjected to HF in pyridine to

cleave the silyl ether followed by treatment with trichloroacetonitrile in presence of catalytic NaH to give GlcA donors **17-19** which were used to perform the second set of glycosylations.



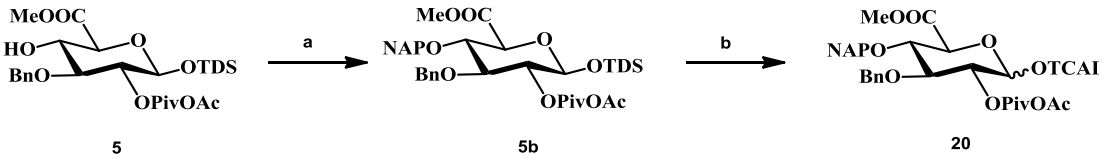
Scheme 2.5: Synthesis of Glucuronic acid donors with C4 benzyl ether

Reagents and conditions: (a) Et_3SiH,, $PhBCl_3$, 3Å Molecular sieves, DCM, -78 ^{0}C (91%); (b) (i) TEMPO, BAIB, rt, DCM, H_2O; (ii) CH_2N_2, Et_2O, (84%); (c) (i) Ac_2O, Pyridine (**14**); (ii) Levulinic acid, DCC, DMAP, rt, DCM, (89%, **15**); (iii) PivOAc-Cl, DMAP, Pyr., (88%, **16**); (d) (i) HF:Pyr. THF; (ii) Cl_3CCN, NaH, DCM

Synthesis of disaccharides with C4 benzyl ethers

With the new C4 benzyl substituted donors a series of glycosylations were performed with the glucosazide acceptor under similar coupling conditions (scheme 2.7). The C2 acetyl GlcA donor in this case showed improvement in reactivity with 22% product formation (**26**) along with an unusual side product, a trihalose orthoester (**26a**, experimental data). This side product was isolated and was well characterized to confirm its formation. The replacement of Fmoc ester with a benzyl ether lead to rise in the reactivity of the GlcA donor. Similarly, C2 Lev donor exhibited improvement in

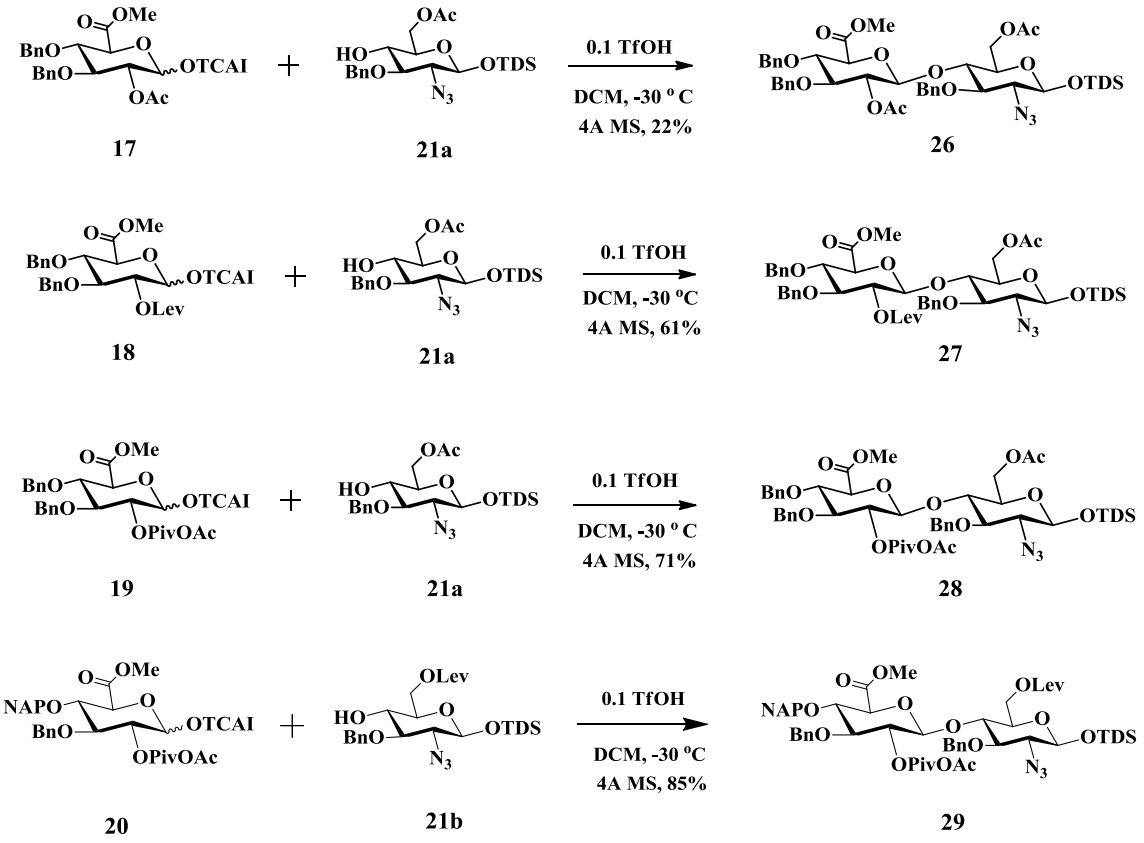
reactivity to give disaccharide **27** with 61% yield. The GlcA donor substituted with C2 PivOAc recorded the best outcome between the three C2 ester functionalities with disaccharide formation (**28**) in 71% yield. The combination of benzyl ether and PivOAc succeeded in improving the reactivity of GlcA donor. The presence of electron donating C4 benzyl group lead to a definite increase in the reactivity but this was attained at a loss of strategic orthogonality. The orthogonal deprotection at the C4 position is needed in the overall design of a modular synthesis to construct larger oligosaccharides. Thus we envisioned the use of a C4 naphthyl ether which had the essential electron donating effect of a benzyl ether and necessary orthogonality of a Fmoc ester. Thus, a GlcA donor with C4 NAP ether was constructed (scheme 2.6). The precursor compound **5** was subjected to C4 napthylation in presence of naphthyl methyl bromide and NaH in DMF. The anomeric TDS group of compound **5b** was cleaved in presence of HF in pyridine followed by treatment with trichloroacetonitrile in DCM to give the trichloroacetimidate donor **20** (scheme 2.6). The resulting GlcA donor with C4 naphthyl ether was then subjected to glycosylation with the glucosamine acceptor **21a** under exactly similar conditions as before to give disaccharide **38** in 85% yield (scheme 2.7).



5 5b 20

Scheme 2.6: Synthesis of Glucuronic donor with C4 napthyl ether

(a) NAPBr, NaH, TBAI, DMF, -20 ^{o}C (**5b**, 70%); (b) (i) HF:Pyr. THF; (ii) Cl_3CCN, NaH, DCM

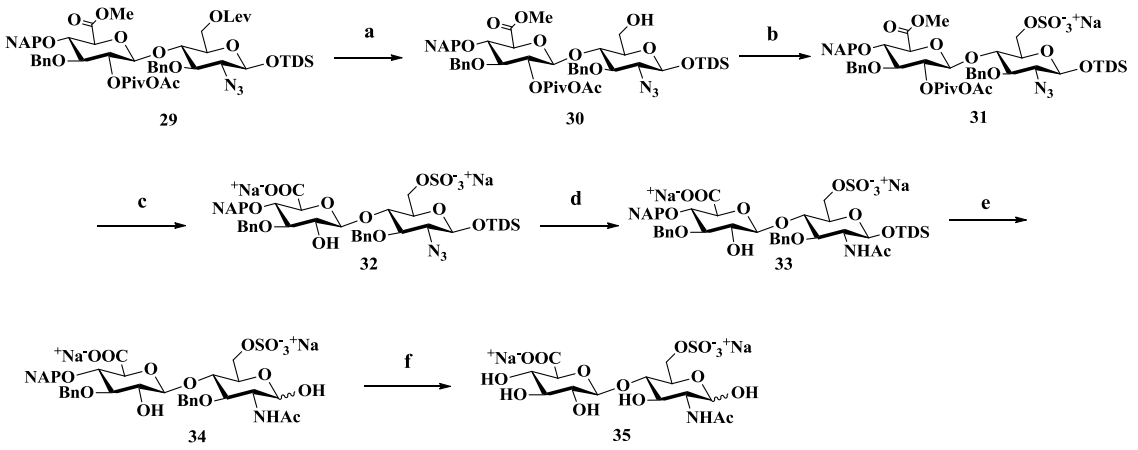


Scheme 2.7: Synthesis of disaccharides compounds with C4 benzyl and naphthyl ethers

Deprotection of disaccharide with TDS

With disaccharide **29** in hand, we moved our focus to the deprotection scheme (scheme 2.8). The orthogonally substituted disaccharide was first subjected to levulinolysis in presence of hydrazine acetate in toluene and ethanol (9:1) at room temperature to give the disaccharide **30** with free C6 alcohol with 76% yield. The free alcohol of de-levulinated disaccharide was then treated with pyridine sulfur trioxide complex to give disaccharide **31** with C6 *O*-sulfate in 71% yield. The crude product was purified with an iatrobeads column and further passed through an ion exchange resin to give the sodium salt of

sulfate. The sulfate group is very labile and thus it was stabilized by the sodium salt which was introduced during the ion-exchange column. After introduction of the sulfate,



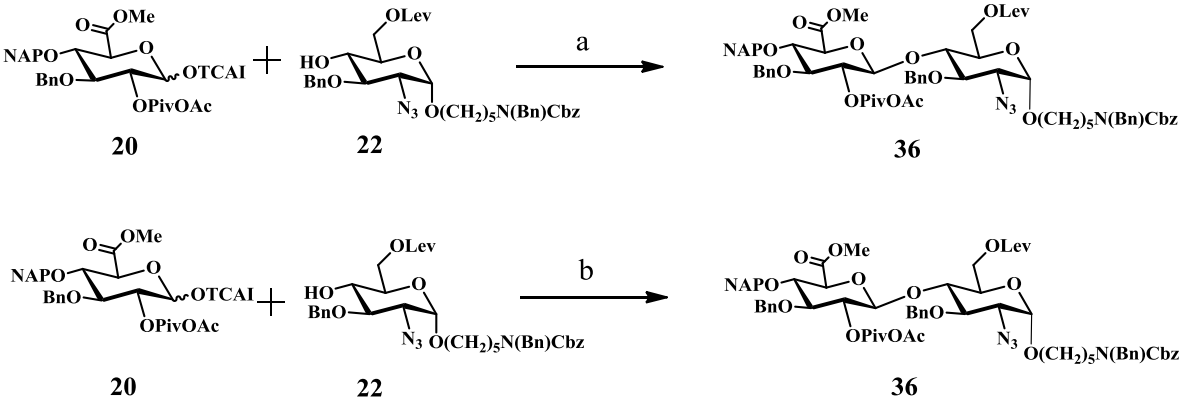
Scheme 2.8: Deprotection of disaccharide with TDS

Reagents and conditions: (a) NH_2NH_3OAc, Tol:EtOH (9:1) (76%); (b) $Pyr.SO_3$, DMF (70%); (c) (i) LiOH, H_2O_2, THF (ii) NaOH, MeOH (43%, over 2 steps); (d) (i) PMe_3, THF, NaOH; (ii) Ac_2O, Pyr, MeOH (72%); (e) HF/Pyr, THF, H_2O (60%); (f) Pd/C, H_2 (71%)

compound **31** was treated with hydrogen peroxide and lithium hydroxide in THF to saponify the methyl ester of the carboxylic acid to give free carboxylic acid. Without purification the crude acid was treated with NaOH in MeOH to deprotect the C2 PivOAc ester to give compound **32** in 43% yield over two steps. The saponification protocol gave yields lower than expected. On performing further analysis we found that TDS group was cleaved in presence of the basic conditions which caused the drop in yield of the reaction. Due to the presence of the TDS group the solubility of the compound was also compromised. Taking the above discussed points into account we proceeded with the remainder of the deprotection scheme. Next we subjected saponified compound **32** for

azide reduction in presence of trimethyl phosphine (PMe_3) and NaOH in THF. The resulting free amine was reacted with acetic anhydride in pyridine and MeOH to give disaccharide compound **33** with NHAc functionality with 72% yield. Compound **33** was then treated with HF in pyridine to cleave the silyl ether to give lactol compound **34** in 60% yield as an anomeric mixture. The lactol is finally subjected to hydrogenolysis with palladium on charcoal and H_2 gas to give completely deprotected compound **35** with 71% yield as an anomeric mixture.

At this point we revisited the problem of undesired TDS cleavage by changing our glucosamine acceptor with anomeric TDS to anomeric masked amino pentyl linker (scheme 2.7). The new glucosamine acceptor with linker **22** was synthesized based on our previous work.[2] With acceptor **22** in hand we performed its glycosylation with GlcA donor **20** with C4 NAP and C2 PivOAc in presence of TfOH in DCM to obtain disaccharide **36** in 44% yield. The lower yields were attributed to the presence of the lengthy amino pentyl linker. The linker amine can in theory



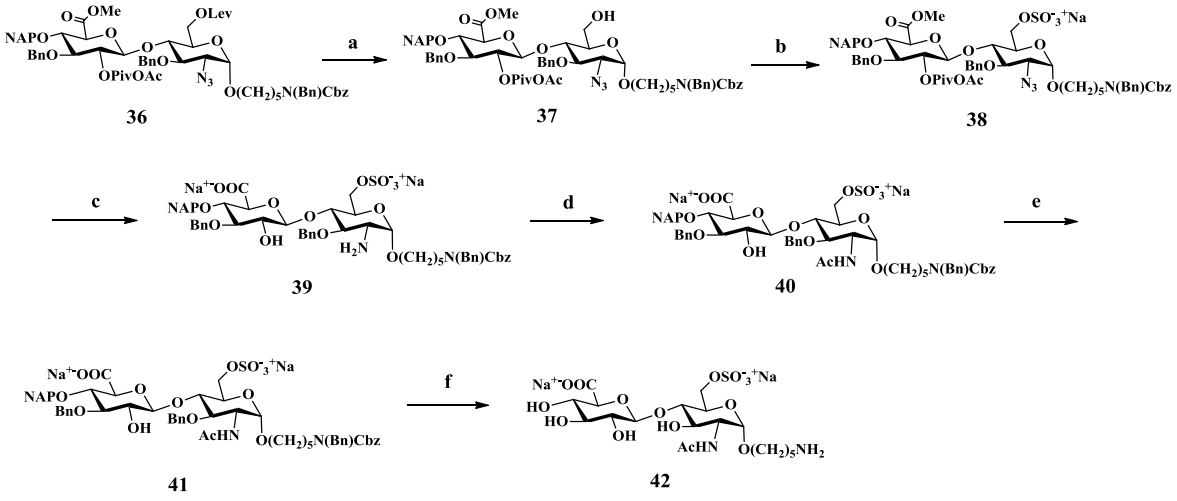
Scheme 2.9: Synthesis of disaccharide with amino pentyl linker

(a) 0.1 TfOH, DCM, -30 ^{o}C, 4Å MS, (44%, **36**), (a) 0.4 TfOH, DCM, -30 ^{o}C, 4Å MS, (64%, **36**)

quench the acid catalyst in the coupling reaction hampering proper activation and subsequently the yield of the reaction.[2] Therefore with above reasoning we repeated the glycosylation reaction with increased dosage of TfOH to observe improvement and obtain compound **36** in 64% yield. Next we set out to perform the deprotection of compound **36.**

Deprotection scheme with Linker

Disaccharide **36** was subjected to levulinolysis in presence of hydrazine acetate in toluene and ethanol (9:1) at room temperature to give the disaccharide **37** with free C6 alcohol with 65% yield. Resulting C6 alcohol was then treated with pyridine sulfur trioxide complex to give disaccharide **38** with C6 *O*-sulfate with 77% yield. The sulfated crude product was purified by passing through iatrobeads column and further it was passed through an ion exchange resin to give the stable sodium salt of sulfate. The sulfated compound **38** was subsequently treated with hydrogen peroxide and lithium hydroxide in THF to saponify the methyl ester of the carboxylic acid to give free carboxylic acid. Without purification the crude acid is treated with NaOH in MeOH to deprotect the C2 PivOAc. Due to the presence of the linker in place of TDS, an improved yield of 84% for compound **39** over two steps was observed. Next we subjected saponified compound **39** for azide reduction in presence of trimethyl phosphine (PMe_3) and NaOH in THF to obtain free amine **40** in 72% yield. The resulting free amine was reacted with acetic anhydride in pyridine and methanol to give disaccharide compound **41** with NHAc functionality with 83% yield. Compound **41** was finally subjected to a two-step hydrogenolysis with Palladium on charcoal and H_2 followed by palladium hydroxide on carbon with H_2 in water to give completely deprotected compound **42** with 86% yield.



Scheme 2.10: Deprotection of disaccharide with amino pentyl linker

Reagents and conditions: (a) NH_2NH_3OAc, Tol:EtOH (9:1) (65%); (b) $Pyr.SO_3$, DMF (77%); (c) (i) LiOH, H_2O_2, THF (ii) NaOH, MeOH (84%); (d) PMe_3, THF, NaOH (72%); (e) Ac_2O, Pyr, MeOH (83%); (f) Pd/C, H_2 ; $Pd(OH)_2$ (86%).

Summary and future outlook

We have performed a novel study to understand the effect of protecting groups on glycosylation outcomes. The competitive experiments towards the utility of PivOAc as a C2 participating functionality on GlcA donors in comparison to Ac and Lev proved effective and valuable. The occurrence of both ortho-esters and threhalose-ortho-esters were observed in presence of the classic Ac group at the C2 position of GlcA donors. Levulinoate esters at C2 position of GlcA did not show occurrence ortho-ester leading to product formation with decent yields. This is helpful as Lev group at C2 presents the opportunity to introduce sulfation in GlcA if required. PivOAc proved to be the best of the lot showcasing best yields without any trace of formation of ortho-esters. PivOAc was helpful in inducing excellent stereoselectivity in products and it was also suitable for the

required late stage deprotection and functionalization. In our strategy we included the popular Fmoc group at the C4 position for their convenience of orthogonality. The aim was to design a compound with access to chain extension at the non-reducing end. The presence of Fmoc esters on the C4 position hampered the yields of the glycosylations. The electron withdrawing carbamate functionality had a disarming effect on the already deactivated GlcA donor leading to coupling inefficiency. At this point we modified our building block design by replacing the Fmoc ester with a NAP ether which directly resulted with improvement in yields without loss of strategic orthogonality. In our late stage deprotection we encountered another unexpected problem with the saponification of the methyl ester of the carboxylic acid along with the removal of the PivOAc group. The presence of the anomeric TDS group affected the solubility of the compound which lead to longer reaction times under strongly basic conditions which resulted in the partial cleavage of TDS group itself. Although we completed the deprotection scheme for this particular disaccharide we were presented with an issue that called for revisiting our strategic design. We decided to replace the TDS ether with aminopentyl spacer in an effort to circumvent the inefficient saponification. Our efforts in doing so resulted in acheiving excellent yields in the saponification along with the remainders of the deprotections reactions. Further the disaccharide compounds can be converted into donors and acceptors by removing TDS and NAP groups respectively which can be employed for making larger HS oligosaccharides.

Experimental information

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 ~150oC or by spraying with a solution of $(NH_4)_6Mo_7O_{24}$ .H_2O (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150^oC. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å) 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. Mass spectra were recorded on Mass spectra were recorded on an ABISciex 5800 MALDi-TOF-TOF or Shimadzu LCMS-IT-TOF mass spectrometer. The matrix used was 2,5-dihydroxy-benzoicacid (DHB) and ultramark 1621 as the internal standard.

Dimethylthexylsilyl 2-*O*-levulinoyl -3-*O*-benzyl-4,6-*O*-benzylidene -β-D-glucopyranoside (**1**): A suspension of DCC (1.2 g, 5.990 mmol) and DMAP (0.012 g, 0.09 mmol) in DCM (5 mL) was added to a solution of starting material (1.0 g, 1.99

mmol) and levulinilic acid (0.46 g, 3.99 mmol) in DCM (5 mL) at 0 ^{0}C. After stirring for 2 h at ambient temperature TLC (hexanes/EtOAc, 70/30, v/v) indicated the total consumption of the starting material. The mixture was filtered over pad of celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as an oil (1.07 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.08 (m, 10H, CH Aromatic), 5.44 (s, 1H, CH benzylidene), 4.85 (t, *J* = 8.8, 7.5 Hz, 1H, H2), 4.73 (d, *J* = 12.1 Hz, 1H, C*H*HBn), 4.62 – 4.51 (m, 2H, H1 CH*H*Bn), 4.18 (dd, *J* = 10.5, 5.0 Hz, 1H, H6a), 3.73 – 3.53 (m, 3H, H3, H5, H6b), 3.32 (dt, *J* = 9.4, 4.8 Hz, 1H, H4), 2.66 – 2.34 (m, 4H, 2xCH_2 Lev), 2.04 (s, 3H, CH_3 Lev), 1.55 – 1.41 (m, 1H, $CH(CH_3)_2$), 0.76 – 0.67 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.01 (d, *J* = 6.3 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.39, 128.47, 129.95, 128.27, 101.78, 75.56, 74.23, 96.45, 74.23, 68.98, 68.96, 81.79, 78.68, 66.50, 37.99, 28.07, 30.05, 34.06, 18.97. HRMS: ($M+Na^+$) found 598.2958, observed 598.2962

Dimethylthexylsilyl*O*-methyl-2-*O*-levulinoyl-3-*O*-benzyl-β-D-glucupyranosyluronate (**4**): Starting material (1.0 g, 1.671 mmol) was dissolved in DCM:TFA:H_2O (0.06 M) (10/1/0.1, v/v) at 0 ^{0}C. After stirring for 1hr TLC (Hex/EtOAc, 50/50, v/v) indicated the complete consumption of the starting material. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* and was dried on the membrane pump. A suspension of TEMPO (0.052 g, 0.334 mmol) and BAIB (1.34 g, 4.177 mmol) were added to a stirred solution of the crude diol in DCM/water (3/1 v/v, 2 mL). After stirring the biphasic solution vigorously for 1hr at ambient temperature TLC (hexanes/EtOAc, 60/40, v/v)

indicated the consumption of the starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M, 10 mL) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried (MgSO4) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (5 mL) followed by addition of a freshly prepared solution of diazomethane in Et_2O (2 mL). After stirring for five minutes, the excess diazomethane was quenched with a few drops of acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (0.61 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 – 7.10 (m, 5H, CH Aromatic), 4.80 (dd, *J* = 9.5, 7.5 Hz, 1H, H4), 4.72 – 4.57 (m, 2H, C*H*HBn, CH*H*Bn), 4.54 (d, *J* = 7.6 Hz, 1H, H1), 3.85 (dd, *J* = 9.8 Hz, 1H, H4), 3.69 (d, *J* = 9.5 Hz, 4H, H5, CH_3 $COOCH_3$), 3.40 (dd, *J* = 9.5 Hz, 1H, H3), 2.68 – 2.26 (m, 4H, 2xCH_2 Lev), 2.02 (s, 3H, CH_3 Lev), 1.44 (q, *J* = 6.9 Hz, 1H, $CH(CH_3)_2$), 0.76 – 0.59 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.01 (d, *J* = 8.0 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.54, 96.31, 81.35, 74.59, 74.37, 74.19, 72.23, 54.08, 38.05, 34.10, 30.17, 28.14, 20.12, 0.80. HRMS: ($M+Na^+$) found 561.1480, observed 561.1483.

Dimethylthexylsilyl *O*-methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucupyranosyluronate (6): A suspension of Fmoc-Cl (1.2 g, 4.475 mmol) and DMAP (1 mg, 0.074 mmol) were added to a stirred solution of starting material (0.4 g, 0.745 mmol) in Pyridine (7.5 mL) at ambient temperature. TLC indicated complete consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by

silica gel column chromatography using a gradient of hexanes/EtOAc (6/1 v/v) to obtain product (0.49 g, 87%) as oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 – 7.70 (m, 2H, CH Aromatic), 7.65 – 7.55 (m, 2H, CH Aromatic), 7.39 (tt, *J* = 7.7, 3.8 Hz, 2H, CH Aromatic), 7.33 – 7.18 (m, 5H, CH Aromatic), 5.19 – 5.08 (t, 1H, H4), 5.04 (t, *J* = 9.1, 7.5, 1.0 Hz, 1H, H2), 4.75 – 4.68 (m, 1H, H1), 4.70 – 4.58 (m, 2H, CH*H*Bn, C*H*HBn), 4.44 (m, *J* = 10.4, 7.2, 1.0 Hz, 1H, C*H*H Fmoc), 4.34 (ddd, *J* = 10.3, 7.4, 1.0 Hz, 1H, CH*H* Fmoc), 4.28 – 4.19 (m, 1H, CH_2C*H* Fmoc),), 4.03 (dd, *J* = 10.0, 1.0 Hz, 1H, H5), 3.81 – 3.71 (m, 1H, H3), 3.69 (d, *J* = 1.0 Hz, 3H, CH_3 $COOCH_3$), 2.78 – 2.41 (m, 4H, 2xCH_2 Lev), 2.16 (d, *J* = 1.0 Hz, 3H, CH_3 Lev), 1.67 – 1.49 (m, 1H, $CH(CH_3)_2$), 0.92 – 0.75 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.16 (dd, *J* = 11.4, 1.0 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 127.24, 125.20, 120.71, 95.90, 79.33, 74.97, 74.40, 74.04, 72.42, 70.36, 70.35, 52.45, 46.65, 37.84, 33.99, 29.82, 27.95, 18.46, 19.91, 1.11. HRMS: ($M+Na^+$) found 783.3618, observed 783.3626

Trichloroacetimidate *O*-methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucupyranosyluronate (8): To a stirred solution of starting material (92 mg, 0.1210 mmol) in THF (1.2 mL), HF in pyridine (220 μL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with $NaHCO_3$ (Satd.), H_2O and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. Cl_3CCN (61 μL, 0.605 mmol) and NaH (60%) (~1 mg, 0.012 mmol) were added to a solution of the crude material in DCM (1.2 mL). After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel

column chromatography using a gradient of hexanes/EtOAc (2/1 v/v) to give product which was used directly for glycosylation.

Dimethylthexylsilyl *O*-Methyl-2-*O*-acetyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucupyranosyluronate (8): Hydrazine acetate (18 mg, 0.128 mmol) was added to a solution of starting compound (100 mg, 0.128 mmol) in Tol/EtOH (2/1, v/v, 4.2 mL) and the reaction mixture was stirred at ambient temperature. After 2 h, TLC analysis showed total consumption of the starting material. The reaction mixture was diluted with dichloromethane, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. A solution of crude compound in pyridine/acetic anhydride (4/1, v/v, 0.2 M) was stirred for 6 hr at ambient temperature. The mixture was co-evaporated with toluene *in vacuo* and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (3/1 v/v) to obtain product as oil. (74 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (t, *J* = 6.8 Hz, 2H, CH Aromatic), 7.63 (dd, J = 10.6, 7.4 Hz, 2H, CH Aromatic), 7.41 (p, J = 9.4, 8.2 Hz, 3H, CH Aromatic), 7.36 – 7.29 (m, 5H, CH Aromatic), 5.15 (t, *J* = 9.6 Hz, 1H, H4), 5.06 (dd, *J* = 9.4, 7.3 Hz, 1H, H2), 4.75 – 4.67 (m, 2H, H1, CH*H*Bn), 4.60 (d, *J* = 11.8 Hz, 1H, C*H*HBn), 4.47 (dd, *J* = 10.5, 7.1 Hz, 1H, CH*H* Fmoc), 4.37 (dd, *J* = 10.7, 7.5 Hz, 1H, C*H*H Fmoc), 4.26 (t, *J* = 7.3 Hz, 1H, CH_2C*H* Fmoc), 4.06 (d, *J* = 9.8 Hz, 1H, H5), 3.75 (d, J = 32.7 Hz, 3H, CH_3 $COOCH_3$), 3.68 (m, 1H, H3), 1.98 (s, 3H, CH_3 Ac), 1.63 (q, J = 6. 9 Hz, 1H, $CH(CH_3)_2$), 1.00 – 0.82 (m, 16H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (s, 3H), 0.16 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 119.84, 125.01, 127.69, 127.02, 127.95, 127.68, 75.00, 73.71, 95.83, 73.79, 73.78, 70.23, 70.28, 46.58, 72.28, 79.05, 53.77, 52.65, 20.91, 29.60, 18.57, 19.90, 0.19. HRMS: ($M+Na^+$) found 727.3016, observed 727.3011.

Methyl-2-*O*-acetyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucupyranosyluronate (8a): To a stirred solution of crude material in THF (1.3 mL), HF in pyridine (30%, 233 μL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with $NaHCO_3$ (Satd.), H_2O and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. The resulting residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (2/1 v/v) to obtain product as oil (48 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (dt, *J* = 7.7 Hz, 2H CH Aromatic), 7.66 – 7.60 (m, 2H), 7.46 – 7.39 (m, 2H), 7.35 – 7.23 (m, 7H, CH Aromatic), 5.58 (dd, *J* = 4.7, Hz, 1H, H1), 5.12 (t, *J* = 9.2 Hz, 0.25H, H2β), 5.07 (dd, *J* = 9.4, 8.6 Hz, 1H, H2α), 4.97 – 4.90 (m, 1H, H2α), 4.77 – 4.69 (m, 2H, C*H*HBn, CH*H*Bn), 4.68 – 4.63 (m, 1H, H5α), 4.47 (ddd, *J* = 10.4, Hz, 1H, C*H*H Fmoc), 4.41 – 4.34 (m, 1H, CH*H* Fmoc), 4.26 (t, *J* = 7.3 Hz, 1H, CH_2C*H* Fmoc), 4.20 – 4.11 (m, 1.25H, H3α, H3β), 3.95 (dd, *J* = 9.3, 1.6 Hz, 0.25H, OHβ), 3.85 (t, *J* = 9.0 Hz, 0.25H, H3β), 3.70 (s, 0.75H, CH_3 $COOCH_3$), 3.67 (s, 3H, CH_3 $COOCH_3$), 3.53 (dd, *J* = 4.4 Hz, 1H, OHα), 2.06 (d, *J* = 2.2 Hz, 3H, CH_3, Ac) ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.33, 127.46, 125.80, 125.69, 125.32, 128.19, 127.45, 128.24, 127.45, 127.84, 128.61, 127.82, 127.87, 90.45, 75.04, 74.61, 74.97, 74.54, 72.38, 72.38, 75.30, 74.94, 75.30, 95.96, 74.84, 75.29, 74.84, 68.94, 70.66, 70.66, 46.87, 46.54, 76.17, 76.17, 73.08, 60.69, 78.62, 53.21, 53.06, 21.02. HRMS: ($M+Na^+$) found 585.1059, observed 585.1066.

Trichloroacetimidate *O*-methyl-2-*O*- acetyl -3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)- α/β -D-glucupyranosyluronate (9): To a stirred solution

of the starting material (48 mg, 0.111 mmol) in DCM (1.1 mL), Cl_3CCN (56 μL, 0.558 mmol) and NaH (60%) (~1 mg, 0.011mol) were added. After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography using a gradient of hexanes/EtOAc (2/1 v/v) to give product which was used directly for glycosylation.

Dimethylthexylsilyl2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4,6-*O*-benzylidene - β -D-glucopyranoside (**2**): To a stirring solution of starting material (2.7 g, 5.335 mmol) in pyridine (18 mL), DMAP (0.7 g, 5.535 mmol) and PivOAc-Cl (1.3 mL, 10.67 mmol) was added at 0 ^{0}C. After stirring for 4hr at ambient temperature TLC (hexanes/EtOAc, 70/30, v/v) indicated the consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The mixture was concentrated *in vacuo* and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (3.0 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.42 (m, 2H, CH Aromatic), 7.41 – 7.32 (m, 4H, CH Aromatic), 7.32 – 7.20 (m, 4H, CH Aromatic), 5.56 (s, 1H, CH benzylidene), 5.02 (t, *J* = 8.8, 7.2, 1.3 Hz, 1H, H2), 4.92 (d, *J* = 11.6 Hz, 1H, CH*H*Bn), 4.81 (dd, *J* = 7.1, 1.2 Hz, 1H, H1), 4.63 (d, *J* = 11.7 Hz, 1H, C*H*HBn), 4.32 (dd, *J* = 10.5, 4.8 Hz, 1H, H4), 4.07 (t, *J* = 7.2, 2.4 Hz, 2H, CH_2 PivOAc), 3.88 – 3.73 (m, 3H, H3, H6a, H6b), 3.49 (m, *J* = 5.0 Hz, 1H, H5), 1.97 (d, *J* = 1.3 Hz, 3H, CH_3 PivOAc), 1.85 (t, *J* = 7.4 Hz, 2H, CH_2 PivOAc), 1.62 (d, *J* = 6.8 Hz, 1H, 1H, $CH(CH_3)_2$), 1.18 (t, *J* = 2.6 Hz, 7H, 2xCH_3 PivOAc), 0.92 – 0.80 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 – 0.11 (m, 6H, $Si(CH_3)_2$). 13C NMR (151 MHz, CDCl3) δ 129.68, 129.11, 128.01, 127.49, 124.85, 101.76, 96.44, 81.25, 79.51, 74.01,

73.82, 73.71, 69.15, 68.26, 66.08, 62.20, 38.63, 34.55, 24.65, 21.51, 19.22, -1.81, -2.24. HRMS: ($M+Na^+$) found 679. 2346, observed 679.2346

Dimethylthexylsilyl 2-*O*-(4-acetoxy-2,2-dimethylbunoate) -3-*O*-benzyl - β -D-glucopyranoside (**3b**): EtSH (1.7 g, 27.419 mmol) and TsOH (0.55 g, 2.75 mmol) were added to a stirred solution of starting material (3 g, 4.569 mmol) in DCM. After stirring at ambient temperature for 1h TLC (Hex/EtOAc, 75/25, v/v) indicated the complete consumption of the starting material. The reaction mixture was quenched with Et_3N and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (3/1 v/v) to obtain product as oil (2.0 g, 77%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 5H, CH Aromatic), 4.99 (t, *J* = 9.5, 1H, H2), 4.81 – 4.74 (m, 2H, CH*H*Bn, H1), 4.64 (d, *J* = 11.6 Hz, 1H, C*H*HBn), 4.14 (t, *J* = 7.4 Hz, 2H, CH_2 PivOAc), 3.88 (dd, *J* = 11.8, 4.1 Hz, 1H, H6a), 3.82 – 3.69 (m, 2H, H6b, H3), 3.57 (t, *J* = 9.2 Hz, 1H, H4), 3.40 (m, *J* = 9.2, Hz, 1H, H3), 2.01 (s, 3H, CH_3 PivOAc), 1.97 – 1.88 (m, 2H, CH_2 PivOAc), 1.69 – 1.55 (m, 1H, $CH(CH_3)_2$), 1.30 – 1.23 (m, 6H, 2xCH_3 PivOAc), 0.92 – 0.82 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.16 (d, *J* = 16.2 Hz, 7H, $Si(CH_3)_2$),. ^{13}C NMR (126 MHz, $CDCl_3$) δ 129.00, 127.99, 126.36, 96.15, 83.11, 75.16, 74.58, 73.80, 73.75, 73.76, 70.50, 62.69, 62.68, 61.31, 50.75, 37.96, 33.54, 26.67, 25.17, 22.92, 21.05, 20.70, 19.52, 18.48, -1.64, -2.18. HRMS: ($M+Na^+$) found 591. 3984, observed 591.3984.

Dimethylthexylsilyl *O*-methyl-2-*O*- (4-acetoxy-2,2-dimethylbunoate) -3-*O*-benzyl-β-D-glucopyranosyluronate (5): A suspension of TEMPO (66 mg, 0.422 mmol) and BAIB (1.69 g, 5.277 mmol) were added to a stirred solution of starting material (1.2 g, 2.110 mmol) in DCM/water (2/1 v/v, 10 mL). After stirring the biphasic solution

vigorously for 1hr at ambient temperature TLC (hexanes/EtOAc, 60/40, v/v) indicated the complete consumption of the starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (6 mL) followed by addition of a freshly prepared solution of diazomethane in Et_2O (4 mL). After stirring for five minutes, the excess diazomethane was quenched with acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient of hexane/EtOAc (6/1 v/v) to obtain product as oil (1.10 g, 88%). 1H NMR (500 MHz, $CDCl_3$) δ 7.20 – 7.08 (m, 10H, CH Aromatic), 4.83 (t, J = 9.3, 1H, H3), 4.74 (d, J = 11.5 Hz, 1H, CH*H*Bn), 4.62 (d, J = 7.3 Hz, 1H, H1), 4.54 (d, J = 11.5 Hz, 1H, C*H*HBn), 3.99 – 3.86 (m, 3H, H4, CH_2 PivOAc), 3.70 (d, 4H, H5, CH_3 $COOCH_3$), 3.46 (t, J = 9.0 Hz, 1H, H3), 1.83 (s, 2H, CH_3 PivOAc,), 1.53 – 1.43 (m, 1H, $CH(CH_3)_2$), 1.05 (s, 6H, 2xCH_3 PivOAc), 0.75 – 0.66 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.02 (d, J = 19.5 Hz, 6H, $Si(CH_3)_2$).^{13}C NMR (126 MHz, $CDCl_3$) δ 128.55, 128.53, 127.48, 128.84, 127.77, 74.22, 74.08, 96.44, 74.08, 61.56, 72.12, 74.14, 52.90, 81.75, 21.18, 38.32, 33.99, 25.40, 18.68, 20.71, 20.15, -1.84, -2.70. HRMS: ($M+Na^+$) found 619.2037, observed 619.2047.

Dimethylthexylsilyl*O*-methyl-2-*O*-(4-acetoxy-2,2dimethylbunoate)-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)- β -D-glucupyranosyluronate (7): A suspension of Fmoc-Cl (0.78 g, 3.017 mmol) and DMAP (~1.0 mg, 0.0051 mmol) were added to a stirred solution of starting material (0.3 g, 0.502 mmol) in Pyridine (5 mL) at ambient temperature. TLC (hexanes/EtOAc, 75/25, v/v) indicated complete consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed

with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography with a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as an oil (0.313 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 3H, CH Aromatic), 7.72 – 7.42 (m, 7H, CH Aromatic), 7.55 – 7.29 (m, 4H, CH Aromatic), 5.24 (t, *J* = 9.6 Hz, 1H, H4), 5.11 (dd, *J* = 8.7, 6.9 Hz, 1H, H2), 4.84 (d, *J* = 6.9 Hz, 1H, H1), 4.74 – 4.62 (m, 2H, C*H*HBn, CH*H*Bn), 4.52 – 4.37 (m, 1H, CH*H* Fmoc), 4.36 – 4.18 (m, 2H, C*H*H Fmoc, CH Fmoc), 4.16 – 4.06 (m, 3H, CH_2 PivOAc), 3.87 (t, *J* = 9.1 Hz, 1H, H3), 3.71 (s, 3H, CH_3 $COOCH_3$), 2.00 (s, 3H, CH_3 PivOAc), 1.89 (t, *J* = 7.4 Hz, 3H, CH_2 PivOAc), 1.59 (s, 1H, $CH(CH_3)_2$), 1.22 (s, 6H, 2xCH_3 PivOAc), 0.91 – 0.85 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.25 – 0.16 (m, 6H, $Si(CH_3)_2$).^{13}C NMR (151 MHz, $CDCl_3$) δ 127.97, 127.73, 125.06, 120.11, 158.37, 95.86, 79.40, 74.58, 73.78, 73.20, 72.11, 70.27, 61.13, 52.54, 46.35, 37.97, 33.69, 29.39, 25.10, 20.76, 19.51, -9.66. HRMS: ($M+Na^+$) found 841.20, observed 841.2122.

Trichloroacetimidate *O*-methyl-2-*O*- (4-acetoxy-2,2-dimethylbunoate) -3-*O*- benzyl -4-*O*- (9- fluorenylmethoxycarbonyl) -α/β-D-glucupyranosyluronate (11): To a stirred solution of starting material (140 mg, 0.17 mmol) in THF (1.7 mL), 30% HF in pyridine (0.34 mL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with water, $NaHCO_3$ (Satd.), and brine. The organic phase was dried (MgSO4), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. Cl_3CCN (200 μL, 0.200 mmol) and NaH (60%) (~1.0 mg, 0.020 mmol) were added to a solution of the crude material in DCM (1.7 mL). After

stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

Dimethylthexylsilyl -3,4-*O*-benzyl-β-D-glucopyranoside (**12**): A solution of starting material (1.0 g, 1.998 mmol) and activated molecular sieves (3Å, 1.0 g) in dichloromethane (20.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -78 ^{o}C followed by addition of Et_3SiH (576 µl, 5.994 mmol) and $PhBCl_2$ (1.5 mL, 6.993 mmol). After being stirred for 1 h at -78 ^{o}C, Et_3N (3 mL) and MeOH (3 mL) were added successively, and the mixture was diluted with $CHCl_3$ and washed with $NaHCO_3$ (satd.), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of $CHCl_3$/MeOH (95/5 v/v) to obtain product as oil (0.91 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 – 7.20 (m, 10H, CH Aromatic), 4.98 (d, *J* = 11.2 Hz, 1H, C*H*HBn), 4.89 (dd, *J* = 18.4, 11.1 Hz, 2H, CH*H*Bn, C*H*HBn), 4.67 (d, *J* = 11.0 Hz, 1H, CH*H*Bn), 4.58 (d, *J* = 7.5 Hz, 1H, H1), 3.86 (dd, *J* = 11.9, 2.9 Hz, 1H, H6a), 3.76 – 3.53 (m, 3H, H6b, H4, H3), 3.52 – 3.37 (m, 2H, H2, H5), 1.68 (p, *J* = 6.9 Hz, 1H, $CH(CH_3)_2$), 0.91 (d, *J* = 5.0 Hz, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.21 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.00, 97.67, 84.18, 76.76, 76.71, 75.45, 75.16, 75.14, 75.03, 62.32, 62.28, 34.25, 18.48, -1.95. HRMS: ($M+Na^+$) found 525.2862, observed 525.2855.

Dimethylthexylsilyl *O*-methyl-3,4-*O*-benzyl-β-D-glucopyranosyluronate (13): A suspension of TEMPO (30 mg, 0.199) and BAIB (640 mg, 1.99 mmol) were added to a stirred solution of starting material (0.40 g, 0.796 mmol) in DCM/water (3/1 v/v, 5.4 mL). After stirring the biphasic solution vigorously for 1hr at ambient temperature TLC

(hexanes/EtOAc, 60/40, v/v) indicated the consumption of the starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M, 2.5 mL) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (1.5 mL) followed by addition of a freshly prepared solution of diazomethane in Et_2O (2 mL). After stirring for five minutes, the excess diazomethane was quenched with a few drops of acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient of hexane/EtOAc (3/1 v/v) to obtain product as oil (355 mg, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.33 (m, 6H, CH Aromatic), 7.37 – 7.27 (m, 5H, CH Aromatic), 4.92 (d, J = 11.3 Hz, 1H, CH*H*Bn), 4.86 – 4.80 (m, 2H, C*H*HBn, CH*H*Bn), 4.62 (d, J = 11.3 Hz, 1H, CH*H*Bn), 4.53 (d, J = 7.4 Hz, 1H, H1), 3.92 – 3.87 (m, 1H, H5), 3.84 (d, J = 8.9 Hz, 1H, H4), 3.74 – 3.70 (m, 3H, CH_3 $COOCH_3$), 3.59 (t, J = 9.0 Hz, 1H, H3), 3.54 – 3.48 (m, 1H, H2), 1.55 (d, J = 2.8 Hz, 1H, $CH(CH_3)_2$), 0.91 – 0.82 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.17 (d, J = 4.2 Hz, 7H, $Si(CH_3)_2$), 13C NMR (151 MHz, CDCl3) δ 126.73, 126.73, 126.07, 125.59, 98.03, 82.72, 78.05, 76.93, 74.25, 74.17, 73.44, 56.37, 51.81, 33.08, 29.14, 20.78, 19.58, -0.29. HRMS: ($M+Na^+$) found 553.1355, observed 553.1364.

Methyl-2-*O*-acetyl-3,4-*O*-benzyl-α/β-D-glucupyranosyluronate (14): A solution of starting material (90 mg, 0.169 mmol) in pyridine:acetic anhydride (4/1,v/v, 0.2 M) was stirred for 2 hr at ambient temperature. The mixture was co-evaporated with toluene *in vacuo* and dried on the membrane pump for 3 hours. To a stirred solution of the resulting crude material in THF (1.5 mL), 30% HF in pyridine (340 µL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting

material. The reaction mixture was subsequently diluted with DCM, washed with water, $NaHCO_3$ (Satd.), and brine. The organic phase was dried (MgSO4), filtered, and concentrated under reduced pressure and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (2/1 v/v) to obtain product as oil (57 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 10H, CH Aromatic), 5.50 (d, *J* = 3.6 Hz, 1H, H1), 4.97 – 4.71 (m, 5H, H2, CH*H*Bn, CH*H*Bn, CH*H*Bn,), 4.66 (d, *J* = 10.9 Hz, 1H CH*H*Bn,), 4.53 (d, *J* = 9.3 Hz, 1H, H5), 4.09 (s, 1H, H3), 3.88 (s, 1H, H4), 3.76 (d, *J* = 6.2 Hz, 3H, CH_3, $COOCH_3$), 2.05 (d, *J* = 9.4 Hz, 3H, CH_3, Ac) ^{13}C NMR (126 MHz, $cdcl_3$) δ 129.81, 128.36, 128.33, 127.67, 127.66, 90.81, 79.18, 78.68, 75.43, 75.36, 75.03, 74.49, 72.96, 70.61, 51.91, 21.52. HRMS: ($M+Na^+$) found 453.1531, observed 453.1540.

Trichloroacetimidate *O*-methyl-2-*O*-acetyl-3,4-*O*-benzyl-α/β-D-glucupyranosyluronate (17): To a stirred solution of starting material (57 mg, 0.132 mmol) in DCM (1.3 mL), Cl_3CCN (66 μL, 0.662 mmol) and NaH (60%) (~1 mg, 0.013 mol) were added. After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

Dimethylthexylsilyl*O*-methyl-2-*O*-levulinoyl-3,4-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of DCC (117 mg, 0.565 mmol) and DMAP (1.1 mg, 0.01 mmol) was added to a solution of starting material (100 mg, 0.188 mmol) and levulinilic acid (39 µL, 0.377 mmol) in DCM (1.2 mL) at 0 ^{0}C. After stirring for 6 h at ambient temperature TLC (hexanes/EtOAc, 70/30, v/v) indicated the consumption of the starting material. The mixture was filtered over pad of celite and the filtrate was

concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (3/1 v/v) to obtain product as oil (105 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 11H, CH Aromatic), 4.99 (t, *J* = 9.3, 7.2 Hz, 1H, H2), 4.79 (dd, *J* = 11.5, 5.2 Hz, 2H, CH*H*Bn, C*H*HBn), 4.75 – 4.61 (m, 3H, H1, CH*H*Bn, CH*H*Bn), 3.94 (d, *J* = 7.4 Hz, 2H, H5, H4), 3.75 (s, 3H, CH_3 $COOCH_3$), 3.68 (t, *J* = 8.4 Hz, 1H, H3), 2.68 (dt, *J* = 17.5, 6.8 Hz, 2H, CH_2 Lev), 2.49 (t, *J* = 6.8 Hz, 2H, CH_2,Lev), 2.17 (s, 3H, CH_3 Lev), 1.31 (s, 1H, $CH(CH_3)_2$), 0.88 – 0.81 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.15 (d, *J* = 9.8 Hz, 6H, $Si(CH_3)_2$), ^{13}C NMR (151 MHz, $CDCl_3$) δ 128.16, 127.95, 127.89, 127.88, 127.83, 127.71, 96.13, 82.01, 79.00, 74.91, 74.83, 74.82, 74.50, 74.76, 74..46, 37.73, 35, 52, 30.01, 29.41, 27.79, 19.05, 1.28, 1.25. HRMS: ($M+Na^+$) found 651.2019, observed 651.2011.

Trichloroacetimidate-*O*-methyl-2-*O*-levulinoyl-3,4-*O*-benzyl-β-D-glucupyranosyluronate (18): To a stirred solution of starting material (105 mg, 0.167 mmol) in THF (1.6 mL), HF in pyridine (30%, 303 μL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with water, $NaHCO_3$ (Satd.), and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. Cl_3CCN (83 μL, 0.835 mmol) and NaH (60%) (~1.0 mg, 0.016 mmol) were added to a solution of the crude material in DCM (1.7 mL). After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

Dimethylthexylsilyl *O*-methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3,4-*O*-benzyl-β-D-glucupyranosyluronate (16): To a stirring solution of starting material (100 mg, 0.188 mmol) in pyridine (1.25 mL), DMAP (30 mg, 0.188 mmol) and PivOAc-Cl (45 μL, 0.377 mmol) was added at 0 ^{0}C. After stirring for 4hr at ambient temperature TLC (hexanes/EtOAc, 70/30, v/v) indicated the total consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The mixture was concentrated *in vacuo* and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (114 mg, 88%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.14 (m, 10H, CH Aromatic), 5.06 – 4.92 (m, 1H, H2), 4.85 – 4.59 (m, 5H, CH*H*Bn, CH*H*Bn, CH*H*Bn, CH*H*Bn, H1), 4.12 – 4.04 (t, 2H, CH_2 PivOAc), 3.75 – 3.66 (m, 4H, CH_3 COOMe, H4), 3.46 – 3.40 (t, 1H, H3), 1.98 – 1.93 (m, 3H, CH_3,PivOAc), 1.85 (t, *J* = 7.7, 2.5 Hz, 2H, CH_2 PivOAc), 1.61 (m, *J* = 2.9 Hz, 1H, $CH(CH_3)_2$), 1.18 (d, *J* = 4.7 Hz, 6H, 2xCH_3 PivOAc), 0.88 – 0.81 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.18 – 0.11 (m, 7H, $Si(CH_3)_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 128.41, 128.20, 127.74, 127.59, 127. 42, 127.35, 95.85, 82.63, 77.58, 75.31, 75.23, 74.39, 74.74, 74.42, 74.40, 61.22, 52.32, 20.92, 38.04, 33.70, 25.19, 19.71. HRMS: ($M+Na^+$) found 709.1469, observed 709.1477.

Trichloroacetimidate *O*-methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3,4-*O*-benzyl-β-D-glucupyranosyluronate (19): To a stirred solution of starting material (114 mg, 0.165 mmol) in THF (1.6 mL), HF in pyridine (30%, 332 μL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with water,

$NaHCO_3$ (Satd.), and brine. The organic phase was dried (MgSO4), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. Cl_3CCN (83 μL, 0.829 mmol) and NaH (60%) (~1.0 mg, 0.016 mmol) were added to a solution of the crude material in DCM (1.7 mL). After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

Dimethylthexylsilyl *O*-methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate (5b): To stirring solution of starting material (300 mg, 0.5029 mmol) in DMF (5.1 mL), 2-naphthylmethyl bromide (277 mg, 1.257 mmol) TBAI (1.8 mg, 0.005 mmol) and NaH (12 mg, 0.502 mmol) were added at -20 ^oC. After stirring for 30 mins at -20 ^oC TLC indicated total consumption of starting material. The reaction mixture was quenched with MeOH and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (5/1 v/v) to obtain product as oil (259 mg, 70%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 – 7.57 (m, 4H, CH Aromatic), 7.32 (dt, *J* = 7.5, 1.8 Hz, 3H, CH Aromatic), 7.22 – 7.05 (m, 7H, CH Aromatic), 4.91 (t, *J* = 8.5, 1H, H2), 4.64 (m, *J* =11.1 Hz, 6H, C*H*HNAP, CH*H*NAP, C*H*HBn, CH*H*Bn, H1), 4.02 – 3.80 (m, 4H, H4, H5, CH_2 PivOAc), 3.65 – 3.51 (m, 4H, H3, CH_3 $COOCH_3$), 1.83 (d, *J* = 1.5 Hz, 3H, CH_3 PivOAc), 1.73 (dd, *J* = 8.0, 6.3 Hz, 2H, CH_2 PivOAc), 1.57 – 1.38 (m, 1H, $CH(CH_3)_2$), 1.06 (s, 6H, 2x CH_2 PivOAc), 0.79 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.09 – -0.06 (m, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.34, 127.96, 127.10, 127.07, 126.73, 125.97, 125.85, 96.09, 82.29, 78.98, 74.88, 74.86, 74.86, 74.75,74.36, 74.31,

74.30, 74.28, 61.33, 52.44, 38.04, 38.02, 33.77, 26.06, 25.26, 25.23, 24.68, 24.06,20.93, 18.56, 19.96. HRMS: ($M+Na^+$) found 759.2667, observed 759.2660.

Trichloroacetimidate *O*-methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate (20): To a stirred solution of starting material (240 mg, 0.326 mmol) in THF (3.3 mL), HF in pyridine (30%, 1.1 mL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with water, $NaHCO_3$ (Satd.), and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. Cl_3CCN (163 μL, 1.63 mmol) and NaH (60%) (1.0 mg, 0.032 mmol) were added to a solution of the crude material in DCM (3.2 mL). After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

Dimethylthexylsilyl *O*-(methyl-2-*O*-acetyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-β -D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (23): A suspension of glucuronic acid donor (50 mg, 0.0708 mmol), glucoszide acceptor (28 mg, 0.0590 mmol) and activated molecular sieves (4Å crushed, 80 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.0 µl, 0.0708 mmol). The TLC analysis showed complete consumption of the donor.

Dimethylthexylsilyl *O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-

3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (24): A suspension of glucuronic acid donor (60 mg, 0.07865 mmol), glucoszide acceptor (31 mg, 0.06554 mmol) and activated molecular sieves (4Å crushed, 90 mg) in dichloromethane (1.6 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TfOH (1.0 µl, 0.007865 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (6/1 v/v) to obtain product as oil (19 mg, 27%). ^{1}H NMR (500 MHz, CDCl3) δ 7.75 (t, J = 7.2 Hz, 2H, CH Aromatic), 7.60 – 7.54 (m, 2H, CH Aromatic), 7.43 – 7.18 (m, 16H, CH Aromatic), 5.04 (d, J = 19.1, 9.6 Hz, 2H, H2′, H4′), 4.97 (d, J = 11.6 Hz, 1H, *CHHBn*), 4.80 (d, J = 11.6 Hz, 1H, *CHHBn*), 4.67 – 4.57 (m, 3H, *CHHBn* , *CHHBn* , H1′), 4.49 (d, J = 7.7 Hz, 1H, H1), 4.44 – 4.37 (m, 1H, H6a), 4.31 (dd, J = 10.5, 7.5 Hz, 2H, CH2 Fmoc), 4.20 (t, J = 7.1 Hz, 1H, CH2 Fmoc), 4.12 (dd, J = 11.8, 6.1 Hz, 1H, H6b), 3.85 (d, J = 9.8 Hz, 1H, H5′), 3.76 – 3.66 (m, 2H, H3′, H4′), 3.58 (dd, J = 14.1, 7.8 Hz, 1H, H5), 3.47 (s, 3H, CH3 COOMe), 3.43 – 3.37 (m, 1H, H3), 3.29 (dd, J = 9.8, 7.8 Hz, 1H, H2), 2.79 – 2.70 (m, 1H, CH2 Lev), 2.68 – 2.51 (m, 3H, CH2 Lev), 2.41 – 2.33 (m, 1H, CH2 Lev), 2.15 (d, J = 6.1 Hz, 3H, CH3 Lev), 2.08 (d, J = 9.7 Hz, 3H, CH3 Ac), 1.64 (dt, J = 13.8, 6.8 Hz, 1H, CH CH(CH3)2), 0.88 (dd, J = 13.2, 6.3 Hz, 12H, C(CH3)2 and CH(CH3)2), 0.28 – 0.09 (m, 6H, Si(CH3)2). 13C NMR (126 MHz, CDCl3) δ 120.36, 125.31, 127.49, 127.50, 128.57, 127.99, 73.03, 75.26, 75.08, 75.08, 74.66, 101.27, 74.67, 97.03, 70.68, 62.96, 70.67, 46.87, 62.96, 72.79, 72.81, 79.68,

78.98, 72.67, 52.92, 81.27, 68.97, 68.93, 30.04, 21.05, 29.95, 18.65, 20.20. HRMS: ($M+Na^+$) found 1102.2998, observed 1102.2984.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2 dimethylbunoate)-3-*O*- benzyl-4-*O*- (9- fluorenyl methoxycarbonyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (25): A suspension of glucuronic acid donor (70 mg, 0.0830 mmol), glucoszide acceptor (33 mg, 0.06916 mmol) and activated molecular sieves (4Å crushed, 100 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.0 µl, 0.0083 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (6/1 v/v) to obtain product as oil (28 mg, 36%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 – 7.73 (m, 2H, CH Aromatic), 7.62 – 7.54 (m, 2H, CH Aromatic), 7.47 – 7.18 (m, 15H, CH Aromatic), 5.22 – 5.10 (m, 2H, H4′, H2′), 5.02 (d, J = 11.2 Hz, 1H, *CH*H*Bn*), 4.80 (d, J = 11.5 Hz, 1H, *CH*H*Bn*), 4.73 – 4.60 (m, 3H, H1′, CH_2 Fmoc), 4.53 (d, J = 7.5 Hz, 1H, H1), 4.42 (dd, J = 10.4, 7.1 Hz, 1H, H6a), 4.34 (dd, J = 11.8, 1.8 Hz, 1H, *CH*H*Bn*), 4.28 (dd, J = 10.4, 7.6 Hz, 1H, H6b), 4.25 – 4.17 (m, 2H, *CH*H*Bn* , CH Fmoc), 4.12 – 3.96 (m, 3H, CH_2 PivOAc, H5′), 3.82 (m, J = 9.1, 5.5 Hz, 2H, H3′, H4), 3.57 (s, 3H, CH_3 COOMe), 3.51 (dd, J = 8.4, 4.0 Hz, 1H, H5), 3.43 (t, J = 9.1 Hz, 1H, H3), 3.32 (dd, J = 10.0, 7.6 Hz, 1H, H2), 2.11 (s, 3H, CH_3 PivOAc), 2.04 – 1.98 (m, 3H, CH_3 Ac), 1.88 (dd, J = 14.7, 7.7 Hz, 2H, CH_2 PivOAc), 1.75 – 1.61 (m, 1H,

CH, $CH(CH_3)_2$), 1.23 – 1.14 (m, 6H, 2 X CH_3 PivOAc), 0.94 – 0.86 (m, 12H $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). 13C NMR (126 MHz, $CDCl_3$) δ 120.46, 120.04, 125.08, 127.87, 128.23, 127.19, 128.27, 127.67, 127.16, 127.14, 74.73, 72.28, 75.02, 75.01, 75.01, 75.02, 99.88, 73.73, 96.69, 70.47, 62.61, 70.48, 62.61, 46.55, 61.16, 72.75, 79.37, 76.84, 52.73, 52.73, 53.15, 72.48, 80.61, 68.54, 20.78, 20.93, 38.17, 33.93, 29.68, 24.89, 25.33, 25.30, 18.44, 19.94, 19.92, -3.17 HRMS: ($M+Na^+$) found 1160.2460, observed 1160.2469.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate) -3-*O*-benzyl-4-*O*-(9-fluorenyl methoxycarbonyl)-β-D- glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (25): A suspension of glucuronic acid donor (60 mg, 0.0711 mmol), glucoszide acceptor (28 mg, 0.0593 mmol) and activated molecular sieves (4Å crushed, 100 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^oC followed by addition of TfOH (1.0 µl, 0.0071 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µL) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (4/1 v/v) to obtain product as oil (26 mg, 39%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 – 7.73 (m, 2H, CH Aromatic), 7.62 – 7.54 (m, 2H, CH Aromatic), 7.47 – 7.18 (m, 12H, CH Aromatic), 5.22 – 5.10 (m, 2H, H4′, H2′), 5.02 (d, J = 11.2 Hz, 1H, *CH*H*Bn*), 4.80 (d, J = 11.5 Hz, 1H, *CH**HBn*), 4.73 – 4.60 (m, 3H, H1′, CH_2 Fmoc), 4.53 (d, J = 7.5 Hz, 1H, H1), 4.42 (dd, J = 10.4, 7.1 Hz, 1H, H6a), 4.34 (dd, J

= 11.8, 1.8 Hz, 1H, *CH*H*Bn*), 4.28 (dd, J = 10.4, 7.6 Hz, 1H, H6b), 4.25 – 4.17 (m, 2H, *CH**HBn* , CH Fmoc), 4.12 – 3.96 (m, 3H, CH_2 PivOAc, H5′), 3.82 (m, J = 9.1, 5.5 Hz, 2H, H3′, H4), 3.57 (s, 3H, CH_3 COOMe), 3.51 (dd, J = 8.4, 4.0 Hz, 1H, H5), 3.43 (t, J = 9.1 Hz, 1H, H3), 3.32 (dd, J = 10.0, 7.6 Hz, 1H, H2), 2.11 (s, 3H, CH_3 PivOAc), 2.04 – 1.98 (m, 3H, CH_3 Ac), 1.88 (dd, J = 14.7, 7.7 Hz, 2H, CH_2 PivOAc), 1.75 – 1.61 (m, 1H, CH, $CH(CH_3)_2$), 1.23 – 1.14 (m, 6H, 2 X CH_3 PivOAc), 0.94 – 0.86 (m, 12H $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). 13C NMR (126 MHz, $CDCl_3$) δ 120.46, 120.04, 125.08, 127.87, 128.23, 127.19, 128.27, 127.67, 127.16, 127.14, 74.73, 72.28, 75.02, 75.01, 75.01, 75.02, 99.88, 73.73, 96.69, 70.47, 62.61, 70.48, 62.61, 46.55, 61.16, 72.75, 79.37, 76.84, 52.73, 52.73, 53.15, 72.48, 80.61, 68.54, 20.78, 20.93, 38.17, 33.93, 29.68, 24.89, 25.33, 25.30, 18.44, 19.94, 19.92, -3.17. HRMS: ($M+Na^+$) found 1160.2460, observed 1160.2472.

Dimethylthexylsilyl *O*-(methyl-2-*O*-acetyl-3,4-*O*-benzyl-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (26): A suspension of glucuronic acid donor (55 mg, 0.09598 mmol), glucoszide acceptor (38 mg, 0.1322 mmol) and activated molecular sieves (4Å crushed, 90 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.0 µl, 0.09598 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (6/1

v/v) to obtain product as oil (16 mg, 22%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.17 (m, 15H, CH Aromatic), 5.05 – 4.99 (m, 1H, H2′), 4.95 (d, J = 11.2 Hz, 1H, C*H*HBn), 4.78 (d, J = 11.5 Hz, 1H, CH*H*Bn), 4.72 (d, J = 11.0 Hz, 2H, C*H*HBn, CH*H*Bn), 4.63 (d, J = 11.5 Hz, 1H, C*H*HBn), 4.60 – 4.55 (m, 2H, CH*H*Bn, H1′), 4.45 (d, J = 7.7 Hz, 1H, H1), 4.38 (dd, J = 11.6, 1.9 Hz, 1H, H6a), 4.08 (dd, J = 11.6, 6.4 Hz, 1H, H6b), 3.91 (t, J = 9.1 Hz, 1H, H4′), 3.84 (d, J = 9.5 Hz, 1H, H5′), 3.70 – 3.59 (m, 2H, H4, H3′), 3.53 (s, 3H, CH_3 $COOCH_3$), 3.43 (d, J = 6.9 Hz, 1H, H4), 3.38 – 3.31 (m, 1H, H3), 3.27 (dd, J = 9.8, 7.7 Hz, 1H, H2), 2.10 – 2.02 (m, 3H, CH_3 Ac), 1.94 (d, J = 11.9 Hz, 3H CH_3 Ac), 1.71 – 1.59 (m, 1H, $CH(CH_2)_3$), 0.93 – 0.82 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.17 (dd, J = 18.0, 6.2 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.96, 128.76, 128.45, 128.38, 127.90, 127.70, 127.58, 127.46, 101.32, 96.73, 81.94, 80.85, 79.30, 78.48, 75.59, 75.57, 75.09, 75.09, 75.08, 75.03, 75.01, 75.01, 75.00, 74.99, 74.54, 73.04, 72.68, 68.59, 63.29, 62.51, 62.49, 52.43, 33.92, 29.68, 20.83, 20.81, 20.26, 20.25, 19.94, 19.37, 18.55, 18.49, 17.92. HRMS: ($M+Na^+$) found 914.2568, observed 914.2577.

Trihalose-ortho-ester (26a): 1H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.02 (m, 20H, CH Aromatic), 6.29 (d, J = 3.6 Hz, 1H, H1), 5.02 (dd, J = 9.0, 7.8 Hz, 1H, H2′), 4.82 – 4.67 (m, 6H, *CH*H*Bn* , C*HHBn* , *CH*H*Bn* , C*HHBn* , *CH*H*Bn* , H1′), 4.62 (dd, J = 11.1, 6.2 Hz, 2H, *CH*H*Bn* , C*HHBn*), 4.53 (d, J = 10.8 Hz, 1H, C*HHBn*), 4.22 (d, J = 10.0 Hz, 1H, H5), 3.96 – 3.83 (m, 4H, H2, H3, H4′, H5′), 3.80 – 3.73 (m, 4H, H4, CH_3 COOMe), 3.69 (s, 3H, CH_3′ COOMe), 3.61 (t, J = 8.8 Hz, 1H, H3′), 2.10 (s, 3H CH_3 Ac), 1.64 (s, 3H CH_3′ Ac). 13C NMR (126 MHz, $CDCl_3$) δ 127.00, 127.46, 128.59, 128.60, 128.58, 129.01, 128.54, 128.11, 128.12, 128.05, 127.39, 128.12, 128.03, 128.05, 128.14, 128.24, 128.85, 128.21, 128.25, 128.22, 90.94, 72.66, 75.66, 75.12, 75.64, 75.06, 75.63, 75.30,

101.87, 75.29, 75.61, 72.28, 79.28, 80.73, 74.09, 78.55, 79.31, 52.92, 52.83, 82.17, 21.11, 20.84. HRMS: ($M+Na^+$) found 865.1851, observed 865.1852.

Dimethylthexylsilyl *O*-(methyl-2-*O*-levulinoyl-3,4-*O*-benzyl-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (27): A suspension of glucuronic acid donor (100 mg, 0.1587 mmol), glucoszide acceptor (63 mg, 0.1322 mmol) and activated molecular sieves (4Å crushed, 180 mg) in dichloromethane (1.6 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.5 µl, 0.01587 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (4/1 v/v) to obtain product as oil (76 mg, 61%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.17 (m, 15H, CH Aromatic), 5.06 – 4.99 (m, 1H, H2′), 4.96 (d, J = 11.1 Hz, 1H, C*H*HBn), 4.80 – 4.64 (m, 4H, , CH*H*Bn, C*H*HBn, CH*H*Bn, C*H*HBn,), 4.57 (dd, J = 9.4, 3.4 Hz, 2H, CH*H*Bn, H1′), 4.48 (d, J = 7.7 Hz, 1H, H1), 4.42 (dd, J = 11.7, 1.8 Hz, 1H, H6a), 4.14 (dd, J = 11.8, 5.9 Hz, 1H, H6b), 3.94 – 3.88 (m, 1H, H4′), 3.85 (d, J = 9.5 Hz, 1H, H5′), 3.68 (dt, J = 17.8, 9.3 Hz, 2H, H4′, H5′), 3.59 – 3.50 (m, 4H, H5, CH_3 COOMe), 3.39 – 3.32 (m, 1H, H3), 3.27 (dd, J = 9.8, 7.8 Hz, 1H, H2), 2.79 – 2.69 (m, 1H, CH_2 Lev), 2.66 – 2.50 (m, 2H, CH_2 Lev), 2.36 – 2.27 (m, 1H, CH_2 Lev), 2.15 (s, 3H, CH_3 Lev), 2.06 (s, 3H, CH_3 Ac), 1.63 (td, J = 13.7, 6.9 Hz, 1H, CH $CH(CH_3)_2$), 0.93 – 0.82 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.18 (dd, J = 15.3, 6.1 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ

128.71, 128.62, 128.59, 128.29, 128.28, 128.28, 128.27, 128.26, 128.25, 128.25, 128.25, 128.24, 128.23, 128.21, 128.20, 128.14, 128.03, 128.03, 127.99, 127.41, 127.29, 127.25, 127.15, 127.14, 101.46, 96.99, 82.23, 81.16, 79.50, 78.52, 75.30, 75.30, 75.25, 75.25, 75.22, 74.83, 73.62, 72.80, 68.83, 62.85, 62.85, 52.65, 37.76, 37.75, 34.00, 29.90, 20.84, 20.07, 18.39, -2.08, -3.07. HRMS: ($M+Na^+$) found 970.4164, observed 970.4160.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3,4-*O*-benzyl-β-D-glucupyranosyluronate)-(1→4)-*O*- 2-deoxy-2-azido-3-*O*-benzyl-6-*O*-acetyl-β-D-glucopyranoside (28): A suspension of glucuronic acid donor (109 mg, 0.1586 mmol), glucoszide acceptor (63 mg, 0.1336 mmol) and activated molecular sieves (4Å crushed, 180 mg) in dichloromethane (1.6 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.4 µl, 0.01586 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (6/1 v/v) to obtain product 524 (95 mg, 71%) as oil. ^1H NMR (500 MHz, CDCl3) δ 7.34 – 6.96 (m, 15H, CH Aromatic), 4.98 – 4.89 (t, 1H, 1H, H2′), 4.82 (d, J = 10.8 Hz, 1H, *CH*H*Bn*), 4.64 (d, J = 11.4 Hz, 1H, C*H**HBn***), 4.59 – 4.40 (m, 4H, *CH*H*Bn* , C*H**HBn*** , *CH*H*Bn* , C*H**HBn*** 1H, H1′), 4.35 (d, J = 7.7 Hz, 1H, H1), 4.17 (dd, J = 11.7, 1.8 Hz, 1H, H6a), 4.03 (dd, J = 11.8, 5.9 Hz, 1H, H6b), 3.86 (dd, J = 16.5, 8.2 Hz, 3H, H4′, CH_2 PivOAc), 3.76 (d, J = 9.3 Hz, 1H, H5′), 3.68 (dd, J = 9.8, 8.7 Hz, 1H, H4), 3.53 (t, J = 8.6 Hz, 1H H3′), 3.45 (s, 3H, CH_3 COOMe), 3.36 – 3.29 (m, 1H, H5), 3.24 – 3.17 (t, 1H,

H3), 3.12 (m, J = 9.9, 7.7 Hz, 1H, H2), 1.92 (d, J = 5.6 Hz, 3H, CH3 PivOAc), 1.82 (d, J = 4.1 Hz, 3H CH3 Ac), 1.77 – 1.63 (m, 2H, CH_2 PivOAc), 1.50 (dd, J = 20.6, 13.7 Hz, 1H, CH $CH(CH_3)_2$), 1.00 (d, J = 12.6 Hz, 6H 2 X CH_3 PivOAc), 0.72 (dd, J = 14.4, 7.5 Hz, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.09 – -0.05 (m, 6H, $Si(CH_3)_2$). 13C NMR (126 MHz, $CDCl_3$) δ 127.89, 128.40, 128.46, 127.80, 128.47, 127.78, 128.10, 127.81, 128.60, 128.14, 127.81, 127.24, 127.94, 127.31, 127.66, 127.30, 128.13, 127.71, 128.13, 73.34, 75.46, 74.81, 75.47, 75.02, 74.79, 100.23, 75.03, 96.92, 62.85, 62.85, 61.50, 79.45, 74.99, 76.48, 81.97, 52.75, 72.81, 80.80, 68.66, -9.83, 31.14, 20.92, 21.17, 38.53, 34.17, 25.20, 18.62, 20.19, -3.00, -1.97. HRMS: ($M+Na^+$) found 1028.2480, observed 1028.2488.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-β-D-glucopyranoside (29): A suspension of glucuronic acid donor (83 mg, 0.112 mmol), glucosazide acceptor (50 mg, 0.09345 mmol) and activated molecular sieves (4Å crushed, 150 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TfOH (1.0 µl, 0.011 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µL) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4). The organic phase was then filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (4/1 v/v) to obtain product as oil (88 mg, 85%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 2H, CH Aromatic), 7.62 (d, *J* = 14.7 Hz, 1H, CH Aromatic), 7.51 – 7.15 (m, 13H, CH Aromatic), 5.20 – 5.10 (t, 1H,

H2′), 5.03 (dt, *J* = 14.9, 5.3 Hz, 1H, *CHHBn*), 4.84 (t, *J* = 12.3 Hz, 2H, CH napthylidene, *CHHBn*), 4.80 – 4.67 (m, 3H, CH napthylidene, *CHHBn* , *CHHBn* , H1′), 4.56 – 4.50 (d, 1H, H1), 4.36 – 4.22 (m, 2H, $H6_{A,}$, $H6_B$), 4.15 – 4.00 (m, 2H, H5′, H4′), 3.95 – 3.85 (m, 2H, H3′, H4), 3.57 (s, 3H, $COOCH_3$), 3.52 – 3.46 (m, 1H, H5), 3.40 – 3.26 (m, 2H, H3, H2), 2.90 – 2.71 (m, 2H, CH_2 Lev), 2.69 – 2.53 (m, 2H, CH_2 Lev), 2.24 – 2.19 (s, 3H, CH_3 Lev), 1.99 (s, 3H, CH_3), 1.95 – 1.83 (m, 2H, CH_2 PivOAc), 1.71 – 1.62 (m, 1H), 1.59 (M, 1H, C*H*$(CH_3)_2$), 1.20 (dd, *J* = 3.5, 1.3 Hz, 6H, 2xCH_3 PivOAc), 0.91 (d, 12H, 12H, C(C*H*$_3)_2$ and CH(C*H*$_3)_2$), 0.24 – 0.13 (m, 6H, Si(C*H*$_3)_2$). ^{13}C NMR (126 MHz, $cdcl_3$) δ 127.65, 127.95, 126.56, 126.06, 128.24, 128.80, 128.25, 125.76, 128.33, 127.54, 127.07, 129.00, 125.31, 73.13, 75.37, 79.25, 75.36, 74.70, 74.73, 74.73, 99.93, 74.65, 75.36, 96.74, 62.59, 62.59, 62.58, 74.52, 79.45, 79.44, 61.33, 76.04, 81.73, 51.97, 52.49, 72.46, 80.57, 80.57, 68.42, 37.90, 28.04, 21.63, 29.69, 30.25, 20.87, 38.35, 34.02, 29.73, 25.30, 25.90, 18.23, 19.89, 3.14, 1.95. HRMS: ($M+Na^+$) found 1134.5242, observed 1134.5249.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-β-D-glucopyranoside (30): Hydrazine acetate (9.5 mg, 0.104 mmol) was added to a solution of starting material (**29**) (70 mg, 0.069 mmol) in toluene and ethanol (2/1, v/v, 2.2 mL) and the reaction mixture was stirred at ambient temperature. After 2 h, TLC analysis showed complete consumption of the starting material. The reaction mixture was diluted with dichloromethane, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene/EtOAc 4/1 v/v) to obtain product as oil (48mg, 76%). ^{1}H NMR (600 MHz,

$CDCl_3$) δ 7.79 (d, *J* = 9.7 Hz, 1H, CH Aromatic), 7.75 (dd, *J* = 8.8, 4.4 Hz, 2H, CH Aromatic), 7.48 – 7.39 (m, 4H, C*H* Aromatic), 7.36 – 7.19 (m, 12H, C*H* Aromatic), 5.13 (t, *J* = 9.0, 7.7 Hz, 1H, H2′), 4.99 (d, *J* = 10.6 Hz, 1H, *CH*H*Bn*), 4.82 (dd, *J* = 11.3, 8.7 Hz, 2H, CH*H* NAP, *CH*H*Bn*), 4.74 (dd, *J* = 9.4, 4.4 Hz, 2H, H1′, C*H*H*Bn*), 4.67 (dd, *J* = 11.1, 6.5 Hz, 2H, C*H*H NAP, C*H*H*Bn*), 4.52 (d, *J* = 7.9 Hz, 1H, H1), 4.15 – 3.96 (m, 4H, H4′, H5′, CH_2 PivOAc), 3.92 (t, *J* = 9.8, 8.9 Hz, 1H, H4), 3.87 – 3.81 (m, 1H, H6a), 3.73 (t, *J* = 8.8 Hz, 2H, H3′, H6b), 3.55 (s, 3H, $COOCH_3$), 3.35 (t, *J* = 9.9, 8.9 Hz, 1H, H3), 3.32 – 3.27 (m, 1H, H5), 3.24 (t, *J* = 9.8, 7.8 Hz, 1H, H2), 1.96 (s, 3H, $COCH_3$), 1.67 – 1.60 (m, 2H, CH_2 PivOAc), 1.56 (s, 1H, $CH(CH_3)_2$), 1.19 – 1.15 (s, 6H, 2xCH_3 PivOAc), 0.89 – 0.84 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.16 (t, *J* = 7.1 Hz, 9H, $Si(CH_3)_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ -9.82, -9.82, 127.76, 128.01, 126.71, 125.96, 128.30, 128.28, 125.80, 128.44, 125.84, 128.07, 127.65, 126.91, -9.82, 53.20, 73.03, 73.02, 73.02, 75.18, 75.16, 74.69, 74.73, 100.08, 74.81, 100.04, 74.82, 74.81, 75.16, 74.71, 96.66, 96.63, 79.47, 79.46, 61.31, 61.31, 74.61, 74.59, 75.53, 75.52, 61.25, 61.25, 81.99, 81.96, 81.94, 61.24, 52.47, 52.53, 80.41, 80.38, 74.70, 74.69, 68.46, 68.45, 68.43, 21.10, 20.91, -9.82, 38.27, 38.20, 33.85, 29.57, 25.19, 26.91, 18.56, 19.88, 21.45, 19.88, -2.44, -3.95, -2.42. HRMS: ($M+Na^+$) found 1036.4762, observed 1036.4760.

Dimethylthexylsilyl *O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside sodium salt (31): Sulfur trioxide pyridine complex (45 mg, 0.284 mmol) was added to a solution of the starting material (**30**) (48 mg, 0.047 mmol) in DMF (4.5 mL) and the resulting mixture was stirred for 2 h at ambient temperature. TLC analysis ($CHCl_3/CH_3OH$ 9/1 v/v) indicated complete

consumption of starting material. Further, $NaHCO_3$ (47 mg, 0.568 mmol) was added to the reaction mixture and it was continued to stir for additional 10 min. The crude mixture was filtered with a syringe filter and concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was passed through a column of iatrobeads ($CHCl_3$/CH_3OH 97/3 to 70/30 v/v). The fractions containing product were concentrated *in vacuo* (bath temperature 20 °C), and the residue was immediately passed through a column of biorad resin (50 × 8 Na+ 0.6 × 5 cm, CH_3OH), providing the product as oil (37 mg, 70%). 1H NMR (600 MHz, CD_3OD) δ 7.82 – 7.77 (m, 1H, C*H* Aromatic), 7.77 – 7.72 (m, 2H, C*H* Aromatic), 7.64 (s, 1H, C*H* Aromatic), 7.47 – 7.41 (m, 4H, C*H* Aromatic), 7.33 – 7.28 (m, 3H, C*H* Aromatic), 7.26 – 7.18 (m, 6H, C*H* Aromatic), 5.14 (d, J = 8.2 Hz, 1H, H1′), 5.04 – 4.99 (m, 2H, H2′, C*H*H*Bn*), 4.91 – 4.68 (m, 4H, C*H*H NAP, CH*H* NAP, C*H*H*Bn,* C*H*H*Bn*), 4.63 – 4.55 (m, 2H, C*H*H*Bn* , H1), 4.32 – 4.27 (dd, 1H, H6a), 4.27 – 4.23 (d, 1H, H5′), 4.18 – 4.12 (dd, 1H, H6b), 4.06 – 3.90 (m, 5H, H3′, H4′, H4, CH_2 PivOAc), 3.58 (s, 3H, CH_3 COOMe), 3.54 (m, J = 7.2, 4.7, 1.9 Hz, 1H, H5), 3.38 – 3.33 (m, 1H, H3), 3.16 – 3.10 (m, 1H, H2), 1.96 (s, 3H, CH_3 PivOAc), 1.92 – 1.86 (t, 2H, CH_2 PivOAc), 1.70 – 1.61 (m, 1H, $CH(CH_3)_2$), 1.21 (d, J = 6.9 Hz, 3H, CH_3 PivOAc), 1.20 – 1.14 (m, 3H, CH_3 PivOAc), 0.93 – 0.85 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.18 (d, J = 2.3 Hz, 6H, $Si(CH_3)_2$). 13C NMR (151 MHz, CD_3OD) δ 127.54, 127.80, 126.39, 126.15, 127.30, 127.23, 127.21, 99.33, 72.98, 75.48, 73.17, 75.54, 73.26, 74.64, 74.47, 74.41, 74.36, 74.44, 74.62, 74.80, 74.91, 74.95, 75.07, 96.56, 65.18, 74.28, 65.21, 63.71, 74.88, 61.54, 81.64, 80.45, 80.48, 53.85, 49.14, 48.83, 54.27, 51.94, 72.91, 80.42, 47.93, 48.20, 68.35, 68.47, 37.62, 38.07, 39.98, 35.90, 32.24, 32.57, 30.22, 19.09, 38.22, 26.86, 27.24, 24.96,

22.07, 22.78, 19.90, 12.37. HRMS ESI-TOF: (M-1Na$^+$+1H$^+$) found 1093.2651, observed 1093.2644.

Dimethylthexylsilyl 3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside disodium salt (32): A premixed solution of aqueous H_2O_2 (50%, 188 µL, 3.31 mmol) and 1 M LiOH (1.65 mL, 1.65 mmol) were added to a solution of compound **31** (37 mg, 0.0331 mmol) in THF (2.0 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to mixture (pH 14). The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column 250 mg, H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm) using CH_3OH as eluent, providing compound **32** as an oil (14 mg, 43%). 1H NMR (500 MHz,) δ 7.62 – 7.50 (m, 2H, CH Aromatic), 7.45 (s, 1H, CH Aromatic), 7.29 – 6.92 (m, 10H, CH Aromatic), 4.86 (t, J = 9.6 Hz, 1H, C*H*H*Bn*), 4.78 (t, J = 11.5 Hz, 1H, *CH*H NAP), 4.69 – 4.54 (m, 17H, C*H*H*Bn* , *CH*H*Bn* , H1′, CH*H* NAP), 4.47 – 4.40 (m, 1H, *CH*H*Bn*), 4.38 – 4.33 (m, 1H, H1), 4.24 (dd, J = 10.7, 3.6 Hz, 1H, H6a), 4.12 – 4.04 (m, 1H, H6b), 3.80 (d, J = 9.8 Hz, 1H, H5′), 3.76 – 3.68 (m, 1H, H4), 3.57 (t, J = 9.2 Hz, 1H, H4′), 3.49 (t, J = 9.0 Hz, 1H, H3′), 3.42 – 3.35 (m, 1H, H5), 3.28 – 3.19 (m, 2H, H3, H2′), 3.14 – 3.04 (m, 12H), 2.97 (dd, J = 10.0, 7.6 Hz, 1H, H2), 1.52 – 1.41 (m, 1H, $CH(CH_3)_2$), 0.76 – 0.62 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.05 – -0.06 (m, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, CD_3OD)

δ 127.27, 125.84, 125.42, 127.68, 127.53, 74.80, 75.03, 74.20, 102.96, 74.22, 74.21, 75.01, 74.80, 96.52, 65.67, 65.68, 74.72, 76.95, 79.76, 84.23, 73.46, 81.23, 47.72, 68.70, 33.95, 17.73, 18.98, 4.67, 2.63. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 922.1922, observed 922.1928.

Dimethylthexylsilyl 3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-acetamido-3-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside disodium salt (33): A solution of 1 M PMe_3 in THF (92 μL, 0.091 mmol) was added to a stirred solution of starting material (**32**) (20 mg, 0.017 mmol), 0.1 M NaOH (367 μL, 0.036 mmol) in THF (2.0 mL) at ambient temperature. After 3 h, TLC analysis showed complete consumption of the starting material. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (H_2O/CH_3OH 9/1 to 5/5 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH) to obtain product as oil (14 mg, 72%). Acetic anhydride (15.0 μL, 0. 131 mmol) was added to a solution of starting material (14.0 mg, 0.012 mmol) in CH_3OH (1 mL) and Et_3N (26.6 μL, 0.263 mmol) at 0 °C. After stirring for 1 h at ambient temperature, the mixture was co-evaporated with toluene under reduced pressure (bath temperature 20 °C). The residue was passed through a RP-18 column (H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH), to obtain product as oil (12 mg, 83%). ^1H NMR (500 MHz, D_2O) δ 8.16 – 7.96 (m, 5H, C*H* Aromatic), 7.83 – 7.50 (m, 14H, C*H* Aromatic), 5.56 (d, J = 10.7 Hz, 1H, CH*H*

NAP), 5.33 – 5.27 (m, 2H, CH*H* Bn, CH*H* Bn), 5.25 – 5.07 (m, 4H, C*H*H Bn, H1′, H1, C*H*H Bn), 4.98 (dd, J = 13.9, 8.1 Hz, 1H, C*H*H NAP), 4.86 – 4.78 (m, 1H, H6a), 4.65 (dd, J = 11.0, 2.6 Hz, 1H, H6b), 4.49 – 4.42 (m, 1H, H4), 4.39 (d, J = 8.8 Hz, 1H, H5′), 4.14 – 4.04 (m, 3H, H4′, H3′, H5), 3.99 (dt, J = 6.8, 6.3 Hz, 1H, H3), 3.83 – 3.75 (m, 1H, H2′), 3.27 (dd, J = 10.0, 7.7 Hz, 1H, H2), 2.06 – 1.96 (s, 3H, CH_3 Ac), 1.28 – 1.16 (m, 1H, C*H*$(CH_3)_2$), 0.76 – 0.62 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.05 – 0.04 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (126 MHz, D_2O) δ 126.57, 127.39, 125.40, 128.01, 127.60, 127.05, 74.42, 74.35, 74.96, 74.52, 103.49, 96.14, 74.34, 66.76, 78.13, 80.92, 77.89, 81.35, 57.22, 73.92, 84.63, 75.22, 48.10, 22.29, 17.76, 19.84. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 938.3438, observed 938.3431.

3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-acetamido-3-*O*-benzyl-6-*O*-sulfonato-α/β-D-glucopyranoside disodium salt (34): Compound **33** (7 mg, 0.00705 mmol) was dissolved in pyridine (825 μL), THF (100 μL) and H_2O (35 μL). The reaction was cooled to 0 °C followed by addition of HF:pyridine (38 μL) and it was slowly warmed to rt overnight. The reaction mixture was concentrated *in vacuo* (bath temperature 20 °C) and was then applied to a RP-18 column (H_2O/CH_3OH 9/1 to 5/5 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH) to obtain product **34** as an oil (3.5 mg, 60%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 – 7.71 (m, 3H, C*H* Aromatic), 7.68 (s, 1H, C*H* Aromatic), 7.45 – 7.17 (m, 10H, C*H* Aromatic), 5.08 – 5.01 (m, 2H, C*H**HBn* , H1α), 4.96 (dd, *J* = 11.3, 3.0 Hz, 1H, C*H*H NAP), 4.89 – 4.76 (m, 4H, C*H**HBn* ,*CH*HBn, CH*H* NAP, H1′), 4.65 – 4.61 (m, 1H, H1β), 4.58 (dd, *J* = 11.6, 2.0 Hz, 1H, *CH*H*Bn*), 4.52 (dd, *J* = 10.8, 3.8 Hz, 1H, H6aα), 4.41

(ddd, *J* = 13.1, 10.8, 3.2 Hz, 1H, H6aβ, H6bβ), 4.26 (dd, *J* = 10.7, 2.0 Hz, 1H, H6bα), 4.16 (ddd, *J* = 9.8, 3.9, 2.1 Hz, 1H, H5α), 4.05 (ddd, *J* = 13.0, 6.2, 2.9 Hz, 1H, H2α), 3.99 – 3.89 (m, 2H, H5′, H4α), 3.88 – 3.82 (m, 1H, H3α), 3.80 (dd, *J* = 11.9, 6.7 Hz, 1H, H4′), 3.73 – 3.63 (m, 1H, H3′), 3.52 – 3.45 (m, 1H, H2′), 3.30 (dt, *J* = 3.3, 1.6 Hz, 7H), 1.83 (s, 2H, CH_3α NHAc), 1.80 (s, 1H, CH_3β NHAc). 13C NMR (126 MHz, CD_3OD) δ 126.33, 124.80, 124.32, 126.51, 126.38, 72.52, 89.82, 73.71, 72.86, 101.82, 73.63, 72.58, 64.80, 64.75, 67.56, 74.48, 76.33, 78.68, 83.11, 73.54, 46.24, 19.53, 19.70, 206.06, 171.50, 170.02, 169.06, 161.71, 138.30, 137.92, 129.05, 128.35, 128.33, 127.71, 126.91, 126.06, 100.65, 93.77, 77.58, 77.36, 77.16, 76.83, 76.74, 74.82, 71.59, 71.08, 70.67, 69.64, 67.87, 66.77, 66.38, 66.14, 57.24, 52.11, 37.85, 34.18, 29.86, 27.84, 24.90, 20.87, 20.36, 20.19, 18.80, 18.73, 3.08. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 796.1066, observed 796.1061.

β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-acetamido-6-*O*-sulfonato-α/β-D-glucopyranoside disodium salt (35): A suspension of Pd/C (7.0 mg) was added to a solution of the starting material (**34**) (3.5 mg) in CH_3OH (0.8 mL) and H_2O (250 μL). The mixture was placed under an atmosphere of hydrogen, and the 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_3COOH, 3/5/3/1, v/v/v). The hydrogenation was stopped when TLC indicated the disappearance of the starting material. The residue was passed through a short column of Biorad 50 × 8 Na+ resin (0.6 × 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze dried to provide the final product (1.5 mg, 71%). ^{1}H NMR (800 MHz, D_2O) δ 5.20 (dd, *J* = 2.4, 0.8 Hz, 0.48H, H1α), 4.74 – 4.70 (m, 0.52H, H1β), 4.57 (dd, *J* = 7.9, 3.6 Hz, 1H, H1′), 4.42 – 4.25 (m, 2H, H6a, H6b), 4.19 – 4.14 (m,

1H, H5α), 3.92 – 3.86 (m, 1H, H3β, H2α), 3.80 (ddt, *J* = 6.9, 5.3, 1.9 Hz, 05H, H5α), 3.78 – 3.74 (m, 1H, H5′), 3.74 – 3.68 (m, 1H, H2β, H4β, H3α, H4α), 3.56 – 3.46 (m, 2H, H3′, H4′), 3.33 (dt, *J* = 9.3, 8.2 Hz, 1H, H2′), 2.05 – 1.99 (m, 3H, C*H*$_3$ Ac). ^{13}C NMR (201 MHz, D_2O) δ 93.07, 97.51, 104.77, 69.16, 69.17, 69.18, 70.78, 56.20, 71.72, 75.19, 78.31, 81.52, 58.66, 74.90, 77.83, 74.34, 75.63, 104.58. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 476.0010, observed 476.0002.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)- *O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside (36):** A suspension of glucuronic acid donor (48 mg, 0.0649 mmol), glucosazide acceptor (38 mg, 0.0541 mmol) and activated molecular sieves (4Å crushed, 80 mg) in dichloromethane (0.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TfOH (1.0 µl, 0.00649 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4). The organic phase was then filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /EtOAc 4/1 v/v) to obtain product as oil (31 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 3H, CH Aromatic), 7.64 (s, 1H CH Aromatic), 7.53 – 7.43 (m, 3H, CH Aromatic), 7.42 – 7.16 (m, 20H, CH Aromatic), 5.28 – 5.14 (m, 4H, C*H*$_2$ Cbz, H2′, C*H*HNAP), 4.93 – 4.65 (m, 7H, C*H*HNAP, C*H*HBn, CH*H*Bn, H1′, H1), 4.58 – 4.49 (m, 2H, C*H*$_2$NBn), 4.46 – 4.39 (m, 1H, H6a), 4.25 (dd, J = 14.6, 10.5 Hz, 2H, H6b, H5′), 4.08 (dt, J = 15.6, 8.4 Hz, 3H, H4′,

CH_2, PivOAc), 4.02 – 3.87 (m, 3H, H4, H3′, H3), 3.85 – 3.77 (m, 1H, H5), 3.73 – 3.57 (m, 1H, OC*H*H Linker), 3.53 (s, 3H, CH_3 $COOCH_3$,), 3.49 – 3.17 (m, 4H, OC*H*H Linker, C*H*2N Linker, H2), 2.94 – 2.83 (m, 1H, CH_2 Lev), 2.79 – 2.50 (m, 3H, CH_2 Lev), 2.22 (s, 3H, CH_3 Lev), 1.98 (s, 3H, CH_3 PivOAc), 1.90 (q, J = 6.9 Hz, 2H, CH_2 PivOAc), 1.70 – 1.47 (m, 4H, 2x CH_2 Linker), 1.41 – 1.25 (m, 2H, CH_2 Linker), 1.20 (d, J = 6.2 Hz, 6H, 2x CH_3 PivOAc). ^{13}C NMR (126 MHz, CDCl3) δ 128.3, 128.14, 127.96, 127.81, 127.47, 127.35, 127.25, 126.69, 125.96, 125.88, 100.24, 97.71, 81.67, 79.57, 79.56, 77.76, 76.61, 75.4, 75.38, 75.37, 75.35, 74.71, 74.7, 74.61, 74.58, 74.54, 74.52, 73.28, 68.61, 68.33, 68.32, 68.32, 67.18, 67.17, 62.78, 62.46, 62.46, 61.22, 52.46, 50.99, 50.84, 50.41, 46.99, 46.44, 38.32, 37.95, 37.94, 30.03, 29.06, 28.54, 28.03, 28.03, 25.41, 23.91, 23.38, 20.95. HRMS: ($M+Na^+$) found 1301.4755, observed 1301.4748.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside (36):** A suspension of glucuronic acid donor (164 mg, 0.222 mmol), glucoszide acceptor (130 mg, 0.1850 mmol) and activated molecular sieves (4Å crushed, 300 mg) in dichloromethane (1.8 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TfOH (6.0 µl, 0.0666 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~25 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4). The organic phase was then filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc

(4/1 v/v) to obtain product as oil (152 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 3H, CH Aromatic), 7.64 (s, 1H CH Aromatic), 7.53 – 7.43 (m, 3H, CH Aromatic), 7.42 – 7.16 (m, 20H, CH Aromatic), 5.28 – 5.14 (m, 4H, C*H*$_2$ Cbz, H2′, C*H*HNAP), 4.93 – 4.65 (m, 7H, C*H*HNAP, C*H*HBn, CH*H*Bn, H1′, H1), 4.58 – 4.49 (m, 2H, C*H*$_2$NBn), 4.46 – 4.39 (m, 1H, H6a), 4.25 (dd, J = 14.6, 10.5 Hz, 2H, H6b, H5′), 4.08 (dt, J = 15.6, 8.4 Hz, 3H, H4′, CH_2, PivOAc), 4.02 – 3.87 (m, 3H, H4, H3′, H3), 3.85 – 3.77 (m, 1H, H5), 3.73 – 3.57 (m, 1H, OC*H*H Linker), 3.53 (s, 3H, CH_3 $COOCH_3$,), 3.49 – 3.17 (m, 4H, OC*H*H Linker, C*H*2N Linker, H2), 2.94 – 2.83 (m, 1H, C*H*$_2$ Lev), 2.79 – 2.50 (m, 3H, C*H*$_2$ Lev), 2.22 (s, 3H, C*H*$_3$ Lev), 1.98 (s, 3H, C*H*$_3$ PivOAc), 1.90 (q, J = 6.9 Hz, 2H, C*H*$_2$ PivOAc), 1.70 – 1.47 (m, 4H, 2x C*H*$_2$ Linker), 1.41 – 1.25 (m, 2H, C*H*$_2$ Linker), 1.20 (d, J = 6.2 Hz, 6H, 2x C*H*$_3$ PivOAc). ^{13}C NMR (126 MHz, CDCl3) δ 128.3, 128.14, 127.96, 127.81, 127.47, 127.35, 127.25, 126.69, 125.96, 125.88, 100.24, 97.71, 81.67, 79.57, 79.56, 77.76, 76.61, 75.4, 75.38, 75.37, 75.35, 74.71, 74.7, 74.61, 74.58, 74.54, 74.52, 73.28, 68.61, 68.33, 68.32, 68.32, 67.18, 67.17, 62.78, 62.46, 62.46, 61.22, 52.46, 50.99, 50.84, 50.41, 46.99, 46.44, 38.32, 37.95, 37.94, 30.03, 29.06, 28.54, 28.03, 28.03, 25.41, 23.91, 23.38, 20.95. HRMS: ($M+Na^+$) found 1301.4755, observed 1301.4761.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O*-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)--*O*-2-deoxy-2-azido-3-*O*-benzyl-α-D-glucopyranoside (37):** Hydrazine acetate (9.5 mg, 0.1041 mmol) was added to a solution of compound **36** (90 mg, 0.06942 mmol) in ethanol and toluene (2/1, v/v, 2.2 mL) and the reaction mixture was stirred at ambient temperature. After 2 h, TLC analysis showed complete consumption of the starting

material. The reaction mixture was diluted with dichloromethane, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc (4/1 v/v) to obtain product as oil (70 mg, 85%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 – 7.73 (m, 3H, CH Aromatic), 7.61 (s, 1H, CH Aromatic), 7.49 – 7.40 (m, 4H, CH Aromatic), 7.40 – 7.20 (m, 18H, CH Aromatic), 7.17 (d, J = 7.2 Hz, 1H, CH Aromatic), 5.23 – 5.09 (m, 4H, C*H*$_2$ Cbz, H2′, C*H*HNAP), 4.88 – 4.62 (m, 7H, C*H*HNAP, C*H*HBn, CH*H*Bn, H1′, H1), 4.50 (d, J = 11.8 Hz, 2H, C*H*$_2$NBn), 4.15 – 3.98 (m, 5H, C*H*$_2$ PivOAc, H6a, H5′, H4′), 3.97 – 3.87 (m, 2H, H4, H3′), 3.85 – 3.70 (m, 2H, H6b, H3), 3.66 – 3.55 (m, 3H, OC*H*H Linker, H5), 3.53 (s, 3H, CH_3 $COOCH_3$), 3.43 – 3.13 (m, 4H, OCH*H* Linker, C*H*$_2$NBn, H2), 2.00 (d, J = 5.5 Hz, 3H, C*H*$_3$ PivOAc), 1.86 (t, J = 7.4 Hz, 2H, C*H*$_2$ PivOAc), 1.70 – 1.45 (m, 4H, 2x C*H*$_2$ Linker), 1.41 – 1.23 (m, 2H, C*H*$_2$ Linker), 1.17 (d, J = 3.6 Hz, 6H, 2x C*H*$_3$ PivOAc), ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.23, 128.05, 127.92, 127.76, 127.52, 127.22, 126.69, 125.95, 125.84, 100.4, 97.84, 81.86, 79.42, 77.04 , 76.64, 75.06, 74.82, 74.75, 74.73, 74.7, 74.62, 74.57, 74.57, 73.23, 70.88, 68.19, 68.18, 68.11, 67.11, 62.91, 61.18, 61.04, 54.75, 52.49, 51.02, 50.37, 46.43, 38.2, 28.94, 25.36, 23.88, 23.25, 20.87. HRMS: ($M+Na^+$) found 1203.4303, observed 1203.4311.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O* -(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)- *O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-sulfonato-α-D-glucopyranoside sodium salt (38):** Sulfur trioxide pyridine complex (68 mg, 0.05761 mmol) was added to a solution of the compound **37** (55 mg 0.3456 mmol,) in DMF (6.0 mL) and the resulting mixture was stirred for 2 h at ambient temperature. TLC analysis ($CHCl_3/CH_3OH$ 9/1

v/v) indicated complete consumption of starting material. Further, $NaHCO_3$ (0.6913 mmol, 58 mg) was added to the reaction mixture and it was continued to stir for additional 10 min. The crude mixture was filtered with a syringe filter and concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was passed through a column of iatrobeads ($CHCl_3$/CH_3OH 97/3 to 90/10 v/v). The fractions containing product were concentrated *in vacuo* (bath temperature 20 °C), and the residue was immediately passed through a column of biorad resin (50 × 8 Na+ 0.6 × 5 cm, CH_3OH), providing the product as an oil (57 mg, 77%). ^1H NMR (500 MHz, CD_3OD) δ 7.85 – 7.80 (m, 1H, CH Aromatic), 7.78 (dd, J = 7.2, 3.5 Hz, 2H, CH Aromatic), 7.67 (d, J = 1.5 Hz, 1H, CH Aromatic), 7.50 – 7.19 (m, 24H, CH Aromatic), 5.25 – 5.03 (m, 5H, H1′, H2′, C*H*$_2$ Cbz, C*H*HNAP), 4.87 (s, 4H, C*H*HBn, CH*H*Bn, C*H*HBn, CH*H*Bn, H1), 4.64 – 4.57 (m, 1H, C*H*HNAP), 4.54 (d, J = 4.2 Hz, 2H, C*H*$_2$NBn), 4.40 (d, J = 10.8 Hz, 1H, H6a), 4.28 (d, J = 9.2 Hz, 1H, H5′), 4.17 (s, 1H, H6b), 4.12 – 3.96 (m, 5H CH_2 PivOAc, H4, H3′, H4′, OCH*H* Linker), 3.88 – 3.65 (m, 3H), 3.58 (s, 3H), 3.33 (p, J = 1.6 Hz, 4H, H2, OCH*H* Linker, C*H*$_2$NBn), 1.95 (s, 3H, CH_3 PivOAc), 1.88 (t, J= 6.7 Hz, 2H, CH_2 PivOAc), 1.56 (d, J = 3.7 Hz, 4H, 2x C*H*$_2$ Linker), 1.43 – 1.27 (m, 2H, C*H*$_2$ Linker. ^{13}C NMR (126 MHz, CD_3OD) δ 128.08, 127.64, 127.53, 127.31, 125.84, 125.82, 98.71, 97.55, 80.87, 77.18, 75.93, 74.65, 74.57, 74.36, 74.28, 74.27, 74.01, 69.61, 66.41, 64.91, 64.62, 61.7, 60.98, 51.31, 49.7, 47.7, 46, 43.54, 38.5, 28.33, 23.61, 18.87. HRMS ESI-TOF: (M-1Na^++1H^+) found 1260.3029, observed 1260.3032.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O*-3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)-*O*-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-sulfonato-α-D-glucopyranoside disodium salt (39):** A premixed solution of aqueous

H_2O_2 (50%, 110 μL, 3.90 mmol) and 1 M LiOH (1.90 mL, 1.90 mmol) were added to a solution of starting material (**38**) (50 mg, 0.039 mmol) in THF (4.0 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to the mixture (pH 14). The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH) to obtain product as an oil (37 mg, 84%). ^{1}NMR (500 MHz, CD_3OD) δ 7.83 – 7.79 (m, 1H, CH Aromatic), 7.79 – 7.72 (m, 3H, CH Aromatic), 7.54 – 7.50 (m, 2H, CH Aromatic), 7.50 – 7.38 (m, 5H, CH Aromatic), 7.37 – 7.18 (m, 15H, CH Aromatic), 5.27 – 5.13 (m, 3H, C*H*$_2$ Cbz, C*H*HNAP), 4.97 (dd, J = 17.3, 11.1 Hz, 2H, C*H*HBn, CH*H*Bn), 4.87 (s, 4H, C*H*HBn, CH*H*Bn, H1, H1′), 4.69 (d, J = 11.3 Hz, 1H, C*H*HNAP), 4.54 (s, 3H, NC*H*$_2$ Ph, H6a), 4.35 (d, J = 11.0 Hz, 1H, H6b), 3.99 – 3.86 (m, 5H, H5, H5′, H4′, H3, H4), 3.66 (td, J = 7.8, 6.6, 2.0 Hz, 2H, OCH*H* Linker, H3′), 3.55 (dd, J = 9.2, 7.8 Hz, 1H, H2′), 3.48 – 3.38 (m, 1H, OCH*H* Linker), 3.33 (dt, J = 3.3, 1.6 Hz, 3H, C*H*$_2$N linker, H2), 1.58 (d, J = 33.0 Hz, 4H, 2x CH_2 Linker), 1.46 – 1.28 (m, 2H, CH_2 Linker). ^{13}C NMR (126 MHz, CD_3OD) δ 128.60, 128.48, 127.91, 127.64, 127.25, 127.23, 127.12, 127.01, 126.11, 125.89,125.27, 103.65, 97.45, 84.47, 80.66, 79.47, 77.98, 77.83, 74.90, 74.53, 74.52, 74.44, 74.43, 74.10, 69.67, 69.62, 67.41, 67.37, 67.04, 65.89, 65.89, 62.60, 51.52, 50.03, 47.95, 46.37, 28.19, 27.91, 27.25, 22.88. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 1089.2254, observed 1089.2268.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O* -3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)- *O*-2-deoxy-2-amino-3-*O*-benzyl-6-*O*-sulfonato-α-D-glucopyranoside disodium salt (40):** A solution of 1 M PMe_3 in THF (92 μL, 0.091 mmol) was added to a stirred solution of starting material (**39**) (20 mg, 0.017 mmol), 0.1 M NaOH (367 μL, 0.036 mmol) in THF (2.0 mL) at ambient temperature. After 3 h, TLC analysis showed complete consumption of the starting material. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (H_2O/CH_3OH 9/1 to 5/5 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH) to obtain product as oil (14 mg, 72%). ^{1}H NMR (500 MHz, CD_3OD) ^{1}H NMR (500 MHz, CD_3OD) δ 7.83 – 7.72 (m, 4H, CH Aromatic), 7.51 – 7.19 (m, 24H, CH Aromatic), 5.25 – 5.14 (m, 3H, C*H*$_2$ Cbz, CH*H*NAP), 4.97 (dd, *J* = 13.8, 11.3 Hz, 2H, , C*H*HBn, CH*H*Bn), 4.87 (s, 2H, , C*H*HBn, CH*H*Bn), 4.80 – 4.70 (m, 1H, H1′), 4.65 (d, *J* = 11.8 Hz, 1H, C*H*HNAP), 4.55 (s, 3H, NC*H*$_2$ Ph, H6a), 4.33 (dd, *J* = 10.9, 2.2 Hz, 1H, H6b), 4.02 – 3.80 (m, 4H, H5, H5′, H3, OCH*H* Linker), 3.78 – 3.58 (m, 3H, H4′), 3.54 (dd, *J* = 9.2, 7.8 Hz, 1H, H2′), 3.33 (dt, *J* = 3.3, 1.6 Hz, 3H, H2 OC*H*H Linker, CH_2N linker), 2.71 (s, 1H), 1.57 (dd, *J* = 32.7, 11.8 Hz, 4H, 2x C*H*$_2$ linker), 1.42 – 1.22 (m, 2H, C*H*$_2$ linker). ^{13}C NMR (126 MHz, CD_3OD) δ 127.6, 127.3, 127.18, 126.02, 125.18, 103.74, 84.62, 84.43, 80.61, 78.02, 77.69 74.88, 74.78, 74.64, 74.39, 74.24, 70.37, 66.91, 65.84, 65.83, 50.01, 47.86, 46.25. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 1063.2358, observed 1063.2366.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O* -3-*O*-benzyl-4-*O*-(2-methyl-napthyl)-β-D-glucupyranosyluronate)-(1→4)- *O*-2-deoxy-2-acetamido-3-*O*-benzyl-6-*O*-sulfonato-α-D-glucopyranoside disodium salt (41):** Acetic anhydride (15.0 μL, 0. 131 mmol) was added to a solution of starting material (**40**) (14.0 mg, 0.012 mmol) in CH_3OH (1 mL) and Et_3N (26.6 μL, 0.263 mmol) at 0 °C. After stirring for 1 h at ambient temperature, the mixture was co-evaporated with toluene under reduced pressure (bath temperature 20 °C). The residue was passed through a RP-18 column (H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm, CH_3OH), to obtain product as oil (12 mg, 83%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.89 – 7.12 (m, 26H, CH Aromatic), 5.18 – 4.99 (m, 3H, CH*H*NAP C*H*$_2$Bn), 5.00 – 4.91 (dd, *J* = 36.4, 17.1 Hz, 1H, C*H*HBn), 4.99 – 4.72 (m, 2H, H1, C*H*HNAP), 4.69 (d, *J* = 17.4 Hz, 1H, H1′), 4.61 (d, *J* = 11.2 Hz, 1H, C*H*HBn), 4.54 (s, 2H, C*H*$_2$Bn Cbz), 4.51 – 4.41 (m, 1H, H6a), 4.31 – 4.20 (m, 1H, H6b), 4.08 (m, 1H, H2), 3.92 – 3.66 (m, 5H, H3, H4, H5, H4′, H5′), 3.66 – 3.54 (m, 2H, H3′, OC*H*H linker), 3.50 (t, *J* = 6.0 Hz, 1H, H2′), 3.38 – 3.29 (m, 3H, OC*H*H linker, C*H*$_2$NBn linker,), 1.96 – 1.67 (m, 3H, CH_3 Ac), 1.67 – 1.14 (m, 4H, 2x C*H*$_2$ linker), 1.41 – 0.93 (m, 2H, C*H*$_2$ linker). ^{13}C NMR (126 MHz, CD_3OD) δ 127.9, 127.2, 127.2, 126.8, 125.5, 103.5, 97.1, 84.6, 80.5, 77.8, 77.2, 75.0, 74.8, 74.7, 74.6, 74.3, 74.2, 74.2, 74.0, 70.1, 69.6, 68.3, 67.5, 67.1, 66.3, 66.2, 52.8, 50.1, 48.5, 48.0, 46.5, 29.4, 28.0, 23.0, 21.2. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 1063.2358, observed 1063.2300.

5-aminopentyl-*O*-β-D-glucupyranosyluronate-(1→4)- *O*-2-deoxy-2-acetamido-6-*O*-sulfonato-α-D-glucopyranoside disodium salt (42): A suspension of Pd/C (10%, 15

mg) was added to a solution of starting material (**41**) (10.0 mg, 0.008 mmol) in CH_3OH, H_2O, CH_3CO_2H (1/1/0.02, v/v/v, 3 mL). The mixture was stirred for 12 h under an atmosphere of hydrogen and then filtered through a PTFE syringe filter (0.2 mm, 13 mm), washed with a mixture of CH_3OH and H_2O (1/1, v/v, 2 mL), and the solvents were concentrated under reduced pressure. The residue was dissolved in distilled water, CH_3CO_2H (1/0.01, v/v, 3 mL), and $Pd(OH)_2$ on carbon (Degussa type, 20%, 15 mg) was added. The mixture was stirred for 12 h under an atmosphere of hydrogen and then filtered through a PTFE syringe filter. The residue was washed with H_2O (2 mL) and after freezing drying the filtrate, the residue was dissolved in H_2O and passed through a column of Biorad 50 × 8 Na+ resin (0.6 × 2.5 cm). The appropriate fractions were freeze dried to obtain product as white solid (3.9 mg, 86.0 %). ^{1}H NMR (500 MHz, D_2O) δ 4.79 (d, J = 3.4 Hz, 1H, H1), 4.48 (d, J = 6.2 Hz, 1H, H1′), 4.35 (dd, J = 11.2, 2.0 Hz, 1H, H6a), 4.17 (m, 1H, H6b), 3.97 (m, 1H, H5), 3.85 – 3.78 (m, 2H, H2, H3), 3.71 – 3.52 (m, 3H, H4, H4′, OC*H*H linker), 3.48 – 3.35 (m, 3H, H3′, H5′, OC*H*H linker), 3.24 (t, J = 3.4 Hz, 1H, H2′), 2.98 – 2.86 (t, J = 7.7 Hz, 2H, CH_2NBn linker), 1.97 – 1.90 (s, 3H, CH_3 Ac), 1.68 – 1.47 (m, 4H, 2x CH_2 linker), 1.42 – 1.30 (m, 2H, CH_2 linker). ^{13}C NMR (126 MHz, D_2O) δ 102.3, 96.4, 79.5, 75.7, 75.3, 73.0, 71.7, 69.4, 68.6, 68.0, 66.8, 53.2, 39.4, 28.0, 21.9, 26.2, 22.3. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 561.0791, observed 561.0786.

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CHAPTER 3

SYNTHESIS OF DERMATAN SULFATE DISACCHARIDES AS BIOMARKERS FOR DIAGNOSIS OF MUCOPOLYSACCHARIDOSES

Mucopolysaccharidoses belong to the family of lysosomal storage diseases which occur by unregulated accumulation of glycosaminoglycans (GAGs) caused by incomplete degradations due to dysfunctional enzymes. In the lysosome, the enzymes are programmed to breakdown GAGs in a progressive sequential manner. If along the process an enzyme is inactivated it will halt the remaining degradation process and lead to the accumulation and storage of GAG compounds. This undesirable storage of heparan sulfate and dermatan sulfate related by-products results in occurrence of the Sanfilippo disease. Sanfilippo is subtype of the mucopolysaccharidoses diseases which cause severe neuropathology in children. The presence of dermatan sulfate in the Sanfilippo patients in elevated levels presents an opportunity to use them as biomarkers for disease diagnosis. In order to customize and establish diagnostic biomarker for the disease, extensive standard values need to be obtained. Towards this end, we propose a modular synthesis of dermatan sulfate disaccharides. A set of orthogonally protected idouronyl donors and galactoamine acceptors can be coupled to obtain a disaccharide scaffold which can then be converted into several target disaccharide compounds. Sulfation can be achieved selectively at required separate locations or globally at all allowed

locations. This modular strategy gives us access to an array of dermatan sulfate disaccharide compounds in a rapid and highly efficient manner.

Introduction

Heparan sulfate, chondroitin sulfate, and dermatan sulfate found on mammalian cells belong to the GAG family.[1] These GAGs are degraded through a series of enzymatic transformations in the lysosome. The lysosome is commonly referred to as the cell's recycling center because it processes unwanted material into substances that the cell can utilize. Here hydrolytic enzymes such as sulfatases and glycosidases remove sulfate groups and monosaccharides from the nonreducing end of the polysaccharides in a very specific sequential manner.[2] A halt in this sequential degradation may result in the accumulation of non-degraded GAGs in the lysosomes. This unnecessary storage of GAG fragments leads to severe diseases known as the mucopolysaccharidoses (MPS) diseases. MPS comes under the bigger family of lysosomal storage diseases (LSD) which occur as a result of unwanted accumulation or storage of GAGs, glycoproteins and lipoproteins. Several different types of MPS have been identified *viz*. MPS I, II, IIIA–D, and VII. Although each mucopolysaccharidosis differs clinically, most patients generally experience a period of normal development followed by a decline in physical and/or mental function. The occurrence of mucopolysaccharidoses may be apparent at birth but it develops as the storage of GAGs increases over time, which affects bone and skeletal structure, connective tissues, and organs. It may lead to neurological problems which may include damage to neurons along with compromised motor function. The subtype MPS IIIA–D is referred to as Sanfilippo syndrome/ disease. The disorder is

named after Sylvester Sanfilippo, the pediatrician who first described the disease. Within the class of MPS diseases, the Sanfilippo type (MPS IIIA-D) results from an

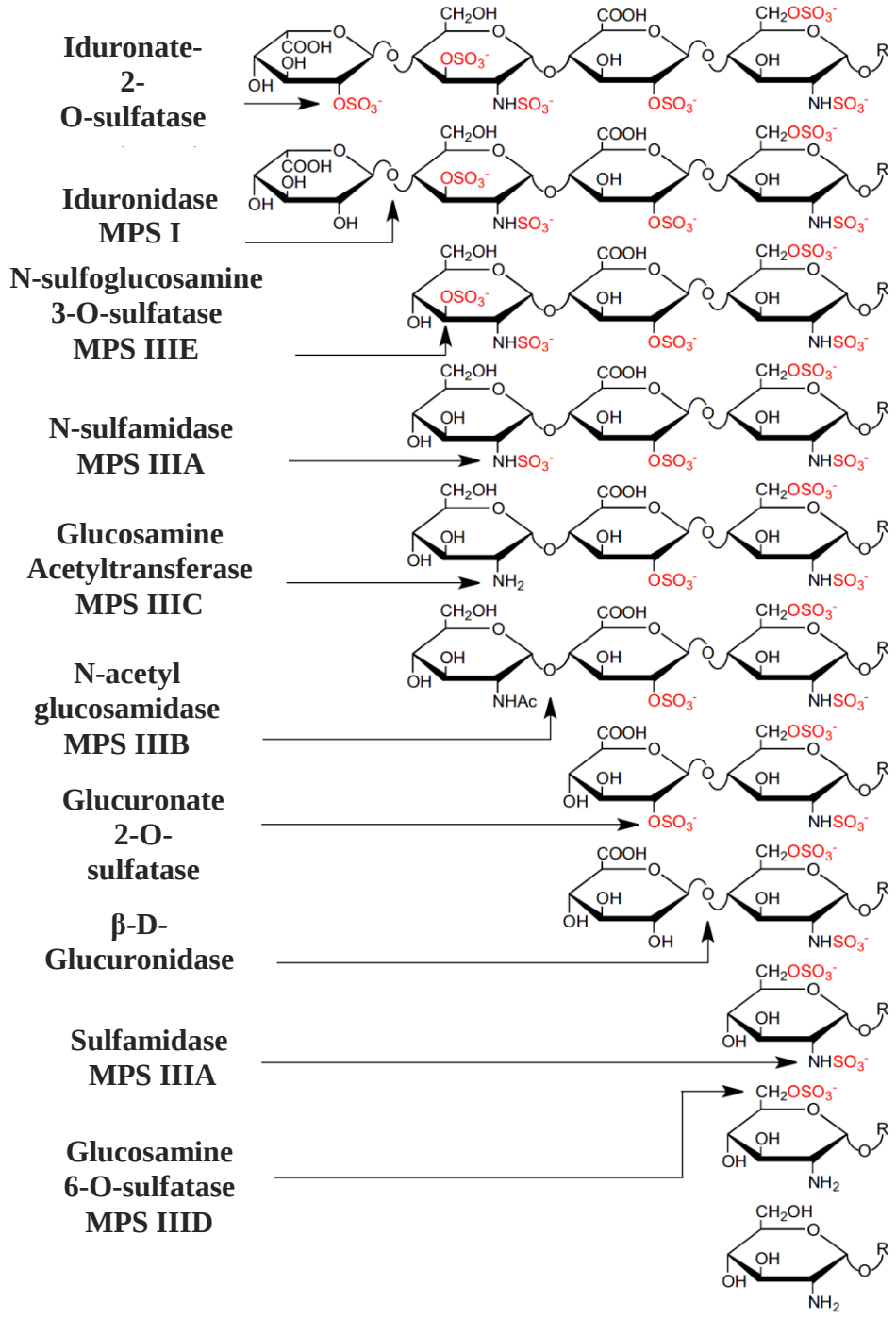


Figure 3.1 Enymatic degradation of heparan sulfate.[3]

inefficient catabolism of heparan sulfate which is an outcome of the genetic impairment of lysosomal enzymes (Figure 3.1).[3] There are four specific enzymes which are responsible for each subtype of the Sanfilippo diseases namely sulfamidase (MPS IIIA), α-*N*-acetyl-glucosaminidase (MPS IIIB), α-glucosaminide *N*-acetyltransferase (MPS IIIC) and *N*-acetylglucosamine-6-sulfatase (MPS IIID).[2] According to the reported cases, all the subtypes of Sanfilippo disease are associated with severe neuropathological disorders caused by the lysosomal storage of heparansulfate and related secondary metabolites in the CNS. [3,16]

In a recent study, Esko and coworkers provided evidence of the accumulation of dermatan sulfate as a secondary metabolite in Sanfilippo patient cells.[2] Iduronate 2-sulfatase is an enzyme that is known to remove the 2-*O*-sulfate group on the iduronic acid residue and thus helps with degradation of heparan sulfate. Excessive accumulation of heparan sulfate in the lysosomes could have a depreciating effect on iduronate 2-sulfatase enzyme leading to its inactivation. Dermatan sulfate has a high percentage of iduronic acid residues and thus inhibition of iduronate 2-sulfatase would lead to its accumulation as well, thus suggesting that primary storage of heparan sulfate lead to the inhibition of iduronate 2-sulfatase which resulted in secondary accumulation of dermatan sulfate. Thus collective accumulation of heparan sulfate and dermatan sulfate in Sanfilippo patient cells may be responsible for the occurrence of the disease. But the rationale to explain the link between the accumulation of these two carbohydrates and the neuro-pathological state of the disease has not been reported so far. The elevation of levels of these GAGs can be measured and thus can prove to have diagnostic significance. These accumulating

GAGs can, in theory, function as biomarkers for the identification of the Sanfilippo disease and the broader MPS diseases.

The use of biomarkers for the early diagnosis of the MPS diseases is crucial to gauge their pathological status, allowing for the opportunity to initiate therapy and to monitor the patient's responsiveness to the therapy.[4] Both primary and secondary accumulating metabolites can potentially be used as diagnostic analytical tools for detecting MPS disease and can be excellentcandidates as biomarkers.[5] Protocols exist for detecting the urinary total composition of GAGs towards the diagnosis of MPS using dye-spectrometric methods such as dimethylmethylene blue[6] and alcian blue[7]. Although effective, these dye-spectrometric methods cannot be used to sample blood or tissue extracts without prior digestion with protease, nuclease or hyaluronidase to avoid unnecessary background detection.[6] HPLC is a sensitive and accurate method but not appropriate for mass screening since it is time consuming and costly to analyze samples.[5,8] In designing a diagnostic experiment the inaccessibility towards sufficient quantities of the secondary metabolites is major concern and therefore a limitation to perform necessary screenings. A challenge in this field is to develop new diagnostic techniques to analyze the occurrence of the faintly elevated levels of GAG chains with accuracy.

A few therapies are available for the treatment of MPS diseases like the enzyme replacement therapy (ERT)[9] and hematopoietic stem cell transplantation[10]. However due to the lack of diagnostic biomarkers, the progression of therapy becomes challenging and thus it becomes difficult to monitor disease state. Performing quantitative and qualitative analysis of the excessive GAG accumulation is definitely a promising option but it faces

two major challenges. First, it is very difficult to attain standard values for GAG analysis in healthy subjects as carbohydrates are present in varying amounts which creates a high background.[11] Second, the GAGs that accumulate in MPS patients are of varying sizes (mono/di/tri-saccharides), substitution patterns (*O*-sulfation and *N*-acetylation/ sulfation), sugar composition and orientation (glcA/idoA) thus making diagnosis very challenging.[12-14]

Therefore, it is quite challenging to develop techniques and perform diagnostic tests to detect and identify analytes present only in MPS patients. Esko and co-workers have reported a highly sensitive mass spectrometric method for analyzing the accumulating disaccharide subunit structures at the non-reducing end of GAG chains based on stable isotope tagging with aniline.[15] This mass spectrometric diagnostic approach is able to detect both dermatan sulfate and heparan sulfate residues in Sanfilippo, thus acting as an indication of disease progression and prognosis. In designing such diagnostic test, having access to well-defined library of disaccharide standards is extremely crucial. Thus there is an urgency to initiate synthetic protocols to prospectively generate a library of these accumulating GAGs for diagnosis and validation of MPS diseases. Several efficient strategies have been reported for the chemical synthesis of heparan sulfate oligosaccharides[17] but the same is not true for dermatan sulfate oligosaccharides

Dermatan Sulfate

Dermatan Sulfate (DS) is a poly-anionic linear polysaccharide composed of a repeating disaccharide unit of β 1,3- linked iduronic acid (IdoA) and N-acetyl galactosamine (GalNAc). DS is also commonly known as chondroitin sulfate B (CS-B), because of the presence of GalNAc residues along its backbone. But what differentiates it from CS is the

occurrence of iduronic acid (IdoA) along its backbone and therefore it co-relates structurally more to heparin and HS, both of which too contain IdoA as a major residue. These polysaccharides are characterized with varying degrees of chain lengths along with diverse substitution pattern due to placement of sulfate groups at different locations along its backbone, which imparts them a great amount of complexity.[18] Due to the presence of negatively charged sulfates and carboxylates, DS is characterized by a high negative charge density, thus allowing it to interact with a wide array of different protein substrates. Sulfation in DS can occur at three locations mainly. the C2 of the IdoA residue (similar to HS or heparin) and the C4 and C6 of the galactosamine (similar to CS-A and CS-C).[18, 19] Herein we report a novel approach towards the synthesis of dermatan sulfate disaccharide motifs. Differentially protected monosaccharide building blocks were coupled to generate a modular disaccharide compound which may be employed to obtain several different target disaccharide compounds. To show the attractiveness of our synthetic strategy all the possible disaccharide structures attainable from our common modular disaccharide motif are shown in the Figure 3.2. Sulfation may be performed selectively or globally at C2 of the IdoA residue, C4 and C6 of the galactosamine residue by selective delevulination and regioselective ring opening on C4 or C6 respectively. The methyl ester of the carboxylic acid can be saponified to achieve the sodium salt of carboxylic acid. The trichloroacetamido-(NHTCA) can be reduced to get *N*-acetyl (NHAc) and the dimethylthexylsilyl group (TDS) can be removed to give the hemiacetal.

Results and discussion

We have developed a modular synthesis of dermatan disaccharide, which may well be used repeatedly for the preparation of a library of dermatan oligosaccharides. In the

present study we synthesized two disaccharides to be used in the diagnosis of MPS related disorders particularly the SanFilippo disease. The key to the synthesis was the preparation of strategically chosen monosaccharide building blocks; iduronic acid donor and *N*-acetyl galactosamine acceptor. The synthesis of idosyl building block was

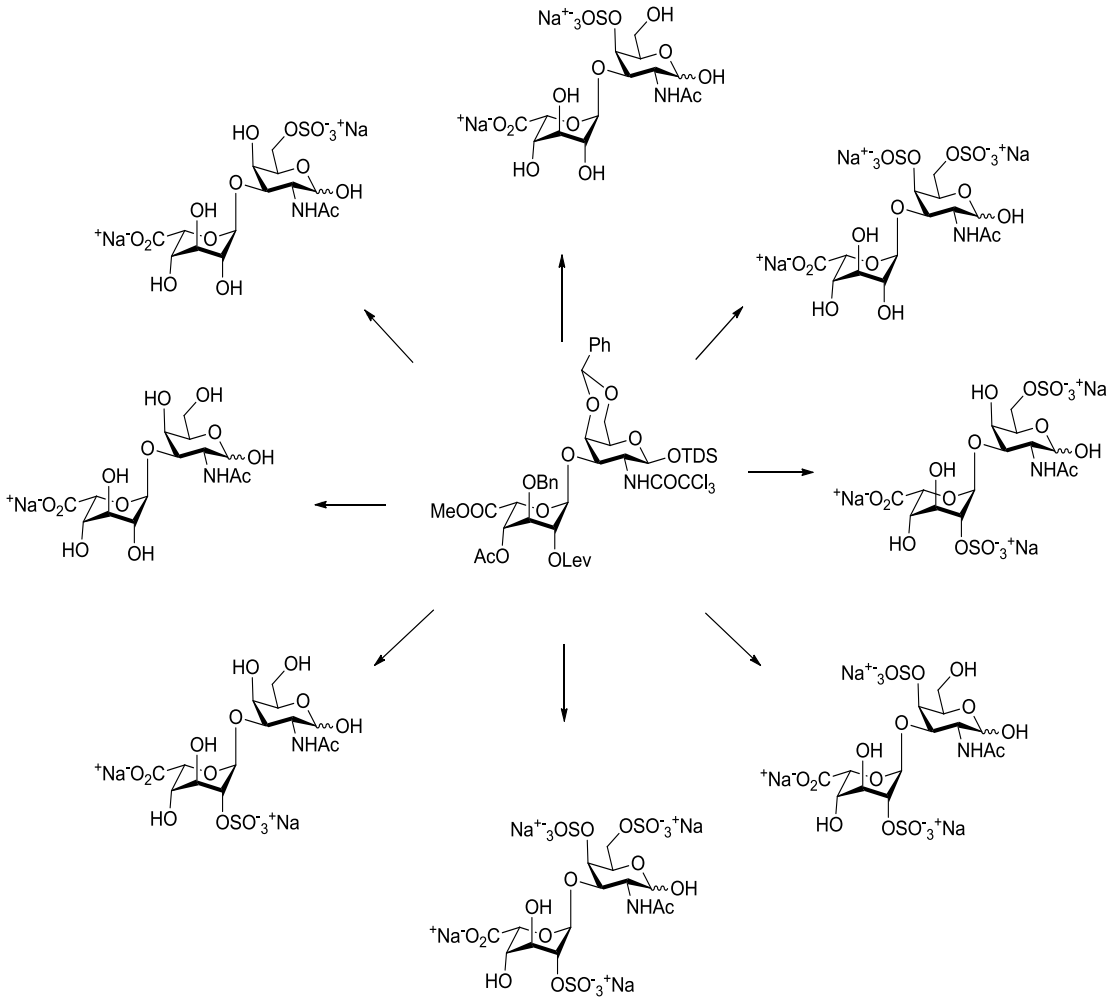
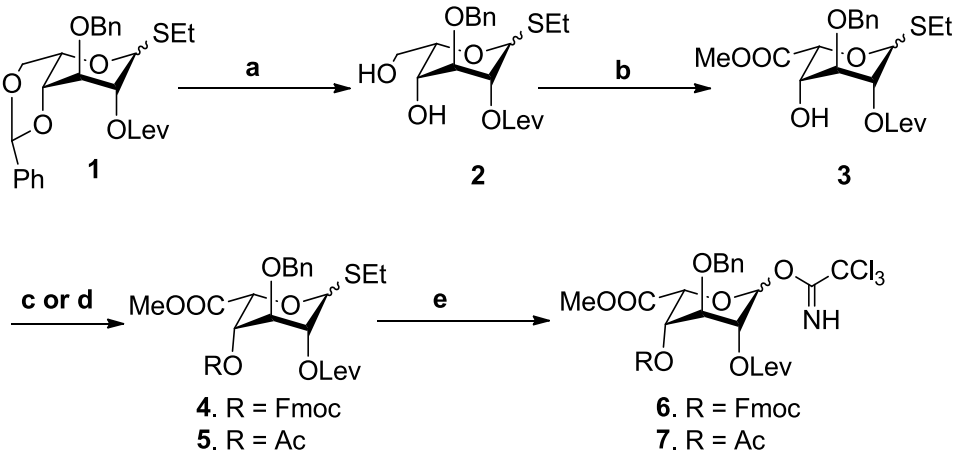


Figure 3.2: Dermatan Sulfate disaccharide motifs

essentially based on our previous work.[17] The benzylidene protection was removed using 80% aq. acetic acid (AcOH) at 60^{o}C to give **2** with 94% yield (Scheme 3.1). The **2** was then oxidized to an acid with 2,2,6,6-tetramethyl-1-piperdinyloxy/ bis-acetoxy

iodobenzene (TEMPO/BAIB) in DCM/H_2O, which was then protected as its methyl ester by treating it with diazomethane (CH_2N_2) in tetrahydrofuran (THF) to give **3** with 87% yield. Since, the idea was to synthesis of modular building blocks agreeable for the synthesis of larger oligosaccharides, the C4 of the **3** was protected with two different groups i.e., fluorenylmethyloxycarbonyl-(Fmoc) and acetyl by treating it with fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) and acetic anhydride to afford the thioglycoside idosyl donors **4** and **5** respectively with good yields. The thioglycoside moiety of the idosyl donors **4** and **5** can be deprotected at the anomeric position using *N*-bromosuccinimide (NBS)/acetone, which on treatment with trichloroacetonitrile in



Scheme 3.1: Synthesis of thioethyl and trichloroacetimidate Idosyl Donor

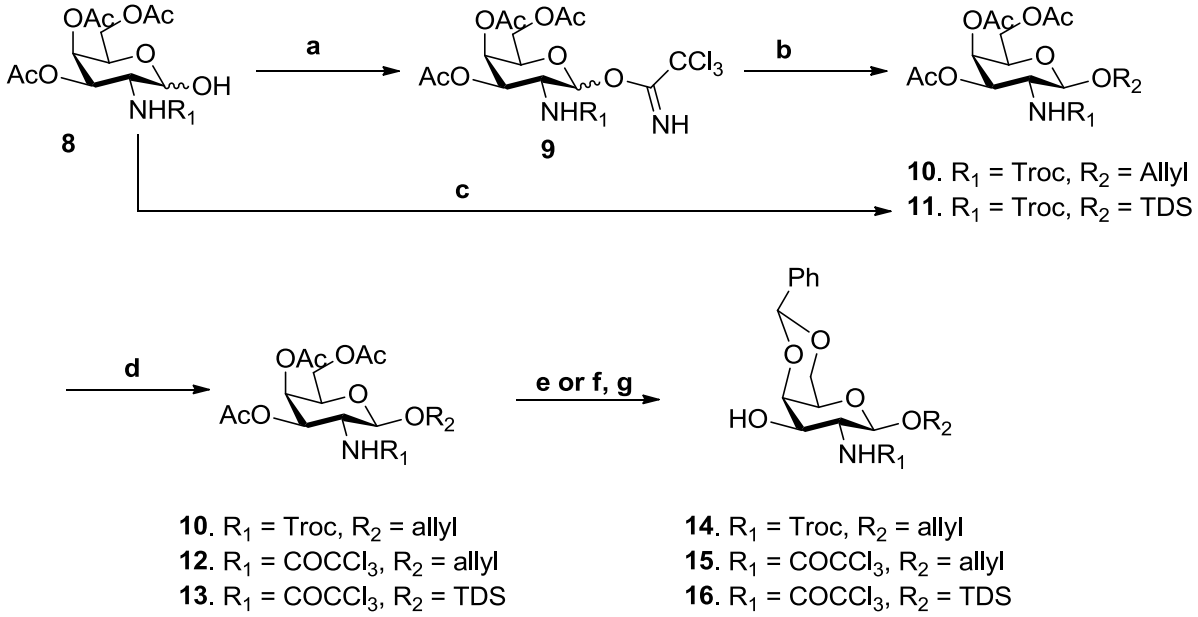
Reagents and conditions: (a) 80% aq. AcOH, 60 ^oC, 2h 94%; (b) (i) TEMPO, BIAB, DCM, H_2O, 1h; (ii) CH_2N_2, THF 87%; (c) FmocCl, Py, DMAP, 0 ^oC to r.t. 77%; (d) Ac_2O, Py, DMAP, 0 ^oC to r.t. 86%; (e) (i) NBS, Acetone:H_2O, r.t., 2h; (ii) Cs_2CO_3, CCl_3CN, DCM

presence of cesium carbonate (Cs_2CO_3) to give the trichloroacetimidate donor **6** and **7** respectively.

Having the idosyl donor in hand, we focused our attention towards the synthesis of acceptor. Since, the target disaccharide would be subjected to isotope tagging with radio-labeled aniline[15], the placement of a strategic orthogonal group at the anomeric position became even more important. We envisioned placement of an allyl group at the anomeric position as it can be selectively removed by treating with Grubb's second generation catalyst in presence of H_2.[20]

Thus, **8** (Scheme 3.2) was converted to trichloroacetimidate donor by treating it with trichloroacetonitrile/Cs_2CO_3 to give **9**, which was coupled with allyl alcohol by using trifluromethanesulfonic acid (TfOH) as promoter to give **10** with 75% yield. The **10** was deacetylated with guanidine hydrochloride with sodium methoxide (NaOMe) in DCM:MeOH and subsequently protected as 4,6-*O*-benylidene acetal by treating it with ($PhCH(OMe)_2$, using camphorsulfonic acid (CSA) in dimethylformamide (DMF) at 60^oC to give compound **14** with 61% yield. With the preparation of monosaccharide blocks viz., idosyl donor and glycosyl acceptor, the next step was their coupling to prepare the modular disaccharide. The (NIS/TfOH (0.2 eq), mediated coupling of **4** and **14** at -40 ^oC gave hydrolysis of donor. We then decided to use trichloroacetimidate idosyl donor **6** for the glycosylation, thus, the coupling of **6** with glycoyl acceptor **14** mediated by TMSOTf in DCM at -40 ^oC lead to hydrolysis of the donor as well. The reaction of **14** with **6** under the same conditions at 0 ^oC, resulted in the same outcome. We envisaged replacing NH-Troc with NH-TCA (Cl_3CCO-) at C2 of acceptor, we reasoned that probably the bulk of -Troc group is causing steric hindrance and hence loss in reactivity. Jacquinet and co-workers have reported successful use of NHTCA group in their synthesis of chondroitin sulfate oligosaccharides.[21] Moreover, since the target disaccharides have NH-Acetyl at C-

2, the TCA can be easily reduced to the acetamido. To synthesize the TCA acceptor with anomeric allyl (**15**), the Troc group was removed by treating it with Zinc (Zn) dust in acetic acid at r.t., followed by of NHTCA group in their synthesis of chondroitin sulfate



Scheme 3.2: Synthesis of the galactosamine acceptor

Reagents and conditions: (a) Cs_2CO_3, CCl_3CN, DCM; (b) TfOH (0.2 eq), All-OH, 0 ^oC (75%, **10**); (d) TDSCl, imidazole, DCM (71%, **11**); (d) (i) Zn dust, AcOH, r.t.; (ii) Cl_3CCOCl, Et_3N, 0 ^oC to r.t. (78%, **12**); (f) Guanidine.HCl, 1M NaOH, DCM:MeOH; (g) $PhCH(OMe)_2$, CSA, DMF, 60 ^oC (61%, **14**); (64%, **15**); (g) NaOMe, MeOH, r.t. ; (g) $PhCH(OMe)_2$, CSA, DMF, 60 ^oC, (54%, **16**)

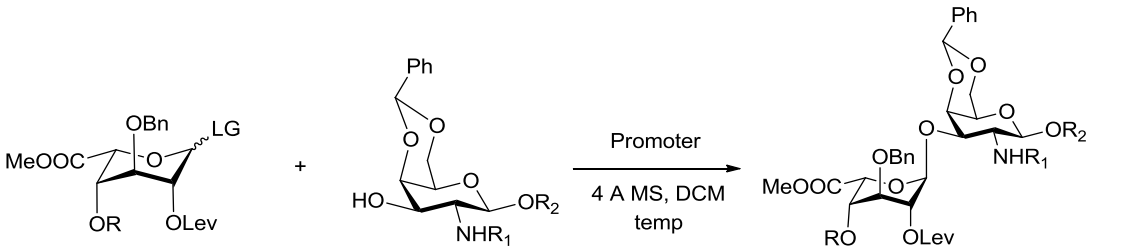
oligosaccharides.[21] Moreover, since the target disaccharides have NH-Acetyl at C-2, the TCA can be easily reduced to the acetamido. To synthesize the TCA acceptor with anomeric allyl (**15**), the Troc group was removed by treating it with Zinc (Zn) dust in acetic acid at r.t., followed by protection with TCA using trichloroacetyl chloride (TrocCl) in presence of triethyl amine to give **12** with 78% yield. The acetyl groups of **12**

were removed by treating it with basic conditions and the resulting compound was then protected as 4,6-O-benzylidene acetal to give acceptor **15** with 64% yield (Scheme 3.2).

The next step was coupling of acceptor **15** having NHTCA at C-2 and idosyl donor **6**. The coupling mediated by trimethylsilyltrifluoromethane sulfonate (TMSOTf) at 0^{o}C went smoothly to give **18** in significantly improved yields (61%). Notably the TfOH/NIS mediated coupling of **15** with **4** gave comparatively lower yields (47%). From our previous work we knew that TDS can be an ideal group at the anomeric position, which can be orthogonally deprotected.[17] The TDS acceptor (**16**) was synthesized from **8** by treating it with TDS-Cl in presence of imidazole to give **11** with 71% yield, which was then subjected to similar steps as that of **15** to give acceptor **16** with decent yield. The coupling of the TDS acceptor **16** with **7** mediated through TfOH at 0 ^{o}C gave disaccharide compound **19** in 57% yields. However, the donor seemed to undergo hydrolysis to a small extent during the course of the reaction, which led to moderate yields of the desired product. We reasoned that a slow reacting donor might help to address the issue, so we shifted our attention back to the thioglycoside donor **5**, which on coupling with **16** activated by TfOH and NIS gave the corresponding disaccharide product **19** in 83% yield. The various attempts at glycosylation are shown in Table 3.1.

Thus, with a well-placed strategy in hand we focused our attention to the synthesis of the target disaccharides. The deprotection strategy was inspired by previous work done in our lab.[17] The first step now involved the removal of the C-2 group on idosyl moiety by treating it with hydrazine acetate in EtOH:toluene to give disaccharide compound **20** with 92% yield. Since the target disaccharides had two differently placed *O*-sulfates on glucosamine moiety i.e, 4 and 6- position of the reducing sugar, it was imperative to

diversify the disaccharide at this stage, though the remaining deprotection steps essentially remained the same. Thus, the treatment of **20** with Et_3SiH in presence of TfOH lead to the opening of benzylidene ring at C6 of galactosamine moiety to give **21**



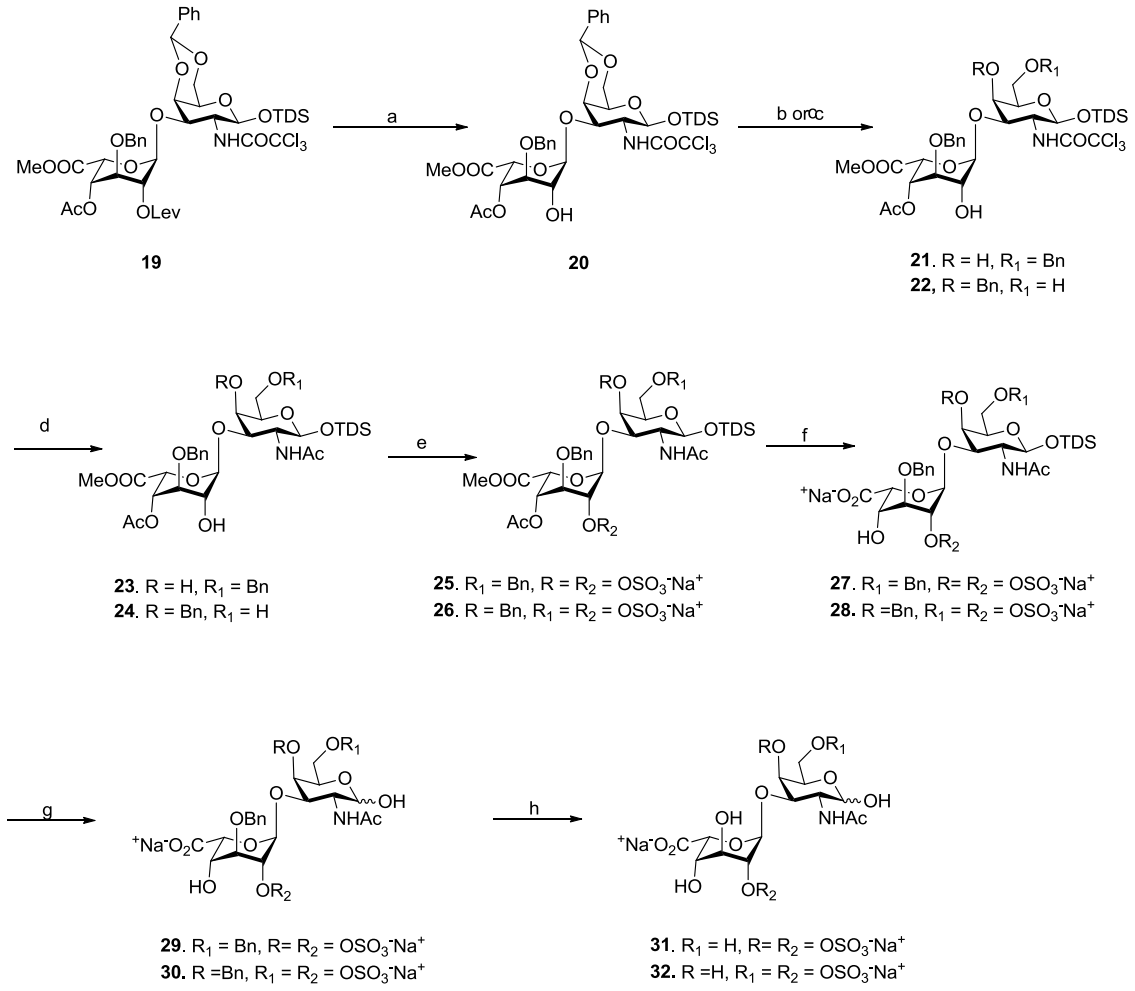
4. R= Fmoc, LG = SEt
6. R= Fmoc, LG = $OC(=NH)CCl_3$
5. R= Ac, LG = SEt
7. R= Ac, LG = $OC(=NH)CCl_3$

14. R_1 = Troc, R_2 = allyl
15. R_1 = $COCCl_3$, R_2 = allyl
16. R_1 = $COCCl_3$, R_2 = TDS

17. R= Fmoc, R_1 = Troc, R_2 = allyl
18. R= Fmoc, R_1 = $COCCl_3$, R_2 = allyl
19. R= Fmoc, R_1 = $COCCl_3$, R_2 = TDS

Entry	Donor	Acceptor	Promoter	Temp	Product	Yield (%)
1	**4**	**14**	TfOH/NIS	-40 ^oC	**17**	Donor hydrolysed
2	**6**	**14**	TMSOTf	-40 ^oC	**17**	Donor hydrolysed
3	**6**	**14**	TMSOTf	0 ^oC	**17**	Donor hydrolysed
4	**6**	**15**	TMSOTf	0 ^oC	**18**	61
5	**4**	**15**	TfOH/NIS	0 ^oC	**18**	47
6	**7**	**16**	TfOH	0 ^oC	**19**	57
7	**5**	**16**	TfOH/NIS	0 ^oC	**19**	83

Table 3.1: Glycosylation reaction to obtain disaccharide derivatives.



19

20

21. R = H, R_1 = Bn
22, R = Bn, R_1 = H

23. R = H, R_1 = Bn
24. R = Bn, R_1 = H

25. R_1 = Bn, R = R_2 = $OSO_3^-Na^+$
26. R = Bn, R_1 = R_2 = $OSO_3^-Na^+$

27. R_1 = Bn, R= R_2 = $OSO_3^-Na^+$
28. R =Bn, R_1 = R_2 = $OSO_3^-Na^+$

29. R_1 = Bn, R= R_2 = $OSO_3^-Na^+$
30. R =Bn, R_1 = R_2 = $OSO_3^-Na^+$

31. R_1 = H, R= R_2 = $OSO_3^-Na^+$
32. R =H, R_1 = R_2 = $OSO_3^-Na^+$

Scheme 3.3: Deprotection of disaccharide compounds.

Reagents and conditions: (a) $NH_2NH_2.OAc$,toluene/EtOH; 92%; (b) Et_3SiH, TfOH, -78 ^oC (61%, **21**); (c) Et_3SiH, $PhBCl_2$,-78 ^oC (81%, **22**); (d) Zn-Cu couple, AcOH, r.t. (64%, **23**); (72%, **24**); (e) $Py.SO_3$, DMF, (81%, **25**); (92%, **26**) (f) (i) LiOH, H_2O_2, THF (ii) 4M NaOH, MeOH (93%, **27**); (74%, **28**); (iii) HF.Py, pyridine, r.t. (81%, **29**); (75%, **30**); (iv) $Pd(OH)_2/C$, H_2, H_2O, (78%, **31**); (81%, **32**)

with 61% yield with a free hydroxyl group at C4. Similarly to prepare **22** with 81% yield having a free hydroxyl group at C6, **20** was treated with Et_3SiH in presence of $PhBCl_2$ to

afford **22**. The reduction of the tricloroacetamido group to the corresponding acetate functionality using the conventional method of Bu3SnH/azobisisobutyronitile (AIBN) proved to be a roadblock. The reaction never proceeded to completion and always resulted in the formation of a mixture of products. A breakthrough was achieved by switching to Zn-Cu[22] couple in AcOH which gave the desired product in good yields. The disaccharides **23** and **24** were then treated with SO_3.Py in DMF to give **25** and **26** with 81% and 92% yields respectively. After the installation of *O*-sulfates at desired position, we hydrolyzed the methyl ester to corresponding sodium salt by treating it with $LiOH/H_2O_2$ in presence of NaOH and MeOH to obtain compounds **27** and **28** with high yields. Next removal of the anomeric TDS was performed deftly with the aid of HF.Pyr in the presence of pyridine to give disaccharides **29** and **31** with 81% and 75% yields respectively. Controlling the amount of HF.Pyr added to the reaction mixture was estential as excess reagent can cleave the disaccharide. The last step was the removal of two Bn groups, which was facilitated through hydrogenation with H_2 in presence of Pd/C in MeOH:H_2O to give our target disaccharides **31** and **32** with 78% and 81% yields respectively.

The above synthesized disaccharide derivatives of DS will be used as mass spectrometric standards to facilitate qualitative and quantitative analysis of unknown DS fragments isolated from Sanfilippo patients. A traditional LC/MS based approach may prove to be non-quantitative due to intrinsic molecular differences and extrinsic effects of solvents and impurities on the ionization. Therefore a Glycan reductive isotope labeling (GRIL) coupled LC/MS method described by Esko and co-workers will be employed to modify the known DS derivatives with [$^{12}C_6$] and [$^{13}C_6$] aniline tags at the reducing ends.[15,23] The

partially purified GAGS fragments in the test sample are first subjected to enzymatic depolymerization followed by reductive amination at the reducing ends of the disaccharide with aniline tags to form secondary amine derivatives. Derivatization of the disaccharides with aniline tags has shown to improve liquid chromatographic resolution along with better detection by mass spectrometry. The peak assignments for the unknown DS disaccharides will be based on the comparative peak assignments of the known aniline tagged DS disaccharides. The benefit of using the differentially mass tagged aniline GAG derivatives (12C6 or 13C6) is that the different isotopically labeled anilines can be mixed and simultaneously analyzed to perform quantitative comparisons between the unknown and known disaccharides. The addition of the [$^{12}C_6$] aniline tag adds 77 atomic mass units to the mass of the free molecular ion as well as to any adducts or decomposition products, whereas [$^{13}C_6$] aniline group adds 83 atomic mass units. The difference of 6 atomic mass units between [$^{12}C_6$] aniline- and [$^{13}C_6$] aniline-tagged disaccharides is readily detected by mass spectrometry.[23] This tested GRIL-LC/MS method would provide more robust, reliable, and sensitive means towards the qualitative and quantitative assessment of DS based disaccharide compositions.

Summary and future outlook

An efficient and highly modular strategy for the synthesis of dermatan sulfate disaccharide has been discussed above. A set of orthogonally protected iduronyl donors and galactoamine acceptors were coupled to obtain a common disaccharide derivative in excellent yields. Further this common disaccharide was subjected to our rapid deprotections approach to obtain two differentially sulfated i.e., 2S4S and 2S6S disaccharide compounds with *N*-acetates. The design of our disaccharide motif

is such that with slight modification several possible sulfated derivatives may be obtained. Sulfation can be achieved selectively at required separate locations or globally at all allowed locations. This modular strategy gives us access to an array of dermatan sulfate disaccharide compounds in a rapid manner. We have capped our reducing end (C1) and non-reducing end (C4) with orthogonal protecting groups thus giving us the opportunity to further extend our strategy to construct larger fragments of dermatan sulfate oligosaccharides.

Experimental information

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 ~150oC or by spraying with a solution of $(NH_4)_6Mo_7O_{24}$ .H_2O (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150^oC. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å) 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-dihydroxy-benzoicacid (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.

Ethyl 2-levulinoyl-3-benzyl-1-thio- α/β -D-idopyranoside (2): Compound **1**[17] (5.2g, 10.4 mmol) was dissolved in aqueous acetic acid (80%, 100 mL) and heated at 60°C for 2 h. TLC analysis showed complete consumption of the starting material. The solvents were evaporated, after which traces of acetic acid and water were removed by repeated co-evaporation with toluene and the crude diol was purified by silica gel column chromatography (hexane/EtOAc 2/1 v/v) to afford compound **2** as a colorless oil (4.0 g, 94%). ^{1}H NMR (300 MHz,) δ 7.52 (dd, *J* = 6.6, 3.4 Hz, 2H), 7.43 – 7.20 (m, 7H), 5.51 (s, 1H), 5.42 (s, 1H), 5.12 – 5.04 (m, 1H), 4.71 (dd, *J* = 77.5, 11.9 Hz, 2H), 4.20 (ddd, *J* = 14.3, 13.8, 2.3 Hz, 2H), 3.97 (s, 1H), 3.76 – 3.66 (m, 1H), 2.77 – 2.46 (m, 5H), 2.00 (s, 3H), 1.80 (d, *J* = 11.6 Hz, 1H), 1.31 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) 172.34, 171.81, 170.57, 170.32, 169.56, 168.10, 137.19, 128.84, 128.74, 128.66, 128.51, 128.40, 128.33, 128.30, 128.22, 128.09, 127.48, 127.16, 82.67, 81.54, 77.10, 77.47, 77.05, 74.52, 73.08, 72.74, 72.65, 72.00, 69.54, 68.36, 62.80, 58.61, 37.89, 37.83, 29.98, 25.73, 16.20, 14.43. HRMS MALDI-TOF: ($M+Na^+$) found 435.1852, observed 435.1858.

Ethyl *O*-methyl-2-*O*-levulinoyl-3-benzyl-1-thio-α/β-L-idopyranosyluronate (3): A suspension of TEMPO (14 mg, 0.092 mmol) and BAIB (370 mg, 1.15 mmol) were added to a stirred solution of compound **2** (190mg, 0.46 mmol) in DCM/water (3/1 v/v, 2 mL) at ambient temperature. The biphasic mixture was stirred vigorously for 30 min. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M, 1.5 mL). The layers were separated and the aqueous layer was acidified with aqueous HCl (1M, 0.5 mL), and extracted thrice with DCM. The combined organic layers were dried (MgSO4) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (1.5 mL) followed by addition of solution of diazomethane in Et_2O (1 mL). After stirring for five minutes, the yellow solution was treated with a few drops of acetic acid until the mixture turned colorless. The solvents were evaporated *in vacuo* and the residue was purified by silica gel column chromatography (hexane/EtOAc 3/1 v/v) to afford compound **3** as an oil (176 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.24 (m, 6H), 5.37 (s, 1H), 5.20 (d, *J* = 1.6 Hz, 1H), 5.09 (dt, *J* = 2.4, 1.1 Hz, 1H), 4.81 – 4.52 (m, 2H), 4.09 (dt, *J* = 22.0, 8.2 Hz, 2H), 3.80 (d, *J* = 8.2 Hz, 3H), 3.77 (d, *J* = 3.5 Hz, 1H), 3.71 (td, *J* = 2.9, 1.2 Hz, 1H), 2.95 (s, 1H), 2.88 (d, *J* = 0.6 Hz, 1H), 2.84 – 2.52 (m, 7H), 2.19 (s, 3H), 2.04 (s, 1H), 1.36 – 1.21 (m, 4H), -0.00 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) 172.21, 171.76, 170.20, 170.02, 169.10, 168.10, 137.32, 137.19, 128.84, 128.70, 128.64, 128.57, 128.50, 128.37, 128.33, 128.12, 128.03, 127.98, 127.76, 82.77, 81.51, 77.90, 77.47, 77.05, 74.52, 73.08, 72.74, 72.65, 72.00, 69.14, 68.97, 68.06, 66.89, 66.69, 53.78, 52.57, 52.56, 37.89, 37.83, 29.93, 29.91, 28.03 26.82, 25.93, 15.20, 15.03. HRMS MALDI-TOF: ($M+Na^+$) found 463.0118, observed 463.0112.

Ethyl *O*-methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-Fluorenylmethyloxycarbonyl)-1-thio-α/β-L-idopyranosyluronate (4): A suspension of Fmoc-Cl (7.60 g, 5.90 mmol) and DMAP (7.20 mg, 0.059 mmol) were added to a stirred solution of compound **3** (2.60 g, 0.46 mmol) in Pyridine (59 mL) at ambient temperature. TLC indicated complete consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes/EtOAc 5/1 v/v) to afford compound **3** (3.05 g, 77%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.5 Hz, 2H, *CH* Aromatic), 7.59 (d, J = 7.2 Hz, 2H, *CH* Aromatic), 7.44 – 7.21 (m, 10H, *CH* Aromatic), 5.42 (s, 1H, H1), 5.29 (d, J = 2.1 Hz, 1H, H5), 5.09 (s, 1H, H4), 5.01 (d, J = 1.3 Hz, 1H, H2), 4.73 (dd, J = 31.1, 11.7 Hz, 2H, C*H*HBn, CH*H*Bn), 4.51 (dd, J = 10.4, 7.2 Hz, 1H, CH*H* Fmoc), 4.41 – 4.31 (m, 1H, C*H*H Fmoc), 4.23 (d, J = 7.2 Hz, 1H C*H* Fmoc), 3.86 (s, 1H, H3), 3.77 (s, 3H, C*H*$_3$ COOMe), 2.83 – 2.38 (m, 8H, 2 X C*H*$_2$ Lev, C*H*$_2$ SEt), 2.04 (d, J = 3.3 Hz, 3H, C*H*$_3$ Lev), 1.30 (t, J = 7.4 Hz, 2H, C*H*$_2$ SEt). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.73, 128.23, 128.20, 128.04, 127.48, 127.47, 126.90, 125.46, 125.45, 120.38, 119.73, 82.86, 72.94, 72.93, 72.12, 71.48, 70.36, 70.32, 70.33, 68.68, 55.55, 52.71, 46.84, 37.79, 37.76, 37.75, 29.94, 29.72, 28.17, 26.90, 26.90, 15.01, 15.00. HRMS MALDI-TOF: ($M+Na^+$) found 658.1290, observed 358.1298.

Ethyl *O*-methyl-2-*O*-levulinoyl-3-benzyl-4-acetyl-1-thio-α/β-L-idopyranosyluronate (5) A solution of compound **3** (1.0 g, 2.27 mmol) in pyridine: acetic anhydride (4/1, v/v, 0.2 M) was stirred for 6 hr at ambient temperature. TLC indicated complete consumption of the starting material, after which the mixture was concentrated *in vacuo*. The residue

was purified by silica gel column chromatography (hexanes/EtOAc 4/1 v/v) to afford compound **5** (0.94 g, 86%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 5H, *CH* Aromatic), 5.36 (s, 1H, H1α), 5.23 – 5.21 (m, 1H, H5α), 5.20 (d, *J* = 2.0 Hz, 1H, H4β), 5.15 (s, 1H, H4α), 4.96 (d, *J* = 1.4 Hz, 1H, H1β), 4.90 (d, *J* = 5.9 Hz, 2H, H2α, H2β), 4.73 - 4.53 (m, 4H, CH*H*Bn, C*H*HBn, CH*H*Bn, C*H*HBn), 3.83 (t, *J* = 2.5 Hz, 1H, H3β), 3.72 (d, *J* = 3.4 Hz, 1H, H3α), 3.69 (s, 3H, C*H*$_3$ COOMe), 2.83 – 2.41 (m, 7H, 2 x C*H*$_2$ Lev, C*H*$_2$ SEt), 2.11 (d, *J* = 5.4 Hz, 3H, C*H*$_3$ Lev), 2.01 (d, *J* = 4.5 Hz, 3H, C*H*$_2$ Ac), 1.23 (td, *J* = 7.4, 4.4 Hz, 3H, C*H*$_3$ SEt). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.21, 171.76, 170.20, 170.02, 169.10, 168.10, 137.32, 137.19, 128.84, 128.70, 128.64, 128.57, 128.50, 128.37, 128.33, 128.12, 128.03, 127.98, 127.76, 82.77, 81.51, 77.90, 77.47, 77.05, 74.52, 73.08, 72.74, 72.65, 72.00, 69.14, 68.97, 68.06, 66.89, 66.69, 53.78, 52.57, 52.56, 37.89, 37.83, 29.93, 29.91, 28.03, 27.98, 26.82, 25.93, 20.91, 15.20, 15.03. HRMS MALDI-TOF: ($M+Na^+$) found 658.1290, observed 658.1282.

Allyl 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-[(2, 2, 2-trichloroethoxy) carbonyl] amino]-*β*-D-glucopyranoside (10): Trichloroacetonitrile (2.08ml, 10.00mmol) and DBU (.119ml, 0.76mmol) were added to a solution of starting material (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 purified by passing through a short silica gel column chromatography using a gradient of (hexane/EtOAc 2:1). The imidate compound (1.87g, 2.99mmol) was then dissolved in anhydrous DCM (30ml) followed by addition of TMSOTf (0.052 ml, 0.190mmol) and allyl alcohol (1.02 ml, 20.51mmol) and was stirred at -20 ^{o}C for 1hr. Upon completion the reaction was quenched with triethylamine and concentrated *in vacuo*. The crude residue was purified by silica gel column

chromatography using a gradient of hexane/EtOAc (3:1) to obtain compound **10** as an oil (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_3CCH_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. HRMS MALDI-TOF: ($M+Na^+$) found 542.0929, observed 542.0937.

Allyl 2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-4,6-*O*-benzylidene-β-D-galactopyranoside (14): A solution of starting material (5.00g, 12.69mmol) in a Guanidine/Guanidinium Chloride NaOMe solution (50ml) was stirred at rt for 30min. Dowex 50X8-200 resin was added to quench the reaction and was allowed to stir an additional 30 min. The mixture was filtered and the solvent removed *in vacuo* to afford **5** (3.89 g, 9.87mmol 77%). This compound was dried over vacuum pump overnight. Camphor sulfonic acid (0.292g, 1.26 mmol) was added to a solution of benzaldehyde dimethyl acetal (2.47ml, 16.02 mmol) and above crude compound in anhydrous CH_3CN (40ml). After stirring at ambient temperature for 12 h TLC showed complete consumption of starting material. The reaction was diluted with ethyl acetate and washed with H_2O, aqueous $NaHCO_3$ (satd), and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using a gradient of hexane/EtOAc (4:1) to obtain compound **14** as an oil (3.76 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 – 7.29 (m, C*H* Aromatic), 6.01 – 5.80 (m, 1H, C*H*$=CH_2$), 5.58 (s, 1H, C*H* benzylidene), 5.39 – 5.08 (m, 2H, CH_2=CH), 4.78-

4.62 (m, 3H, C*H*$_2$ Troc, H1), 4.47 – 4.31 (m, 2H, H6a, CH*H* Allyl), 4.29 – 4.19 (m, 1H, H4), 4.16 – 3.95 (m, 3H, H6b, H3, C*H*H Allyl), 3.69 (s, 1H, H2), 3.49 (dd, *J* = 3.1, 1.7 Hz, 1H, H5). ^{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. HRMS MALDI-TOF: ($M+Na^+$) found 504.5380, observed 504.5384.

Allyl 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-trichloracetamido-*β*-D-galactopyranoside (12): A suspension of zinc dust (14,66g, 222.400 mmol) was added to a solution of compound **10** (4.157g, 8.010 mmol) in AcOH (61 ml). After stirring at ambient temperature for 2 h TLC showed complete consumption of starting material. The reaction mixture was filtered over a pad of celite and was co-evaporated with toluene and dried at vacuum pump overnight. The crude amine was dispersed in 40ml DCM and cooled to 0 ^{0}C. Trichloroacetyl chloride (5.66 ml, 50.5) and Et_3N (9.39ml, 67.40) were added to this stirred solution. After stirring at 0 ^{o}C for 30 mins TLC showed complete consumption of starting material. The reaction mixture was then diluted with DCM and was washed with aqueous $NaHCO_3$ (satd), water and brine. The organic layer was dried (MgSO4) and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using a gradient of hexane/EtOAc (3:1) to obtain compound **12** as an oil (3.08 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (d, *J* = 9.1 Hz, 1H, NH), 5.82 (ddt, *J* = 17.1, 10.4, 5.2 Hz, 1H, C*H*=CH_2), 5.30 – 5.16 (m, 3H, H4, H3, CH=CH*H*), 5.11 (ddd, *J* = 10.4, 3.3, 1.5 Hz, 1H, CH=C*H*H), 4.70 (d, *J* = 8.4 Hz, 1H, H1), 4.26 – 4.16 (m, 1H, CH*H* Allyl), 4.13 – 3.87 (m, 6H, C*H*H Allyl, H6a, H6b, H2, H5), 2.11 (s,3H, C*H*$_3$ Ac), 1.98 (s, 3H, C*H*$_3$ Ac), 1.87 (s, 3H, C*H*$_3$ Ac). ^{13}C NMR (75 MHz, $CDCl_3$) 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. HRMS MALDI-TOF: ($M+Na^+$) found 512.1335, observed 512.1339.

Allyl 2-deoxy-2-trichloroacetamido-4, 6-*O*-benzylidene-β-D-galactopyranoside (15): Compound **12** (2.2 g, 4.224 mmol) was dissolved in a solution of methanol (24 mL) and aq. ammonia (28%, 2.4 mL). After stirring at ambient temperature for 12 h, TLC analysis showed complete consumption of starting material. The solvents were evaporated *in vacuo* to give crude triol. The crude compound co-evaporated with toluene and dried on the vacuum pump over night and was dissolved in CH_3CN (40ml) followed by addition of camphor sulfonic acid (0.090 g, 0.39 mmol) and benzaldehyde dimethyl acetal (0.837ml, 7.90 mmol). The reaction mixture was stirred at ambient temperature under the atmosphere of Ar for 16 h. TLC analysis showed complete consumption of starting material. The mixture was diluted with ethyl acetate and was washed with aq. $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc 3:1) to afford compound **15** as an oil (1.18 g, 67%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.30 (m, 5H, C*H* Aromatic), 6.86 (d, *J* = 6.8 Hz, 1H, NH), 5.89 (ddd, *J* = 16.7, 11.0, 6.2 Hz, 1H, C*H*=CH_2), 5.60 (s, 1H, C*H* Aromatic), 5.29 (dd, *J* = 17.2, 1.6 Hz, 1H, CH=C*H*H), 5.20 (dd, *J* = 10.3, 1.5 Hz, 1H, CH=C*H*H), 4.86 (d, *J* = 8.3 Hz, 1H, H1), 4.46 – 4.30 (m, 2H, H6a, C*H*H Allyl), 4.28-4.18 (m, 2H, H4, H3), 4.16 – 3.99 (m, 2H, , H6b, CH*H* Allyl), 3.88-3.76 (m, 1H, H2), 3.54 (s, 1H, H5). ^{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. HRMS MALDI-TOF: ($M+Na^+$) found 474.5074, observed 474.5071.

Dimethylthexylsilane 3,4,6-tri-*O*-acetyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino-*β*-D-galactopyranoside (11): A suspension of dimethylthexylsilane chloride (11.60ml, 71.60mmol) and imidazole (6.60g, 97.50mmol) were added to a solution of starting material (18.00 g, 37.50 mmol) in DMF (90ml) at ambient temperature and stirred under Ar for 16 h. TLC analysis showed complete consumption of starting material. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc 4/1 v/v) to afford compound **11** as an oil (16.52g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.21 (dd, J = 3.5, 1.3 Hz, 1H, H4), 5.02 (d, J = 10.7 Hz, 1H, H3), 4.93 (d, J = 8.6 Hz, 1H, NH), 4.65 – 4.42 (m, 3H, H1, C*H*$_2$ Troc), 4.06 – 3.90 (m, 2H, H6a, H6b), 3.79 – 3.70 (m, 1H, H5), 3.70 – 3.57 (m, 1H, H2), 2.02 (d, *J* = 2.8 Hz, 3H, C*H*$_3$ Ac), 1.90 (s, 3H, C*H*$_3$ Ac), 1.85 (s, 3H, C*H*$_3$ Ac), 1.53 – 1.42 (m, 1H, C*H*$(CH_3)_2$), 0.78 – 0.65 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 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. HRMS MALDI-TOF: ($M+Na^+$) found 644.6901, observed 644.6897.

Dimethylthexylsilane 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloracetamido-*β*-D-galactopyranoside (13): A suspension of Zinc dust (71.29 g, 1090.00mmol) was added to a solution of compound **11** (24.66 g, 38.90 mmol) in AcOH (294 ml) at ambient temperature and stirred for 2 h under an atmosphere of Ar. TLC analysis showed complete consumption of starting material. The mixture was then filtered through a pad of Celite and the filtrate was concentrated *in vacuo* followed by co-evaporated with

toluene to remove traces of AcOH and dried under vacuum overnight. The crude amine was dissolved in DCM (200 ml) and cooled to 0 ^{0}C followed by addition of trichloroacetyl chloride (21.83 ml, 120.10 mmol) and TEA (32.57 ml, 320 mmol). TLC analysis showed complete consumption of starting material with 30 min. The mixture was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc 2:1) to afford compound **13** as an oil (17.93g, 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, H4), 5.26 (dd, *J* = 11.3, 3.4 Hz, 1H, H3), 4.90 (d, *J* = 7.9 Hz, 1H, H1), 4.22 – 4.00 (m, 3H, H6a, H6b, H2), 3.93 (t, *J* = 6.8 Hz, 1H, H5), 2.18 (s, 3H, C*H*$_3$ Ac), 2.05 (s, 3H, C*H*$_3$ Ac), 1.99 (s, 3H, C*H*$_3$ Ac), 1.62 (m,1H, C*H*$(CH_3)_2$), 0.87-0.84 (m, 12H, $(C(CH_3)_2$ and $CH(CH_3)_2$), 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. HRMS MALDI-TOF: ($M+Na^+$) found 614.1096, observed 614.1091.

Dimethylthexylsilane-2-deoxy-2-trichloroacetamido-4,6-*O*-benzylidene-β-D-galactopyranoside (16): Compound **13** was dissolved (2.11 g, 5.8 mmol) in a solution of methanol (21 mL) and aq. ammonia (28%, 2.14 mL) and stirred at ambient temperature for 12 h. TLC analysis showed complete consumption of starting material. The solvents were evaporated *in vacuo* to give crude triol. The crude compound co-evaporated with toluene and dried on the vacuum pump over night and was dissolved in CH_3CN (20ml) followed by addition of camphor sulfonic acid (0.249g, 1.07 mmol) and benzaldehyde dimethyl acetal (1.61ml, 10.70 mmol). The reaction mixture was stirred at ambient temperature under the atmosphere of Ar for 16 h. TLC analysis showed complete

consumption of starting material. The mixture was diluted with ethyl acetate and was washed with aq. $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc 3:1) to afford compound **16** as an oil (1.61g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 – 7.29 (m, 5H, C*H* Aromatic), 6.79 (d, *J* = 7.6 Hz, 1H, NH), 5.58 (s, 1H, C*H* Benzylidene), 4.99 (d, *J* = 7.9 Hz, 1H, H1) , 4.32 – 4.25 (m, 1H, H6a), 4.23 (d, *J* = 3.7 Hz, 1H, H4), 4.14 – 4.02 (m, 2H, H6b, H3), 3.88 – 3.75 (m, 1H, H2), 3.51(s, 1H, H5), 1.72 – 1.58 (m, 1H, C*H*$(CH_3)_2$), 0.90 – 0.81 (m, 12H, CH$(CH_3)_2$ and C$(CH_3)_2$), 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. HRMS MALDI-TOF: ($M+Na^+$) found 576.7151, observed 576.7159.

Allyl (methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-(9-Fluorenylmethyloxycarbonyl)-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-[(2,2,2-trichloroethoxy)carbonyl]amino--β-D-galactopyranoside (17): A suspension of iduronic acid donor **4** (900 mg, 1.860 mmol), galactosyl acceptor **10** (860 mg, 1.550 mmol) and activated molecular sieves (4Å crushed, 1.5 g) in dichloromethane (19 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -40 ^{o}C followed by addition of NIS (833 mg, 3.720 mmol) TfOH (17 μl, 0.186 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete hydrolysis of the donor.

Allyl (methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-(9-Fluorenylmethyloxycarbonyl)-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-[(2,2,2-trichloroethoxy)carbonyl]amino--β-D-galactopyranoside (17): A suspension of

iduronic acid donor **6** (110 mg, 0.145 mmol), galactosyl acceptor **10** (105 mg, 0.217 mmol) and activated molecular sieves (4Å crushed, 220 mg) in dichloromethane (2 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -40 ^{o}C followed by addition of TMSOTf (5 μl, 0.029 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 30 min TLC analysis showed complete hydrolysis of the donor.

Allyl (methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-(9-Fluorenylmethyloxycarbonyl)-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-[(2,2,2-trichloroethoxy)carbonyl]amino--β-D-galactopyranoside (17): A suspension of iduronic acid donor **6** (150 mg, 0.198 mmol), galactosyl acceptor **10** (73 mg, 0.152 mmol) and activated molecular sieves (4Å crushed, 220 mg) in dichloromethane (2 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^{o}C followed by addition of TMSOTf (7 μl, 0.039 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete hydrolysis of the donor.

Allyl *O*-(methyl-2-*O*-levulinoyl-3-O-benzyl-4-*O*-(9-Fluorenylmethyloxycarbonyl)-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (18): A suspension of iduronic acid donor **6** (190 mg, 0.250 mmol), galactoside acceptor **10** (94 mg, 0.208 mmol) and activated molecular sieves (4Å crushed, 300 mg) in dichloromethane (1.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^{o}C followed by addition of TMS-OTf (9 μl, 0.050 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete consumption of the donor. The reaction

mixture was neutralized with pyridine (~15 μl) and filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /EtOAc 4/1 v/v) to afford compound **18** (161 mg, 61 %) as an oil. ^{1}H NMR (600 MHz,) δ 7.76 – 7.62 (m, 3H, CH Aromatic), 7.60 – 7.19 (m, 14H, CH Aromatic), 6.78 (dd, *J* = 7.3, 2.4 Hz, 1H, N*H*), 5.90 – 5.78 (m, 1H, C*H* Allyl), 5.50 – 5.43 (m, 1H C*H* Benzylidene), 5.29 – 5.17 (m, 2H, CH*H* Allyl, H5′), 5.17 – 4.95 (m, 2H, C*H*H Allyl, H5′), 4.93 (s, 1H, H2′), 4.75 (dd, *J* = 12.1, 2.3 Hz, 1H, C*H*HBn), 4.70 – 4.59 (m, 2H, CH*H*Bn, H4), 4.55 (dt, *J* = 11.0, 3.1 Hz, 1H, H3), 4.44 (ddd, *J* = 10.4, 7.1, 2.4 Hz, 1H, CH*H* Fmoc), 4.40 – 4.23 (m, 2H, C*H*H Fmoc, H6a), 4.17 (t, *J* = 7.5 Hz, 1H, C*H* Fmoc) , 4.12 – 3.97 (m, 3H, H6b, CH_2 Allyl), 3.85 – 3.68 (m, 2H, H2, H3′), 3.50 (s, 1H, H5), 3.40 – 3.26 (m, 3H, CH_3 COOMe), 2.70 – 2.61 (m, 2H, CH_2 Lev), 2.53 – 2.30 (m, 2H, CH_2 Lev), 2.19 – 2.08 (m, 3H, CH_3 Lev), 2.03 – 1.91 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 208.44, 207.04, 206.20, 206.15, 200.63, 171.38, 171.34, 171.29, 168.58, 168.52, 161.81, 156.73, 154.21, 154.17, 149.51, 145.55, 145.43, 143.31, 143.25, 143.19, 143.13, 143.05, 142.85, 142.76, 141.21, 140.24, 140.21, 140.18, 137.79, 137.73, 137.47, 137.23, 136.18, 133.65, 133.63, 133.47, 131.01, 128.84, 128.66, 128.56, 128.28, 128.22, 128.10, 127.95, 127.89, 127.84, 127.74, 127.68, 127.63, 127.13, 127.07, 127.04, 126.83, 126.30, 125.90, 125.84, 125.63, 125.18, 125.12, 125.02, 121.34, 120.79, 120.74, 120.09, 120.02, 119.99, 118.36, 117.76, 117.72, 110.84, 108.03, 100.45, 100.43, 100.38, 97.80, 97.76, 92.25, 90.69, 77.28, 77.18, 77.13, 77.07, 76.93, 76.85, 75.93, 74.58, 74.55, 71.55, 71.51, 71.29, 71.26, 71.23, 71.08, 71.03, 70.13, 70.06, 70.01, 69.99, 69.63, 69.37, 69.25, 66.56, 66.53, 66.44, 66.34, 66.32, 65.73, 65.68, 54.72, 53.46, 53.42, 52.62, 52.07, 50.71, 46.53, 37.59, 31.89, 30.92, 29.76, 29.67, 29.63, 29.59, 29.50, 29.45, 29.42, 29.33, 29.28,

29.12, 27.88, 27.19, 22.67, 14.12, 13.09. HRMS MALDI-TOF: ($M+Na^+$) found 1074.1350, observed 1074.1345.

Allyl *O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-Fluorenylmethyloxycarbonyl)-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (18): A suspension of iduronic acid donor **4** (80 mg, 0.105 mmol), galactoside acceptor **10** (40 mg, 0.087 mmol) and activated molecular sieves (4Å crushed, 120 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The reaction mixture was cooled to 0 ^oC followed by addition of NIS (47mg, 0.210 mmol) and TfOH (2 μl, 0.021 mmol). The reaction mixture was allowed to warm to 5 ^oC and after 15 min TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 μl) and quenched with aqueous $Na_2S_2O_3$ (satd). The organic layer was isolated, dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /EtOAc 4/1 v/v) to afford product **18** (43 mg, 47%) as an oil. ^1H NMR (600 MHz,) δ 7.76 – 7.62 (m, 3H, CH Aromatic), 7.60 – 7.19 (m, 14H, CH Aromatic), 6.78 (dd, *J* = 7.3, 2.4 Hz, 1H, N*H*), 5.90 – 5.78 (m, 1H, C*H* Allyl), 5.50 – 5.43 (m, 1H C*H* Benzylidene), 5.29 – 5.17 (m, 2H, CH*H* Allyl, H5′), 5.17 – 4.95 (m, 2H, C*H*H Allyl, H5′), 4.93 (s, 1H, H2′), 4.75 (dd, *J* = 12.1, 2.3 Hz, 1H, C*H*HBn), 4.70 – 4.59 (m, 2H, CH*H*Bn, H4), 4.55 (dt, *J* = 11.0, 3.1 Hz, 1H, H3), 4.44 (ddd, *J* = 10.4, 7.1, 2.4 Hz, 1H, CH*H* Fmoc), 4.40 – 4.23 (m, 2H, C*H*H Fmoc, H6a), 4.17 (t, *J* = 7.5 Hz, 1H, C*H* Fmoc) , 4.12 – 3.97 (m, 3H, H6b, CH_2 Allyl), 3.85 – 3.68 (m, 2H, H2, H3′), 3.50 (s, 1H, H5), 3.40 – 3.26 (m, 3H, CH_3 COOMe), 2.70 – 2.61 (m, 2H, CH_2 Lev), 2.53 – 2.30 (m, 2H, CH_2 Lev), 2.19 – 2.08 (m, 3H, CH_3 Lev), 2.03 – 1.91 (m, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 208.44, 207.04, 206.20, 206.15, 200.63, 171.38, 171.34, 171.29, 168.58, 168.52, 161.81, 156.73, 154.21, 154.17, 149.51, 145.55, 145.43, 143.31, 143.25, 143.19, 143.13, 143.05, 142.85, 142.76, 141.21, 140.24, 140.21, 140.18, 137.79, 137.73, 137.47, 137.23, 136.18, 133.65, 133.63, 133.47, 131.01, 128.84, 128.66, 128.56, 128.28, 128.22, 128.10, 127.95, 127.89, 127.84, 127.74, 127.68, 127.63, 127.13, 127.07, 127.04, 126.83, 126.30, 125.90, 125.84, 125.63, 125.18, 125.12, 125.02, 121.34, 120.79, 120.74, 120.09, 120.02, 119.99, 118.36, 117.76, 117.72, 110.84, 108.03, 100.45, 100.43, 100.38, 97.80, 97.76, 92.25, 90.69, 77.28, 77.18, 77.13, 77.07, 76.93, 76.85, 75.93, 74.58, 74.55, 71.55, 71.51, 71.29, 71.26, 71.23, 71.08, 71.03, 70.13, 70.06, 70.01, 69.99, 69.63, 69.37, 69.25, 66.56, 66.53, 66.44, 66.34, 66.32, 65.73, 65.68, 54.72, 53.46, 53.42, 52.62, 52.07, 50.71, 46.53, 37.59, 31.89, 30.92, 29.76, 29.67, 29.63, 29.59, 29.50, 29.45, 29.42, 29.33, 29.28, 29.12, 27.88, 27.19, 22.67, 14.12, 13.09. HRMS MALDI-TOF: ($M+Na^+$) found 1074.1350, observed 1074.1357.

Dimethylthexylsilyl *O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (19): A suspension of iduronic acid donor **5** (300 mg, 0.510 mmol), galactoside acceptor **16** (233 mg, 0.420 mmol) and activated molecular sieves (4Å crushed, 530 mg) in dichloromethane (1.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^{o}C followed by addition of TfOH (9 μl, 0.102 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~15 μl) and filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography

(hexane /EtOAc 4/1 v/v) to afford compound **19** (233 mg, 57%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.46 (m, 1H, CH Aromatic), 7.38 – 7.24 (m, 7H, CH Aromatic), 6.62 (d, *J* = 6.7 Hz, 1H, NH), 5.47 (s, 1H, C*H* benzylidene), 5.27 (d, *J* = 7.9 Hz, 1H, H1′), 5.22 (d, *J* = 1.8 Hz, 1H, H5), 5.15 (d, *J* = 2.6 Hz, 1H,), 5.12 (s, 1H, H4′), 4.91 (dd, *J* = 2.5, 1.2 Hz, 1H, H2), 4.79 – 4.68 (m, 2H, CH_2Bn), 4.60 – 4.53 (m, 2H, H4, H3), 4.27 – 4.19 (m, 1H, H6a), 4.09 (dd, *J* = 8.7, 3.8 Hz, 1H, , H6b), 3.79 (d, *J* = 2.3 Hz, 1H′), 3.73 (dd, *J* = 4.3, 2.0 Hz, 1H, H3), 3.68 – 3.55 (m, 1H H2′), 3.49 (s, 1H, H5′), 3.37 (s, 3H, , COCH_3), 2.80 (m, *J* = 18.0, 8.0, 5.8 Hz, 2H, CH_2 Lev), 2.68 – 2.37 (m, 2H, CH_2 Lev), 2.21 – 2.12 (m, 3H CH_3 Lev), 2.04 (d, *J* = 1.6 Hz, 3H, CH_3 Ac), 1.63 (dt, *J* = 13.7, 7.0 Hz, 1H, C*H*(CH_3)$_2$), 0.97 – 0.75 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.26 – -0.05 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (300 MHz, $CDCl_3$) δ 206.06, 171.50, 170.02, 169.06, 161.71, 138.30, 137.92, 129.05, 128.35, 128.33, 127.71, 126.91, 126.06, 100.65, 93.77, 77.58, 77.36, 77.16, 76.83, 76.74, 74.82, 71.59, 71.08, 70.67, 69.64, 67.87, 66.77, 66.38, 66.14, 57.24, 52.11, 37.85, 34.18, 29.86, 27.84, 24.90, 20.87, 20.36, 20.19, 18.80, 18.73, 3.08. HRMS MALDI-TOF: ($M+Na^+$) found 996.2548, observed 996.2541.

Dimethylthexylsilyl (methyl-2-*O*-levulinoyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (19): A suspension of iduronic acid donor **5** (900 mg, 1.860 mmol), galactosyl acceptor **16** (860 mg, 1.550 mmol) and activated molecular sieves (4Å crushed, 1.5 g) in dichloromethane (19 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^{o}C followed by addition of NIS (833 mg, 3.720 mmol) TfOH (17 μl, 0.186 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete consumption of the donor.

The reaction mixture was neutralized with pyridine (~30 μl), quenched with aqueous $Na_2S_2O_3$ (satd). The organic layer was isolated, dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /EtOAc 4/1 v/v) to afford compound **19** (1.49 g, 83%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.46 (m, 1H, CH Aromatic), 7.38 – 7.24 (m, 7H, CH Aromatic), 6.62 (d, *J* = 6.7 Hz, 1H, NH), 5.47 (s, 1H, C*H* benzylidene), 5.27 (d, *J* = 7.9 Hz, 1H, H1′), 5.22 (d, *J* = 1.8 Hz, 1H, H5), 5.15 (d, *J* = 2.6 Hz, 1H,), 5.12 (s, 1H, H4′), 4.91 (dd, *J* = 2.5, 1.2 Hz, 1H, H2), 4.79 – 4.68 (m, 2H, CH_2Bn), 4.60 – 4.53 (m, 2H, H4, H3), 4.27 – 4.19 (m, 1H, H6a), 4.09 (dd, *J* = 8.7, 3.8 Hz, 1H, , H6b), 3.79 (d, *J* = 2.3 Hz, 1H′), 3.73 (dd, *J* = 4.3, 2.0 Hz, 1H, H3), 3.68 – 3.55 (m, 1H H2′), 3.49 (s, 1H, H5′), 3.37 (s, 3H, , COCH_3), 2.80 (m, *J* = 18.0, 8.0, 5.8 Hz, 2H, CH_2 Lev), 2.68 – 2.37 (m, 2H, CH_2 Lev), 2.21 – 2.12 (m, 3H CH_3 Lev), 2.04 (d, *J* = 1.6 Hz, 3H, CH_3 Ac), 1.63 (dt, *J* = 13.7, 7.0 Hz, 1H, C*H*$(CH_3)_2$), 0.97 – 0.75 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.26 – -0.05 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (300 MHz, $CDCl_3$) δ 206.06, 171.50, 170.02, 169.06, 161.71, 138.30, 137.92, 129.05, 128.35, 128.33, 127.71, 126.91, 126.06, 100.65, 93.77, 77.58, 77.36, 77.16, 76.83, 76.74, 74.82, 71.59, 71.08, 70.67, 69.64, 67.87, 66.77, 66.38, 66.14, 57.24, 52.11, 37.85, 34.18, 29.86, 27.84, 24.90, 20.87, 20.36, 20.19, 18.80, 18.73, 3.08. HRMS MALDI-TOF: ($M+Na^+$) found 996.2548, observed 996.2540.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (20): Hydrazine acetate (221 mg, 2.40 mmol) was added to a solution of compound **19** (471 mg, 0.48 mmol) in ethanol and toluene (2/1, v/v, 6 mL) and the reaction mixture was stirred at ambient temperature. After 2 h, TLC analysis showed complete consumption of

the starting material. The reaction mixture was diluted with dichloromethane, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene/EtOAc 4/1 v/v) to afford compound **20** as an oil (434mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 – 6.98 (m, 10H, CH Aromatic), 5.47 (d, *J* = 5.1 Hz, 1H, C*H* benzylidene), 5.32 – 5.24 (m, 2H, H1′, H4), 5.22 (t, *J* = 2.9 Hz, 1H, H5), 5.16 (d, *J* = 6.7 Hz, 1H, H1), 4.69 – 4.61 (m, 4H, CH_2Ph, H3′, H4′), 4.60 – 4.55 (m, 1H, 3H), 4.28 – 4.04 (m, 2H, H6a ,H6b), 3.81 – 3.58 (m, 3H, H3, H2, H2′), 3.55 – 3.46 (s, 1H, H5′), 3.36 (d, *J* = 4.8 Hz, 3H, CH_3 COOMe), 2.66 – 2.57 (m, 5H), 2.35 (s, 3H), 2.05 – 1.98 (m, 3H, CH_3 Ac), 1.83 (s, 1H), 1.69 – 1.55 (m, 1H, C*H*$(CH_3)_2$), 1.26 (s, 1H), 0.91 – 0.79 (m, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.21 – 0.10 (m, 6H, Si$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.88, 169.23, 169.20, 161.68, 137.98, 137.94, 137.90, 129.11, 128.96, 128.42, 128.35, 128.31, 128.24, 127.84, 127.08, 126.05, 125.38, 103.44, 100.55, 93.80, 92.48, 77.58, 77.16, 77.04, 76.74, 74.80, 74.11, 71.45, 69.51, 69.04, 67.23, 66.59, 66.36, 57.04, 52.10, 34.09, 29.60, 25.10, 24.84, 21.54, 20.80, 20.27, 20.14, 18.72, 18.67, -1.67. HRMS MALDI-TOF: ($M+Na^+$) found 898.2179, observed 898.2185.

Dimethylthexylsilyl *O*-(methyl-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (21): A suspension of compound **20** (220 mg, 0.247 mmol) and activated molecular sieves (4Å, 220 mg) in dichloromethane (3.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -78 ^{o}C followed by addition of Et_3SiH (118 µl, 0.740 mmol) and TfOH (74 µl, 0.840 mmol). After being stirred for 1 h at -78 ^{o}C, Et_3N (1 mL) and MeOH (1 mL) were added successively, and the mixture was

diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ (10%), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/ MeOH 95/5 v/v) to afford compound **21** as an oil (135mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 10H, C*H* Aromatic), 6.93 (d, *J* = 7.8 Hz, 1H, NH), 5.29 (s, 1H), 5.27 – 5.20 (m, 2H, H1′, H5′), 5.02 (dd, *J* = 10.7, 5.8 Hz, 2H, H1, H4′), 4.76 – 4.63 (m, 2H, CH_2 Bn), 4.58 – 4.54 (s, 2H, CH_2 Bn), 4.28 (dd, *J* = 10.8, 3.3 Hz, 1H, H3), 4.14 (t, *J* = 3.6 Hz, 1H, H4), 3.82 – 3.65 (m, 9H, H2′, H3′, H2, H5, H6a, , H6b, CH_3 COOMe), 2.06 – 1.98 (m, 3H, CH_3 Ac), 1.70 – 1.53 (m, 1H, C*H*$(CH_3)_2$), 0.92 – 0.78 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.23 – 0.10 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.60, 165.21, 141.29, 141.04, 131.81, 131.73, 131.26, 131.01, 130.98, 130.92, 105.44, 97.91, 95.88, 81.42, 80.80, 80.58, 80.38, 79.95, 78.61, 76.97, 76.45, 76.16, 72.99, 72.69, 72.23, 71.81, 71.62, 60.36, 55.78, 37.25, 28.07, 24.06, 23.48, 23.30, 21.96, 21.88, 1.63. HRMS MALDI-TOF: ($M+Na^+$) found 900.2335, observed 900.2331.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (23): A suspension of Zn-Cu couple (2 g) was added to a solution of disaccharide **21** (138 mg, 0.157 mmol) in acetic acid (3.0 mL) under an atmosphere of Ar and the resulting mixture was stirred for 5 h. The mixture was then filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/ MeOH 95/5 v/v) to afford compound **23** as an oil (88 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.22 (m, 10H, CH Aromatic), 5.84 (d, *J* = 7.8 Hz, 1H, N*H*), 5.27 – 5.14 (m, 2H, H1′, H5′), 5.01 – 4.84 (m, 2H, H1, H5), 4.82 – 4.64 (m, 2H, CH_2Bn), 4.59 – 4.51 (s, 2H, CH_2Bn), 4.28 – 4.01 (m, 2H, H3, H4), 3.83 – 3.56 (m, 9H, H2′, H3′, H2^A, H5,

H6a, , H6b, CH_3 COOMe), 2.10 – 1.87 (m, 3H, CH_3 Ac), 1.61 (m, *J* = 7.0 Hz, 1H, CH(CH_3)$_2$), 0.94 – 0.75 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.23 – 0.09 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.11, 171.38, 169.60, 169.58, 138.25, 138.14, 128.59, 128.51, 128.47, 127.89, 127.82, 127.77, 127.70, 102.39, 95.37, 78.96, 77.58, 77.16, 76.85, 76.74, 73.66, 73.57, 73.38, 70.75, 70.15, 69.84, 69.41, 68.78, 60.54, 55.82, 53.56, 52.42, 34.19, 24.92, 23.73, 21.18, 20.89, 20.81, 20.22, 20.18, 18.69, 18.67, 14.32, 1.56. HRMS MALDI-TOF: ($M+Na^+$) found 798.3502, observed 798.3511.

Dimethylthexylsilyl *O*-(methyl-2-*O*-sulfonato-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-sulfonato-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside disodium salt (25): Sulfur trioxide pyridine complex (110 mg, 0.690 mmol) was added to a solution of the compound **23** (0.058 mmol, 45 mg) in DMF (1.5 mL) and the resulting mixture was stirred for 2 h at ambient temperature. TLC analysis ($CHCl_3$/CH_3OH 9/1 v/v) indicated complete consumption of starting material. Further, pyridine (0.2 mL) and methanol (0.5 mL) were added to the reaction mixture and it was continued to stir for additional 30 min. The mixture was concentrated *in vacuo* (bath temperature 20 ^{o}C), and the residue was passed through a column of iatrobeads (1.5 g, CH_3OH/$CHCl_3$ 96/4 to 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 resin (50 × 8 Na+ 0.6 × 5 cm, CH_3OH), providing the compound **25** as an oil (46 mg, 81%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.82 (d, *J* = 13.2 Hz, 1H, *CH* Aromatic), 7.73 (d, *J* = 2.6 Hz, 1H, *CH* Aromatic), 7.28 (t, *J* = 8.9 Hz, 2H, *CH* Aromatic), 7.16 (ddd, *J* = 15.3, 14.2, 7.5 Hz, 6H, *CH* Aromatic), 7.10 – 7.04 (m, 2H, *CH* Aromatic), 5.49 (d, *J* = 2.3 Hz, 1H, H5′), 5.05 (s, 1H, H1′), 4.96 (dd, *J* =

7.9, 5.3 Hz, 2H, H4′), 4.66 (dd, *J* = 20.0, 7.6 Hz, 2H, H1,CH*H*Bn), 4.54 – 4.48 (m, 1H, C*H*HBn), 4.44 – 4.34 (m, 2H, CH*H*Bn, C*H*HBn), 4.22 (d, *J* = 2.3 Hz, 1H, H2′), 3.82 (t, *J* = 2.9 Hz, 1H, H3′), 3.69 (ddd, *J* = 17.9, 10.6, 5.6 Hz, 2H, H6a, H6b), 3.61 (dd, *J* = 7.5, 4.0 Hz, 1H, H5), 3.55 (d, *J* = 10.3 Hz, 3H, C*H*$_3$ COOMe), 3.17 (d, *J* = 6.5 Hz, 1H), 1.88 – 1.78 (m, 7H, C*H*$_3$ Ac, C*H*$_3$ AcN), 1.50 – 1.41 (m, 1H, CH(CH$_2$)$_3$), 0.76 – 0.65 (m, 13H, C(CH$_2$)$_3$ and CH(CH$_2$)$_3$), 0.04 – -0.04 (m, 7H, Si(CH$_2$)$_3$). ^{13}C NMR (125 MHz, CD$_3$OD) δ 155.34, 149.28, 127.95, 127.44, 127.14, 102.71, 76.09, 73.73, 72.82, 71.88, 71.44, 71.43, 71.42, 71.41, 70.84, 70.42, 70.40, 67.61, 66.38, 51.31, 51.03, 51.37, 33.94, 29.31, 22.10, 22.08, 19.35, 19.30, 19.25, 19.21, 18.86, 17.70, 9.91. HRMS ESI-TOF: (M-2Na$^+$+2H$^+$) found 956.2490, observed 956.2487.

Dimethylthexylsilyl (2-O-sulfonato-3-O-benzyl-α-L-idopyranosyluronate)-(1→3)- 4-O- sulfonato-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (27): A premixed solution of aqueous H_2O_2 (30%, 251 μL, 10.20 mmol) and 1 M LiOH (2.25 mL, 2.25 mmol) were added to a solution of compound **25** (45 mg, 0.045 mmol) in THF (1.0 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to mixture (pH 14). The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (3.5 g, H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm) using CH_3OH as eluent, providing compound **27** as an oil (40 mg, 93%). ^{1}H NMR (600 MHz, CD$_3$OD) δ 7.30 (d, *J* = 7.5 Hz, 2H, *CH* Aromatic), 7.20 – 7.04 (m,

7H, *CH* Aromatic), 5.11 (s, 1H, H1′), 4.74 (dd, *J* = 4.4, 2.2 Hz, 2H, H5′, H4), 4.63 (t, *J* = 14.2 Hz, 2H, CH*H*Bn, H1), 4.53 (d, *J* = 12.4 Hz, 1H, C*H*HBn), 4.39 (q, *J* = 11.8 Hz, 2H, , CH*H*Bn, C*H*HBn), 4.23 (t, *J* = 2.2 Hz, 1H, H2′), 3.91 (t, *J* = 2.7 Hz, 1H, H4′), 3.87 – 3.78 (m, 2H, H2, H3), 3.75 – 3.62 (m, 4H, H3′, H5, H6a, H6b), 3.14 (p, *J* = 1.5 Hz, 3H, C*H*$_3$ COOMe), 1.77 (s, 3H C*H*$_3$ Ac), 1.45 (hept, *J* = 6.9 Hz, 1H, CH(CH$_2$)$_3$), 0.75 – 0.66 (m, 11H, C(CH$_2$)$_3$ and CH(CH$_2$)$_3$). 0.18 (d, 6H, Si(CH$_2$)$_3$). ^{13}C NMR (151 MHz,) δ 179.49, 177.52, 153.29, 143.22, 142.37, 132.78, 132.76, 132.54, 132.52, 132.51, 132.50, 132.48, 132.10, 132.06, 132.04, 131.69, 105.18, 105.08, 100.87, 99.71, 82.83, 80.75, 80.67, 79.73, 79.58, 78.28, 78.21, 77.51, 76.59, 76.52, 76.35, 76.27, 76.25, 76.17, 75.20, 74.32, 74.17, 71.65, 71.52, 58.29, 52.42, 52.37, 52.32, 52.27, 52.24, 52.21, 52.18, 52.13, 52.04, 52.00, 51.95, 51.93, 51.92, 51.91, 51.89, 51.85, 51.82, 51.81, 51.80, 51.78, 51.76, 51.73, 51.73, 38.70, 38.67, 29.11, 26.83, 26.76, 23.98, 23.95, 23.91, 22.41, 22.36, 1.96, 1.92, 1.87, 1.83, 0.14, 0.09, 0.05. HRMS ESI-TOF: (M-3Na$^+$+1H$^+$) found 956.2490, observed 956.2485.

2-*O*-sulfonato-3-*O*-benzyl-α-L-idopyranosyluronate-(1→3)-4-*O*-sulfonato-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (29): Compound **27** (40 mg, 0.042 mmol) was dissolved in pyridine (825 μL), THF (412 μL) and H_2O (100 μL). The reaction was cooled to 0 °C followed by addition of HF:pyridine (229 μL) and it was slowly warmed to rt overnight. The mixture was passed through a Sephadex LH-20 column (CH_2Cl_2/MeOH 1/1 v/v) concentrated *in vacuo* and was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9/1 v/v) to afford compound **29** as a white solid (27 mg, 81%). 1H NMR (500 MHz, D_2O) δ 7.33 – 7.07 (m, 10H, CH Aromatic), 5.16 (d, *J* = 15.9 Hz, 2H, H1′, H1α), 4.67 (d, *J* = 10.6 Hz, 4H, H4α, H4β, H1β, C*H*HBn),

4.64 – 4.57 (m, 1H, CH*H*Bn), 4.52 (dd, *J* = 20.4, 11.2 Hz, 2H, C*H*HBn, CH*H*Bn), 4.45 – 4.32 (m, 2H, H5α, H2α), 4.20 (dd, *J* = 7.7, 4.2 Hz, 1H, H5′), 4.10 (dd, *J* = 12.7, 9.6 Hz, 3H, H2′, H3′, H5′), 3.89 (dd, *J* = 21.0, 6.6 Hz, 2H, H5β, H3β), 3.82 (m, *J* = 18.9, 11.2, 5.3 Hz, 3H, H6a, H6b, H4′), 1.99 (t, *J* = 8.3 Hz, 3H C*H*$_3$ AcN).^{13}C NMR (126 MHz, D_2O) δ 128.93, 128.66, 130.32, 128.68, 129.07, 101.09, 101.09, 91.66, 68.56, 95.39, 72.39, 73.95, 72.38, 72.41, 74.74, 74.76, 49.86, 69.46, 71.03, 74.77, 74.74, 69.05, 66.75, 53.25, 76.37, 76.38, 75.25, 79.10, 76.38, 67.76, 75.44, 72.92, 67.74, 49.05, 24.08, 22.46. HRMS ESI-TOF: ($M-2Na^+ + 1H^+$) found 757.9000, observed 757.9007.

2-*O*-sulfonato-α-L-idopyranosyluronate-(1→3)-4-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (31): A suspension of Pd/C (24 mg) was added to a solution of the starting material **29** (12 mg) in CH_3OH (2.5 mL) and H_2O (0.75 mL). The mixture was placed under an atmosphere of hydrogen, and the 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_3COOH, 3/5/3/1, v/v/v). The hydrogenation was stopped when TLC indicated the disappearance of the starting material. The residue was passed through a short column of Biorad 50 × 8 Na+ resin (0.6 × 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze dried to provide the final product **31** (7.3 mg, 78%). ^{1}H NMR (800 MHz, D_2O) δ 5.29-5.25 (d, 1H, H1′α,β), 5.21 (d, J = 3.6 Hz, 1H, H1α), 4.91 (dd, J = 5.1, 1.8 Hz, 1H, H5′), 4.79 – 4.77 (m, 1H, H1β), 4.76 (d, J = 2.6 Hz, 1H, H4α), 4.70 (s, 1H, H4β), 4.39 (dd, J = 11.1, 3.6 Hz, 1H, H2α), 4.27 (dd, J = 8.2, 4.1 Hz, 1H, H5α), 4.22 – 4.17 (m, 2H, H3α, H2′α,β), 4.06 (d, J = 4.9 Hz, 2H, H4′α,β, H3β,H2β), 4.00 (s, 1H, H3′α,β), 3.84 (dd, J = 8.0, 4.2 Hz, 1H, H5β), 3.81 – 3.70 (m, 3H, H6a,α,β, H6b,α,β), 2.08 (d, J = 1.0 Hz, 3H, C*H*$_3$ AcN). ^{13}C NMR (800 MHz, D_2O) δ 91.56,

101.39, 101.45, 95.45, 68.23, 68.71, 49.06, 67.80, 74.10, 68.66, 76.80, 52.50, 69.06, 73.12, 79.94, 79.74, 22.28. HRMS ESI-TOF: ($M-3Na^{+}+1H^{+}$) found 577.9139, observed 577.9144.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (22): A suspension of compound **20** (214 mg, 0.243 mmol) and activated molecular sieves (4Å, 220 mg) in dichloromethane (3.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -78 ^{o}C followed by addition of Et_3SiH (118 µl, 0.740 mmol) and $PhBCl_2$ (110 µl, 0.840 mmol). After being stirred for 1 h at -78 ^{o}C, Et_3N (1 mL) and MeOH (1 mL) were added successively, and the mixture was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ (satd), dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel column chromatography (DCM/MeOH 95/5 v/v) to afford compound **22** as an oil (173mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.11 (m, 10H, CH Aromatic), 6.95 (d, *J* = 8.1 Hz), 5.23 – 5.04 (m, 2H, H1′, H5′), 4.82 (ddd, *J* = 32.1, 16.4, 5.2 Hz, 3H, H1, H4′, CH*H*Bn), 4.57 (ddd, *J* = 17.8, 13.4, 10.1 Hz, 3H, CH*HBn*), 4.31 – 3.83 (m, 3H, H3, H2), 3.78 (d, *J* = 2.8 Hz, 1H, H4), 3.69 – 3.54 (m, 4H, H3′, H2′, H5, H6a), 3.42 (d, *J* = 4.2 Hz, 1H, H6b), 2.03 – 1.87 (m, 3H C*H*$_3$ Ac), 1.62 – 1.42 (m, 1H, C*H*$(CH_3)_2$), 0.84 – 0.67 (m, 12H, C(C*H*$_3)_2$ and CH(C*H*$_3)_2$), 0.08 – 0.01 (m, 6H, Si(C*H*$_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.82, 171.40, 169.55, 169.44, 162.19, 138.39, 137.60, 133.88, 133.76, 128.69, 128.58, 128.50, 128.37, 128.24, 128.08, 128.04, 127.97, 127.89, 127.85, 127.69, 102.76, 95.15, 92.70, 79.30, 77.58, 77.16, 76.74, 75.79, 75.52, 75.16, 74.91, 74.54, 74.05, 73.35, 69.82, 69.59, 68.78,

61.88, 60.56, 57.63, 52.59, 52.55, 33.94, 29.65, 24.81, 21.17, 20.79, 20.23, 20.11, 18.69, 18.66, 14.30, -1.42. HRMS MALDI-TOF: ($M+Na^+$) found 900.2333, observed 900.2338.

Dimethylthexylsilyl *O*-(methyl-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (24): A suspension of Zn-Cu couple (2 g) was added to a solution of disaccharide **22** (140 mg, 0.157 mmol) in acetic acid (3.0 mL) under an atmosphere of Ar and the resulting mixture was stirred for 5 h. The mixture was then filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH 95/5 v/v) to afford compound **24** as an oil (100 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.22 (m, 10H, C*H* Aromatic), 6.11 (d, *J* = 7.9 Hz, 1H, N*H*), 5.24 (d, *J* = 5.3 Hz, 1H, H1′), 5.17 (t, *J* = 4.7 z, 1H, H4′), 4.94 (d, *J* = 7.7 Hz, 1H, H1), 4.90 – 4.55 (m, 5H, H5′, C*H*HBn CH*H*Bn), 4.12 (q, *J* = 7.2 Hz, 1H, H3), 3.97 – 3.63 (m, 5H, H2′, H3′, H4, H5, H6a), 3.59 – 3.43 (m, 1H, H6b), 2.32 – 1.84 (m, 6H, CH_3 Ac, CH_3 AcN), 1.70 – 1.49 (m, 1H, C*H*$(CH_3)_2$), 0.96 – 0.71 (m, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.25 – 0.10 (m, 6H, Si$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.84, 179.22, 176.61, 173.76, 170.54, 170.00, 169.97, 169.90, 169.81, 169.51, 158.67, 149.17, 138.50, 138.31, 138.23, 138.05, 137.82, 129.40, 128.62, 128.58, 128.56, 128.53, 128.29, 128.17, 128.08, 128.02, 127.98, 127.94, 117.17, 103.53, 102.83, 96.30, 95.32, 90.38, 81.72, 79.87, 77.58, 77.37, 77.16, 77.02, 76.86, 76.74, 74.95, 74.89, 74.67, 74.29, 74.18, 73.84, 73.74, 73.19, 70.98, 70.35, 70.31, 70.19, 69.78, 69.54, 61.94, 61.83, 60.10, 59.41, 56.79, 52.82, 52.66, 37.60, 34.16, 34.09, 34.04, 32.07, 29.85, 24.96, 24.93, 23.83, 22.84, 21.21, 21.00, 20.89, 20.85, 20.57, 20.26, 20.22, 20.14, 20.09, 19.53, 18.71, 18.68, 18.63, 18.57, 14.34, 14.27, 0.14, -1.42. HRMS MALDI-TOF: ($M+Na^+$) found798.3500, observed 798.3509.

Dimethylthexylsilyl (methyl-2-*O*-sulfonato-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-sulfonato-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside disodium salt (26): Sulfur trioxide pyridine complex (367 mg, 2.31 mmol) was added to a stirred solution of compound **24** (0.115 mmol, 90 mg) in DMF (4.0 mL) at ambient temperature for 2 h. TLC analysis ($CHCl_3$, CH_3OH 90/ 10, v/v) indicated complete consumption of starting material. Further, pyridine (0.2 mL) and methanol (0.5 mL) were added to the reaction mixture and it was continued to stir for additional 30 min. The mixture was concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was passed through a column of iatrobeads (1.5 g, $CH_3OH/CHCl_3$ 96/4 to 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 resin (50 × 8 Na+ 0.6 × 5 cm, CH_3OH), providing the compound **26** as an oil (104 mg, 92%). ^1H NMR (500 MHz, CD_3OD) δ 7.4 – 7.17 (d, 2H, CH Aromatic), 7.12 – 6.97 (m, 8H, CH Aromatic), 5.15 (s, 1H, H1′), 4.96 (t, *J* = 9.0 Hz, 1H, H5′), 4.86 (d, *J* = 1.9 Hz, 1H, H4′), 4.78 (m, 2H, H1, CH*H*Bn), 4.64 – 4.54 (d, 1H, C*H*HBn), 4.47 – 4.35 (m, 3H, C*H*HBn, CH*H*Bn, H2′), 4.03 – 3.87 (m, 4H, H3′, H6a, H6b, H3), 3.79 – 3.75 (m, 1H, H4), 3.69 (t, *J* = 6.8 Hz, 1H, H5), 3.54 (s, 4H, CH_3 COOMe, H2), 1.96 – 1.81 (m, 7H, CH_3 Ac, CH_3 AcN), 1.47 (dt, *J* = 13.8, 7.0 Hz, 1H, $CH(CH_2)_3$), 0.79 – 0.65 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.09 – -0.07 (m, 7H, $Si(CH_3)_2$). ^{13}C NMR (300 MHz, CD_3OD) δ 127.82, 127.52, 67.98, 66.27, 72.07, 72.06, 74.35, 70.10, 72.35, 63.79, 63.81, 63.80, 75.75, 75.67, 72.87, 51.89, 53.94, 48.65, 48.15, 35.92, 30.61, 22.71, 19.66, 14.18, 14.20, 18.13, 18.09, 19.60. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 956.2488, observed 956.2481.

Dimethylthexylsilyl (2-*O*-sulfonato-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-sulfonato-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (28): A premixed solution of aqueous H_2O_2 (30%, 579 μL, 10.20 mmol) and 1 M LiOH (122 mg, 5.10 mmol) were added to a solution of compound **26** (100 mg, 0.102 mmol) in THF (1.5 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to mixture until pH 14. The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (3.5 g), which was eluted with a stepwise gradient of H_2O and CH_3OH (9/1 to 7/3, v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of Biorad resin (50 × 8, Na, 0.6 × 5 cm) using CH_3OH as eluent, providing compound **28** as an oil (71 mg, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.21 – 7.17 (m, 2H, C*H* Aromatic), 7.09 – 6.96 (m, 7H C*H* Aromatic), 5.23 (s, 1H, H1′), 4.70 (d, *J* = 3.6 Hz, 3H, H5′, CH*H*Bn, H1), 4.54 m, *J* = 11.8 Hz, 2H, C*H*HBn, C*H*HBn), 4.45 – 4.32 (m, 2H, H2′, CH*H*Bn), 4.23 – 4.12 (m, 2H, H4′, H2), 3.99 (d, *J* = 7.1 Hz, 2H, H3′, H3), 3.97 – 3.91 (m, 1H, H6a), 3.84 – 3.80 (m, 2H, H4, H5), 3.76 (dd, *J* = 11.7, 4.8 Hz, 1H, H6b), 1.95 (s, 3H, C*H*$_3$ Ac), 1.53 – 1.41 (m, 1H, CH(C*H*$_3$)$_2$), 0.71 (q, *J* = 7.1 Hz, 12H, C(C*H*$_3$)$_2$ and CH(C*H*$_3$)$_2$), 0.01 (d, *J* = 11.5 Hz, 7H, Si(C*H*$_3$)$_2$). ^{13}C NMR (126 MHz, CD_3OD) δ 128.09, 127.30, 128.84, 128.07, 127.28, 127.40, 128.19, 128.97, 128.18, 127.39, 128.20, 127.33, 126.75, 128.77, 127.92, 127.85, 128.71, 126.14, 126.89, 128.44, 101.73, 75.60, 74.81, 74.86, 96.76, 69.27, 70.03, 68.51, 71.45, 72.22, 71.51, 70.74, 71.81, 70.21, 69.43, 72.18, 71.41, 70.74, 71.49, 72.19, 74.81, 75.55, 74.88, 67.88, 68.65, 67.12,

66.22, 76.31, 75.54, 74.78, 75.80, 76.55, 77.30, 72.88, 73.63, 72.13, 48.08, 22.74, 23.55, 20.39, 34.05, 34.79, 34.06, 34.08, 34.80, 34.09, 34.10, 18.71, 17.91, 34.11, 18.72, 17.93, 17.11, 20.25, 19.44. HRMS ESI-TOF: ($M-2Na^{+}+2H^{+}$) found 956.2488, observed 956.2481.

2-*O*-sulfonato-3-*O*-benzyl-α-L-idopyranosyluronate-(1→3)-6-*O*-sulfonato-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (30): Compound **28** (33mg, 0.035 mmol) was dissolved in pyridine (600 μL), THF (300 μL) and H_2O (100 μL). The reaction was cooled to 0 °C followed by addition of HF;pyridine (229 μL) and it was slowly warmed to rt overnight. The mixture was passed through a Sephadex LH-20 column (CH_2Cl_2/MeOH 1/1 v/v) concentrated *in vacuo* and was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9/1 v/v) to afford compound **30** as a white solid (21.0 mg, 75%). ^{1}H NMR (500 MHz, D_2O) δ 7.31 – 7.12 (m, 8H, C*H* Aromatic), 7.09 (td, *J* = 7.4, 6.1, 3.0 Hz, 3H, C*H* Aromatic), 5.15 (d, *J* = 15.9 Hz, 1H, H1′α/β), 5.05 (d, *J* = 3.6 Hz, 1H, H1α), 4.67 (m, 2H, H5′α/β, C*H*HBn,), 4.64 – 4.45 (m, 4H, H1β, C*H*HBn, C*H*HBn, CH*H*Bn), 4.44 – 4.30 (m, 3H, H2α, H2′α/β, CH*H*Bn), 4.18 (dd, *J* = 7.9, 4.2 Hz, 1H, H5α/β, H4′α/β, H2β), 4.14 – 4.04 (m, 3H, H2β, H3α, H4′α/β), 3.93 – 3.74 (m, 6H, H3′α/β, H4α/β, H5α/β, H6a), 3.70 – 3.61 (m, 1H, H6b), 1.98 (d, *J* = 3.9 Hz, 4H, C*H*$_3$ Ac) ^{13}C NMR (126 MHz, D_2O) δ 128.70, 128.44, 130.09, 128.45, 127.31, 128.84, 100.88, 100.88, 91.45, 68.36, 70.68, 95.18, 72.19, 72.18, 72.20, 74.53, 74.55, 49.67, 69.27, 70.83, 74.57, 74.54, 68.85, 64.93, 66.55, 53.06, 76.17, 76.17, 75.04, 78.89, 76.17, 67.56, 75.24, 72.72, 67.54, 67.50, 48.86, 2069, 22.29. HRMS ESI-TOF: ($M-2Na^{+}+2H^{+}$) found 757.9000, observed 757.9004.

2-*O*-sulfonato-α-L-idopyranosyluronate-(1→3)-6-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (32): A suspension of Pd/C (8.0 mg) was added to a solution of the starting material **30** (4 mg) in CH_3OH (0.8 mL) and H_2O (0.25 mL). The mixture was placed under an atmosphere of hydrogen, and the 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_3COOH, 3/5/3/1, v/v/v). The hydrogenation was stopped when TLC indicated the disappearance of the starting material. The residue was passed through a short column of Biorad 50 × 8 Na+ resin (0.6 × 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze dried to provide the final product **32** (4.8 mg, 81%). ^{1}H NMR (800 MHz, D_2O) δ 5.21 (d, *J* = 21.7 Hz, 1H), 5.17 (d, *J* = 3.6 Hz, 1H), 4.87 (dd, *J* = 5.1, 1.8 Hz, 1H), 4.75 – 4.74 (m, 1H), 4.73 (d, *J* = 2.6 Hz, 1H), 4.67 (s, 1H), 4.36 (dd, *J* = 11.1, 3.6 Hz, 1H), 4.23 (dd, *J* = 8.2, 4.1 Hz, 1H), 4.19 – 4.13 (m, 2H), 4.02 (d, *J* = 4.9 Hz, 2H), 3.97 (s, 1H), 3.81 (dd, *J* = 8.0, 4.2 Hz, 1H), 3.77 – 3.66 (m, 3H), 2.05 (d, *J* = 1.0 Hz, 3H). ^{13}C NMR (800 MHz, D_2O) δ 100.45, 100.53, 91.46, 67.88, 94.99, 77.09, 76.09, 49.88, 49.88, 70.51, 73.90, 73.15, 72.96, 53.46, 76.61, 68.77, 68.73, 74.62, 61.26, 61.31, 61.33, 61.32, 21.16, 22.75. HRMS ESI-TOF: ($M-2Na^+ + 2H^+$) found 577.9144, observed 577.9147.

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CHAPTER 4

DEVELOPMENT OF A MODULAR APPROACH TOWARDS THE STEREOSELECTIVE SYNTHESIS OF HEPARAN SULFATE OLIGOSACCHARIDES

Heparan sulfate (HS) has the binding affinity towards more than a hundred different proteins giving rise to an important class of biomolecules known as the heparan sulfate proteoglycans (HSPGs). These HSPGs reside on the plasma membrane of cells and they participate in a wide range of physiological processes like metabolism, transport and regulation. Many of these proteins have been extensively studied and characterized, but the HS structures that mediate these important interactions have been rarely pursued and seldom understood. The incapability to perform necessary structure-activity-relationship studies is mainly due to the lack of novel synthetic technologies to access a library of well-defined HS oligosaccharides. This difficulty can be attributed to the structural complexity associated with natural HS which in turn arises from an equally complex biosynthetic pathway. To address this problem, we have designed a modular strategy for the stereoselective synthesis of HS oligosaccharides that employs a fairly small number of orthogonally protected monosaccharide and disaccharide building blocks, which can easily be converted into glycosyl donors and acceptors. Further, these donors and acceptors can be coupled in a parallel combinatorial fashion to generate modular disaccharide and tetrasaccharide scaffolds. Late stage modification utilizes a standard set

of conditions for the selective installation of *O*-sulfates, saponification of esters, *N*-sulfation or *N*-acetylation.

Introduction

Heparin and Heparan Sulfate (HS) belong to a family of macromolecules called glycosaminoglycans (GAGs). Other members of this family include chondroitin sulfate, dermatan sulfate, keratan sulfate and hyaluronic acid. HS is a repeating linear copolymer of glucosamine α-1-4 linked to uronic acids (Figure 4.1). Uronic acid consists of two sugars, glucuronic acid (GlcA) and Iduronic acid (IdoA). The linear polysaccharide of HS is polydisperse, unbranched and highly *O*- and *N*- sulfated and primarily exists as a helical structure.[1] However, unlike proteins, HS is not known to exist in any kind of tertiary structure. The average chain length varies between 50 to 200 disaccharide units (25-100 KDa). HS is decorated with *O*- and or *N*- sulfate groups at several different positions, imparting it a high degree of structural diversity. The IdoA residue has conformational flexibility that allows it to adopt different forms, thereby influencing the spatial display of sulfate, carboxylate and hydroxyl groups attached to itself and adjacents sugars. Due to the structural diversity attributed to HS generated via an equally complex biosynthetic pathway, it is known to interact with more than hundred different proteins. The resulting heparan sulfate proteoglycans play important roles in several biological recognition processes, including blood coagulation, virus infection, cell

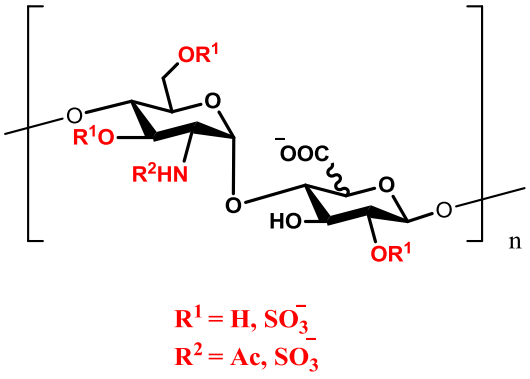
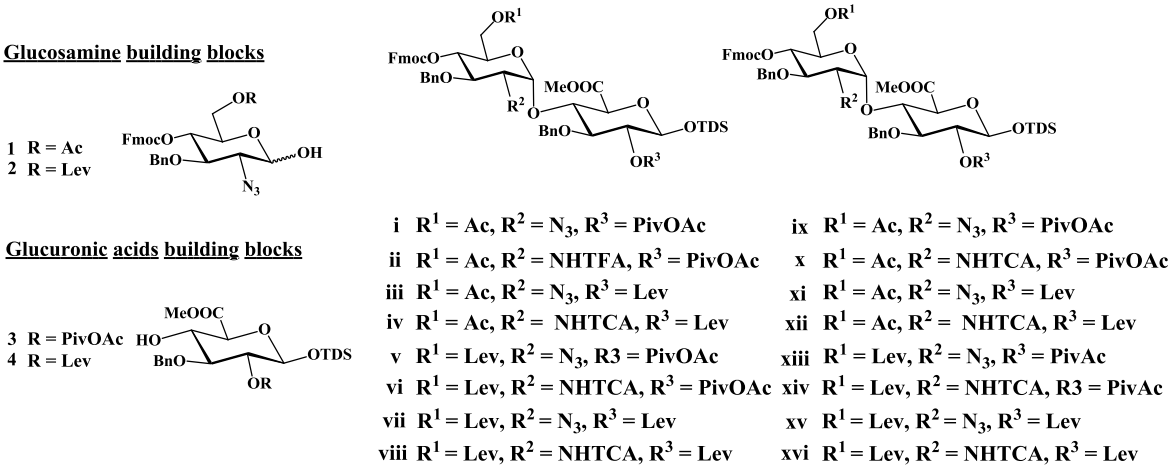


Figure 4.1 HS disaccharide

growth, cell-cell communication, inflammation, wound healing, tumor metastasis, lipid metabolism, and disease of the nervous system.[1] The HS polysaccharides bear specific sequences within their length that modulate selective interactions with proteins and these interactions result in regulation of protein activation. Currently more than hundred different proteins having a binding affinity towards HS have been identified. Some of these proteins have been extensively studied, but the specific oligosaccharide sequences that mediate these interactions have not been characterised[1]

A major hurdle in performing structure activity relationship (SAR) studies between HS and different biologically relevant proteins is the availability of well-defined HS oligosaccharides. The generation of a library of such well-defined oligosaccharides of HS is extremely difficult due to the absence of effective and feasible synthetic strategies. Our group has reported several synthetic strategies toward the synthesis of HS oligosaccharides. All these strategies were helpful in generation of extensive library of compounds.[2] Although very attractive, there were a few limitations in the reported strategies. For example, the orthogonal introduction of *N*- acetate and *N*-sulfate functionalities on the same oligosaccharide compound was not possible. Also, the installation of the aminopentanol linker gave a mixture of anomers which being difficult to separate resulted in loss of valuable material. The synthetic procedures pertaining to uronic acid building blocks, like benzylidine ring cleavage, C6 oxidation and esterification were completed on the disaccharide compounds which led to further loss of valuable material. In an effort to resolve these issues we proposed a modular approach of HS synthesis which entails the use of orthogonally protected monosaccharide building blocks. Installation of the acid functionality was performed at the monosaccharide level

employing the regioselective C6 oxidation condition of 2,2,6,6-tetramethyl-1-piperdinyloxy (TEMPO) and bisacetoxy iodo benzene (BAIB).[3] A set of four differentially protected monosaccharide building blocks **1-4** were prepared (Scheme 4.1). These monosaccharides were divided into two groups, namely 2-deoxy-2-azido-D-glucopyranoside donors **1** and **2b**; and glucuronic acid acceptors **3** and **4**. Further these donors and acceptors can be strategically coupled to generate a library of disaccharide molecules. To show the attractiveness of the strategy, all possible disaccharide structures have been shown in Scheme 4.1. The disaccharide of 2-deoxy-2-azido-D-glucoside α-

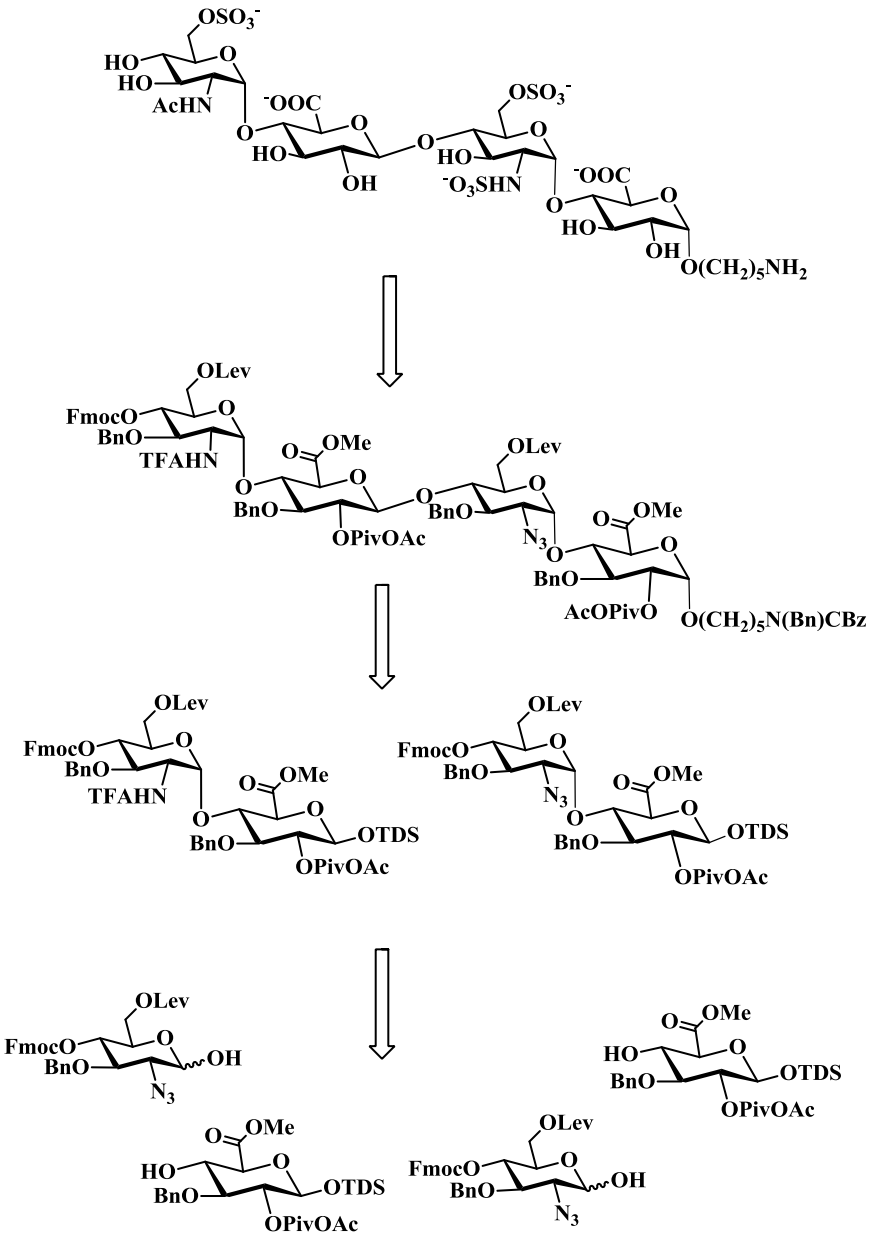


Scheme 4.1: Building blocks for HS synthesis

1,4- linked to an uronic acid residue will used as a common scaffold to generate differentially functionalized disaccharide modules. A common disaccharide motif with an azido moiety can be converted to $NHSO_3^-$ and the other disaccharide was modified to have a *N*-trichloroacetyl or *N*-trifluoroacetyl moiety which will be converted to NHAc (Scheme 4.2). The *N*-trichloroacetamido group can be installed by reducing the azido functionality to an amine followed by treatment with *N*-trichloroacetyl chloride.[4] The

anomeric center of the disaccharide moiety was protected as a TDS ether and the C4 with an Fmoc group.

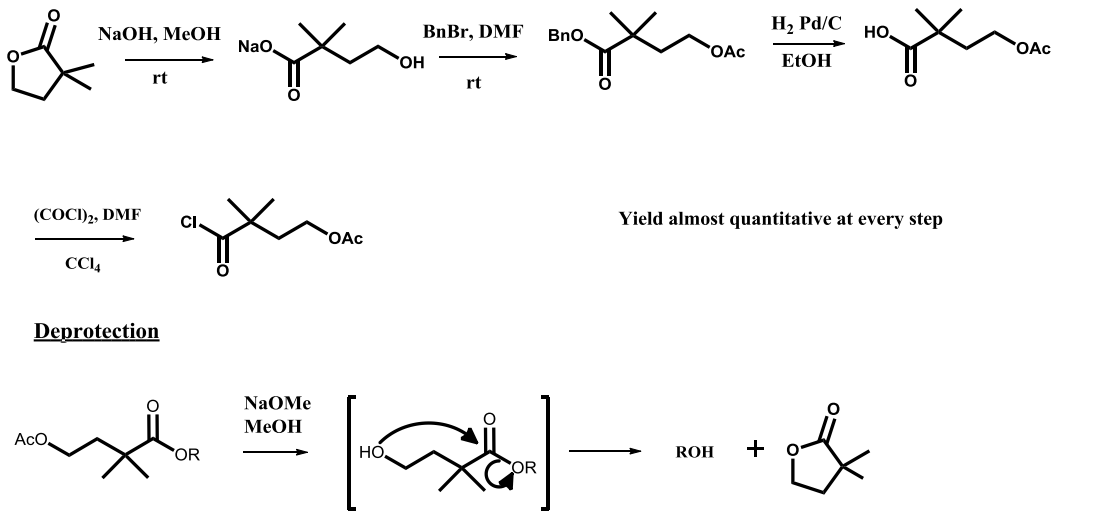
The removal of the TDS group followed by the introduction of the anomeric



Scheme 4.2: Scheme for the retrosynthesis of HS oligosaccharides

trichloroacetimidate group provided a glycosyl donor molecule, while the removal of the Fmoc group provided the glycosylated acceptor molecule. These donors and acceptors were further glycosylated to generate HS tetrasaccharides (Scheme 4.2). In the target compound, the sites which required the presence of sulfate groups were protected with levulinate[5,6] esters, while the sites which require presence of hydroxyl groups were differentially protected with pivaloyl (PivOAc) esters[7] and benzyl ethers. The presence of PivOAc ester protecting group at the C-2 position on the disacharides ensured the formation of 1, 2-trans-glycosidic linkage.[7] Based on our previous results and some model studies, we have observed that use of acetyl groups as C2 protecting groups of hexuronyl donors may lead to formation of an ortho ester. The problem of ortho-ester formation can be circumvented or minimized with the use of benzoyl or pivaloyl esters.[7] However their deprotection employs strong basic conditions which may prove to be detrimental to our synthetic design of HS as sulfate groups can be incompatible with rigorous basic conditions. The above discussed issues presented us with an opportunity to study the application of the new auxiliary PivOAc, in our synthesis which has not yet been reported in the synthesis of glycosaminoglycans to the best our knowledge.[7]

The PivOAc group offers a twofold advantage as it performs neighboring group participation as effectively as a traditional pivaloyl or benzoyl group but can be removed under milder conditions by a relay mechanism, where the extended acetyl group is cleaved under normal deprotection condition followed by intermolecular lactone formation (Scheme 4.3).[7] PivOAc was installed on hydroxyl groups which required no further modifications. The PivOAc protecting group was stable under deprotection conditions for Lev removal, and this orthogonality feature was helpful in facilitating the



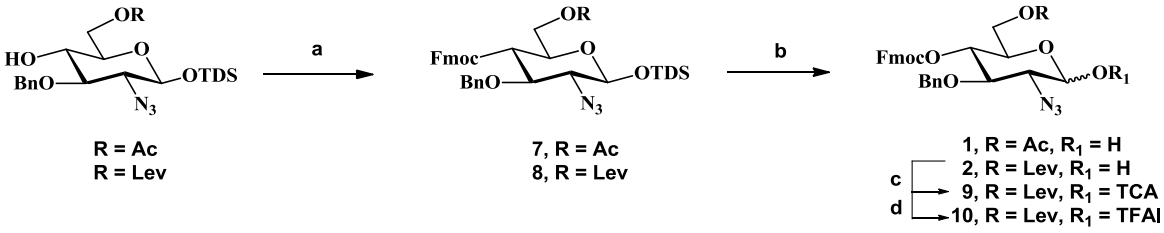
Scheme 4.3: Synthesis and application of PivOAc ester

late stage deprotection and sulfation at required positions. PivOAc was also stable to Fmoc removal condition which was necessary for orthogonal deprotection of Fmoc group followed by glycosidic chain elongation. Use of PivOAc ester group as C2 auxiliary during 1,2 trans stereoselective glycosylations coupled with its orthogonal stability towards required deprotections made it an ideal protecting group for our proposed synthetic strategy.

Results and discussion

Synthesis of 2-deoxy-2-azido glucopyranoside donors

The synthesis of the glucosazide donor was started with the previously reported

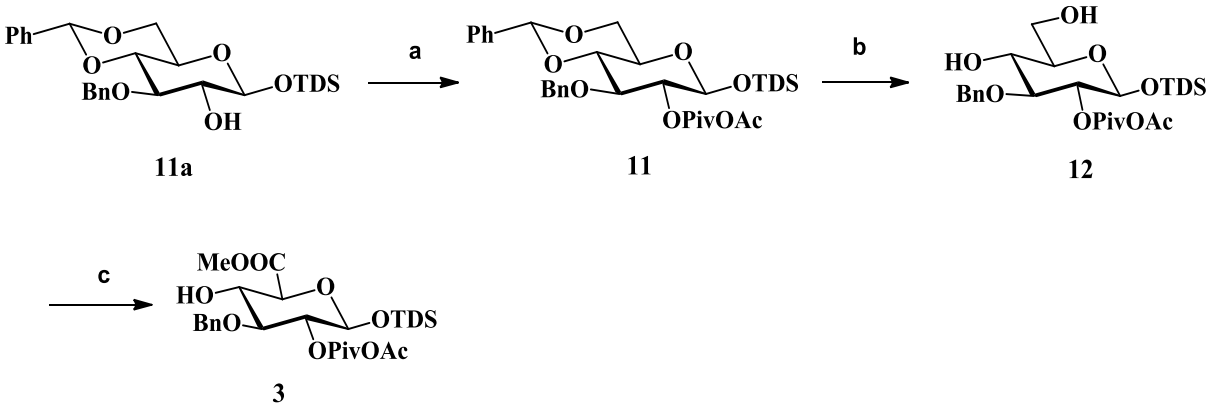


Scheme 4.4: Synthesis of 2-deoxy-2-azido-α-D-glucopyranoside donor

Reagents and conditions: (a) Fmoc-Cl, DMAP, Pyr., (7, 90%) (**8**, 82%); (b) HF in Pyr., THF, (**1**, 78%) (**2**, 72%); (c) Cl_3CCN, NaH, DCM; (d) $Cl(NPh)CCF_3$, NaH, DCM.

derivative with C6 Ac and Lev by our group (Scheme 4.4).[2] The C4 hydroxy of the C6 Ac and Lev compound was separately protected with Fmoc group to give compounds **7** and **8** with 90% and 82% yields respectively**.** The silyl ether of these Fmoc protected derivatives was separately cleaved in presence of HF and pyridine in THF to give the hemiacetal analogs **1** & **2** with 78% and 72% yields respectively as an anomeric mixture. These hemiacetals were directly used as donors for performing dehydrative glycosylations which will be discussed in the later part of the chapter. Compound **2** was treated with trichloroacetonitrile in DCM and catalytic NaH to give the trichloroacetimidate derivative **9** as an anomeric mixture. Compound **2** was also derivatised with trifluoro *N*-phenyl acetimidate at the anomeric position using trifluoro *N*-phenyl acetimidoyl chloride and NaH in DCM to give compound **10** as an anomeric mixture**.** Compounds **9** and **10** were directly used performing glycosylation reactions.

Synthesis of Glucuronic acid acceptor with PivOAc



11a **11** **12** **3**

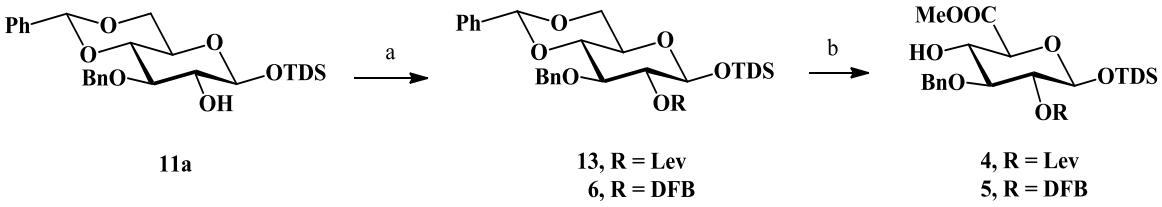
Scheme 4.5: Synthesis of glucuronic acid acceptor with PivOAc.

Reagents and conditions: (a) PivOAc-Cl, DMAP, DCM, 86%; (b) EtSH, TsOH, DCM, 77%; (c) (i) TEMPO, BAIB, DCM, H_2O; (ii) CH_2N_2, THF, 88%.

The synthesis of the glucuronic acceptor began with benzylidene protected glucose derivative **11a** with TDS group at the anomeric position (Scheme 4.5)**.**[8] This compound was treated with PivOAc-Cl in pyridine to introduce PivOAc on the C2 position to give derivative **11** with 86% yield**.** The benzylidine acetal was cleaved by subjecting it to acid catalyzed hydrolysis in presence of EtSH and TsOH in DCM to give the 4,6 diol compound **12** with 77% yield. This diol compound was further treated with TEMPO and BIAB in DCM and H_2O to regioselectively oxidize C6 hydroxy to carboxylic acid. The free acid was then protected by methylation in presence of diazomethane to give compound **3** with 88% yield**.**

Synthesis of glucuronic acid acceptor with Lev and DFB

The synthesis of the glucuronic acceptor began with previously reported benzylidene protected glucose derivative having an anomeric TDS group (Scheme 4.6)**.**[8] This compound was treated with levulinic acid in presence of DCC and DMAP in pyridine to introduce levulinoyl ester on the C2 position to give derivative **13** with 90% yield**.** The benzylidine acetal was cleaved by subjecting it to acid catalyzed hydrolysis in presence



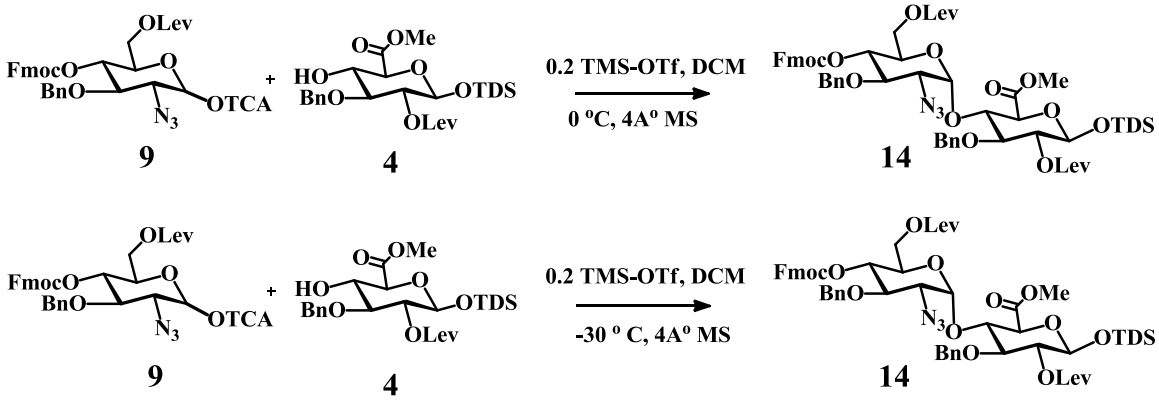
11a | **13, R = Lev** **6, R = DFB** | **4, R = Lev** **5, R = DFB**

Scheme 4.6: Synthesis of glucuronic acid acceptor with C2 Lev and DFB

(a) Levulinic acid, DMAP, DCM, (**13**, 90%); DFB-Cl, DMAP, DCM, (**6**, 86%); (b) (i) TFA, DCM, H_2O, (ii) TEMPO, BAIB, DCM, H_2O, CH_2N_2, THF, (**4**, 68%); (**5**, 71%).

TFA, DCM and H_2O to give the 4,6 diol compound. This diol compound was further treated with TEMPO and BIAB in DCM and H_2O to regioselectively oxidize C6 hydroxy to carboxylic acid. The free acid was then protected by methylation in presence of diazomethane to give compound **4** with 68% yield**.** Similarly for C2 DFB substitution, compound **11a** was treated with DFB-Cl and catalytic DMAP in pyridine to introduce benzoyl ester on the C2 position to give derivative **6** with 86% yield**.** The benzylidine acetal was cleaved by subjecting it to acid catalyzed hydrolysis in presence TFA, DCM and H_2O to give the 4,6 diol compound. This diol compound was further treated with TEMPO and BIAB in DCM and H_2O to regioselectively oxidize C6 hydroxy to carboxylic acid. The free acid was then protected by methylation in presence of diazomethane to give compound **5** with 71% yield**.**

Synthesis of HS Disaccharides



Scheme 4.7: Synthesis of disaccharides

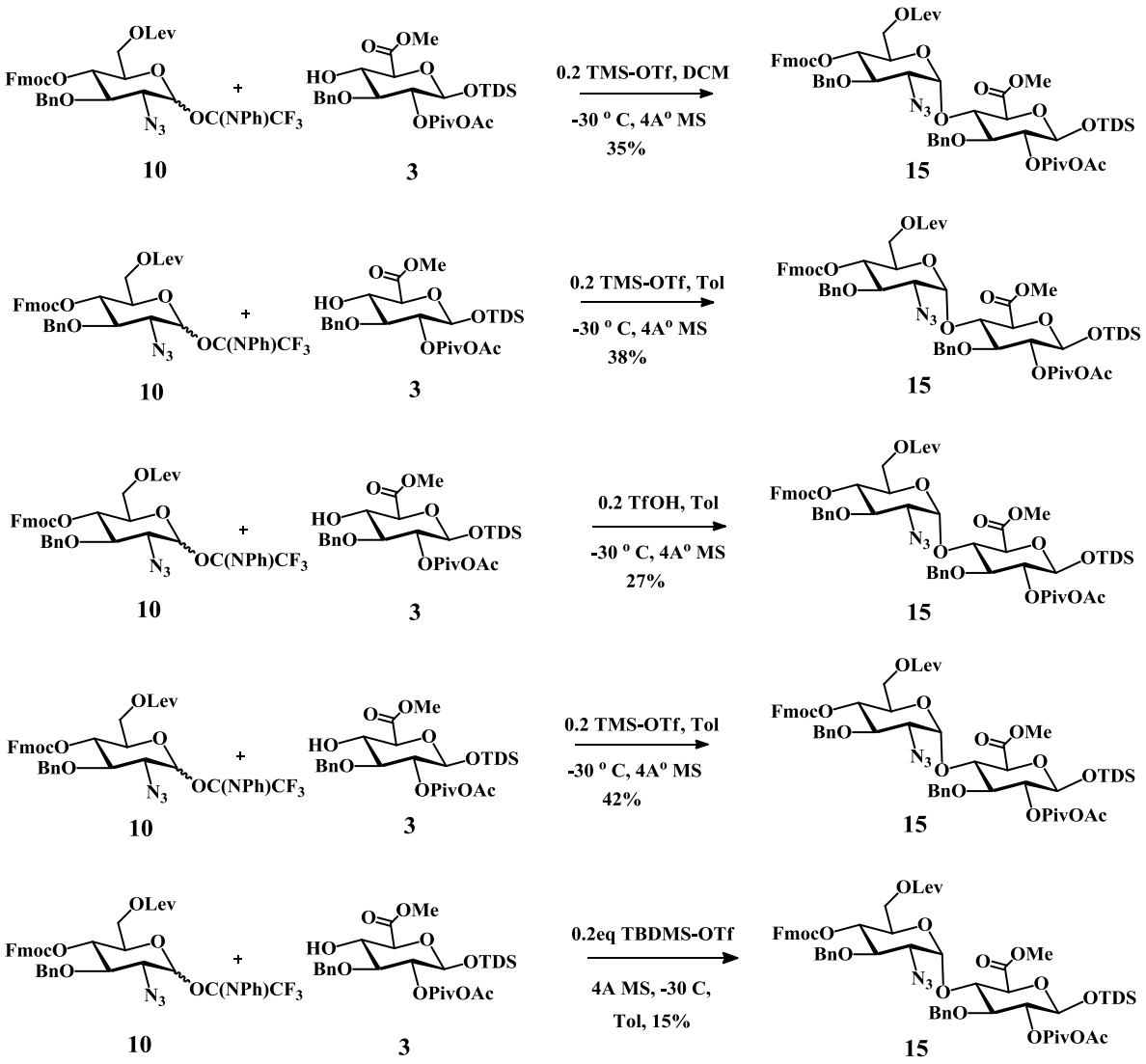
The azido glucose donor with C6 levulinoyl ester and C4 Fmoc carbamate group **9** was reacted with the glucuronic acid acceptor with C2 levulinoyl group **4** in the presence of

TMS-OTf as the acid catalyst, activated 4Å MS in DCM at 0^{o}C. The reaction resulted in giving complete hydrolysis of the imidate donor along with unreacted glucuronic acceptor and no product formation. The same glycosylation reaction was attempted with the same reactants and reagents at -30^{o}C. This gave the same result namely hydrolysed donor, unreacted acceptor and no product formation (Scheme 4.7). The presence of ester functionalities could impose a disarming effect on both the donor and the acceptor compounds, influencing their electrophilicity and nucleophilicity respectively, thereby causing the coupling reaction to fail.[9]

Trichloroacetimidates are known to be very labile making the donor very reactive and susceptible to hydrolysis.[10] In the above glycosylation, glucuronyl donor in its oxidised form is a poor nucleophile and thus having a very reactive donor counterpart would lead to mis-matched reactivity profiles which in turn would be detrimental to the glycosylation reaction. Recently, *N*-phenyl trifluoro acetimidate has gained popularity as more stable imidate functionality.[10] The additional stability comes from the phenyl substitution on the nitrogen. Therefore, we envisioned the use of *N*-phenyl trifluoro acetimidate in our study. The glucosazide donor with *N*-phenyl trifluoro acetimidate **10** (scheme 4.4) was prepared and used in the glycosylation reaction with the glucuronyl acceptor **3** with C2 PivOAc ester functionality (scheme 4.8). The reaction was performed in presence of TMS-OTf as an acid catalyst, activated 4Å molecular sieves and DCM at -30^{o}C. The disaccharide **15** was achieved with 35% yield along with hydrolysed donor. The reaction was successful in terms of generation of disaccharide product albeit in low amounts. The same glycosylation was attempted employing similar reaction conditions, but with toluene as a solvent. The coupling showed slight improvement in the yield (38%). In order to improve

the yield, we decided to manipulate the reaction conditions even further. The next glycosylation was attempted using TfOH as the acid catalyst instead of TMSOTf. Changing the acid catalyst did not improve the yields and therefore it was decided that TMSOTf would be used as the acid catalyst for further glycosylations. The next manipulations involved the amount of donor used in the glycosylation. Initially the glycosylation was performed using 1.5 equivalents of the donor. Increasing the number of equivalents of the donor, while keeping the other reaction conditions same, saw an improvement in the yield. The desired glycoside was obtained in 42% yield (Scheme 4.8). Although an improvement in the coupling efficiency was recorded over several reaction attemps , the yield was still below an acceptable range. With above data in hand we had to move away from conventional glycosylation coupling reactions in an attempt to enhance coupling reaction yields. Saito and co-workers reported the use of TBDMS-OTf as an acid catalyst to achieve the coupling of similar compounds with good yields.[11] Based on their successful attempt we decided to repeat our glycosylation reaction with TBDMS-OTf as an activator keeping all the reactants and solvent same. Our attempt proved futile as poor yield of 15% was recorded (Scheme 4.8).

Literature has shown that most of the glycosylation reactions having an oxidized glucuronyl acceptor showcase poor reactivities and their use in coupling reactions has proved to be quite challenging.[12] We decided to attempt a dehydrative glycosylation protocol developed by David Gin's lab to couple the hemi-acetal glucoazide donor **2** and the C2 levulinoyl substituted glucuronyl acceptor **4**.[13] The protocol follows a preactivation approach were the lactol donor was treated with Tf_2O, Ph_2SO, TTBP, 4Å

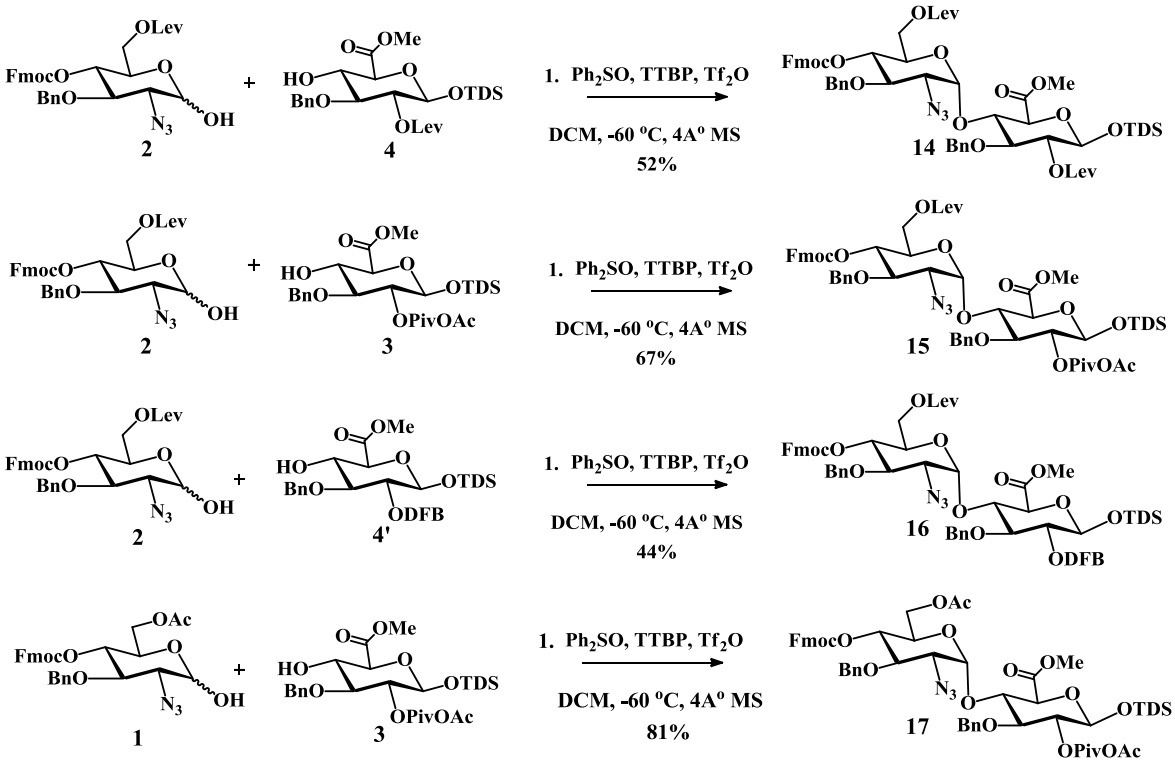


Scheme 4.8: Various glycosylation attempts for the synthesis of disaccharides

MS in DCM at -78 ^{o}C. On activation, the acceptor was introduced to the reaction mixture to afford the disaccharide **14** with 52% yield (Scheme 4.9).

Next the same reaction was attempted with C2 PivOAc substituted glucuronyl acceptor **3** to afford disaccharide **15** with a higher of 67% (Scheme 4.9). With this data in hand we applied the C2 DFB substituted glucuronyl acceptor to this same dehydrative protocol to achieve disaccharide **16** derivative with a lower but acceptable yield of 44%. The difluoro

substituted benzoyl ester can have a dectivating effect on the nucleophilicity of the acceptor species. This lowered nucleophilicity could be responsible for reducing the

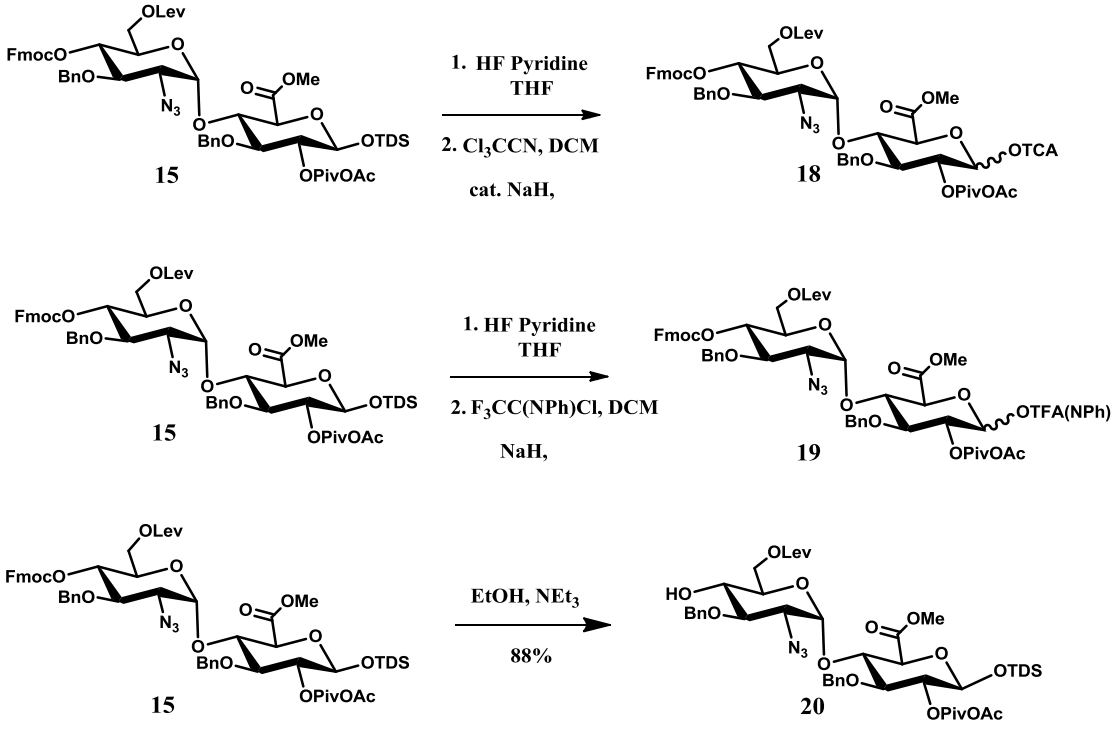


Scheme 4.9: Dehydrative glycosylation for the synthesis of disaccharides

yields of the glycosylation reaction in comparision to C2 levulinoyl and PivOAc substituted glucuronyl derivatives. We knew from previous studies performed in our group that the C6 Ac derivative of glucosazide monosaccharide is more reactive than its C6 levulinoyl counterpart.[2] Thus we decided to use the glucosazide donor with C6 acetyl group instead of the C6 levulinoyl ester for the dehydrative glycosylation protocol with glucuronyl acceptor with C2 PivOAc under the same reactions conditions. Disaccharide **17** was obtained with one our best yields so far of 81% with excellent stereoselectivity (Scheme 4.9).

Synthesis of HS Tetrasaccharides

With the preparation of disaccharide building blocks the next step was to convert the disaccharide scaffold into the corresponding imidate donor as well as the acceptor (Scheme 4.10). Disaccharide **15** was treated with HF in pyridine to cleave the anomeric silyl ether to give the hemiacetal as /β mixture. Further the lactol was subjected to

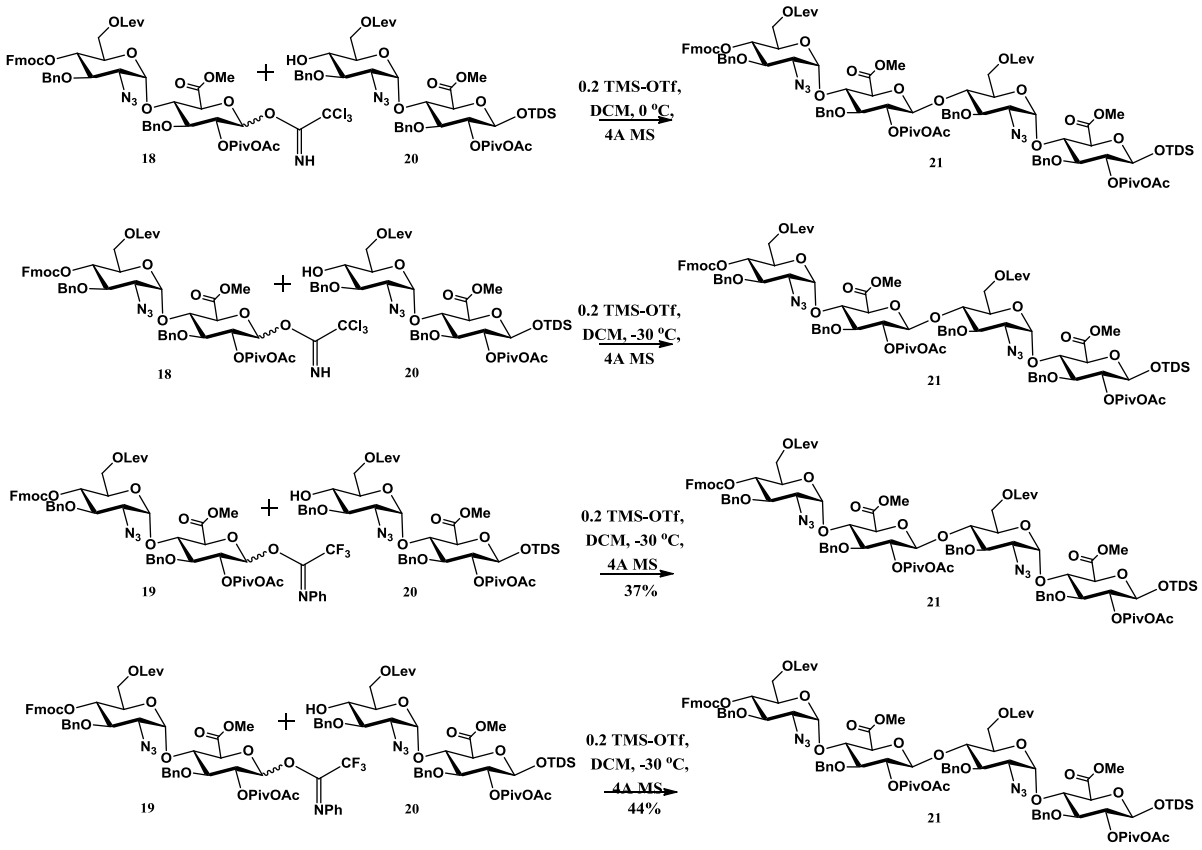


Scheme 4.10: Synthesis of disaccharide donor and acceptor

trichloroacetonitrile in DCM with catalytic NaH to give the trichloroimidate protected disaccharide donor **18**. Another batch of the same disaccharide molecule **15** was then subjected to Fmoc cleavage in presence Et_3N in toluene and ethanol to give C4 free hydroxyl compound **19** which was used as the acceptor for the tetrasaccharide assembly (Scheme 4.10).

The coupling reaction between trichloroimidate donor compound **18** and acceptor **20** was attempted to prepare the modular tetrasaccharide compounds (Scheme 4.11). The TMS-

OTf mediated coupling in DCM at 0 ^{o}C gave hydrolysis of donor with no product formation. The reaction was reattempted with exact same reagents and solvent at a lower temperature of -30 ^{o}C which also resulted in hydrolysis of donor (Scheme 4.11). We then

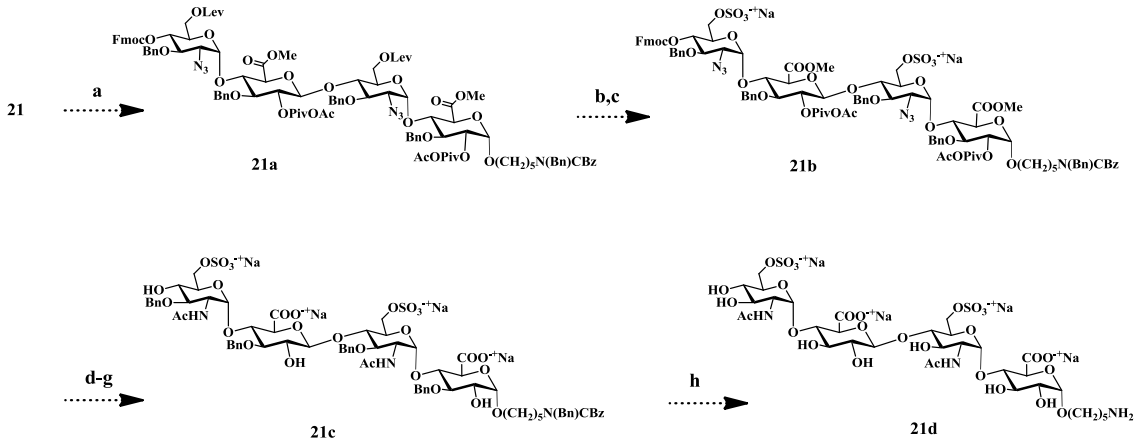


Scheme 4.11: Synthesis of tetrasaccharides

decided to use trifluoroacetimidate protected donor **20** for the glycosylation, with acceptor **9** mediated by TMSOTf in DCM at -30 ^{o}C. It lead to the formation of disaccharide compound **21** with 37% yield. The glycosylation reaction between the same donor **20** and acceptor **9** was reattempted by increasing the equivalence of donor under the same conditions at -30 ^{o}C and the tetrasaccharide compound **21** was obtained with higher yield of 45% (Scheme 4.11).

Deprotection scheme for HS tetrasaccharide

The tetrasaccharide compound **21** will be subjected to a set of orthogonal deprotection conditions along with installation of the sulfate groups at predetermined positions (scheme 4.12). In order to introduce sulfate groups, tetra saccharide **21** treated with HF in pyridine to cleave the anomeric silyl ether followed by installation of an imidate functionality which will be coupled with an aminopentyl spacer to give compound **21a**. This compound will be then subjected to hydrazine acetate in EtOH/toluene solvent mixture to remove both the Lev groups and install sulfate esters using SO_3.Pyr complex in DMF to give compound **21b.** Next the sulfated compound **21b** will be treated with Et_3N in DMF which will result in cleavage of Fmoc ester followed by treatment with LiOH, H_2O_2 in THF and NaOH that will lead to saponification of the methyl ester and removal of PivOAc to give C2 free hydroxyls leading to compound. The azides will be reduced with the help of PMe_3 and NaOH in THF to give compound**.** The free amine will then be acetylated by using Ac_2O in MeOH and Et_3N to give compound **21c**. Then finally



Scheme 4.12: Deprotection strategy for tetrasaccharide compound.

(a) 1. (i) HF/Pyr. THF; (ii) Cl_3CCN, DCM, NaH; 2. $HO(CH_2)_5N(Bn)Cbz$, TMS-OTf, 4A MS, DCM, -30 ^oC; (b). NH_2NH_3OAc, DMF; (c). SO_3Py, DMF; (d) (i) Et_3N, DMF; (ii) LiOH, H_2O_2, THF; (e). 4M NaOH, MeOH; (f) PMe_3, THF, NaOH; (g) Ac_2O, Et_3N, MeOH (h) (i) Pd/C, H_2, MeOH:H_2O (1:1); (ii) $Pd(OH)_2$, H_2O, H_2.

the benzyl and benzyloxycarbonyl groups will be removed by a two-step catalytic hydrogenation procedure to give final deprotected target tetrasaccharide **21d** (scheme 4.12).

Therefore, with similar synthetic approach disaccharide modules **7-22** can be used to synthesize a large number of HS oligosaccharides. The application of novel orthogonal protecting group PivOAc in our synthetic design will ensured the introduction of β stereoselectivity at required linkages. Strategically designed monosaccharides building blocks can be combined to form modular disaccharide motifs which in turn would be coupled in parallel combinatorial manner to generate a library of HS oligosaccharides.

Summary and future outlook

We have designed a novel strategy for the modular synthesis of HS oligosaccharides that employs orthogonally protected monosaccharide and disaccharide building blocks that can easily be converted into glycosyl donors and acceptors. Further these modular donors and acceptors can be utilized for the preparation of library of well-defined HS oligosaccharides. The access to such a collection of compounds can be useful towards performing structure-activity relationship studies for HS binding proteins. The key features of our new strategy includes the use of PivOAc ester for installation of 1,2 trans glycosydic linkages. For the construction of the disaccharide modules pre-oxidized form of glucuronic acids were used. Also in comparison to our old approach, we flipped the

sequence of our disaccharides so that the glucuronic moiety was placed at the reducing end of the synthetic fragment. The 2 + 2 glycosylations were performed in a more stereoselective manner giving rise to only one isomer, due to the anchimeric assistance of PivOAc ester functionality. Like our previous strategy, the current approach also employed:

- the use of Lev esters for those hydroxyls that needed sulfation,
- a Fmoc carbonate as a temporary protecting group for the C4 hydroxyl for the preparation of glycosyl acceptors,
- an anomeric TDS group for glycosyl donor synthesis, and
- benzyl ethers as permanent protecting groups.

The dehydrative and trifluoroacetimidate glycosylation methodologies were employed for the construction of the oligosaccharide assembly.

Furthermore, a few synthetic modifications may be introduced to our proposed synthetic strategy by replacing the C4 Fmoc group on the glucosamine derivative with NAP ether. NAP ether enjoys orthogonality similar to the traditional Fmoc group and being an electron donating functionality it could assist in improving yields of the coupling reactions. Also, the C3 benzyl ethers on the glucosamine derivatives could be replaced with a Lev group in order to incorporate a sulfate group wherever desired during the late stage manipulations. The synthetic compounds will be equipped with an artificial aminopentyl spacer at the reducing end. This would be potentially useful for immobilizing the HS constructs on a microarray plate or a BIACORE® chip. Studies are under way in our laboratory for the construction of such a HS based glyco-chip which could serve to be a unique tool for performing rapid profiling of HS binding proteins**.**

Experimental information

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 ~150oC or by spraying with a solution of $(NH_4)_6Mo_7O_{24}$ .H_2O (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150^oC. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å) 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. Mass spectra were recorded on Mass spectra were recorded on an ABISciex 5800 MALDi-TOF-TOF or Shimadzu LCMS-IT-TOF mass spectrometer. The matrix used was 2,5-dihydroxy-benzoicacid (DHB) and ultramark 1621 as the internal standard.

Dimethylthexylsilyl 2-*O*-levulinoyl -3-*O*-benzyl-4,6-*O*-benzylidene -β-D-glucopyranoside (**13**): A suspension of DCC (1.2 g, 5.990 mmol) and DMAP (0.012 g, 0.09 mmol) in DCM (5 mL) was added to a solution of starting material (1.0 g, 1.99 mmol) and levulinilic acid (0.46 g, 3.99 mmol) in DCM (5 mL) at 0 ^{0}C. After stirring for 2 h at ambient temperature TLC (hexanes/EtOAc, 70/30, v/v) indicated the total consumption of the starting material. The mixture was filtered over pad of celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as an oil (1.07 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.08 (m, 10H, CH Aromatic), 5.44 (s, 1H, CH benzylidene), 4.85 (t, *J* = 8.8, 7.5 Hz, 1H, H2), 4.73 (d, *J* = 12.1 Hz, 1H, C*H*HBn), 4.62 – 4.51 (m, 2H, H1 CH*H*Bn), 4.18 (dd, *J* = 10.5, 5.0 Hz, 1H, H6a), 3.73 – 3.53 (m, 3H, H3, H5, H6b), 3.32 (dt, *J* = 9.4, 4.8 Hz, 1H, H4), 2.66 – 2.34 (m, 4H, 2xCH_2 Lev), 2.04 (s, 3H, CH_3 Lev), 1.55 – 1.41 (m, 1H, $CH(CH_3)_2$), 0.76 – 0.67 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.01 (d, *J* = 6.3 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.39, 128.47, 129.95, 128.27, 128.20, 101.78, 75.56, 74.23, 96.45, 74.23, 68.98, 68.96, 81.79, 78.68, 66.50, 37.99, 28.07, 30.05, 34.06, 18.97, -1.84. HRMS: ($M+Na^+$) found 598.2958, observed 598.2962.

Dimethylthexylsilyl *O*-methyl-2-*O*-levulinoyl-3-*O*-benzyl-β-D-glucopyranosyluronate (4): Starting material (1.0 g, 1.671 mmol) was dissolved in DCM:TFA:H_2O (0.06 M) (10/1/0.1, v/v) at 0 ^{0}C. After stirring for 1hr TLC (Hex/EtOAc, 1/1, v/v) indicated the complete consumption of the starting material. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* and was dried on the membrane pump. A suspension of

TEMPO (52 mg, 0.334 mmol) and BAIB (1.34 g, 4.177 mmol) were added to a stirred solution of the crude diol in DCM/water (3/1 v/v, 2 mL). After stirring the biphasic solution vigorously for 1hr at ambient temperature TLC (hexanes/EtOAc, 6/4, v/v) indicated the consumption of the starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M, 10 mL) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (5 mL) followed by treatment with freshly prepared solution of diazomethane in Et_2O (2 mL). After stirring for five minutes, the excess diazomethane was quenched with a few drops of acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (610 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 – 7.10 (m, 5H, CH Aromatic), 4.80 (dd, *J* = 9.5, 7.5 Hz, 1H, H4), 4.72 – 4.57 (m, 2H, C*H*HBn, CH*H*Bn), 4.54 (d, *J* = 7.6 Hz, 1H, H1), 3.85 (dd, *J* = 9.8 Hz, 1H, H4), 3.69 (d, *J* = 9.5 Hz, 4H, H5, CH_3 $COOCH_3$), 3.40 (dd, *J* = 9.5 Hz, 1H, H3), 2.68 – 2.26 (m, 4H, 2xCH_2 Lev), 2.02 (s, 3H, CH_3 Lev), 1.44 (q, *J* = 6.9 Hz, 1H, $CH(CH_3)_2$), 0.76 – 0.59 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.01 (d, *J* = 8.0 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.54, 96.31, 81.35, 74.59, 74.37, 74.19, 72.23, 54.08, 38.05, 34.10, 30.17, 28.14, 20.12, 0.80. HRMS MALDI-TOF: ($M+Na^+$) found 561.1480, observed 561.1485.

Dimethylthexylsilyl 2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-4,6-*O*-benzylidene -β-D-glucopyranoside (**11**): To a stirring solution of starting material (2.7 g, 5.335 mmol) in pyridine (18 mL), DMAP (0.7 g, 5.535 mmol) and PivOAc-Cl (1.3 mL, 10.67 mmol) was added at 0 ^{0}C under the inert atmosphere of Argon. After stirring

for 4hr at ambient temperature TLC (hexanes/EtOAc, 7/3, v/v) indicated the consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The mixture was concentrated *in vacuo* and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (3.0 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.42 (m, 2H, CH Aromatic), 7.41 – 7.32 (m, 4H, CH Aromatic), 7.32 – 7.20 (m, 4H, CH Aromatic), 5.56 (s, 1H, CH benzylidene), 5.02 (t, *J* = 8.8, 7.2, 1.3 Hz, 1H, H2), 4.92 (d, *J* = 11.6 Hz, 1H, CH*H*Bn), 4.81 (dd, *J* = 7.1, 1.2 Hz, 1H, H1), 4.63 (d, *J* = 11.7 Hz, 1H, C*H*HBn), 4.32 (dd, *J* = 10.5, 4.8 Hz, 1H, H4), 4.07 (t, *J* = 7.2, 2.4 Hz, 2H, CH_2 PivOAc), 3.88 – 3.73 (m, 3H, H3, H6a, H6b), 3.49 (m, *J* = 5.0 Hz, 1H, H5), 1.97 (d, *J* = 1.3 Hz, 3H, CH_3 PivOAc), 1.85 (t, *J* = 7.4 Hz, 2H, CH_2 PivOAc), 1.62 (d, *J* = 6.8 Hz, 1H, 1H, $CH(CH_3)_2$), 1.18 (t, *J* = 2.6 Hz, 7H, 2xCH_3 PivOAc), 0.92 – 0.80 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 – 0.11 (m, 6H, $Si(CH_3)_2$). 13C NMR (151 MHz, CDCl3) δ 129.68, 129.11, 128.01, 127.49, 124.85, 101.76, 96.44, 81.25, 79.51, 74.01, 73.82, 73.71, 69.15, 68.26, 66.08, 62.20, 38.63, 34.55, 24.65, 21.51, 19.22. HRMS MALDI-TOF: ($M+Na^+$) found 679.2346, observed 679.2341.

Dimethylthexylsilyl 2-*O*-(4-acetoxy-2,2-dimethylbunoate) -3-*O*-benzyl -β-D-glucopyranoside (**12**): EtSH (1.7 g, 27.419 mmol) and TsOH (0.55 g, 2.75 mmol) were added to a stirred solution of starting material (3 g, 4.569 mmol). After stirring at ambient temperature for 1h TLC (Hex/EtOAc, 3/1, v/v) indicated the complete consumption of the starting material. The reaction mixture was quenched with Et_3N and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography

using a gradient of hexanes/EtOAc (3/1 v/v) to obtain product as oil (2.0 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 5H, CH Aromatic), 4.99 (t, *J* = 9.5, 1H, H2), 4.81 – 4.74 (m, 2H, CH*H*Bn, H1), 4.64 (d, *J* = 11.6 Hz, 1H, C*H*HBn), 4.14 (t, *J* = 7.4 Hz, 2H, CH_2 PivOAc), 3.88 (dd, *J* = 11.8, 4.1 Hz, 1H, H6a), 3.82 – 3.69 (m, 2H, H6b, H3), 3.57 (t, *J* = 9.2 Hz, 1H, H4), 3.40 (m, *J* = 9.2, Hz, 1H, H3), 2.01 (s, 3H, CH_3 PivOAc), 1.97 – 1.88 (m, 2H, CH_2 PivOAc), 1.69 – 1.55 (m, 1H, $CH(CH_3)_2$), 1.30 – 1.23 (m, 6H, 2xCH_3 PivOAc), 0.92 – 0.82 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.16 (d, *J* = 16.2 Hz, 7H, $Si(CH_3)_2$),. ^{13}C NMR (126 MHz, $CDCl_3$) δ 129.00, 127.99, 126.36, 96.15, 83.11, 75.16, 74.58, 73.80, 73.75, 73.76, 70.50, 62.69, 62.68, 61.31, 50.75, 37.96, 33.54, 26.67, 25.17, 22.92, 21.05, 20.70, 19.52, 18.48. HRMS MALDI-TOF: ($M+Na^+$) found 591.3984, observed 591.3990.

Dimethylthexylsilyl *O*-methyl-2-*O*- (4-acetoxy-2,2-dimethylbunoate) -3-*O*-benzyl-β-D-glucopyranosyluronate (3): A suspension of TEMPO (66 mg, 0.422 mmol) and BAIB (1.69 g, 5.277 mmol) were added to a stirred solution of starting material (1.2 g, 2.110 mmol) in DCM/water (2/1 v/v, 10 mL). After stirring the biphasic solution vigorously for 1hr at ambient temperature TLC (hexanes/EtOAc, 60/40, v/v) indicated the complete consumption of the starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (6 mL) followed by treatment with freshly prepared solution of diazomethane in Et_2O (4 mL). After stirring for five minutes, the excess diazomethane was quenched with acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography

using a gradient of hexane/EtOAc (6/1 v/v) to obtain product as oil (1.10 g, 88%). 1H NMR (500 MHz, $CDCl_3$) δ 7.20 – 7.08 (m, 10H, CH Aromatic), 4.83 (t, J = 9.3, 1H, H3), 4.74 (d, J = 11.5 Hz, 1H, CH*H*Bn), 4.62 (d, J = 7.3 Hz, 1H, H1), 4.54 (d, J = 11.5 Hz, 1H, C*H*HBn), 3.99 – 3.86 (m, 3H, H4, CH_2 PivOAc), 3.70 (d, 4H, H5, CH_3 $COOCH_3$), 3.46 (t, J = 9.0 Hz, 1H, H3), 1.83 (s, 2H, CH_3 PivOAc,), 1.53 – 1.43 (m, 1H, $CH(CH_3)_2$), 1.05 (s, 6H, 2xCH_3 PivOAc), 0.75 – 0.66 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.02 (d, J = 19.5 Hz, 6H, $Si(CH_3)_2$).^{13}C NMR (126 MHz, $CDCl_3$) δ 128.55, 128.53, 127.48, 128.84, 127.77, 74.22, 74.08, 96.44, 74.08, 61.56, 72.12, 74.14, 52.90, 81.75, 21.18, 38.32, 33.99, 25.40, 18.68, 20.71, 20.15, -1.84, -2.70. HRMS MALDI-TOF: ($M+Na^+$) found 619.2037, observed 619.2044.

Dimethylthexylsilyl 2-*O*-(2,5 difluorobenzoyl)-3-*O*-benzyl-4,6-*O*-benzylidene -β-D-glucopyranoside (**6**): To a stirring solution of starting material (723 mg, 1.446 mmol) in pyridine (15 mL), DMAP (144 mg, 0.144 mmol) and DFB-Cl (270 μL, 2.167 mmol) were added under the inert atmosphere of Argon. After stirring for 3h at ambient temperature TLC (hexanes/EtOAc, 5/1, v/v) indicated the consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The mixture was concentrated *in vacuo* and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc 6/1 v/v) to obtain product as oil (840 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 – 8.56 (m, 2H, C*H* Aromatic), 7.66 (tt, *J* = 7.7, 1.9 Hz, 2H, C*H* Aromatic), 7.49 – 7.01 (m, 10H, C*H* Aromatic), 5.54 (s, 1H, CH Benzylidene), 5.21 – 5.14 (m, 1H, H2), 4.83 – 4.76 (m, 2H, H1, C*H*H Bn), 4.62 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.28 (dd, *J* = 10.5, 5.0 Hz, 1H, H4), 3.84 – 3.75 (m, 2H, H6a/b),

3.45 (td, J = 9.5, 4.8 Hz, 1H, H5), 1.46 (h, J = 6.9 Hz, 1H, $CH(CH_3)_2$), 0.75 – 0.66 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.08 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 126.07, 128.01, 128.17, 128.08, 128.11, 129.49, 128.08, 127.30, 101.23, 74.76, 73.64, 96.32, 73.71, 68.75, 68.81, 81.64, 79.09, 66.12, 25.61, 18.48, -0.05,-1.90, -2.93. HRMS MALDI-TOF: ($M+Na^+$) found 640.2641, observed 640.2648.

Dimethylthexylsilyl *O*-methyl-2-*O*- (2,5-difluorobenzoyl) -3-*O*-benzyl-β-D-glucopyranosyluronate (5): Starting material (800 g, 1.248 mmol) was dissolved in DCM:TFA:H_2O (0.07 M) (10/1/0.1, v/v) at 0 ^0C. After stirring for 1hr TLC (Hex/EtOAc, 3/1, v/v) indicated the complete consumption of the starting material. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* and was dried on the membrane pump. A suspension of TEMPO (28 mg, 0.118 mmol) and BAIB (730 mg, 2.264 mmol) were added to a stirred solution of the crude diol in DCM/water (2/1 v/v, 6 mL). After stirring the biphasic solution vigorously for 1hr at ambient temperature TLC (hexanes/EtOAc, 1/1, v/v) indicated the complete consumption of starting material. The reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ (1M, 5 mL) and the resulting mixture was extracted thrice with DCM. The combined organic layers were dried (MgSO4) and evaporated *in vacuo*. This crude residue of glucuronic acid was dissolved in THF (5 mL) followed by treatment with freshly prepared solution of diazomethane in Et_2O (2 mL). After stirring for five minutes, the excess diazomethane was quenched with a few drops of acetic acid. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 v/v) to obtain product as oil (512 mg, 71%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.42 (ddd, J = 8.5, 5.4, 3.3

Hz, 1H, C*H* Aromatic), 7.20 – 6.97 (m, 6H, C*H* Aromatic), 5.10 (dd, *J* = 9.5, 7.6 Hz, 1H, H2), 4.78 – 4.69 (m, 2H, H1,), 4.64 (d, *J* = 11.8 Hz, 1H C*H*H Bn), 3.98 (dd, *J* = 9.8, 8.8 Hz, 1H, H4), 3.78 (d, *J* = 35.6 Hz, 4H, H5, CH_3 COOMe), 3.60 (t, *J* = 9.2 Hz, 1H, H3), 1.50 – 1.38 (m, 1H, C*H*$(CH_3)_2$), 0.73 – 0.59 (m, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.08 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 130.21, 127.19, 129.09, 128.82, 73.93, 74.51, 97.08, 74.99, 72.67, 74.48, 53.38, 82.63, 34.65, 19.12, -1.01), 0.02. HRMS MALDI-TOF: ($M+Na^+$) found 540.2311, observed 540.2304.

Dimethylthexylsilyl -2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7): A suspension of Fmoc-Cl (18.9 g, 73.070 mmol) and DMAP (17 mg, 0.146 mmol) were added to a stirred solution of starting material (7 g, 14.613 mmol) in Pyridine (146 mL) at ambient temperature under the atmosphere of argon. TLC indicated complete consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (6/1 v/v) to obtain product (8.8 g, 87%) as oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 – 6.96 (m, 14H, C*H* Aromatic), 4.72 – 4.37 (m, 3H, H4, CH_2 Bn), 4.37 – 3.89 (m, 7H, H1, CH_2 Fmoc, C*H* Fmoc, H6a/b), 3.42 (ddd, *J* = 9.7, 5.0, 2.1 Hz, 1H, H5), 3.30 – 3.13 (m, 2H, H3, H2), 1.85 (d, *J* = 1.6 Hz, 3H, CH_3 Ac), 1.51 – 1.41 (m, 1H, C*H*$(CH_3)_2$), 0.71 (dd, *J* = 7.2, 1.8 Hz, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.1 (d, 6H, Si$(CH_3)_2$). ^{13}C NMR (75 MHz, $cdcl_3$) δ 120.32, 125.26, 128.17, 127.43, 128.12, 74.95, 75.38, 75.30, 75.30, 75.29, 97.26, 70.30, 70.50, 47.01, 46.94, 62.79, 71.84, 80.17, 68.51,

20.95, 34.17, 20.69, 18.71, 18.15, 17.55, 20.18, 19.54, -3.05, -1.89. HRMS MALDI-TOF: ($M+Na^+$) found 685.3187, observed 685.3183.

Dimethylthexylsilyl -2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-2-deoxy-β-D-glucopyranoside (8): A suspension of Fmoc-Cl (7.3 g, 21.646 mmol) and DMAP (5 mg, 0.004 mmol) were added to a stirred solution of starting material (2.5 g, 4.667 mmol) in Pyridine (45 mL) at ambient temperature under an inert atmosphere of argon. TLC indicated complete consumption of the starting material, after which the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ (10%), H_2O, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (6/1 v/v) to obtain product as an oil (2.9 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.6 Hz, 4H, C*H* Aromatic), 7.53 – 7.35 (m, 5H, C*H* Aromatic), 4.71 – 4.57 (m, 3H, H4, C*H*H Bn), 4.53 – 4.25 (m, 5H, CH*H* Bn, H1, CH_2 Fmoc, C*H* Fmoc), 4.18 (dd, *J* = 10.4, 7.1 Hz, 2H, H6a/b), 3.45 (dd, *J* = 10.0, 4.4 Hz, 1H, H5), 3.34 – 3.15 (m, 2H, H3, H2), 2.63 – 2.29 (m, 7H, 2 x CH_2 Lev), 2.01 (s, 3H, CH_3 Lev), 1.59 – 1.43 (m, 1H, C*H*(CH_3)$_2$), 0.82 – 0.69 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.1 (d, 6H, Si(CH_3)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.02, 121.04, 120.01, 120.12, 125.13, 125.04, 127.91, 61.47, 97.00, 97.01, 62.47, 46.78, 62.72, 46.69, 62.78, 81.30, 68.32, 38.73, 28.00, 29.89, 33.94, 18.41, 19.91, 18.49, 17.55, 19.95, 2.67, 2.70 ,), 0.73 – 0.59. HRMS MALDI-TOF: ($M+Na^+$) found 780.3300, observed 780.3309.

2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-2-deoxy-α/β-D-glucopyranoside (2): To a stirred solution of starting material (2.6 g, 3.430 mmol) in THF (35 mL), HF in pyridine (30%, 6.5 mL) was added at 0 ^{o}C. After stirring at ambient

temperature for 18 h TLC indicated the complete consumption of starting material. The reaction mixture was subsequently diluted with EtOAc, washed with aqueous $NaHCO_3$ (Satd.), H_2O and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Cl_3CCN (330 μL, 3.295 mmol) and NaH (60%) (~1.0 mg, 0.016 mmol) were added to a solution of the crude material (500 mg, 0.659 mmol) in DCM (6 mL). After stirring for 1 h at ambient temperature, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography to give product which was used directly for glycosylation.

2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-acetyl-2-deoxy-α/β-D-glucopyranoside (1): To a stirred solution of starting material (6.5 g, 9.269 mmol) in THF (60 mL), HF in pyridine (30%, 33 mL) was added. After stirring at ambient temperature for 18 h TLC indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with EtOAc, washed with $NaHCO_3$ (Satd.), H_2O and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure and the resulting oil was left *in vacuo* for 2 h. The resulting residue dried on the vacuum pump overnight to obtain crude product as oil.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-β-D-glucupyranosyluronate (14): A suspension of glucuronic acid donor (30 mg, 0.039 mmol), glucoszide acceptor (26 mg, 0.042 mmol) and activated molecular sieves (4Å crushed, 80 mg) in dichloromethane (1.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^oC followed by addition of TMS-OTf (1.5 µl, 0.008 mmol). The TLC analysis showed complete consumption of the

donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-β-D-glucupyranosyluronate (14): A suspension of glucuronic acid donor (130 mg, 0.171 mmol), glucoszide acceptor (111 mg, 0.206 mmol) and activated molecular sieves (4Å crushed, 250 mg) in dichloromethane (2.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (6.0 µl, 0.034 mmol). The TLC analysis showed complete hydrolysis of the donor.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of glucuronic acid donor (158 mg, 0.201 mmol), glucoszide acceptor (80 mg, 0.134 mmol) and activated molecular sieves (4Å crushed, 250 mg) in dichloromethane (1.3 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (7 µL, 0.040 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~15 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (56 mg, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H*

Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 5H, H4′, H1, C*H*HBn, C*H*HBn, C*H*HBn, C*H*HBn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, CH_2 PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, CH_2 Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, CH_2 Lev), 2.00 (s, 2H, CH_3 Lev), 1.84 (s, 3H, CH_3 PivOAc), 1.74 (t, *J* = 7.1, 2.2 Hz, 2H, CH_2 PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48, 68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: ($M+Na^+$) found 1216.4297, observed 1216.4291.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of glucuronic acid donor (158 mg, 0.201 mmol), glucoszide acceptor (80 mg, 0.134 mmol) and activated molecular sieves (4Å crushed, 250 mg) in toluene (1.3 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (7 μL, 0.040 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine

(~15 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (61 mg, 38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H* Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 5H, H4′, H1, C*H*HBn, C*H*HBn, C*H*HBn, C*H*HBn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, CH_2 PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, CH_2 Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, CH_2 Lev), 2.00 (s, 2H, CH_3 Lev), 1.84 (s, 3H, CH_3 PivOAc), 1.74 (t, *J* = 7.1, 2.2 Hz, 2H, CH_2 PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48, 68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: ($M+Na^+$) found 1216.4297, observed 1216.4291.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of

glucuronic acid donor (138 mg, 0.176 mmol), glucoszide acceptor (70 mg, 0.117 mmol) and activated molecular sieves (4Å crushed, 200 mg) in toluene (1.2 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TfOH (3 µL, 0.040 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (38 mg, 27%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H* Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 5H, H4′, H1, C*H*H*Bn,* C*H*H*Bn,* C*H*H*Bn,* C*H*HBn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, CH_2 PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, CH_2 Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, CH_2 Lev), 2.00 (s, 2H, CH_3 Lev), 1.84 (s, 3H, CH_3 PivOAc), 1.74 (t, *J* = 7.1, 2.2 Hz, 2H, CH_2 PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48,

68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: ($M+Na^+$) found 1216.4297, observed 1216.4291.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of glucuronic acid donor (184 mg, 0.234 mmol), glucoszide acceptor (70 mg, 0.117 mmol) and activated molecular sieves (4Å crushed, 250 mg) in toluene (1.2 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^oC followed by addition of TMS-OTf (8.5 μL, 0.046 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~15 μl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (59 mg, 42%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H* Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 5H, H4′, H1, C*H*HBn, CH*H*Bn, C*H*HBn, CH*H*Bn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, CH_2 PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, CH_2 Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, CH_2 Lev), 2.00 (s, 2H, CH_3 Lev), 1.84 (s, 3H, CH_3 PivOAc), 1.74

(t, *J* = 7.1, 2.2 Hz, 2H, C*H*$_2$ PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, CH(CH$_3$)$_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, C(CH$_3$)$_2$ and CH(CH$_3$)$_2$), 0.20 (t, J = 10.6 Hz, 6H, Si(CH$_3$)$_2$). ^{13}C NMR (126 MHz, CDCl$_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48, 68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: (M+Na$^+$) found 1216.4297, observed 1216.4291.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): A suspension of glucuronic acid donor (137 mg, 0.181 mmol), glucoszide acceptor (90 mg, 0.150 mmol) and activated molecular sieves (4Å crushed, 250 mg) in toluene (1.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TBDMS-OTf (8.0 µL, 0.036 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~15 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous NaHCO$_3$ (satd), water, brine, and dried (Mg$_2$SO$_4$), filtered, and the filtrate was concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (27 mg, 15%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H* Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 5H, H4′, H1, C*H*H*Bn,* C*H*H*Bn,* C*H*H*Bn,*

C*H*HBn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, C*H*$_2$ PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, C*H*$_3$ COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, C*H*$_2$ Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, C*H*$_2$ Lev), 2.00 (s, 2H, C*H*$_3$ Lev), 1.84 (s, 3H, C*H*$_3$ PivOAc), 1.74 (t, *J* = 7.1, 2.2 Hz, 2H, C*H*$_2$ PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48, 68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: ($M+Na^+$) found 1216.4297, observed 1216.4291.

General procedure for dehydrative glycosylation

A solution of the glucosazide donor (1.5 equiv to acceptor, 0.02 M in DCM), Ph_2SO (2.8 equiv to donor) and TTBP (3.0 equiv to donor) in DCM was stirred for 45 min over activated 4Å molecular sieves. At -60ºC Tf_2O (1.4 equiv to donor) was added. The temperature was raised to -40ºC and stirred at this temperature for one hour. Then a solution of acceptor (1.0 equiv) in DCM (0.1 M) was added and the reaction mixture was allowed to slowly warm to room temperature. Dry pyridine was added to neutralize the reaction mixture and it was washed with saturated $NaHCO_3$ and water. After drying ($MgSO_4$) and concentration *in vacuo* the residue was purified by silica gel column chromatography.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)6-*O*- levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-levulinoyl-3-*O*-benzyl--β-D-glucupyranosyluronate (14): Glucosazide donor (47 mg, 0.0758 mmol) was condensed with glucuronic acid (34 mg, 0. 0632mmol) following the general dehydrative condensation procedure described above. The crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (6/1 v/v) to obtain product as an oil (37 mg, 52%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.4 Hz, 2H, C*H* Aromatic), 7.23 – 7.03 (m, 10H, C*H* Aromatic), 5.37 (d, *J* = 3.7 Hz, 1H, H1′), 4.90 (dd, *J* = 8.8, 7.1 Hz, 1H, H2), 4.76 – 4.48 (m, 6H, H4′, *CH*H*Bn,CH*H*Bn, CH*H*Bn,CH*H*Bn,*H1′), 4.35 (dd, *J* = 10.4, 6.7 Hz, 1H, C*H*H Fmoc), 4.19 – 3.95 (m, 5H, H4, H6a, H6b, C*H* Fmoc, C*H*H Fmoc), 3.85 (d, *J* = 9.6 Hz, 1H, H5′), 3.77 (dd, *J* = 10.3, 9.1 Hz, 1H, H3′), 3.73 – 3.60 (m, 5H, H3, H5, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.62 – 2.43 (m, 8H, 4 X CH_2 Lev), 2.06 (s, 3H, CH_3 Lev), 1.45 (p, *J* = 6.9 Hz, 1H, $CH(CH_3)_2$), 0.77 – 0.64 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.06 (s, 6H, $Si(CH_3)_2$). NMR (126 MHz, $CDCl_3$) δ 120.39, 125.01, 129.52, 125.08, 131.30, 127.73, 128.27, 97.59, 75.11, 74.60, 74.25, 95.98, 75.74, 74.29, 75.25, 70.54, 70.55, 75.42, 61.90, 46.92, 61.91, 74.43, 77.54, 82.67, 52.97, 68.56, 62.96, 38.01, 38.07, 28.07, 28.15, 29.96, 34.11, 29.85, 18.63, 20.01, -1.80, -3.27. HRMS MALDI-TOF: ($M+Na^+$) found 1135.4715, observed 1135.4709.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (15): Glucosazide donor (103 mg, 0.1667 mmol) was condensed with glucuronic acid (149 mg, 0.250 mmol)

following the general dehydrative condensation procedure described above. Column chromatography provided the α-linked product as an oil (134 mg, 67%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.6, 3.1 Hz, 2H, C*H* Aromatic), 7.44 (dd, *J* = 20.1, 7.5 Hz, 2H, C*H* Aromatic), 7.25 (d, *J* = 14.9 Hz, 2H, C*H* Aromatic), 7.22 – 7.04 (m, 12H, C*H* Aromatic), 5.33 (d, *J* = 3.7 Hz, 1H, H1′), 4.93 (dd, *J* = 8.0, 6.4 Hz, 1H, H2), 4.76 – 4.54 (m, 6H, H4′, H1, C*H**HBn,* C*H**HBn,* C*H*H*Bn,* C*H*HBn), 4.50 (d, *J* = 11.0 Hz, 1H, H6a), 4.35 (dd, *J* = 10.5, 6.7 Hz, 1H, H4), 4.23 (t, *J* = 9.0 Hz, 1H, H6b), 4.19 – 3.91 (m, 7H, CH_2 Fmoc, CH Fmoc, CH_2 PivOAc), 3.88 (d, *J* = 9.5 Hz, 1H, H5), 3.76 (t, *J* = 9.8 Hz, 1H, H3′), 3.67 (d, *J* = 26.9 Hz, 5H, H3, H5′, CH_3 COOMe), 3.21 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 2.58 (q, *J* = 6.8 Hz, 2H, CH_2 Lev), 2.47 (q, *J* = 6.7, 6.2 Hz, 2H, CH_2 Lev), 2.00 (s, 2H, CH_3 Lev), 1.84 (s, 3H, CH_3 PivOAc), 1.74 (t, *J* = 7.1, 2.2 Hz, 2H, CH_2 PivOAc), 1.48 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 1.07 (d, *J* = 2.4 Hz, 6H,), 0.78 – 0.61 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.20 (t, J = 10.6 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.45, 119.96, 125.48, 125.25, 128.30, 128.76, 128.21, 128.67, 128.67, 128.67, 97.61, 74.91, 74.62, 95.96, 73.68, 75.38, 73.35, 75.94, 75.61, 70.56, 75.19, 75.63, 70.68, 62.00, 47.01, 62.31, 61.53, 74.37, 77.72, 82.48, 68.68, 52.94, 63.10, 38.12, 28.70, 30.68, 20.42, 39.51, 25.45, 19.53. HRMS MALDI-TOF: ($M+Na^+$) found 1216.4297, observed 1216.4291.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(2,5 difluorobenzoyl)-3-*O*-benzyl-β-D-glucupyranosyluronate (16): Glucosazide donor (154 mg, 0.1667 mmol) was condensed with glucuronic acid (97 mg, 0.250 mmol) following the general dehydrative condensation procedure described above. The crude residue was purified by

silica gel column chromatography using a gradient of hex:ethyl acetate (5/1 v/v) to obtain product as an oil (87 mg, 44%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 7.6, 3.6 Hz, 2H, C*H* Aromatic), 7.55 – 7.40 (m, 4H, C*H* Aromatic), 7.31 (t, *J* = 7.5 Hz, 3H, C*H* Aromatic), 7.26 – 6.96 (m, 16H, C*H* Aromatic), 5.44 (d, *J* = 3.7 Hz, 1H, H1′), 5.25 – 5.18 (t, 1H, H2), 4.85 – 4.72 (m, 3H, H4′, *CH*H*Bn,*H1′), 4.70 – 4.54 (m, 3H, C*H*HBn *CHHBn, CHHBn,*), 4.47 – 4.37 (m, 1H, H5), 4.28 – 4.03 (m, 5H, H4, H6a, H6b, CH_2 Fmoc), 3.96 (d, *J* = 9.5 Hz, 1H, C*H* Fmoc), 3.93 – 3.67 (m, 7H, H3, H3′, CH_3 COOMe), 3.30 (dd, *J* = 10.4, 3.7 Hz, 1H, H2′), 2.72 – 2.48 (m, 5H, 2 X CH_2 Lev), 2.06 (s, 3H, CH_3 Lev), 1.44 (p, *J* = 6.8 Hz, 1H, CH, $CH(CH_3)_2$), 0.72 – 0.62 (m, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.06 (s, 7H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.34, 125.33, 118.66, 125.16, 128.22, 127.46, 121.69, 128.12, 127.97, 127.91, 118.67, 118.56, 97.71, 76.28, 74.89, 96.56, 74.67, 75.32, 74.25, 75.32, 70.99, 75.81, 70.54, 62.36, 47.41, 62.37, 74.16, 82.68, 77.63, 53.07, 68.68, 63.07, 38.16, 28.15, 30.68, 35.16, 30.68, 31.15, 18.57, -1.71, -3.34. HRMS MALDI-TOF: ($M+Na^+$) found 1141.4612, observed 1141.4606.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*- acetyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (17): Glucosazide donor (100 mg, 0.1676 mmol) was condensed with glucuronic acid (206 mg, 0.335 mmol) following the general dehydrative condensation procedure described above. The crude residue was purified by silica gel column chromatography using a gradient of tol:ethyl acetate (5/1 v/v) to obtain product as an oil (161 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 7.6, 3.3 Hz, 2H, C*H* Aromatic), 7.28 – 7.09 (m, 11H C*H* Aromatic), 5.42 (d, *J* = 3.8 Hz, 1H, H1′), 4.99 (dd, *J* = 7.9, 6.3 Hz, 1H, H2), 4.85 – 4.60 (m, 5H, H4′,

*CH*HBn, H1′, C*H*HBn C*H*HBn), 4.57 (d, *J* = 10.9 Hz, 1H, C*H*HBn), 4.39 (dd, *J* = 10.5, 6.8 Hz, 1H, H6a), 4.31 (dd, *J* = 9.5, 8.5 Hz, 1H, H4), 4.27 – 4.15 (m, 2H, H6b, C*H*H Fmoc), 4.11 (t, *J* = 7.1 Hz, 1H, C*H* Fmoc), 4.07 – 3.98 (m, 3H, C*H*H Fmoc, CH_2 PivOAc), 3.95 (d, *J* = 9.5 Hz, 1H, H5), 3.84 (dd, *J* = 10.3, 9.1 Hz, 1H, H3b), 3.79 – 3.67 (m, 5H, H3, H5′, CH_3 COOMe), 3.28 (dd, *J* = 10.3, 3.7 Hz, 1H, H2′), 1.99 (s, 3H, CH_3 Ac), 1.91 (s, 3H, CH_3 PivOAc), 1.81 (td, *J* = 7.1, 2.4 Hz, 2H, CH_2 PivOAc), 1.66 – 1.54 (m, 1H, CH$(CH_3)_2$), 1.14 (d, *J* = 2.4 Hz, 5H, 2 X CH_3 PivOAc), 0.85 – 0.71 (m, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.08 (d, *J* = 8.8 Hz, 6H, Si$(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.27, 125.02, 129.55, 129.56, 128.66, 128.62, 127.67, 97.52, 74.94, 74.52, 95.85, 73.29, 75.82, 73.28, 75.30, 70.52, 74.96, 70.52, 62.15, 46.91, 61.52, 74.61, 77.65, 82.47, 68.54, 52.90, 63.01, 21.79, 21.15, 39.09, 34.02, 25.86, 18.06, 20.16, -2.23, -2.39. HRMS MALDI-TOF: ($M+Na^+$) found 1137.4871, observed 1137.4866.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (20): Et_3N (0.2 mL) was added to a solution of the starting material (110 mg, 0.092 mmol) in DCM (2.0 mL). After reaction mixture was stirred for 2 h TLC indicated disappearance of starting material. The reaction mixture was concentrated *in vacuo*, and the crude residue was purified by silica gel column chromatography using a gradient of hex:ethyl acetate (2/1 v/v) to obtain product as an oil (78 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.23 (m, 12H, C*H* Aromatic), 5.43 (d, *J* = 4.2 Hz, 1H, H1′), 5.06 (t, *J* = 7.7, 5.6 Hz, 1H, H2), 4.93 – 4.78 (m, 4H, CH*H* Bn, C*H*H Bn, H1, C*H*H Bn), 4.71 (dd, *J* = 11.0, 2.8 Hz, 1H, CH*H* Bn,), 4.62 (d, *J* = 12.4 Hz, 1H, H6a), 4.35 (td, *J* = 8.9, 3.0 Hz, 1H, H4), 4.09 (dd, *J* = 11.4, 4.0 Hz, 3H, H6b, CH_2

PivOAc), 4.03 – 3.97 (m, 1H, H5), 3.83 (td, *J* = 9.0, 8.1, 2.8 Hz, 1H, H3), 3.79 – 3.68 (m, 4H, C*H*$_3$ COOMe, H3′), 3.56 – 3.44 (m, 2H, H4′, H5′), 3.23 (dd, *J* = 9.6, 4.4 Hz, 1H, H2′), 2.76 (q, *J* = 5.3, 4.4 Hz, 2H, C*H*$_2$ Lev), 2.60 (dt, *J* = 7.5, 4.4 Hz, 2H, C*H*$_2$ Lev), 2.16 (d, *J* = 3.1 Hz, 3H, C*H*$_3$ Lev), 1.99 – 1.95 (m, 3H, C*H*$_3$ PivOAc), 1.91 – 1.83 (m, 2H, C*H*$_2$ PivOAc), 1.66 – 1.54 (m, 1H, $CH(CH_3)_2$), 1.20 (d, *J* = 3.5 Hz, 6H, 2 x C*H*$_3$ PivOAc), 0.87 – 0.79 (m, 13H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.14 (dd, *J* = 10.6, 3.4 Hz, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 129.65, 128.76, 129.44, 129.83, 127.41, 98.39, 74.84, 75.99, 96.72, 73.72, 73.34, 73.34, 73.99, 63.53, 74.52, 63.27, 62.23, 74.21, 83.10, 53.41, 79.50, 71.87, 70.89, 63.73, 39.15, 29.02, 38.72, 31.00, 20.67, 39.13, 35.10, 24.82, 20.17, -1.86. HRMS MALDI-TOF: ($M+Na^+$) found 994.3983, observed 994.3990.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxy carbonyl) -6-*O*-levulinoyl-α-D-glucopyranoside-(1→4) -(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate-(1→4)-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (21): A suspension of glucuronic acid donor (49 mg, 0.040 mmol), glucoszide acceptor (33 mg, 0.033 mmol) and activated molecular sieves (4Å crushed, 80 mg) in dichloromethane (0.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 ^{o}C followed by addition of TMS-OTf (1.5 µl, 0.008 mmol). The TLC analysis showed complete hydrolysis of the donor.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxy carbonyl) -6-*O*-levulinoyl-α-D-glucopyranoside-(1→4) -(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate-(1→4)-2-deoxy-2-azido-3-

***O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (21):** A suspension of glucuronic acid donor (51 mg, 0.043 mmol), glucoszide acceptor (35 mg, 0.036 mmol) and activated molecular sieves (4Å crushed, 80 mg) in dichloromethane (0.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (1.5 µl, 0.008 mmol). The TLC analysis showed complete hydrolysis of the donor.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxy carbonyl) -6-*O*-levulinoyl-α-D-glucopyranoside-(1→4) -(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate-(1→4)-2-deoxy-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (21): A suspension of glucuronic acid donor (70 mg, 0.058 mmol), glucoszide acceptor (47 mg, 0.048 mmol) and activated molecular sieves (4Å crushed, 120 mg) in dichloromethane (0.6 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (2 µl, 0.011 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using a gradient of tol:ethyl acetate (5/1 v/v) to obtain product as an oil (36 mg, 37%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 – 7.73 (m, 2H, *CH* Aromatic), 7.55 (ddd, *J* = 28.2, 7.5, 1.1 Hz, 3H, *CH* Aromatic), 7.43 – 7.13 (m, 27H, *CH* Aromatic), 5.38

(d, *J* = 3.8 Hz, 1H, H1^{d}), 5.31 (d, *J* = 3.7 Hz, 1H, H1^{b}), 5.15 (dd, *J* = 8.9, 7.5 Hz, 1H, H2^{c}), 5.09 (d, *J* = 10.7 Hz, 1H, CH*H*Bn), 5.06 – 5.02 (m, 1H, H2^{a}), 4.93 (d, *J* = 10.9 Hz, 1HC*H*HBn), 4.88 – 4.76 (m, 3H, H4^{b}, H1^{a}, CH*H*Bn), 4.72 (d, *J* = 7.5 Hz, 1H, H1^{c}), 4.67 (m, *J* = 6.7, 3.5 Hz, 3H, CH*H*Bn, C*H*HBn, C*H*HBn,), 4.60 (dd, *J* = 18.5, 10.9 Hz, 2H, CH*H*Bn, C*H*HBn), 4.50 (dd, *J* = 10.6, 6.5 Hz, 1H, H6^{d}a), 4.40 – 4.24 (m, 5H, H6^{b}a, 6^{d}b, H4^{a}, H4^{d}, H4^{c},), 4.22 – 3.96 (m, 11H, H6^{b}b, CH*H* Fmoc, C*H*H Fmoc, C*H* Fmoc, 2 X CH_2 PivOAc, H5^{d}, H5^{a}, H5^{c}), 3.91 (dd, *J* = 10.2, 8.8 Hz, 1H, H3^{a}), 3.88 – 3.79 (m, 3H, H3^{b}, H5^{b}), 3.77 – 3.67 (m, 5H, CH_3 COOMe, H3^{c} H3^{d}), 3.56 (s, 3H, CH_3 COOMe), 3.39 (dd, *J* = 10.3, 3.7 Hz, 1H, H2^{b}), 3.27 (dd, *J* = 10.3, 3.8 Hz, 1H, H2^{d}), 2.93 (ddd, *J* = 18.3, 10.5, 4.1 Hz, 2H, CH_2 Lev), 2.74 – 2.59 (m, 4H, CH_2 Lev, CH_2 Lev), 2.59 – 2.53 (m, 2H, CH_2 Lev), 2.26 (3H, CH_3 Lev), 2.22 (s, 3H, CH_3 Lev),1.98 (s, 3H, CH_3 PivOAc), 1.97 (s, 3H, CH_3 PivOAc), 1.92 – 1.82 (m, 4H, 2 X CH_2 PivOAc), 1.61 (h, *J* = 6.9 Hz, 13H, $CH(CH_3)_2$, 4 x CH_3 PivOAc), 0.80 (s, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.13 (d, *J* = 8.6 Hz, 5H, $Si(CH_3)_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120.09, 124.86, 124.78, 128.06, 128.03, 128.26, 128.35, 127.18, 127.07, 127.98, 97.38, 97.31, 72.66, 75.32, 74.49, 73.39, 74.05, 95.61, 72.88, 100.02, 73.19, 74.96, 75.32, 75.12, 70.03, 61.99, 69.99, 76.77, 74.20, 74.31, 75.03, 61.98, 46.64, 61.34, 61.19, 61.22, 81.18, 73.94, 76.09, 77.40, 82.12, 68.28, 77.49, 52.58, 68.96, 52.58, 62.88, 62.70, 37.85, 27.72, 37.85, 37.81, 27.71, 27.77, 27.71, 29.71, 29.75, 20.91, 20.87, 37.97, 37.93, 33.69, 29.63, 25.11, 25.56, 18.52, 19.78. HRMS MALDI-TOF: (M+Na^{+}) found 2027.6011, observed 2027.6017.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxy carbonyl) -6-*O*-levulinoyl-α-D-glucopyranoside-(1→4) -(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate-(1→4)-2-deoxy-2-azido-3-

***O*-benzyl-6-*O*-levulinoyl-α-D-glucopyranoside-(1→4)-(methyl-2-*O*-(4-acetoxy-2,2-dimethylbunoate)-3-*O*-benzyl-β-D-glucupyranosyluronate (21):** A suspension of glucuronic acid donor (66 mg, 0.054 mmol), glucoszide acceptor (35 mg, 0.036 mmol) and activated molecular sieves (4Å crushed, 100 mg) in dichloromethane (0.5 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -30 ^{o}C followed by addition of TMS-OTf (2 µl, 0.01 mmol). The TLC analysis showed complete consumption of the donor. The reaction mixture was neutralized with pyridine (~5 µl) and filtered through a pad of Celite. The filtrate was washed with aqueous $NaHCO_3$ (satd), water, brine, and dried (Mg_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using a gradient of tol:ethyl acetate (5/1 v/v) to obtain product as an oil (32 mg, 44%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 – 7.73 (m, 2H, *CH* Aromatic), 7.55 (ddd, *J* = 28.2, 7.5, 1.1 Hz, 3H, *CH* Aromatic), 7.43 – 7.13 (m, 27H, *CH* Aromatic), 5.38 (d, *J* = 3.8 Hz, 1H, H1^d), 5.31 (d, *J* = 3.7 Hz, 1H, H1^b), 5.15 (dd, *J* = 8.9, 7.5 Hz, 1H, H2^c), 5.09 (d, *J* = 10.7 Hz, 1H, CH*H*Bn), 5.06 – 5.02 (m, 1H, H2^a), 4.93 (d, *J* = 10.9 Hz, 1HC*H*HBn), 4.88 – 4.76 (m, 3H, H4^b, H1^a, CH*H*Bn), 4.72 (d, *J* = 7.5 Hz, 1H, H1^c), 4.67 (m, *J* = 6.7, 3.5 Hz, 3H, CH*H*Bn, C*H*HBn, C*H*HBn,), 4.60 (dd, *J* = 18.5, 10.9 Hz, 2H, CH*H*Bn, C*H*HBn), 4.50 (dd, *J* = 10.6, 6.5 Hz, 1H, H6^{d}a), 4.40 – 4.24 (m, 5H, H6^{b}a, 6^{d}b, H4^a, H4^d, H4^c,), 4.22 – 3.96 (m, 11H, H6^{b}b, CH*H* Fmoc, C*H*H Fmoc, C*H* Fmoc, 2 X CH_2 PivOAc, H5^d, H5^a, H5^c), 3.91 (dd, *J* = 10.2, 8.8 Hz, 1H, H3^a), 3.88 – 3.79 (m, 3H, H3^b, H5^b), 3.77 – 3.67 (m, 5H, C*H*$_3$ COOMe, H3^c H3^d), 3.56 (s, 3H, C*H*$_3$ COOMe), 3.39 (dd, *J* = 10.3, 3.7 Hz, 1H, H2^b), 3.27 (dd, *J* = 10.3, 3.8 Hz, 1H, H2^d), 2.93 (ddd, *J* = 18.3, 10.5, 4.1 Hz, 2H, C*H*$_2$ Lev), 2.74 – 2.59 (m, 4H, C*H*$_2$ Lev, C*H*$_2$ Lev), 2.59 – 2.53 (m, 2H, C*H*$_2$

Lev), 2.26 (3H, C*H*$_3$ Lev), 2.22 (s, 3H, C*H*$_3$ Lev),1.98 (s, 3H, C*H*$_3$ PivOAc), 1.97 (s, 3H, C*H*$_3$ PivOAc), 1.92 – 1.82 (m, 4H, 2 X C*H*$_2$ PivOAc), 1.61 (h, *J* = 6.9 Hz, 13H, CH(CH$_3$)$_2$, 4 x C*H*$_3$ PivOAc), 0.80 (s, 12H, C(CH$_3$)$_2$ and CH(CH$_3$)$_2$), 0.13 (d, *J* = 8.6 Hz, 5H, Si(CH$_3$)$_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120.09, 124.86, 124.78, 128.06, 128.03, 128.26, 128.35, 127.18, 127.07, 127.98, 97.38, 97.31, 72.66, 75.32, 74.49, 73.39, 74.05, 95.61, 72.88, 100.02, 73.19, 74.96, 75.32, 75.12, 70.03, 61.99, 69.99, 76.77, 74.20, 74.31, 75.03, 61.98, 46.64, 61.34, 61.19, 61.22, 81.18, 73.94, 76.09, 77.40, 82.12, 68.28, 77.49, 52.58, 68.96, 52.58, 62.88, 62.70, 37.85, 27.72, 37.85, 37.81, 27.71, 27.77, 27.71, 29.71, 29.75, 20.91, 20.87, 37.97, 37.93, 33.69, 29.63, 25.11, 25.56, 18.52, 19.78. HRMS MALDI-TOF: (M+Na$^+$) found 2027.6011, observed 2027.6017.

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CHAPTER 5

CONCLUSION

We have described new techniques for the stereoselective synthesis of HS and DS oligosaccharides that employ a fairly small number of orthogonally protected building blocks, which can easily be converted into glycosyl donors and acceptors which were utilized to construct respective GAG fragments. We performed a methodological study to test the coupling efficiency of GlcA donors with $GlcN_3$ acceptors to synthesize a library of HS disaccharide compounds. In this study we used a pre-glycosylation oxidation approach. Pivaloyl acetate was utilized for establishing stereoselective β-1,2-trans glycosidic linkages without compromising coupling efficiency. In the modular synthesis of DS, our strategic design allowed us to incorporate sulfates at all the required positions with absolute control and precision. We utilized orthogonal protecting groups for the construction of iduronic acid and galactosamine building blocks which allowed us to rapidly disassemble the derivatives to their final deprotected forms. We also attempted to construct large HS oligosaccharides fragments using a modular strategy by performing a rigourous study to achieve efficient stereoselective α-1,2 cis glycosidic coupling between glucosamine donor and GlcA acceptor using a non-participating azido functionality at the C2 position of the glucosamine donor.

Experiments were designed to understand the effect of protecting groups on glycosylation outcomes. The competitive experiments towards the utility of PivOAc as a C2 participating functionality on GlcA donors in comparison to Ac and Lev proved effective

and valuable. The formation of both ortho-esters and threhalose-ortho-esters were observed in presence of the classic Ac group at the C2 position of GlcA donors. Levulinoate esters at C2 position of GlcA proceeded without the occurrence ortho-ester leading to product formation with decent yields. This is helpful as Lev group at C2 presents the opportunity to introduce sulfation in GlcA if required. PivOAc proved to be the best of the lot showcasing best yields without any trace of formation of ortho-esters. PivOAc was helpful in inducing excellent stereoselectivity in products and it was also suitable for the required late stage deprotection and functionalization. In our strategy we included the popular Fmoc group at the C4 position for their convenience of orthogonality. The aim was to design a compound with access to chain extension at the non-reducing end. The presence of Fmoc esters on the C4 position hampered the yields of the glycosylations. The electron withdrawing carbamate functionality had a disarming effect on the already deactivated GlcA donor leading to coupling inefficiency. At this point we modified our building block design by replacing the Fmoc ester with a NAP ether which directly resulted with improvement in yields without loss of strategic orthogonality.

For the synthesis of dermatan sulfate disaccharides, an efficient and highly modular strategy has been developed. A set of orthogonally protected iduronyl donors and galactoamine acceptors were coupled to obtain a common disaccharide derivative in excellent yields. Further this common disaccharide was subjected to our rapid deprotections approach to obtain two differentially sulfated i.e., 2S4S and 2S6S disaccharide compounds with *N*-acetates. The design of our disaccharide motif is such that with slight modification several possible sulfated derivatives may be obtained.

Sulfation can be achieved selectively at required separate locations or globally at all allowed locations. This modular strategy gives us access to an array of dermatan sulfate disaccharide compounds in a rapid manner. We have capped our reducing end (C1) and non-reducing end (C4) with orthogonal protecting groups thus giving us the opportunity to further extend our strategy to construct larger fragments of dermatan sulfate oligosaccharides. The synthetic disaccharide derivatives of DS will be used as mass spectrometric standards using the GRIL-LC/MS method to facilitate qualitative and quantitative analysis of unknown DS fragments isolated from Sanfilippo patients.

We have attempted to design a novel strategy for the modular synthesis of larger HS oligosaccharides that employs orthogonally protected monosaccharide and disaccharide building blocks that can easily be converted into glycosyl donors and acceptors. Further these modular donors and acceptors can be utilized for the preparation of library of well-defined HS oligosaccharides. The access to such a collection of compounds can be useful towards performing structure-activity relationship studies for HS binding proteins. The key features of our new strategy includes the use of PivOAc ester for installation of 1,2 trans glycosydic linkages. For the construction of the disaccharide modules pre-oxidized form of glucuronic acids were used. Also in comparison to our old approach, we flipped the sequence of our disaccharides so that the glucuronic moiety was placed at the reducing end of the synthetic fragment. The 2 + 2 glycosylations were performed in a more stereoselective manner giving rise to only one isomer, due to the anchimeric assistance of PivOAc ester functionality. Like our previous strategy, the current approach also employed:

- the use of Lev esters for those hydroxyls that needed sulfation,

- a Fmoc carbonate as a temporary protecting group for the C4 hydroxyl for the preparation of glycosyl acceptors,
- an anomeric TDS group for glycosyl donor synthesis, and
- benzyl ethers as permanent protecting groups.

The dehydrative and trifluoroacetimidate glycosylation methodologies were employed for the construction of the oligosaccharide assembly.

Furthermore, a few synthetic modifications may be introduced to our proposed synthetic strategy by replacing the C4 Fmoc group on the glucosamine derivative with NAP ether. NAP ether enjoys orthogonality similar to the traditional Fmoc group and being an electron donating functionality it could assist in improving yields of the coupling reactions. Also, the C3 benzyl ethers on the glucosamine derivatives could be replaced with a Lev group in order to incorporate a sulfate group wherever desired during the late stage manipulations. The synthetic compounds will be equipped with an artificial aminopentyl spacer at the reducing end. This would be potentially useful for immobilizing the HS constructs on a microarray plate or a BIACORE® chip. Studies are under way in our laboratory for the construction of such a HS based glyco-chip which could serve to be a unique tool for performing rapid profiling of HS binding proteins. Thus, we have reported new chemical strategies for the synthetic construction of GAG motifs which will be helpful in addressing the problems associated with the poor reactivity profile of uronic acids and will provide a rapid chemical access to a library of HS and DS compounds. Using the discussed methodologies we set a premise for generating newer strategies for the construction of complex GAG oligosaccharides.