TOWARDS HIGHLY CONVERGENT SYNTHESIS OF PLANT-DERIVED

OLIGOSACCHARIDES

by

ROSHAN BALIGA

(Under the Direction of GEERT-JAN BOONS)

ABSTRACT

Plant cell walls are made up of complex polysaccharides that perform a number of essential functions including control of cell growth, regulation of cell and tissue differentiation as well as providing mechanical support to individual cells. Xyloglucan, which is a hemicellulosic polysaccharide, forms extensive cross links with cellulose microfibrils and the xyloglucan-cellulose network is considered to be the major load bearing structure in plants. Structural modification and reorganization of this network is considered essential for the growth and expansion of plant cells. There is a need for the synthesis of homogenous xyloglucan structures that can be used in the generation of well defined molecular probes. These probes can be used to study the abundances, structures and localization of xyloglucans in the plant wall and help in correlating the biological significance of xyloglucan structures in different plant species.

In chapters II and III of this dissertation we describe our efforts towards the synthesis of tetrasaccharide and hexasaccharide xyloglucan side-chain which contain galacturonic acid. These unique structures are present in the root hairs of *Arabidopsis thaliana* and have found to play an important role in the growth of the root hair. The chemical synthesis of these xyloglucan side-chains will help in the characterization of monoclonal antibodies which can be used in the identification as well as visualization of these structures in different plant species. The key step in the synthesis is the use of xylosyl 1,2-oxathiane donors for stereoselective formation of 1,2-*cis* glycosidic linkage. Sufficient deactivation of the xylosyl donors by electron-withdrawing protecting groups proved to be critical in obtaining good α-stereoselectivity. A late-stage post-glycosylation oxidation strategy was used to install the galacturonic acid in the molecule.

To streamline the process of oligosaccharide synthesis, several groups are pursuing one-pot glycosylation strategies based on chemoselective, orthogonal or pre-activation protocols. But, the synthesis of branched oligosaccharides, using these methods cannot be readily accomplished. In chapter IV , we report the synthesis of novel protecting group that can be deprotected *in situ* in combination with triflic acid promoted glycosylations of trichloroacetimidate donors, paving the way for the synthesis of branched oligosaccharides in an one-pot procedure.

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ABBREVIATIONS

Å ....Angstrom

Ac ....Acetyl

AcOH .... Acetic acid

Ar .... Aromatic

BAIB ....[bis(acetoxy)iodo]benzene

BF$_3$.Et$_2$O ....Borontrifluoride Diethyletherate

Bn .... Benzyl

Bz .... Benzoyl

COSY ....Correlation Spectroscopy

DBU 1,8-Diazabicycloundec-7-ene

DCE ....Dichloroethane

DCM ....Dichloromethane

DDQ 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

dFBz 2,5-Difluorobenzoyl

DHB ....2,5-Dihydroxybenzoic acid

DIC .... *N,N'*-Diisopropylcarbodiimide

DMAP ....4- *N,N'*-Dimethylaminopyridine

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

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

Et$_3$SiH	....	Triethylsilane
EtOAc	....	Ethylacetate
EtOH	....	Ethylalcohol
Gal	....	Galactose
GalN	....	Galactose amine
Glc	....	Glucose
h	....	hour
Hz	....	Hertz
HSQC	....	Heteronuclear Single Quantum Coherence Spectroscopy
IAD	....	Intramolecular Aglycon Delivery
Lev	....	Levulinyl
Le^x	....	Lewis^x
Le^y	....	Lewis^y
mAb	....	Monoclonal antibody
MALDI-TOF	....	Matrix Assisted Laser Desorption Ionization Time of Flight
*m*CPBA	....	*meta*-Chloroperbenzoic acid
MS	....	Molecular Sieves
mmol	....	Millimole
m/z	....	Mass to charge ratio
NaH	....	Sodium hydride
Nap	....	2-Methylnaphthyl
NIS	....	*N*-Iodosuccinimide
NMR	....	Nuclear Magnetic Resonance

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 The Plant Cell Wall

The plant cell wall is a structurally complex, extensively cross linked and metabolically dynamic compartment that contains an array of complex polysaccharides and proteins that encases the plant cell.[1-2] The plant cell wall performs a number of essential functions, including control of cell growth, regulation of cell and tissue differentiation and protection against invading pathogens.[3-4] Moreover, it is essential in providing mechanical support and rigidity to the individual cells and the plant as a whole while allowing for growth and expansion of the cell. The changes in morphology that occur during plant growth are a result of controlled cell division along with structural modification and reorganization of cell wall polysaccharides.[5-6] The plant cell wall is primarily made up of three layers: the middle lamella, the primary cell wall and the secondary cell wall. The middle lamella, which is rich in pectic polysaccharides, is formed soon after mitosis and is a boundary between the two daughter nuclei. After the formation of the middle lamella, a new layer is deposited and continues to be deposited throughout the growth and expansion of the cell, and is called the primary wall. The secondary cell wall is deposited internally to the primary cell wall, once the cell has ceased to grow. However, not all cells have a secondary cell wall. The secondary cell walls are thickened structures containing lignin and usually surround specialized cells such as vessel elements or fiber cells. Polysaccharides make up most of the plant cell wall, the specific type, structure and abundance varying in different plant species, tissue types, developmental stage and the location within a single plant cell.[7] Broadly, cell wall polysaccharides can be grouped into cellulose, hemicelluloses and pectins. While cellulose and hemicelluloses impart rigidity to the wall, pectin provides fluidity through the gelatinous polysaccharide matrix. Plants have played a huge role in human evolution

and cell wall polysaccharides have a lot of economic and commercial significance. Primary walls are the major textural component of many plant derived foods and fermented fruit products contain quantitatively significant amounts of primary wall polysaccharides. Plant cell wall polysaccharides also make up a large portion of industrial and agricultural products, including cotton, linen, paper, lumber, textiles, and are also used as gums, gels and stabilizers.[8] More importantly, cell wall components represent a major renewable source of fixed carbon and will play an important role as a source of biomass for the emerging biofuel industry. Apart from the economic significance, plant cell walls are beneficial to human health due to their ability to bind heavy metals, regulate serum cholesterol levels and stimulate the immune system.[9-11]
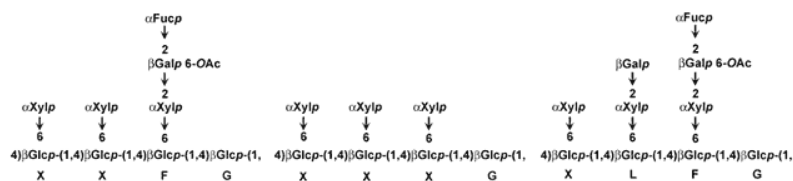
1.2 Plant Primary Cell Wall

The major components of the primary wall of plant cells are polysaccharides (up to 90% of dry weight) together with structural glycoproteins (2-10%), phenolic esters(<2%), ionically and covalently bound minerals (1-5%) and enzymes. Cellulose is the main polysaccharide component present in the primary cell wall accounting for 15-30% of the dry mass of the primary wall. Cellulose is a linear unbranched polymer of 1,4-β-D-glucose residues, which aggregate along their length via hydrogen bonds to form microfibrils.[12] The cellulose microfibrils are highly crystalline and are embedded in a hydrated amorphous matrix of non-cellulosic polysaccharides and glycoproteins that form a network around the cell. Apart from cellulose, the other major polysaccharides present in the primary cell wall are hemicelluloses and pectins. Hemicellulose includes xyloglucans, xylans, glucomannans, and mixed-linkage glucans.[13] Chemically hemicelluloses are homologous to cellulose in that they have a backbone of 1,4-β-D-pyranosyl residues such as glucose, mannose, and xylose, in which the O-4 is in the equatorial position, but unlike cellulose they are usually branched. This structural similarity results in a strong, non-covalent association of hemicellulose with cellulose microfibrils. Along with the cellulose/hemicellulose network there exists another network comprising of the pectic polysaccharides. Pectins consists of 1,4-linked α-D-galacturonic acid and the three major pectic polysaccharides isolated from primary cell wall of plants are homogalacturonan, substituted galacturonan and rhamnogalacturonans.[14] The

cellulose/ hemicellulose network is thought to be responsible for imparting structural rigidity and strength of the cell wall.[1] Enzyme-catalyzed modification of the hemicellulosic component of this network is considered a key process required for wall expansion during cell growth.[15] Therefore, a better understanding of the structure, macromolecular organization and metabolism of this network is essential in understanding the mechanism of cell elongation and the biological regulation of this process.

1.3 Xyloglucan: Structure and Function

Xyloglucan is the major hemicellulosic polysaccharide present in the primary wall of dicotyledonous and non-graminaceous monocotyledonous plants.[16] Structurally, xyloglucans are made up of a backbone of 1,4-β-D-glucosylpyranoside residues, with up to 75% of the Glc*p* substituted at the O-6 position with α-D-xylosyl residue. Furthermore, the Xyl*p* residue can be glycosylated forming oligomeric side chains that contain galactosyl, galactouronosyl, fucosyl, and/or arabinosyl residues.[17] Though the cellulosic backbone of the xyloglucan does not vary, the structure and molecular distribution of the xyloglucan side chains can vary among different plant tissues and species. Therefore, in order to completely and unambiguously assign the structure of a xyloglucan molecule Fry et al. developed a concise nomenclature wherein a xyloglucan molecule is named by partitioning the backbone into segments consisting of a single glucosyl residue and its pendant side chain, and each segment is given a specific code letter depending on the side chain configuration.[18] For example, uppercase **G** and **X** refers to an unbranched β-D-Glc*p* residue and α-D-Xyl*p*-(1,6)-β-D-Glc*p* segment, respectively. The xylosyl residues substituted at O-2 with β-D-Gal*p* is designated **L** or a Glc*p* residue bearing the trisaccharide α-L-Fuc*p*-(1,2)-β-D-Gal*p*-(1,2)-α-D-Xyl*p* at O-6 is designated by **F** (Figure **1.1**).

Figure 1.1: A diagrammatical representation of xyloglucan structural units using the unambiguous nomenclature of Fry et al.[18]
 (Image: http://www.ccrc.uga.edu/~mao/xyloglc/dicxylo.gif)

The primary cell wall can be viewed as a three dimensional lattice made up of cellulose microfibrils, that are coated with xyloglucan on their surface and cross-linked by xyloglucan chains via hydrogen bonds (Figure **1.2**).[19] The cellulose-xyloglucan network is thought to be the major load bearing structure which provides structural rigidity and strength to the wall and hence, xyloglucan metabolism and turnover is thought to play an important role in wall expansion during cell growth.[20-21] For plant cells to expand, cellulose microfibrils need to move apart, and this movement may create possibility of new xyloglucan molecules getting incorporated into the cell wall.[22-23]
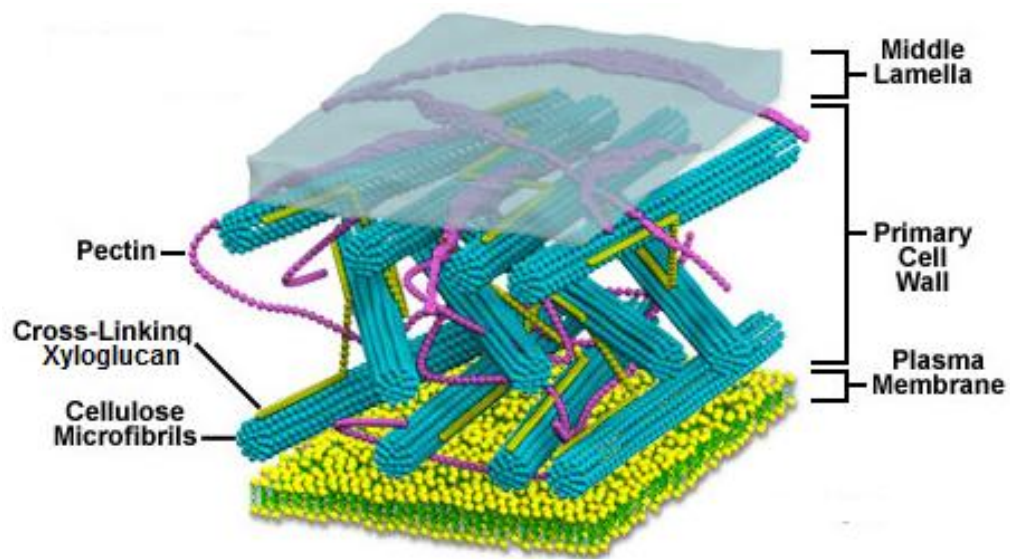
Figure 1.2: Model of the primary cell wall
(Image: http://micro.magnet.fsu.edu/cells/plants/images/cellwallfigure1.jpg)

Enzyme-catalyzed hydrolysis of xyloglucan tethers and simultaneous incorporation of new xyloglucan polymers may allow reversible cell wall loosening in elongating plant cells without compromising the strength of the wall matrix.[23-24] Xyloglucans present in most plant species can be classified as either 'XXXG- type' or 'XXGG-type' based on the number of backbone glucosyl residues that are branched (Figure **1.3**).[25] Xyloglucan structure is found to vary in different plant species, tissues, cell types and even in different parts of the wall surrounding an individual cell.[26] The branching pattern of xyloglucan is of both functional and taxonomic significance. For example, fucosylated xyloglucans with XXXG-type structure, in which four subunits (XXXG, XXFG, XLFG,

and XXLG) constitute the majority of the polymer, are present in the primary wall of gymnosperms, dicotyledonous plants and all monocotyledonous plants with the exception of *Poaceae*, which contain little or no fucose and are less branched than dicotyledon xyloglucans. Also the structure of xyloglucan has been shown to differ in a tissue specific manner. Seed xyloglucans are not fucosylated, while the xyloglucan in other tissues of the same plant usually contain fucose, suggesting that fucose containing side chains may affect the kinetics of the binding of xyloglucan to cellulose microfibrils and is important in the context of the growing cell wall.[27]
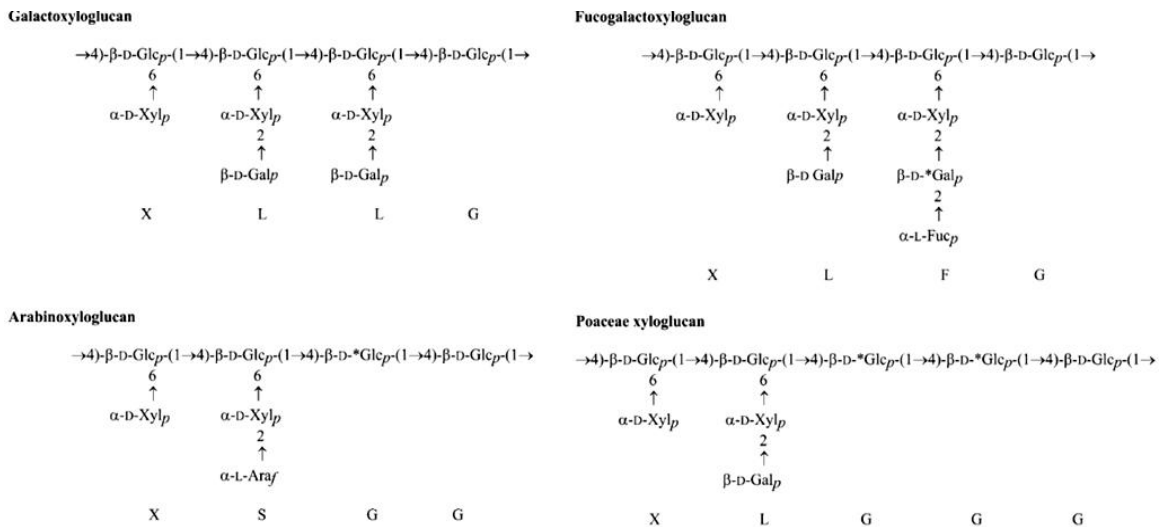
Figure 1.3[28]**:** Structure of xyloglucan oligosaccharide units found in different plant xyloglucans

However, it has been observed that mutant plant lines that are deficient in glycosyl transferase (AtFUT1), that catalyzes transfer of fucose to xyloglucan, grow normally under greenhouse conditions.[29] Furthermore, several members of the *Poaceae* and *Solanaceae*, produce atypical xyloglucans that contain α-L-Ara*f* and/or β-D-Gal*p* residues but lack α-L-Fuc*p* residues.[30] Another major difference among xyloglucan structures is the presence of charged side chains which is found in mosses and liverworts while vascular plants have neutral side chains.[31] It is therefore essential for us to understand and gain more insight regarding the fundamental relationship between the biological functions of xyloglucan and their structures in different plant species. In order to fully understand the role of xyloglucans in plant growth and development, it is not only

important to elucidate the structure of xyloglucans present in the extracellular matrix but also to determine how these components are organized within the matrix, and to monitor changes in structure and composition over time and in response to different stimuli. One important tool that will help in such studies is the use of carbohydrate-specific antibodies as molecular probes to visualize and study stage-specific changes in the distribution of plant cell-wall polysaccharides.

## 1.4 Monoclonal Antibodies: Tools for Cell Wall Characterization

Monoclonal antibodies are specific reagents that have been used to determine primary cell wall composition and organization at the cellular level.[32-33] To date, around 200 monoclonal antibodies have been generated that bind to plant cell wall carbohydrates. For example, monoclonal antibody CCRC-M1 binds to a terminal α-1,2-linked fucosylated xyloglucan.[34] Monoclonal antibodies can either be generated from hybridoma cell culture or by using phage display.[35-36] Though both conventional hybridoma and phage display antibody production exploit the vast diversity of mammalian antibody repertoire, the fundamental difference is that with hybridoma technology antibody production is achieved by immortalized antibody-producing B-cells, while with phage display it is the genes that encode the antibody variable region that is immortalized. Though monoclonal antibodies are specific and sensitive probes, a major problem with generating antibodies to cell wall carbohydrates is that monosaccharides such as arabinose, galactose or fucose are common to many cell wall polysaccharides and immunization with one polymer may result in antibodies that bind to a variety of polysaccharides having similar structural features. In order to generate well characterized antibodies, well defined oligosaccharides having four to seven monosaccharide units need to be used. But, these components occur in complex macromolecular assemblies and their isolation requires chemical or enzymatic treatments that can disrupt covalent bonds making it very difficult to isolate homogenous oligosaccharides in amounts suitable for coupling to immunogenic protein, in which case a polysaccharide is used directly to prepare an immunogen. However, the use of complex polysaccharides makes it difficult to precisely define the carbohydrate epitope in terms of component monosaccharides and linkages, thereby making it difficult

to characterize the antibody in terms of epitope specificity. Determining the binding specificities of the monoclonal antibodies is essential to enhance the value of these tools for functional genomics and immunolocalization studies of plant cell walls. Recent advances in the chemical synthesis of oligosaccharides have made it possible to synthesize well-defined, homogenous glycans that are generally in the order of three to seven sugar units. With the availability of homogenous glycosyl hydrolases, selective fragmentation of plant polysaccharides in combination with competitive ELISA can be used to determine which structural features of the polysaccharide will most likely be recognized by a given antibody. This information can be used to plan the chemical synthesis of plant oligosaccharides having different structural variations for the portion of the polysaccharide that is recognized by the antibody, allowing for the generation of monoclonal antibodies with well defined binding specificities. Moreover, advances in combinatorial approaches for the synthesis of oligosaccharide have resulted in the generation of oligosaccharide libraries that can be displayed on solid-surfaces creating oligosaccharide micro-arrays. These glyco-arrays have a lot of potential for rapid screening of antibodies and determining their binding specificity and epitope characterization. The next section of this dissertation will focus on the challenges in the synthesis of complex oligosaccharides and highlight the methods developed by different research groups to streamline the process of carbohydrate synthesis and also methodologies that have been developed for the stereoselective synthesis of the anomeric bond.

1.5 Oligosaccharide Synthesis

Carbohydrates are the most abundant and diverse biopolymers in nature. Complex carbohydrates, as components of glycoproteins, glycolipids and other conjugates, play a key role in a wide range of biological processes including cell-cell recognition, fertilization, embryogenesis, neuronal development, the proliferation of cells and their organization into specific tissues and tumor cell metastasis.[37-38] Oligosaccharides have also been found to control the development and defense mechanisms in plants.[39] But, a major obstacle to advances in glycobiology is the lack of pure and structurally well-defined carbohydrates and glycoconjugates. These compounds are found in low

concentrations and in micro-heterogeneous forms in nature, thereby complicating their isolation and characterization. The solution to this problem is to obtain well-defined oligosaccharides either by chemical or enzymatic synthesis.[40-41] Unfortunately, unlike the synthesis of biopolymers like peptides or oligonucleotides, there is no general method available for the assembly of complex carbohydrates of biological importance making the synthesis of each target compound challenging and time consuming.[42] The challenges associated with the chemical synthesis of carbohydrates are due to their structural complexity and diversity. Unlike peptides or oligonucleotides, monomeric carbohydrate units can be connected to each other in different ways resulting in highly branched molecules. So, the steps involved in the synthesis of a complex oligosaccharide would first involve synthesis of suitably protected monosaccharide building blocks. Secondly, a series of stereoselective glycosylations are needed to yield a single protected oligosaccharide. Finally, the global deprotection of the oligosaccharide will yield the final biologically active molecule (Scheme **1.1**).
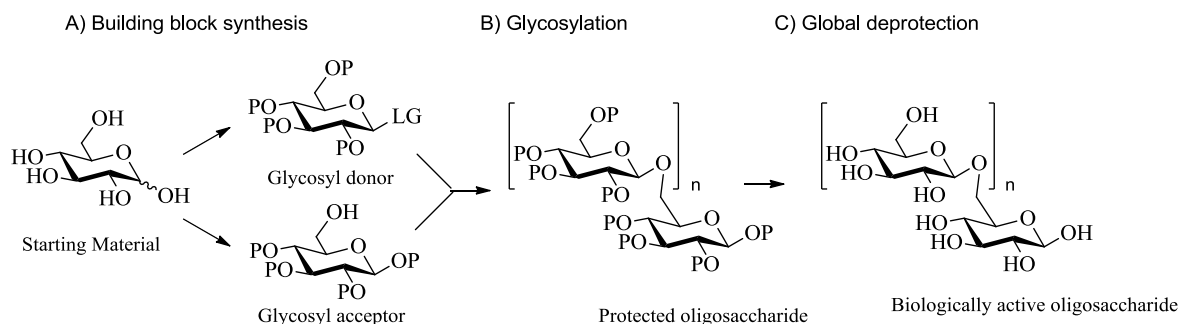
Scheme 1.1: Overview of oligosaccharide synthesis A) Synthesis of monosaccharide building blocks by sequential introduction of protecting groups B) Stereoselective glycosylation to afford a single oligosaccharide C) Global deprotection to afford the target oligosaccharide.

The past few decades have seen a huge amount of progress in the development of oligosaccharide synthetic strategies making it possible to synthesize complex carbohydrates with up to 20 monosaccharide units. Efforts have been directed to identify monosaccharide building blocks that can be repeatedly used for the synthesis of a wide range of target structures.[43-45] Combinatorial and highly regioselective methods are used to develop monosaccharide building blocks with orthogonally removable protecting groups which can then be linked together utilizing a minimum number of steps with

excellent stereoselectivites. To further streamline and speed-up the synthesis of monosaccharide building blocks, protocols for the one-pot multi-step introduction of different protecting groups have also been developed.[46-48] Also, one-pot multistep glycosylations as well as Solid Phase Oligosaccharide Synthesis (SPOS) have been developed which have reduced the number of intermediate purification steps and expedited oligosaccharide assembly.[49-53] Apart from the efforts required to synthesize the monosaccharide building blocks and assemble the target molecule, another major challenge in carbohydrate chemistry is the stereoselective synthesis of the glycosidic bond. A glycosidic linkage is formed by reacting a glycosyl donor, which has a leaving group at the anomeric centre, with a hydroxyl group of a glycosyl acceptor. The formation of the glycosidic bond can result in the formation of two diastereomers which need to be separated to get the oligosaccharide with the desired stereochemistry at the anomeric centre. Often, this is quite painstaking and results in poor yields of the target molecule, as well as limits the use of one-pot multistep glycosylations[54-55] and automated polymer supported synthesis.[56] To address this issue various strategies have been developed to achieve greater control over the stereochemical outcome of glycosylation reactions. 1, 2-*trans*-glycosides can be synthesized by employing groups that can perform neighboring group participation at the C-2 position of the glycosyl donor. Alternatively, 1, 2-*cis* glycosides, which are more challenging to synthesize, can be introduced by using C-2 chiral auxiliaries as well as achiral directing groups. Apart from this, the temperature of the reaction, type of solvent used, anomeric leaving group employed or promoter used in the glycosylations also play an important role in determining the stereochemical outcome of the glycosylation reaction. The next section of this introductory chapter will focus on the various strategies used to streamline oligosaccharide synthesis and control anomeric selectivity.

1.6 Enzymatic and Chemoenzymatic Synthesis

The chemoenzymatic synthesis of carbohydrates has emerged as an efficient route for the synthesis of complex oligosaccharides. The attractiveness of this method is that it combines both the flexibility of chemical synthesis with the ability of the enzymes to catalyze reactions in a highly regio- and stereoselective manner. Enzymatic

transformation occurs under mild conditions and due to their high selectivity, extensive protection-deprotection steps are not necessary and the control of anomeric selectivity is high. In 2007, Chen and co-workers demonstrated a general and efficient method for the synthesis of disaccharides containing sialic acid at the reducing end by using a series of disaccharides, containing galactose (Gal), glucose (Glc) or mannose (Man), α or β linked to the C-2, C-4, C-5 or C-6 hydroxyl group of another mannose residue, as substrates for sialic acid aldolase.[57] The corresponding disaccharides were obtained in yields of 62-85 %. The same lab has also demonstrated the synthesis of polysaccharides with internal sialic acid residues by using a α2,6-sialyltransferase (Pd2,6ST) obtained from *Photobacterium damsel*.[58] Recently, Boons and co-workers reported a systematic and efficient chemo-enzymatic synthesis of asymmetrically branched *N*-glycans starting from a core pentasaccharide. The potential branching points on the pentasaccharide are modified by orthogonal protecting groups to allow for selective attachment of specific saccharide moieties by chemical glycosylation. The resulting decasaccharide is then selectively deprotected and extended using glycosyl transferases to give a library of asymmetrical multi-antennary *N*-glycans.[59]

1.7 Polymer-Supported Oligosaccharide Synthesis

Solid-phase chemistry has greatly improved the synthesis of biopolymers like oligonucleotides and peptides. The major advantage of these automated synthesizers are that the excess reagents that are used to drive the reactions to completion and other side products, can be easily removed by washing the solid resin and the whole process involves a single purification step at the end of the synthesis rather than multiple purifications after each step. The ease and efficiency of these automated solid phase synthesizers have prompted the application of similar technology to oligosaccharide synthesis. In 2001, Seeberger and co-workers introduced the first automated solid phase synthesizer for the synthesis of oligosaccharides, where they showed the synthesis of a series of α (1→2) mannosides **5-7**.[60] A mannose trichloroacetimidate donor **2** was first attached to a solid support **1** *via* an octenediol linker, using TMSOTf-promoted glycosylation. Subsequent glycosylation reactions were carried out by removal of the C-2 acetyl protecting group and addition of tricholoracetimidate donor **2**. After the desired

oligosaccharide has been synthesized it was cleaved from the solid support by olefin cross metathesis using Grubb's catalyst (Scheme **1.2**). The utility of solid-phase oligosaccharide synthesis has also been demonstrated in the synthesis of tumor-associated carbohydrate antigens Gb-3 and Globo-H.[61]
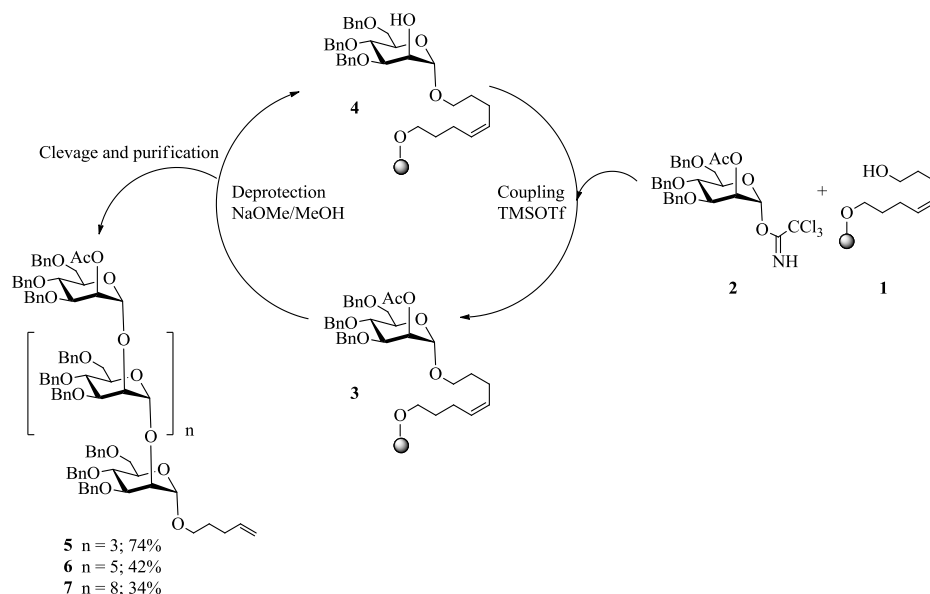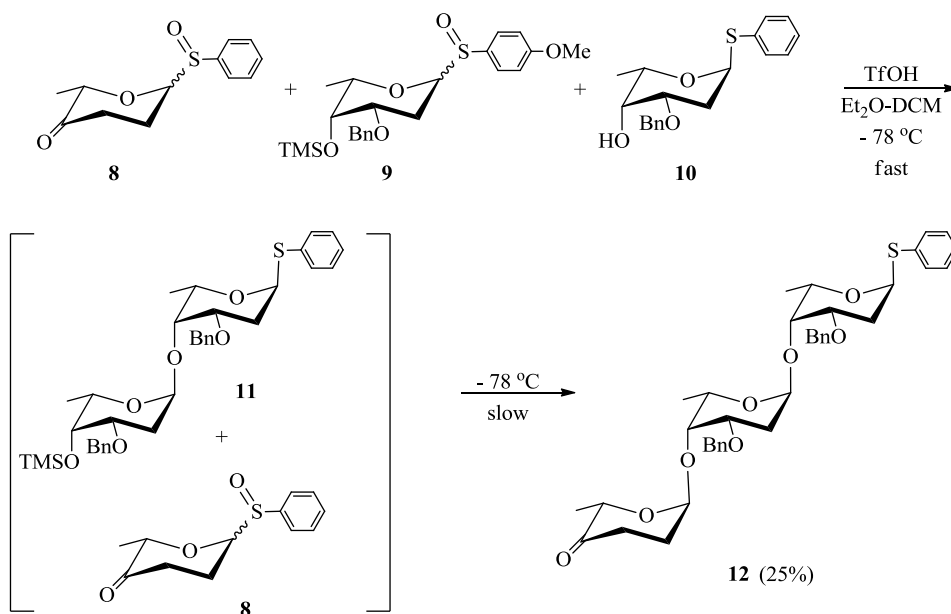

Scheme 1.2: Automated oligosaccharide synthesis with trichloroacetimidates.

Although solid-phase synthesis is an attractive method to rapidly assemble oligosaccharides there are some limitations to this method. Firstly, a large excess of the glycosyl donor is required to drive the reaction to completion. Secondly, all the glycosylation steps need to be high yielding and must go to completion in order to prevent the formation of undesired side products. Also, there is no general protocol for the installation of 1,2-*cis* linkages by solid-phase synthesis. Finally, there is also a need to develop stable linkers that can be readily installed and easily cleaved once the synthesis is complete.

1.8 One-Pot Synthesis of Oligosaccharides

One-pot glycosylations have emerged as another facile and efficient method to rapidly assemble complex oligosaccharides. One-pot glycosylation allows for multiple glycosylation reactions to take place successively in the same reaction flask without the need for protecting group manipulations or isolation and purification of the intermediates.

Kahne and co-workers in 1993 demonstrated the first one-pot synthesis of Ciclamycin trisaccharide **12**.[62] In the synthesis, they utilized the difference in reactivity between the two sulfoxide donors **8** and **9**. Donor **9** which contains a *p*-methoxy substituent can be activated preferentially over donor **8** using catalytic amount of TfOH as the promoter. Donor **9** is activated and reacts with acceptor **10** to form the first glycosidic bond. No self-condensation products are observed as the free hydroxy group of **10** is more nucleophilic than the silyl-protected hydroxy of **9**. After the formation of disaccharide **11**, the silyl ether then reacts with the less reactive donor **8** over a period of 45 min. to give the final trisaccharide **12** in an overall yield of 25% (Scheme **1.3**). The step wise synthesis of ciclamycin trisaccharide reported by the Danishefsky group was achieved in 14 steps with an overall yield of 9%.[63] The one-pot methodology thus provides an efficient route for the synthesis of the ciclamycin trisaccharide.

Scheme 1.3: First reported one-pot glycosylation by Kahne and co-workers

A lot of chemical strategies have been developed for the one-pot multi-step synthesis of oligosaccharides. The most common approaches rely on exploiting the reactivity differences of glycosyl donors and acceptors. Another technique uses pre-activation of the glycosyl donor followed by addition of the acceptor. The Ley group utilized the reactivity difference between two thioethyl donors in the synthesis of trisaccharide **17**, derived from the common polysaccharide antigen of a group **B** *Streptococci* in a one-pot

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synthesis.[64] Thioglycoside **14**, with a 1,2- cyclohexane diacetal protecting group introduces a torsional disarming effect by hampering the formation of a planar oxo-carbenium ion. So, it was possible to activate the benzylated thioglycoside donor **13** chemoselectively and couple it with **14** to give disaccharide **15**. The disaccharide **15** was then activated and coupled with added acceptor **16** to give the final trisaccharide **17** in 62 % overall yield (Scheme **1.4**). Another strategy in one-pot synthesis is the use of orthogonal anomeric leaving groups which can then be activated selectively. The advantage of this technique is that it allows the condensation of building blocks irrespective of the relative reactivity of the different leaving groups and furthermore the leaving groups employed can act as anomeric protecting groups until they are subjected to the right activating conditions.
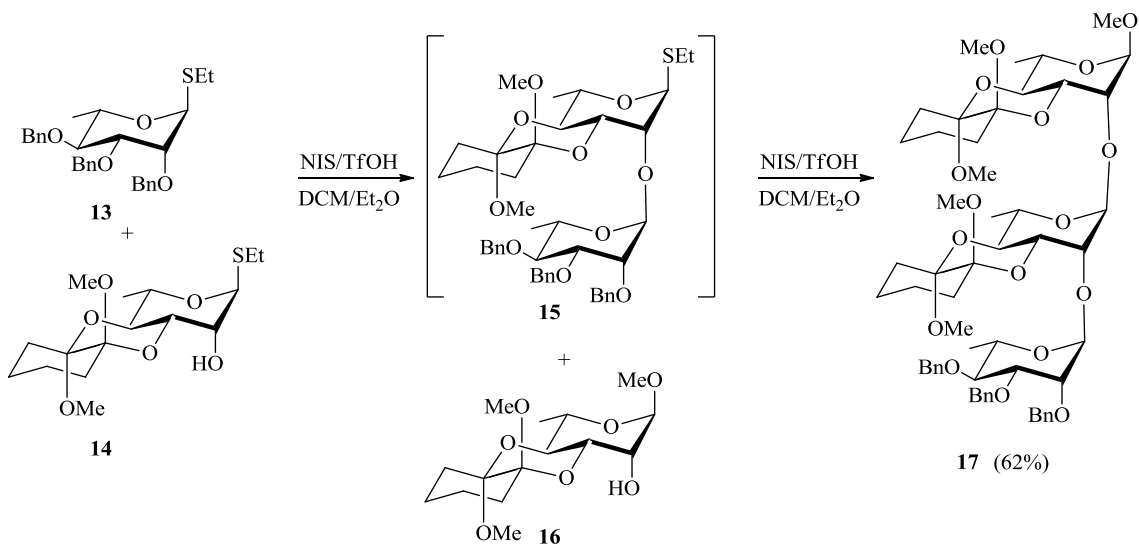
Scheme 1.4: Ley's one-pot synthesis of trisaccharide **17**.

Using a similar strategy, Seeberger and co-workers *s*ynthesized pentasaccharide **21** which showed antibiotic activity against *Helicobacter pylori* by using a one-pot two step glycosylation protocol.[65] Disaccharide trichloroacetimidate donor **18** was coupled with thioglycoside donor **19** which has two potential hydroxyl acceptor groups on C-3 and C-4. But the presence of the C-2 *N*-phthalimido group lowers the reactivity of the C-3 hydroxyl to yield the 1-4 linked trisaccharide. In the next step the disaccharide acceptor **20** was added followed by NIS/TfOH activation of the in situ formed trisaccharide to get the fully protected pentasaccharide **21** in 63% yield (Scheme **1.5**). As mentioned earlier,

oligosaccharides with 1,2-*trans* linkages can easily be installed by using a C-2 participating functionality but in cases where 1,2-*cis* linkages are required the application of one-pot glycosylation is not efficient. In many cases, the optimal conditions required to install these linkages are not compatible with standard one-pot glycosylation conditions.
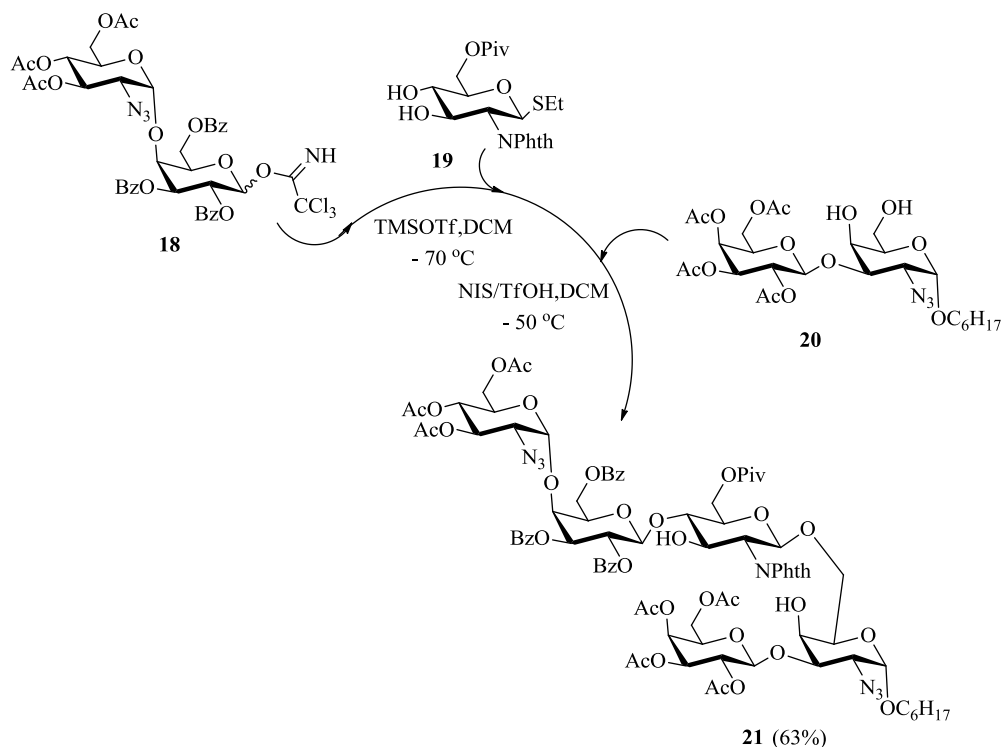
Scheme 1.5: One-pot procedure for the synthesis of pentasaccharide **21**.

Boons and co-workers provided a solution to this problem.[66-67] They reported the synthesis of trisaccharide **27** in a one-pot two step procedure wherein they utilize a (1*S*)-phenyl-2-(phenylsulfanyl)ethyl chiral auxiliary at the C-2 position of the donor **22** to introduce a 1,2-*cis* linkage. Upon activation of the trichloroacetimidate donor **22** and formation of the oxo-carbenium ion **23**, the sulfur on the chiral auxiliary participates to form a stable trans-decalin sulfonium ion intermediate **24** which blocks the β-face thereby allowing acceptor to attack from the α-side leading to the formation 1,2-*cis* linkage. After the formation of the disaccharide the thiophenyl group on the reducing end is activated by the addition of NIS and acceptor **26** is added to form the trisaccharide **27** in 52% yield (Scheme **1.6**). This novel strategy allows for the synthesis of oligosaccharides containing

both 1,2-*cis* and 1,2-*trans* linkages with excellent stereoselectivities in a one-pot two-step glycosylation sequence.
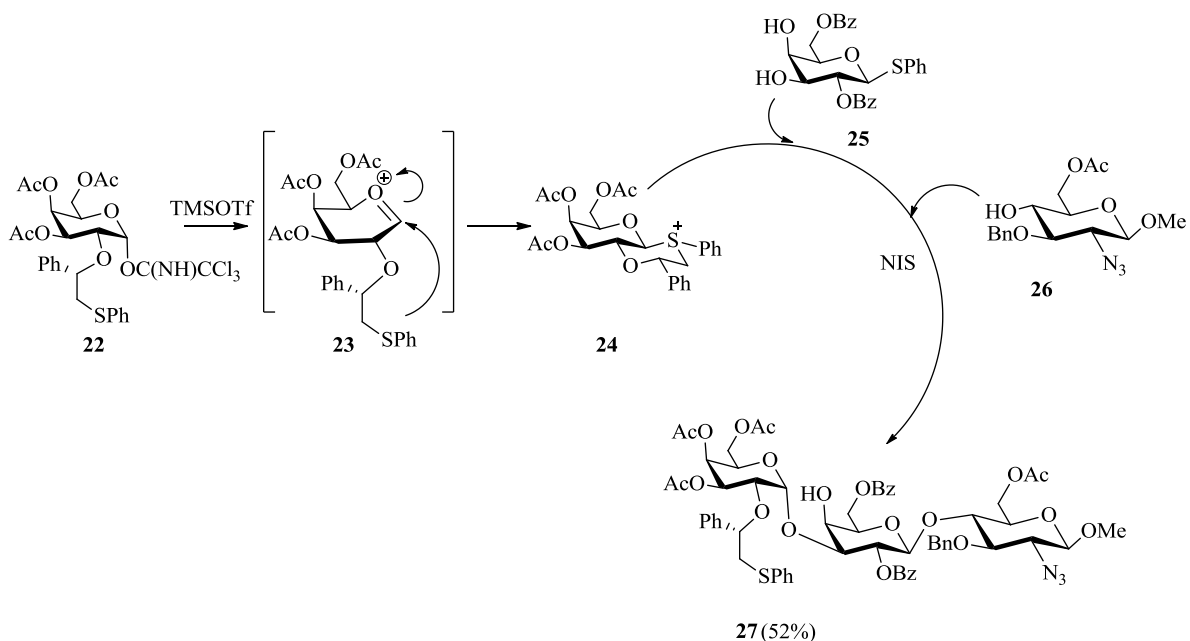
Scheme 1.6: One-pot synthesis of trisaccharide **27** with 1,2-*cis* linkage using C-2 chiral auxilliary.

Another technique used in the one-pot synthesis of oligosaccharides is the pre-activation strategy wherein glycosylations can be carried out independent of the glycosyl donor used. The pre-activation strategy offers greater flexibility in the design of the building blocks allowing for the use of only one type of glycosyl donor which greatly simplifies the synthetic design. It also lets us perform glycosylations, without the need to react the donors in the decreasing order of their anomeric reactivities, thereby offering greater flexibility in choosing protecting groups to match donors with acceptors, leading to improved overall yields. Huang and co-workers reported the synthesis of Globo-H hexasaccharide **32** using pre-activation based iterative one-pot glycosylation strategy.[68] Using the p-tolyl thioglycoside as the donor they were able to sequentially couple four glycosyl building blocks **28**, **29**, **30**, **31** leading to the desired hexasaccharide **32** in 47% overall yield (Scheme **1.7**). The use of one type of glycosyl donor greatly simplified the synthetic design and using the pre-activation strategy they were able to demonstrate that

oligosaccharides containing both α and β linkages within the same molecule can be assembled rapidly in one pot with excellent anomeric selectivity.
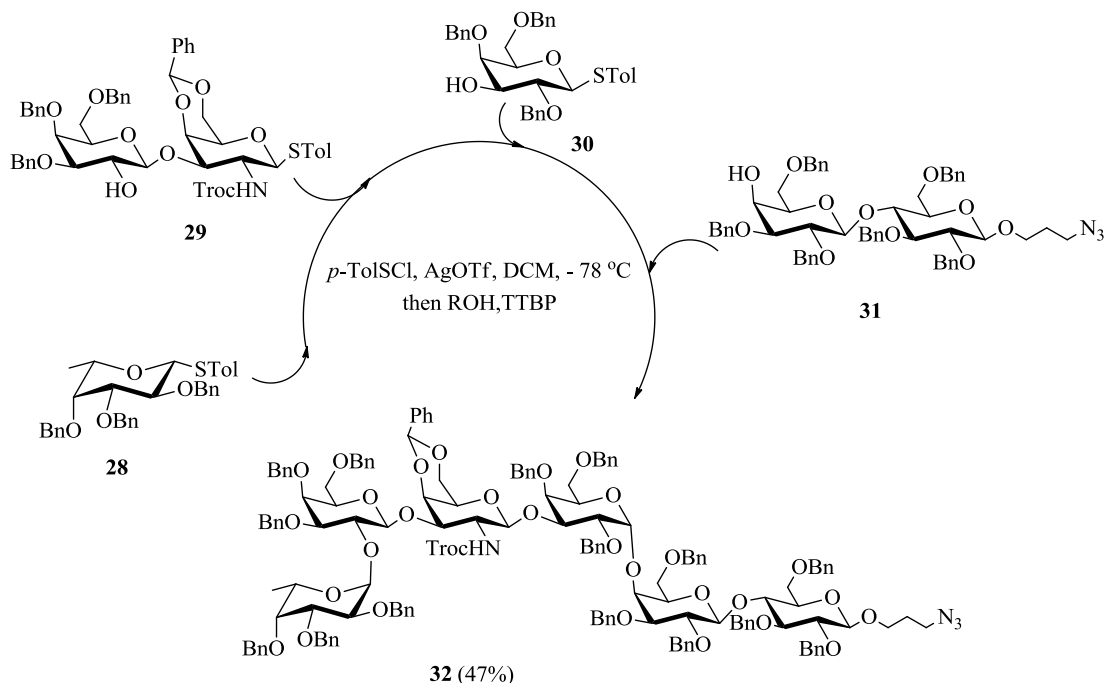

Scheme 1.7: Pre-activation based iterative one-pot synthesis of Globo-H hexasaccharide **32**.

Using a similar strategy, Wong and co-workers developed an efficient one-pot methodology for the synthesis of heparin and heparin sulfate oligosaccharides using thioglycosides as building blocks.[69] The fully protected pentasaccharide **36** was synthesized in one-pot by first coupling azidoglucosyl donor **33** with disaccharide acceptor **34** using NIS/TfOH as the promoter followed by addition of the α-methyl disaccharide acceptor **35** to the reaction mixture (Scheme **1.8**). The utility of the pre-activation based approach was also demonstrated in the synthesis of chitotetroses[70] and hyaluronic acid[71] oligosaccharides in acceptable yields.

Overall, considerable progress has been made in developing methodology to streamline the synthesis of complex oligosaccharides. One-pot glycosylation has contributed immensely to this effect. However, there are still limitations that need to be overcome so that these protocols can have wider applicability. In the case of synthesis of branched oligosaccharides, glycosylation is performed using acceptors having two hydroxyl groups of different reactivities. But, there is always the possibility of the less reactive alcohol

reacting with the donor thereby generating undesired byproducts, lowering the yield and thus making the synthesis inefficient. We have tried to address this issue in chapter IV of this thesis by introducing a novel protecting group that can be used in the one-pot synthesis of branched oligosaccharides.
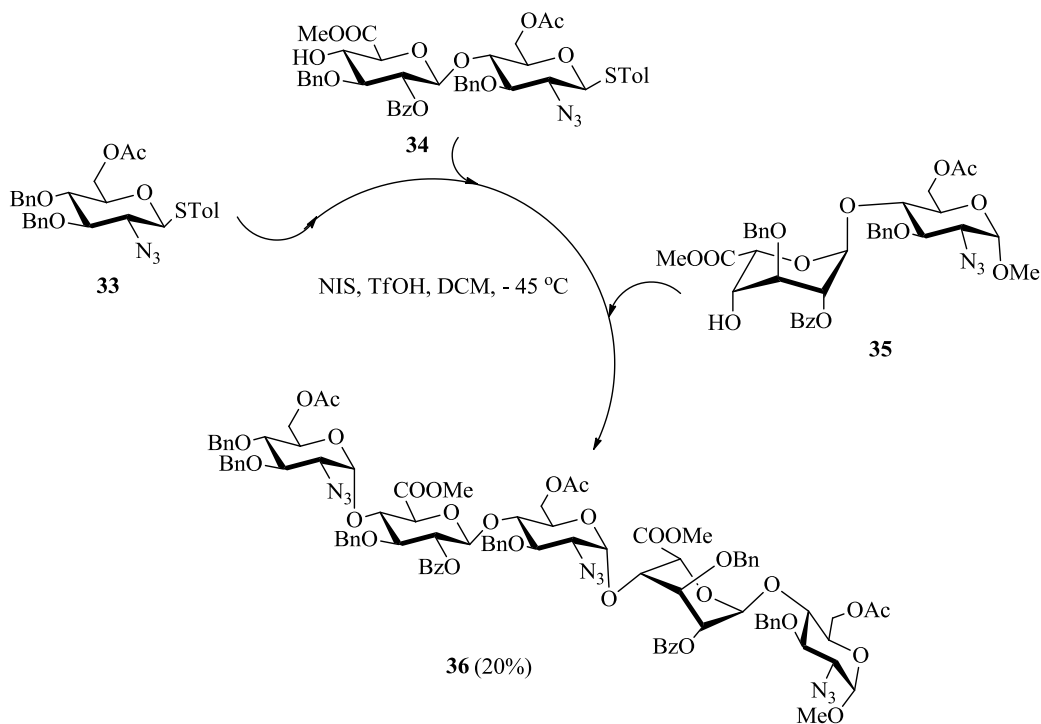

Scheme 1.8: One-pot synthesis of pentasaccharide derivative **36**.

1.9 Control of Anomeric Selectivity in the Formation of Glycosidic Bond

As discussed earlier, one of the biggest challenges in carbohydrate chemistry is the stereoselective formation of the glycosidic bond. The formation of the glycosidic bond involves coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric centre with a suitably protected acceptor that often contains one free hydroxyl group (Scheme **1.9**). More often than not, these reactions lead to the formation of a mixture of two diastereomers that differ in the configuration at the anomeric centre. Separation of these diastereomers requires tedious, time-consuming purification steps resulting in loss of material. It also limits the use of one-pot multistep glycosylations and automated polymer-supported synthesis of oligosaccharides. To address this problem,

different research groups have come up with novel strategies to control anomeric selectivity during glycosylation and the next section will look that some of the methods used to stereoselectively form either 1,2-*trans* or 1,2-*cis* glycosidic bonds.
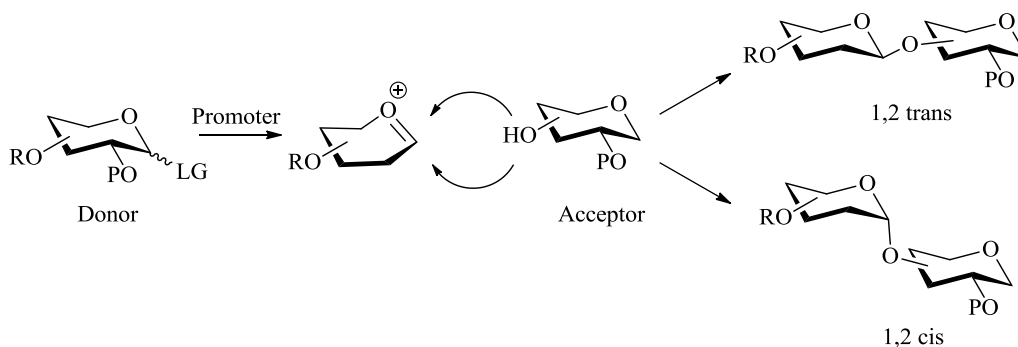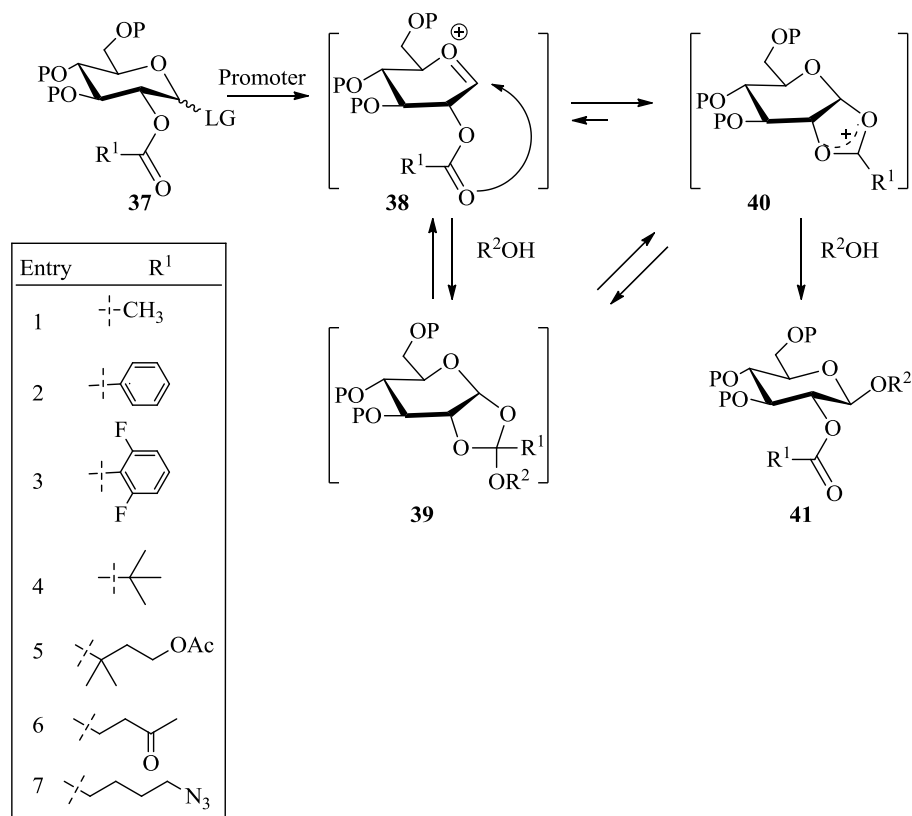

Scheme 1.9: Overview of chemical glycosylation resulting in the formation of α or β anomers.

1.10 Stereoselective Formation of 1,2-*trans* Glycosides

The most reliable method for the stereoselective formation of 1, 2-*trans* glycosides is based on neighboring group participation by C-2 ester functionalities. In these reactions, a promoter activates an anomeric leaving group, resulting in its departure and formation of an intermediate oxacarbenium ion. Now, neighboring group participation by the carbonyl oxygen of the C-2 ester functionality will result in formation of an intermediate five-membered dioxalenium-ion, which can only be formed as a 1,2-*cis* fused system. The incoming nucleophile/acceptor will then attack the anomeric centre from the β-face leading to the stereoselective formation of 1,2-*trans* glycosides. In the case of glucosyl-type donors, β-linked products will be formed and in the case of mannosides α-glycosides will be formed. The most common C-2 participating functionality used is the acyl group (entry **1**, Scheme **1.10**). But, a major drawback of this functionality is the formation of orthoester **39**, which is formed when the nucleophile adds directly to the dioxalenium ion rather than to the anomeric carbon. To circumvent this problem several new C-2 participating functionalities have been developed, which not only eliminate orthoester formation but can be used as orthogonal protecting groups for 1,2-*trans* glycosylations. Pivaloyl ester (trimethyl acetyl ester) (entry **4**, Scheme **1.10**) and 4-acetoxy-2,2-

dimethylbutanoate ester (ADMB)[72] (entry **5**, Scheme **1.10**) are some of the C-2 protecting groups that have been developed to eliminate orthoester formation.

Entry	R^1
1	‐CH$_3$
2	‐⬡ (phenyl)
3	‐⬡ (2,6-difluorophenyl) F F
4	‐C(CH$_3$)$_3$
5	‐C(CH$_3$)$_2$CH$_2$CH$_2$OAc
6	‐C(CH$_3$)$_2$CH$_2$C(O)CH$_3$
7	‐C(CH$_3$)$_2$CH$_2$CH$_2$N$_3$

Scheme 1.10: Neighbouring group participation by C-2-*O*-acyl funtionality to form 1,2-*trans*-glycosides.

Due to increased steric bulk on the α-carbonyl carbon, attack by the incoming nucleophile on the dioxalenium ion is greatly reduced and the geminal methyl substituents promote formation of the five-membered cyclic intermediate by the "gem-dialkyl" or Thrope-Ingold effect.[73] Levulinoyl ester[74] (entry **6**, Scheme **1.10**) and azidobutryl ester[75] (entry **7**, Scheme **1.10**) have also been used as C-2 protecting groups for the formation of 1,2-*trans* glycosides. These groups can easily be removed by intramolecular cyclization to form a five membered lactone or lactam and are orthogonal to most ester protecting groups commonly used in carbohydrate chemistry. Apart from esters, ether protecting groups like diphenylmethyl ether (DPM)[76] (Scheme **1.11a**) and 2-pyridylmethylpicolyl ether[77] (Scheme **1.11b**) have also been developed as C-2 participating functionalities which promote the formation of 1,2-*trans* glycosides. The

bulky DPM group provides anchimeric assistance by blocking the α-face attack of the incoming nucleophile while the 2-pyridylmethylpicolyl acts as an arming participating group resulting in the exclusive formation of β-glucosides. In order to access 2-deoxy-2-amino-glycosides several amino-protecting groups which can perform neighboring group participation have been employed *viz.* phthalimido, tetrachlorophthalimido (TCP), *N,N*-dimethyl maleoyl, trichloroacetyl, allyloxycarbonyl, and trichloroethyloxycarbonyl (Troc).
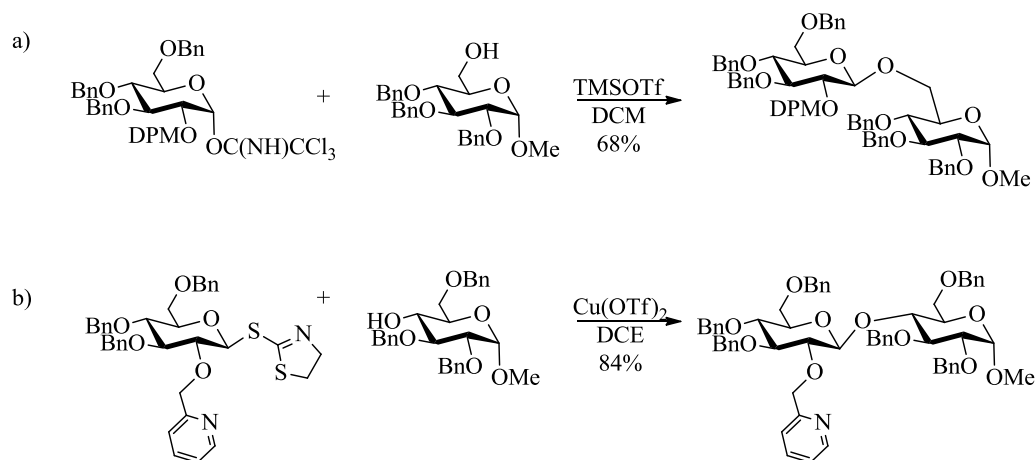

Scheme 1.11: a) Use of 2-diphenylmethyl ether in the synthesis of 1,2-*trans*-glycosides; b) Use of 2-pyridylmethylpicolyl ether for the stereoselective synthesis of 1,2-*trans*-glycosides.

1.11 Stereoselective Formation of 1,2-*cis* Glycosides

The introduction of 1,2-*cis* glycosidic linkages, such as α-glucosides and α-galactosides, requires glycosyl donors which have non-assisting functionality at C-2. These glycosylations require extensive optimization of reaction conditions such as solvent, temperature and promoter as well as constitution of glycosyl donor and acceptor to achieve acceptable anomeric ratios. The stereoselective formation of 1,2-*cis* glycosides, therefore, remains a fundamental challenge for oligosaccharide synthesis. A novel strategy for the synthesis of 1,2-*cis* glycosides was described by Boons and co-workers wherein they use a chiral auxiliary at the C-2 position of a glycosyl donor.[66-67] Upon activation of the donor, the chiral auxiliary is designed to trap the intermediate oxacarbenium ion resulting in the formation of a six-membered *trans*- or *cis*-decalin

bicyclic system. Subsequent displacement of the sulfonium ion by the sugar alcohol will lead to the formation of either α or β anomer. So, the stereochemical outcome of the glycosylation reaction can be controlled by the type of bicyclic system formed which in turn depends on the stereochemistry of the chiral auxiliary used. It was expected that using auxiliary with *S* stereochemistry would lead to formation of the *trans*-decalin (**45**), since the *cis*-fused system (**44**) would place the phenyl substituent in an axial position leading to diaxial steric interactions with the C-3 sugar hydrogen. Alternatively, using the auxiliary with *R* stereochemistry would favor formation of the 1, 2-*cis* (**49**) fused system as the *trans*-decalin system (**50**) in this case would experience unfavorable diaxial interactions (Scheme **1.12**).
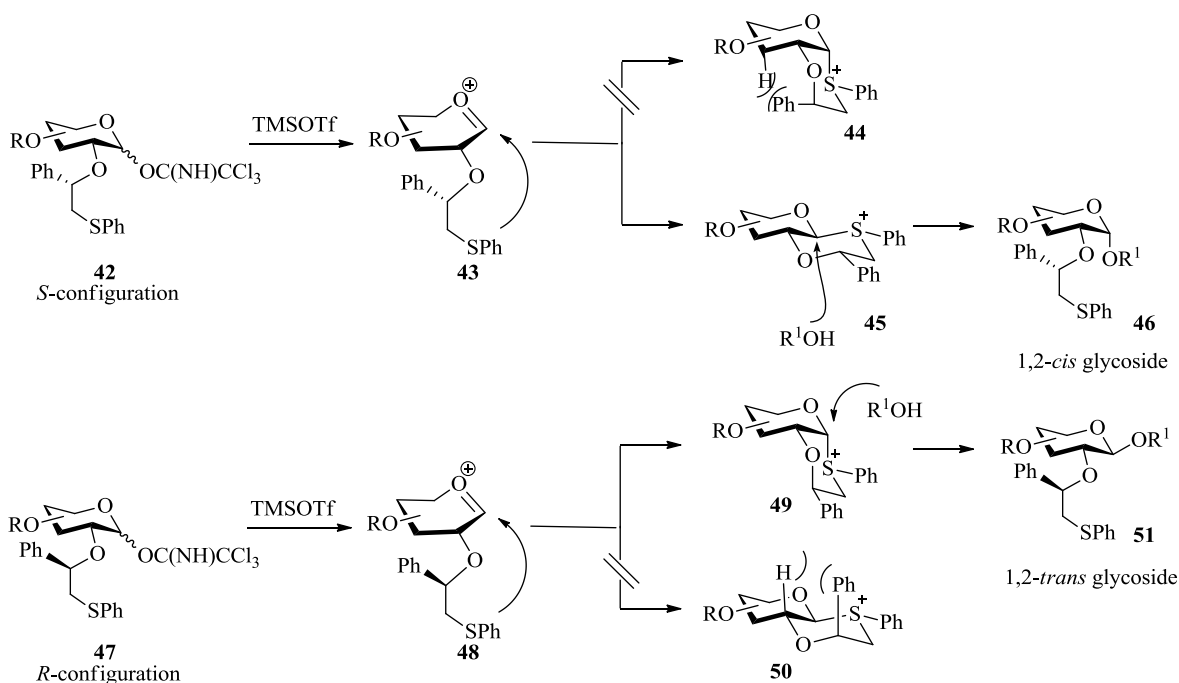
Scheme 1.12: Chiral auxilliaries designed to form *cis* or *trans*-decalin oxonium ion intermediates to afford either 1,2-*cis* or 1,2-*trans*-glycosides respectively.

This new glycosylation approach used the neighboring group participation of a (1*S*)-phenyl-2-(phenylsulfanyl)ethyl moiety at C-2 of the glycosyl donor (**42**). Displacement of the equatorial anomeric sulfonium ion (**45**), by the sugar alcohol led to the formation of 1,2-*cis* glycoside. The glycosylations of trichloroacetimidate donors with (1*S*)-phenyl-2-(phenylsulfanyl)ethyl moiety with different acceptors provided exclusively 1,2-*cis*

glycosides. NMR experiments showed that upon activation of the donor, a single 1,2-*trans* fused six-membered sulfonium ion intermediate was formed. The characteristic anomeric proton signal was shifted upfield after activation of the donor and the coupling constant went from 2.5 Hz to 9.5 Hz clearly indicating formation of the β-linked sulfonium ion intermediate. The HMBC spectrum also showed a correlation between C-1 and H8$_{eq}$ proving that the *trans*-decalin system had formed. The (1*S*)-phenyl-2-(phenylsulfanyl)ethyl moiety can be easily introduced by reaction of sugar alcohol with acetic acid (1*S*)-phenyl-2-(phenylsulfanyl)ethyl ester **53** in the presence of BF$_3$-OEt$_2$.[78] After the glycosylation the auxiliary can be removed by conversion into acetyl ester by treatment with BF$_3$-OEt$_2$ in acetic anhydride. Both reactions proceed *via* an intermediate episulfonium ion with overall retention of configuration (Scheme **1.13**).
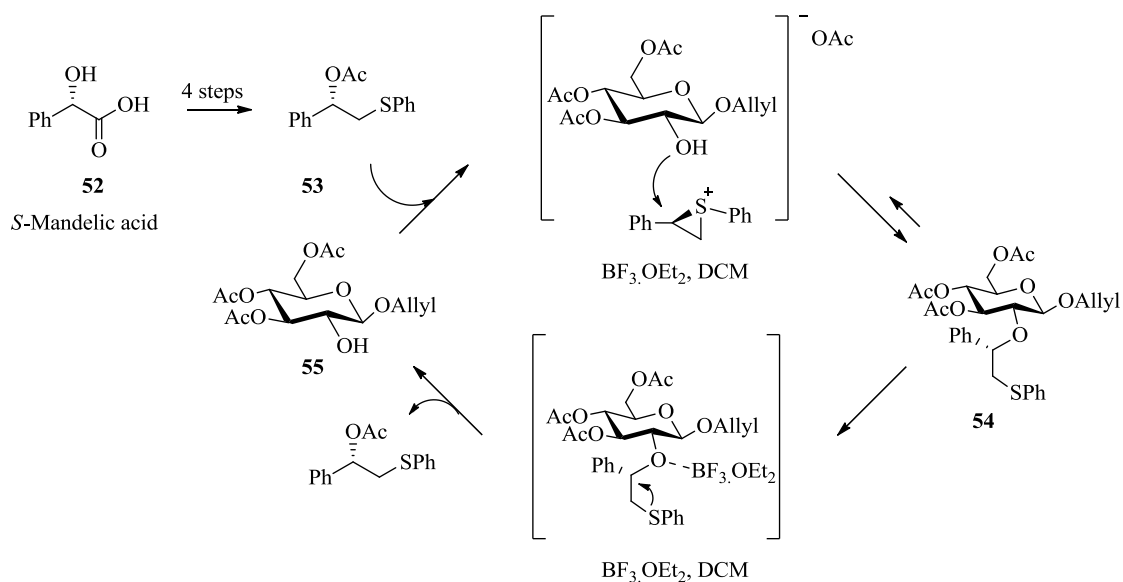
Scheme 1.13: Lewis acid catalyzed stereoselective introduction and removal of the (*S*)-(phenylthiomethyl)benzyl moeity.

The versatility of the chiral-auxilliary mediated 1,2-*cis*-glycosylations using (1*S*)-phenyl-2-(phenylsulfanyl)ethyl moiety was demonstrated in the solid-phase synthesis of a branched 1,2-*cis* linked pentaglucoside.[79] Orthogonal protecting groups 9-fluorenylmethyloxycarbonyl (Fmoc) and allyloxycarbonyl (Alloc) were used, which could be removed during the solid-phase synthesis, to install a branching unit. The synthesis of pentaglucoside **60** was achieved with complete anomeric control with an

overall yield of 25% which corresponds to a yield per step of 90% (Scheme **1.14**). This was the first example of a stereoselective solid-supported synthesis of a branched oligosaccharide containing multiple 1,2-*cis* glycosidic linkages. Recently, Turnbull and co-workers reported the synthesis of a series of oxathiane glycosyl donors that can be prepared in a concise route by cyclization of simple thioglycosides.[80-81] Under acidic conditions, the thioglycoside ketone **62** undergoes intramolecular cyclization to give the corresponding 1,2-oxathiane in high yield and absolute stereoselectivity (Scheme **1.15**). A major advantage of this approach is that it utilizes readily accessible achiral thioglycoside as starting material and the C-2 position of the sugar is selectively modified without the use of any protecting groups thereby reducing the length of the donor synthesis. The sulfoxide **63** obtained by oxidation of the oxathiane ketal was activated for glycosylation using Tf_2O, 1,3,5-trimethoxybenzene and DTBMP. Triflation of the sulfoxide led to formation of sulfonyl triflate **64** which reacted with 1,3,5-trimethoxybenzene by an electrophilic aromatic substitution to afford the aryl sulfonium ion **65**. Glycosylation with various acceptors gave the corresponding 1,2-*cis*-glycosides with absolute selectivity in moderate to high yields (Scheme **1.15**). After the glycosylation the auxiliary could be easily cleaved by using catalytic amount of $BF_3.OEt_2$.
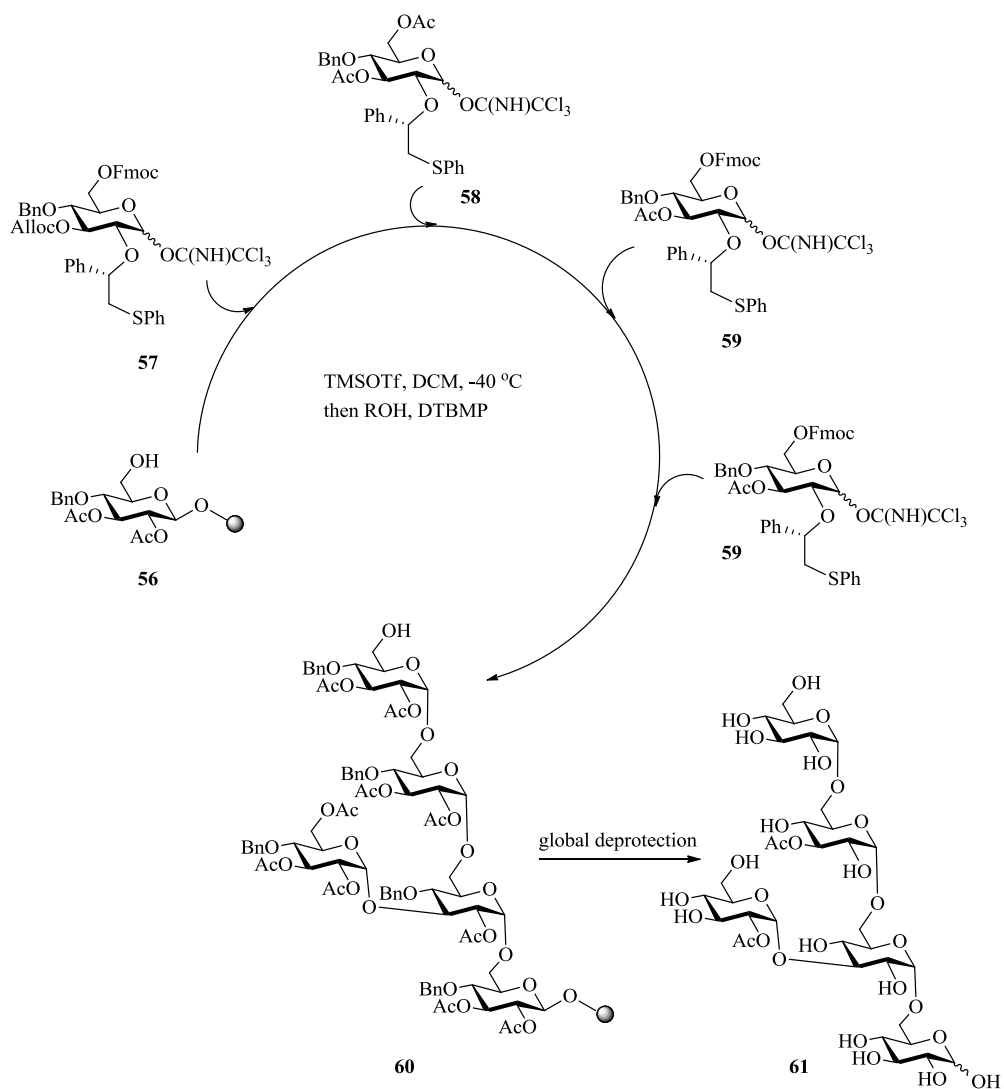
Scheme 1.14: Solid phase synthesis of branched pentaglucoside **61** using C-2 (*S*)-(phenylthiomethyl)benzyl chiral auxilliary.

In order to advance chiral auxiliary mediated glycosylations for the rapid assembly of complex branched oligosaccharides, it is important to establish convenient procedures for the preparation of suitably protected anomeric sulfonium-ion monomeric building blocks. It has been observed that the C-2 (*S*)-(phenylthiomethyl)benzyl ethers or the 1,2-oxathiane ketals are sensitive to moderately strong acidic conditions, thereby complicating a number of important protecting group manipulations essential for the assembly of complex branched α-glucans.
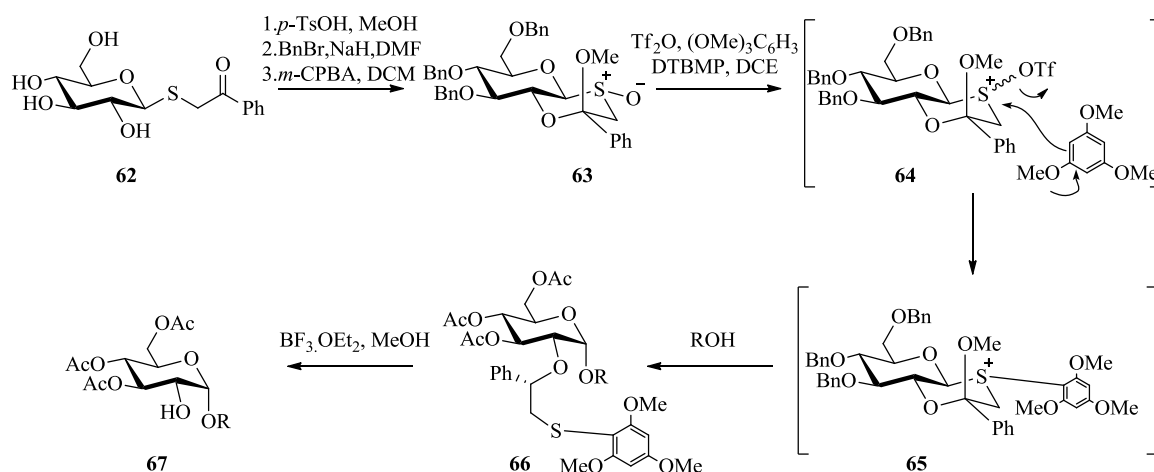
Scheme 1.15: Generation of sulfonium ion by electrophilic aromatic substitution of 1,2-oxathiane ketal glycosyl donor and subsequent glycosylation to form 1,2-*cis* glycosides.

This problem was addressed by using 1,2-oxathiane ether **68** (Scheme **1.16**) as precursors of anomeric sulfonium ion.[82] 1,2-oxathiane ethers are stable under acidic conditions because their ring structure prevents the participation of the sulfur atom in acid catalyzed cleavage of the benzylic ether linkage, thus making it more compatible with a wide variety of protecting group manipulations. A range of 1,2-oxathiane ethers, with different orthogonal protecting groups such as Alloc carbonate, Lev ester, Fmoc and Nap ether were synthesized. These protecting groups can be removed under mild conditions providing opportunity for further functionalization. The selectively protected 1,2-oxathiane ethers, can then be readily converted into anomeric sulfonium ion by oxidative arylation **70** (Scheme **1.16**), for stereoselective 1,2-*cis* glycosylations.[82] The 1, 2-oxathiane building blocks were then employed in the synthesis of a branched α-tetraglucoside **71** by using a latent-active glycosylation strategy. First, the oxathiane was employed as an acceptor for glycosylation with a sulfoxide donor and then the product was oxidized to a sulfoxide for subsequent glycosylations (Scheme **1.16**).
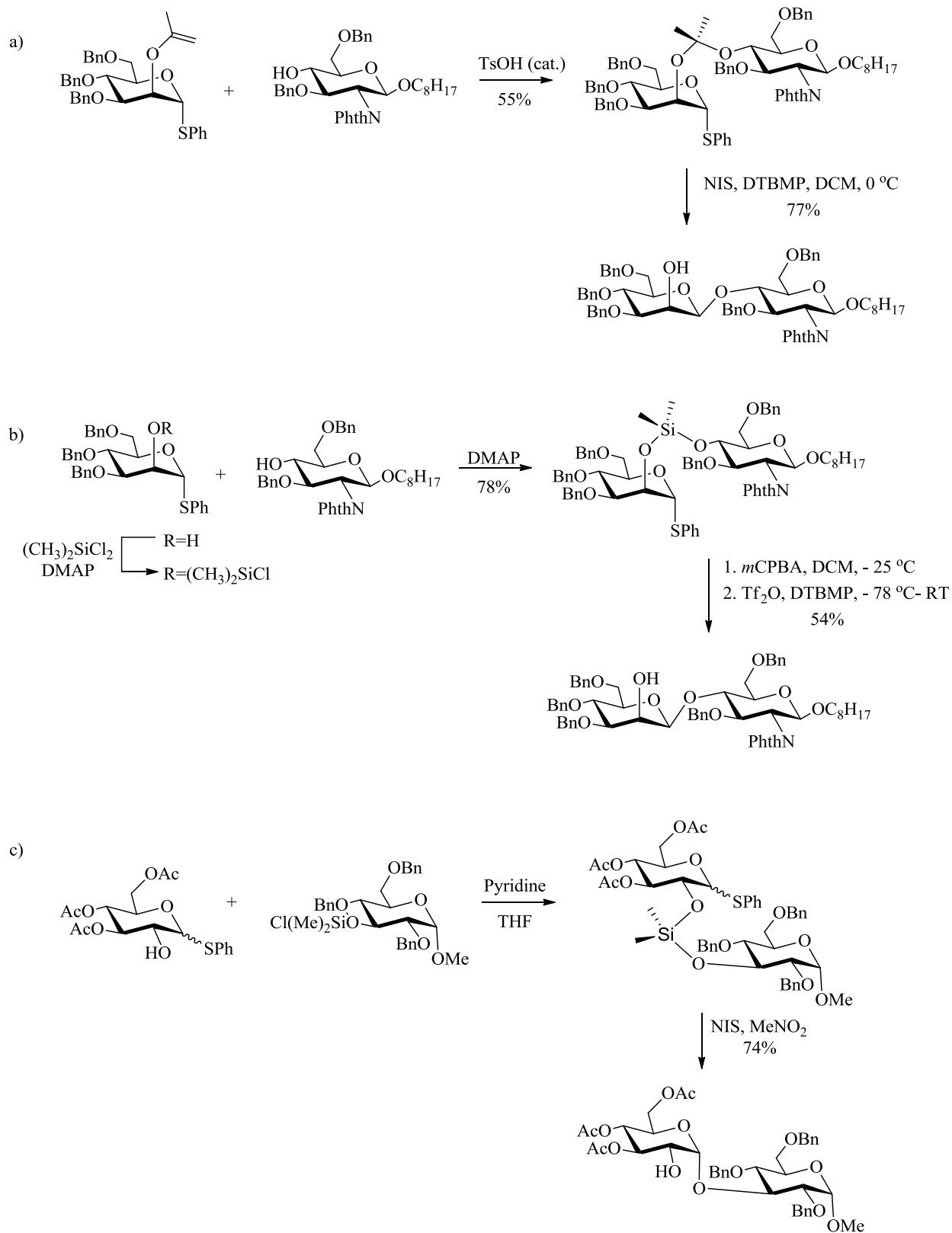
Another innovative approach to synthesize 1,2-*cis* glycosides is using Intramolecular Aglycon Delivery (IAD). In IAD, the stereochemistry at C-2 of the donor can be used to dictate a 1,2-*cis* outcome from a two-step tethering–glycosylation process.
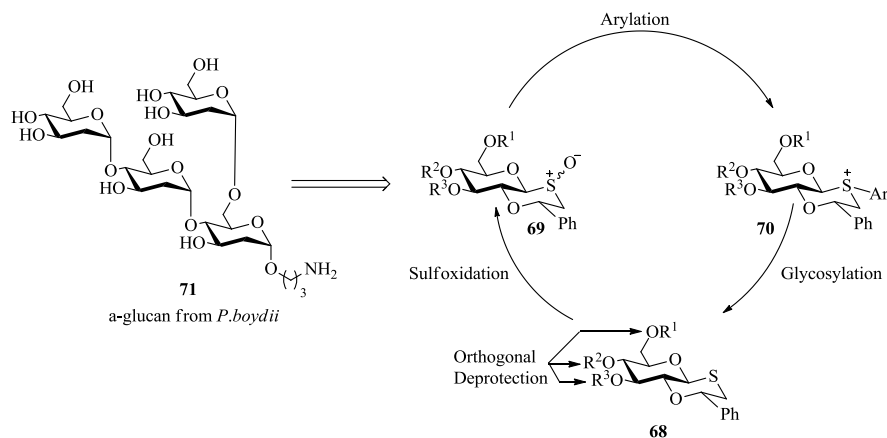
Scheme 1.16: Synthesis of the glycogen-like glucan isolated from *P. boydii* using 1,2-oxathiane ether by a latent-active glycosylation strategy.

The sugar alcohol is first linked *via* silicon or acetal tether to the C-2 position of the donor and subsequent activation of the anomeric centre of this adduct results in the formation of a 5-membered ring transition state. In the next step intramolecular glycosylation occurs with complete stereocontrol of the newly formed glycosidic bond. In the case of glucosyl-type donors, α-linked products will be formed and in the case of mannosides, β-glycosides will be formed. After the glycosylation, the remnant of the tether can be hydrolyzed during the work-up procedure to give the glycoside with a free C-2 hydroxyl. IAD was used for the synthesis of β-mannosides by using a mixed acetal tether. The aglycon alcohol was tethered to the donor using acid catalyst and upon activation of the donor using NIS, the adduct underwent an intramolecular glycosylation to give the β-mannoside as the only product (Scheme **1.17a**).[83] When the reaction was performed in the presence of methanol (1 equiv), no methyl glycoside was obtained, indicating that the glycosylation proceeds via a concerted reaction and not by intermolecular addition of nucleophile to an anomeric oxacarbenium ion. Silicon-tethered IAD using mannosyl sulfoxide donors also gave excellent β-selectivity with a variety of acceptors (Scheme **1.17b**).[84] Bols and co-workers demonstrated the synthesis of α-glucosides and galactosides using the silicon-tethered IAD. Primary, secondary and tertiary alcohols underwent tethering and 1,2-*cis* glycosylation in good yields and excellent anomeric selectivity (Scheme **1.17c**).[85-86]

Scheme 1.17: a) Acid-catalyzed tethering and β-mannosylation using mixed-acetals; b) Stork's silicon-tethered β-mannosylation; c) Bol's silicon-tethered synthesis of α-glucosides.

Another approach to synthesize β-mannosides was discovered by Crich and co-workers, when they found that pre-activation of 4,6-benzylidene protected mannosyl sulfoxide or thioglycoside donors followed by addition of glycosyl acceptors led to stereoselective formation of β-mannosides.[87-88] Apart from pre-activation, the 4,6-benzylidene acetal was found to be indispensable for the high β-selectivity. The 4,6-benzylidene acetal is believed to exert a torsional disarming effect, which disfavors the formation of an intermediate oxacarbenium ion, since it requires rehybridization and flattening of the sugar ring into a half-chair conformation. Due to the torsional disarming effect as well as strong endo-anomeric effect in mannose, the more stable α-triflate **75** is formed, which is displaced by the acceptor in an S_N2-like manner to form the β-mannoside product (Scheme **1.18**).
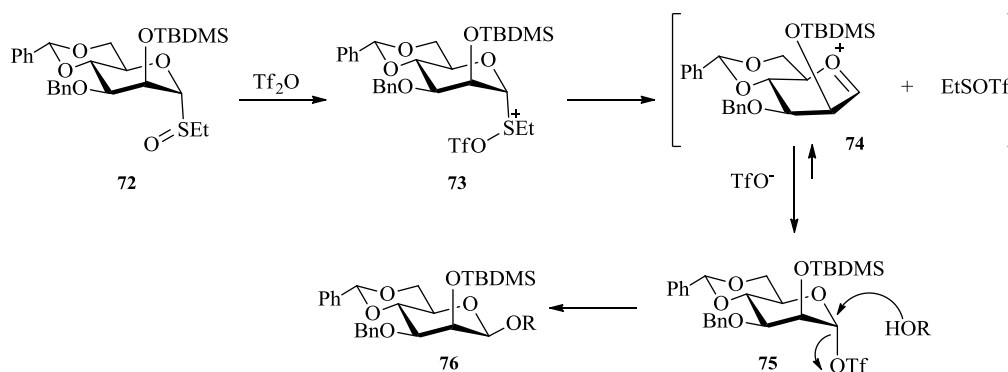
Scheme 1.18: Torsional and electronic disarming of mannosyl donors using 4,6-*O*-benzylidene acetal, leading to the preferential formation of α-triflate intermediate and subsequent β-mannosylation.

Alternatively, Bols and co-workers determined that in addition to the torsional effect of the 4,6-benzylidene acetal, there also exists an electronic effect due to the fixed arrangement of the O-6 substituent.[89] The 4,6-benzylidene acetal places the dipole of the C-6 oxygen atom anti-parallel to the electron deficient oxacarbenium intermediate formed in the transition state, thereby destabilizing it. This electron disarming effect was found to be approximately equal to the torsional disarming effect in 4,6-benzylidene acetal substituted mannose donors, thus giving rise to the high β-selectivity. It has been demonstrated that the use of sterically demanding 4,6-benzylidene acetals can have an influence on the anomeric selectivity of glycosylation reactions.

Kiso and co-workers reported the selective α-galactosylation using 4,6-*O*-di-*tert*-butylsilyl (DTBS) protected galactose donors **77**.[90] The α-selectivity was unaffected by the presence of participating functionalities like NHTroc, NHAc or OBz groups on the C-2 position. The X-ray crystal structure of glycosyl donor showed that the six-membered ring comprising of 4,6-*O*-DTBS acetal and the C4-C5-C6 sugar carbon atoms is in a half-chair conformation placing the *tert*-butyl groups directly over the β-face of the anomeric carbon, thereby blocking attack by the acceptor from the β-face (Scheme **1.19**).
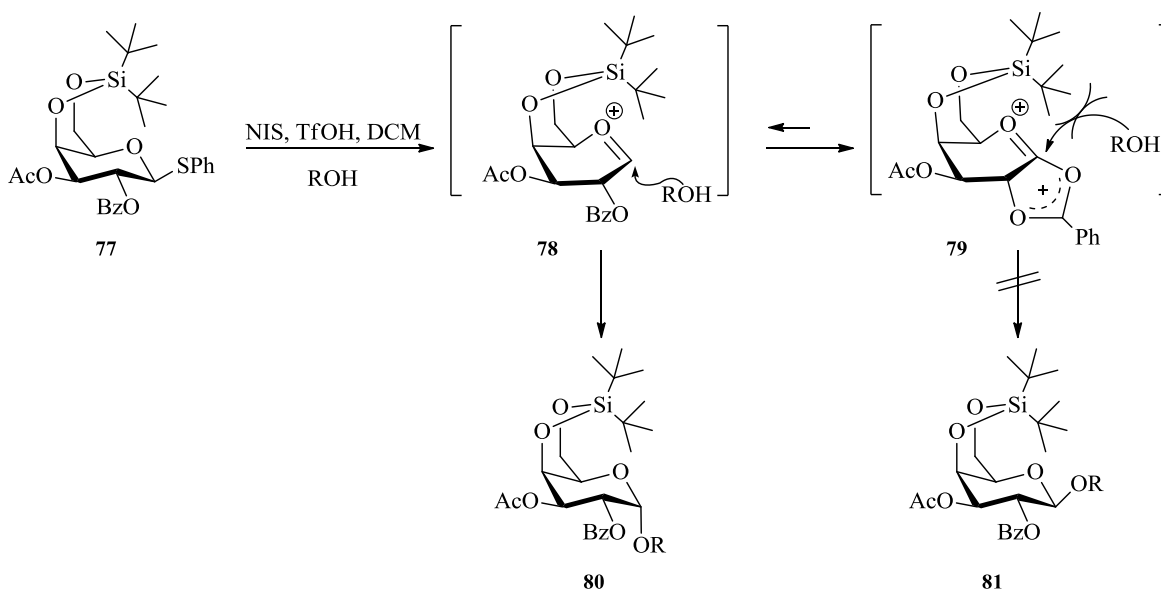
**Scheme 1.19:** Use of DTBS group in α-galactosylation.

The examples described above rely on the use of highly specialized linkages or protecting group patterns for the synthesis of 1,2-*cis* glycosides. Alternatively, it is possible to obtain selectivity in the absence of directing groups by using anomeric leaving groups that undergo S_N2-like displacement. Bennett and co-workers reported the selective synthesis of 1,2-*cis* glycosides by the *in situ* conversion of thioglycosides into reactive glycosyl iodides under mild condition.[91] Phenylthioglycoside **82** was activated using diphenylsulfoxide and triflic anhydride in the presence of a non-nucleophilic base *N*-methylmaleimide. In the presence of excess *tert*-butylammonium iodide, the intermediate glycosyl triflate can be trapped by iodide ion to form the glycosyl iodide intermediate. A rapid equilibrium is established between α and β glycosyl iodide (**84, 83**) with the α-iodide being favored due to stabilization by the endo-anomeric effect. This makes the β-

glycosyl iodide **83** much more reactive than the more stable α-iodide **84** towards nucleophilic attack by an acceptor and glycosylation takes place preferentially on this intermediate in an S_N2 fashion to give the α-glycoside (Scheme **1.20**).
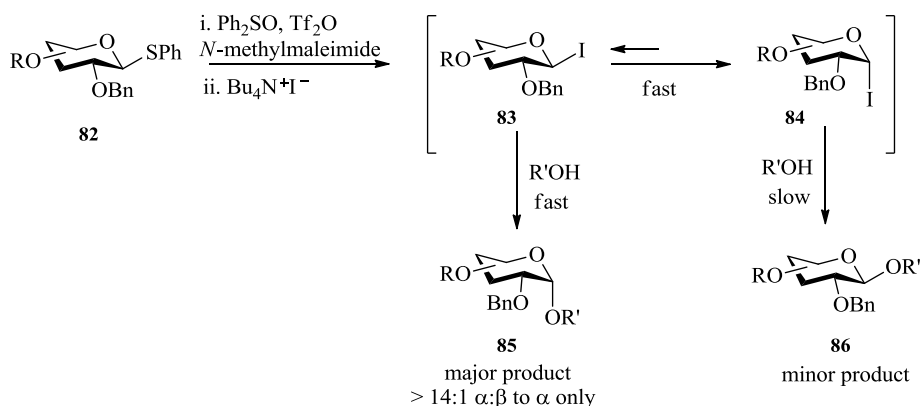
Scheme 1.20: 1,2-*cis*-glycosylation by S_N2 displacement of the β-iodide formed by *in situ* anomerization.

In a similar approach, Boons and co-workers reported the TMSOTf-promoted 1,2-*cis* glycosylations of 2-azido-2-deoxy-glucosyl trichloroacetimidate donors **87** with different acceptors in the presence of PhSEt or thiophene.[92] Activation of the trichloroacetimidate in the presence of either the PhSEt or thiophene results in the formation of the intermediate β-substituted anomeric sulfonium ion **89**. The formation of the β-anomer is favored due to steric factors, and diaplacement of the β-sulfonium ion by the acceptor leads to formation of the α-glycoside (Scheme **1.21**). This method provides an excellent route to the synthesis of 1,2-*cis* 2-deoxy-2-amino-glycosides. The azido precursor is stable under a wide variety of reaction conditions but can be easily reduced to an amine with reagents such as phosphine and thiols, or by catalytic hydrogenation.
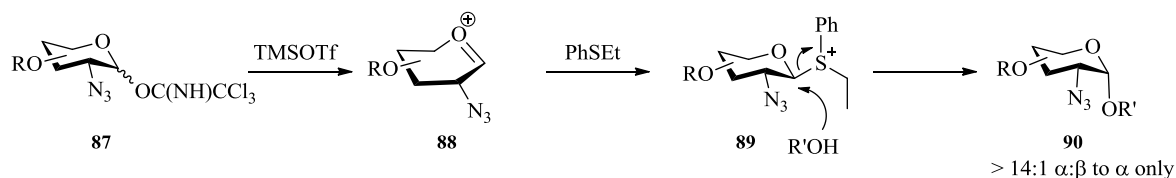
Scheme 1.21: 1,2-*cis*-glycosylation by displacement of an intermediate equatorial sulfonium ion by sugar alcohol.

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In general, a plethora of methods have been developed by different research groups for the stereoselective synthesis of the glycosidic bond. But, for the synthesis of 1,2-*cis* glycosides, most of the methods described are limited, either to a specific type of carbohydrate or protecting group thereby limiting their synthetic applicability. However, the use of a C-2 chiral auxilliary has tremendous potential to become a general method for the synthesis of 1,2-*cis* glycosides. In chapters II and III of this thesis we have demonstrated the use of xylosyl 1,2-oxathiane ether for the stereoselective synthesis of α-xyloglucans in the total synthesis of plant xyloglucan sidechains.

1.12 References

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CHAPTER II

SYNTHESIS OF GALACTURONIC ACID-CONTAINING XYLOGLUCAN SIDE CHAIN INVOLVED IN *ARABIDOPSIS* ROOT HAIR TIP GROWTH*

2.1 Abstract

A unique galacturonic acid (GalA) containing xyloglucan has been identified in the root hairs of *Arabidopsis thaliana*. The xyloglucan containing the GalA subunits are made up of the disaccharide β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→) and the trisaccharide α-L-fucosyl-(1→2)-β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→) in addition to neutral side chains. The acidic xyloglucan was found to be present only in the root hair tips and absent in the walls of the other root cells as well as in the leaf and stem cell walls indicating it plays an important role in the growth of the root hair tip. Herein, we report the first chemical synthesis of tetrasaccharide **25** which forms part of the acidic xyloglucan side chain. The key step in the synthesis involves chiral auxiliary mediated glycosylation of xylosyl 1, 2-oxathiane donor **9** with glucosyl acceptor **12,** to form the α-xyloglucan **19**. The tetrasaccharide is conjugated to biotin and screened against monoclonal antibodies which bind to different epitopes of the xyloglucan oligosaccharide. Screening the antibodies using a fully synthetic xyloglucan sidechain will provide better understanding about the epitope specificity of these monoclonal antibodies and also help in determining if any of the antibodies bind/recognize the unique fucogalcaturonic acid side chain on xyloglucan.
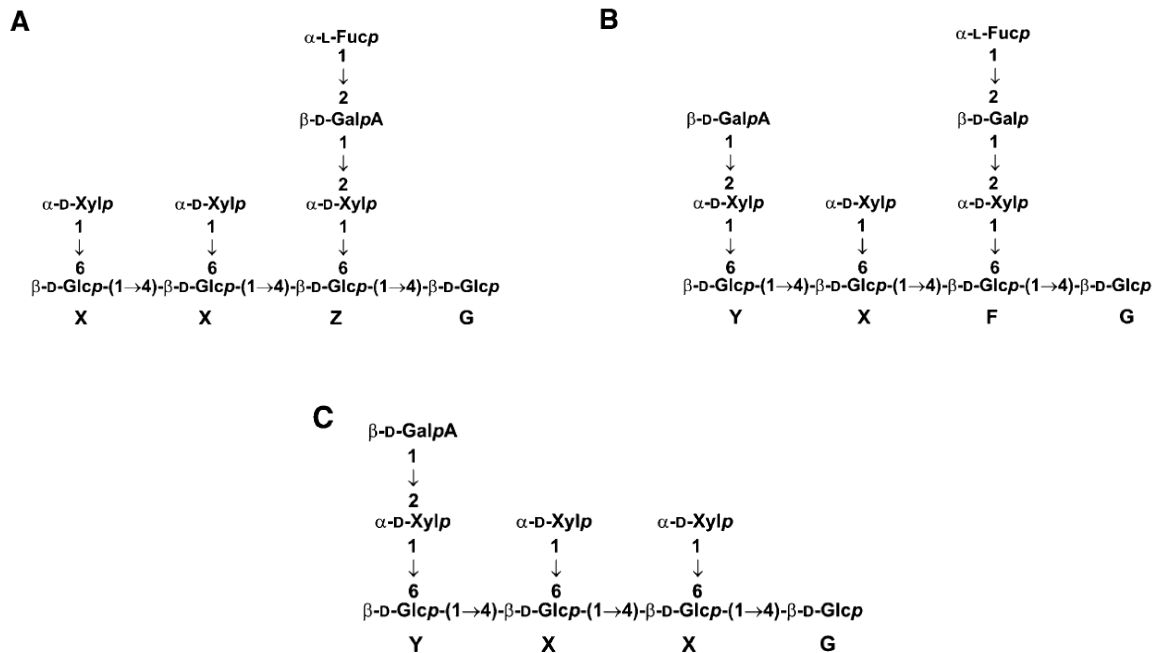
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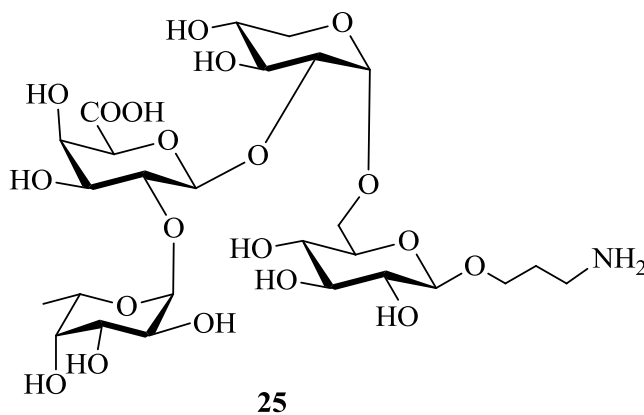
2.2 Introduction

The primary cell wall surrounding all growing cells must be sufficiently strong to prevent the cell from bursting due to internal turgor pressure, yet must accommodate controlled growth and expansion of the cell.[1] The primary walls of plant cells consist of cellulose

microfibrils embedded in a matrix composed of pectins and hemicelluloses together with small amounts of glycoproteins and minerals. In the primary cell wall of most vascular plants, xyloglucan is the most abundant hemicellulose present and it interacts with cellulose microfibrils to form the cellulose-xyloglucan network which forms the major load bearing structure in most plants.[2-3] Enzymatic restructuring of the cellulose-xyloglucan network along with xyloglucan metabolism is therefore considered essential for proper cell wall expansion and growth.[4-5]

Peña and co-workers recently reported that *Arabidopsis thaliana* root hairs contain xyloglucan that is composed of both neutral and acidic subunits.[6] The unique acidic xyloglucan in the root hair cell walls contains a previously unidentified galacturonic acid (GalA) side chain composed of the disaccharide β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→) and the trisaccharide α-L-fucosyl-(1→2)-β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→), which have been assigned the letters **Y** and **Z**, respectively, in addition to neutral side chains (Figure **2.1**). They also found that *Arabidopsis* mutants lacking root hairs do not produce acidic xyloglucan and the root hairs of these mutants are shorter than those of the wild type. Moreover, xyloglucan containing glacturonic acid is present only in the root hairs and is absent in the cell walls of the leaf, stem and also walls of the other roots cells indicating that the acidic xyloglucan plays an important role in the growth of the root hair tip. Unlike, other plant cells which can expand along any axis[4], growth in the root hairs occurs exclusively at the cell apex resulting in an elongated morphology due to tip-growth.[7] It is likely that acidic xyloglucans exhibit different biophysical properties when compared to neutral xyloglucans and such differences affect the interaction of the xyloglucan with other wall components, including cellulose. It has been shown that acidic xyloglucan generated *in vitro* by chemoenzymatic methods, interact weakly with cellulose when compared to neutral xyloglucan.[8] So, the presence of the acidic **Y** and **Z** side chains, affect the interaction between xyloglucan and cellulose microfibrils in expanding tip walls of root hairs. It has been proposed that the weak

A

α-L-Fuc*p*
1
↓
2
β-D-Gal*p*A
1
↓
2
α-D-Xyl*p*   α-D-Xyl*p*   α-D-Xyl*p*
1   1   1
↓   ↓   ↓
6   6   6
β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*
X   X   Z   G

B

α-L-Fuc*p*
1
↓
2
β-D-Gal*p*A   β-D-Gal*p*
1   1
↓   ↓
2   2
α-D-Xyl*p*   α-D-Xyl*p*   α-D-Xyl*p*
1   1   1
↓   ↓   ↓
6   6   6
β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*
Y   X   F   G

C

β-D-Gal*p*A
1
↓
2
α-D-Xyl*p*   α-D-Xyl*p*   α-D-Xyl*p*
1   1   1
↓   ↓   ↓
6   6   6
β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*
Y   X   X   G

Figure 2.1: Structures of the predominant acidic xyloglucan oligosaccharides from root hairs of *Arabidopsis thaliana*

interaction of the acidic xyloglucan with cellulose results in separation and/or random orientation of the cellulose microfibrils at the root tip facilitating locally isotropic wall expansion in that region. On the other hand, cellulose microfibrils have been shown to be highly ordered in the side walls of the root hair when compared to the growing root tip.[9] Since the side walls of the root hairs contain neutral xyloglucan, it contributes to the formation of secondary wall that is deposited on the cytoplasmic side of the primary wall.[10] The formation of this secondary wall thereby limits both lateral and longitudinal expansion of the root hair side walls.[9, 11] *Arabidopsis* produces XXXG-type xyloglucan in which three consecutive glucopyranosyl backbone residues are substituted at the O-6 with a glycosyl side chain.[12] MALDI-TOF MS and ^{1}H-NMR analysis of the oligosaccharide subunits, formed by fragmentation of the xyloglucan backbone with xyloglucan specific endoglucanase (XEG) revealed YXXG and XXZG as the major acidic subunits in the xyloglucan along with small amounts of YXFG, XXZG, YXYG, and YXLG subunits.[6, 13] Together, the acidic subunits account for at least 30% of the total subunits present in root hair tip xyloglucan. In order to understand and correlate the biological functions of acidic xyloglucan with specific structures of the polysaccharide in different species will require techniques for structurally characterizing the glycan and

also sensitive tools for localizing the specific cell wall components in different plant tissues. In this respect, generation of monoclonal antibodies against the **Y** or **Z** side chains of the acidic xyloglucan will not only help in the identification and visualization of these unique structures in the root hairs of different plant species but will also shed more light on the molecular mechanisms underlying the requirement of xyloglucan for the normal expansion of root hairs.[14] But, due to the practical difficulties in the isolation and purification of homogenous, well defined acidic xyloglucan fragments from natural sources, the chemical synthesis of these unique side chains will aid in the generation of well characterized monoclonal antibodies.

We report here the synthesis of α-L-fucosyl-(1→2)-β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→6)-β-D-glucopyranoside tetrasaccharide **25** which forms the **Z** side chain of the acidic xyloglucan identified in the root hairs of *Arabidopsis thaliana*. Key features of the synthesis involve the use of xylosyl 1, 2-oxathiane donor **9** for the stereoselective synthesis of α-xyloside.[15] In addition, the β-galactoside was introduced by employing a C-2 participating levulinyl group along with the use of solvent effects to ensure β-selectivity. Due to the low reactivity of uronic acid donors[16], a late-stage post-glycosylation oxidation strategy was employed to introduce the galacturonic acid functionality into the molecule by using a 4, 6- benzylidene acetal protected galactose donor. After the tetrasaccharide is assembled, the 4, 6-benzylidene acetal is removed and the C-6 hydroxy of the galactose is oxidized to the galacturonic acid using *m*CPBA/BAIB. The tetrasaccharide is equipped with an anomeric aminopropyl spacer which is used for conjugation to biotin. The biotinylated tetrasaccharide was screened against 32 monoclonal antibodies which bind to xyloglucan oligosaccharides using ELISA based screening assay.

2.3 Results and Discussion

Retrosynthetic analysis: Tetrasaccharide **25** can be obtained from its fully protected precursor **23** by first orthogonally deprotecting the 4, 6-benzylidene acetal and oxidizing the C-6 hydroxyl of the galactose to the galacturonic acid using *m*CPBA/BAIB, followed by global deprotection of the ester and ether protecting groups. The fully protected tetrasaccharide **23** can in turn be synthesized by glycosylation of trisaccharide acceptor
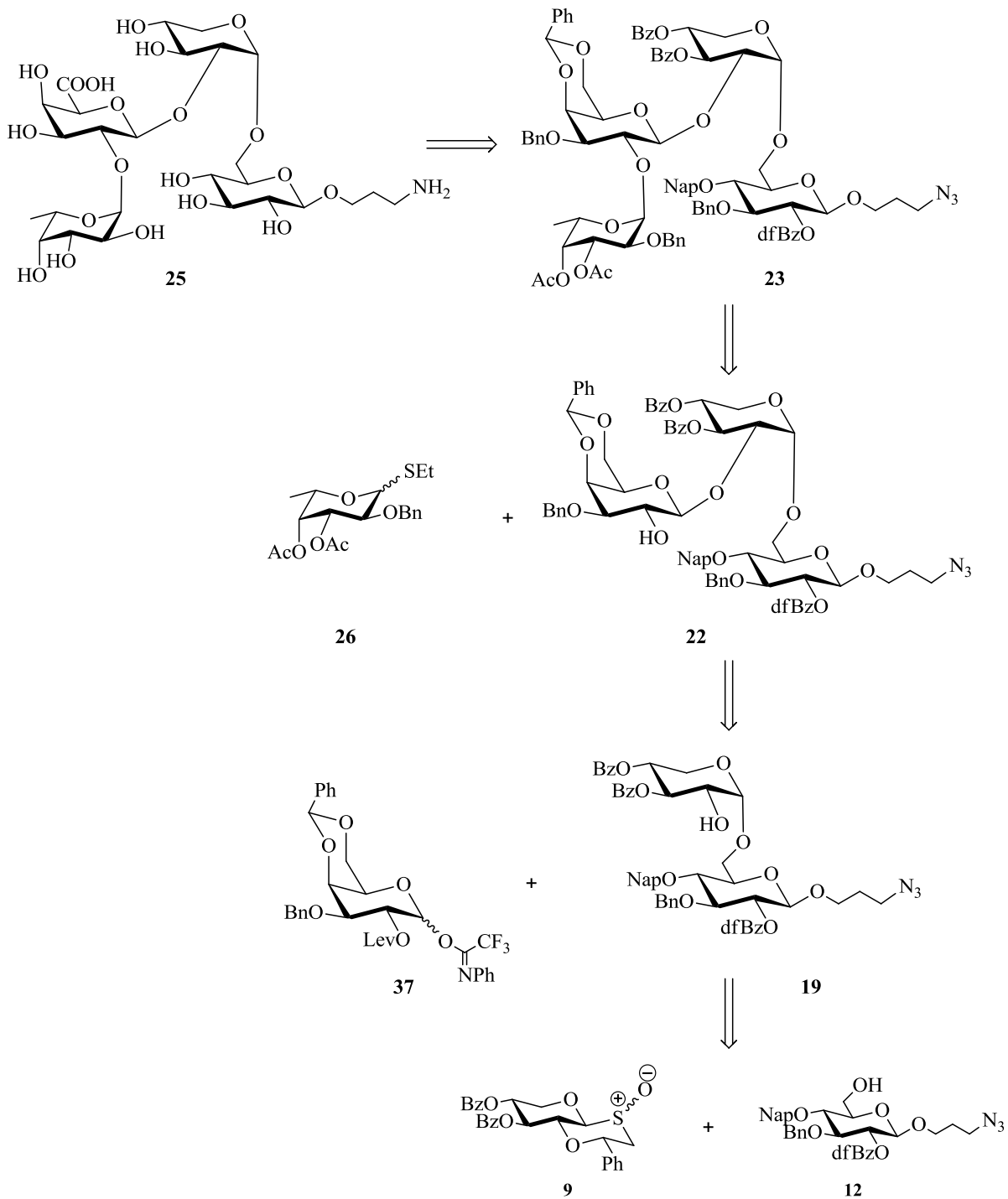
Scheme 2.1: Retrosynthetic analysis.

22 and fucosylthioglycoside donor **26**. Trisaccharide **22** will be synthesized by coupling disaccharide acceptor **19** and galactoysl imidate donor **37**. It was envisaged that stereoselective formation of disaccharide **19** could be achieved by chiral auxiliary

mediated glycosylation using xylosyl 1,2-oxathiane sulfoxide **9**. The sulfoxide **9**, upon reaction with Tf$_2$O and 1,3,5-trimethoxybenzene, can be converted to the aryl sulfonium xylosyl donor, which upon reaction with glucosyl acceptor **12**, will result in attack of the nucleophile from the α-face, to afford the disaccharide **19** in good α-stereoselectivity. Thus, with a practical strategy in place, our effort was focused on the synthesis of the different building blocks required for the assembly of the target tetrasaccharide **25**.

Preparation of the required building blocks- Synthesis of glucose acceptors 12 and 13: Glucosyl acceptors **12** and **13** (Scheme **2.2**) were readily obtained by saponification of the acetyl ester of ethyl 2,3,4-tri-*O*-acetyl thioglucoside **28** using standard conditions followed by selective protection of the 4,6-diol of the resulting compound as a naphthalidene acetal by treatment with 2-(dimethoxymethyl)-naphthalene[17] and camphorsulfonic acid in DMF to afford compound **29** in 60% yield over two steps. In the next step, the C-3 hydroxyl was regioselectively benzylated by first preparing a stannylidene acetal by treatment with dibutyl tin oxide followed by reaction with benzyl bromide in the presence of CsF in DMF to give **30** in 55% yield. Reacting compound **30** with 2,5-difluorobenzoylchloride with catalytic amount of DMAP in pyridine gave the fully protected compound **31** in 79% yield. Thioglycoside **31** was glycosylated with 3-azido-1-propanol[18] with NIS/TMSOTf[19] as promoter in DCM at -20 ^{o}C to provide spacer-containing **32** as only the β-anomer. The 4,6-naphthalidene acetal of compound **32** was reductively opened using dichlorophenylborane and Et$_3$SiH in DCM at -78 ^{o}C to give the glucose acceptor **12** having a C-6 hydoxyl and C-4 benzyl ether in 91% yield.[20] Similarly, reductive opening of 4,6-naphthalidene acetal of compound **31** using the same conditions provided glucose acceptor **13** in 68% yield (Scheme **2.2**).
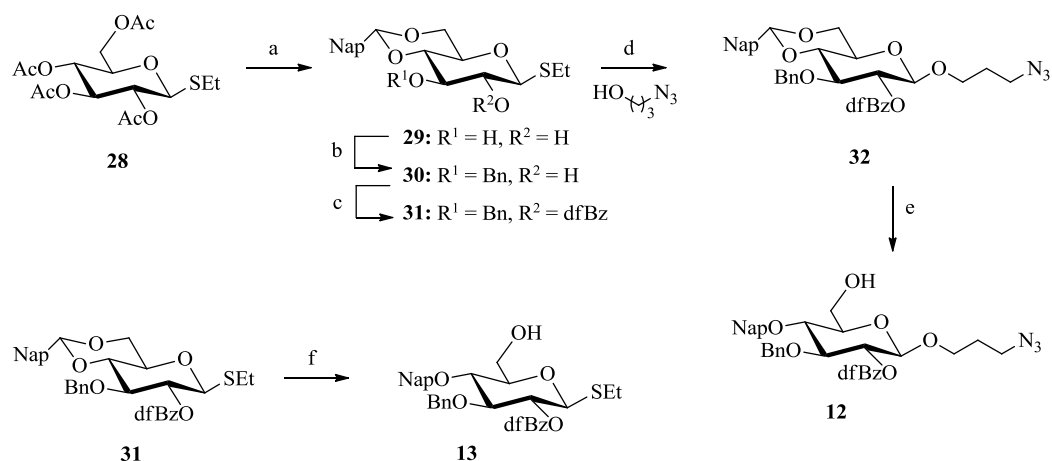
Scheme 2.2: a) i.) NaOMe, MeOH; ii.) 2-(Dimethoxymethyl)-napthalene, CSA, reduced pressure, DMF, 60 $^{\circ}$ C, 18h (60%, 2 steps); b) (Bu$_2$Sn)O, MeOH, 80 $^{\circ}$ C, BnBr, CsF, DMF, 16 h (55%); c) dfBzCl,DMAP, Py (79%); d) NIS, TMSOTf, DCM, - 20 $^{\circ}$ C (66%); e) Et$_3$SiH, PhBCl$_2$, DCM, - 78 $^{\circ}$ C (91%); f) Et$_3$SiH, PhBCl$_2$, DCM, - 78 $^{\circ}$ C (68 %).

Synthesis of disaccharide 19: The key step in the synthesis of tetrasaccharide **25** is the stereoselective synthesis of the α-xyloglucan. Glycosylation of xylosyl trichloacetimidate donor **10** with glucose acceptor **11**[21] gave the corresponding disaccharide **15** in 74% yield and a 1:1 mixture of α/β anomers (Table **2.1**, entry **1**). The poor α-selectivity prompted us to explore the utility of chiral auxiliary mediated glycosylation using glycosyl 1,2-oxathiane donors developed in our lab.[15] We envisaged that xylosyl 1,2-oxathiane ethers, upon conversion into anomeric sulfonium ion by oxidation to sulfoxides followed by arylation with Tf$_2$O and 1,3,5-trimethoxybenzene, can be used as suitable donor for stereoselective 1,2-*cis* glycosylations.[22] To this end, xylosyl 1,2-oxathiane ethers **4-6** were prepared, which were oxidized to the corresponding sulfoxides **7-9** to be used for the generation of sulfonium ions for subsequent glycosylation (Scheme **2.3**). Thus, thioglycoside **2** was prepared from peracetylated xylose **1**. Lewis acid activation of the anomeric acetate in the presence of thiourea provided a β-thiouronium salt which was treated with Et$_3$N and 2-bromoacetophenone *in situ*.[23] The resulting thioglycoside **2** was de-esterified under Zemplén conditions to give the corresponding triol which was subsequently converted into oxathiane **3** by a one-pot, two- step reaction involving the treatment of the xylosyl triol with TMS$_2$O in the presence of TMSOTf to give an intermediate trimethylsilyl acetal, which was reduced by the addition of Et$_3$SiH.[24]
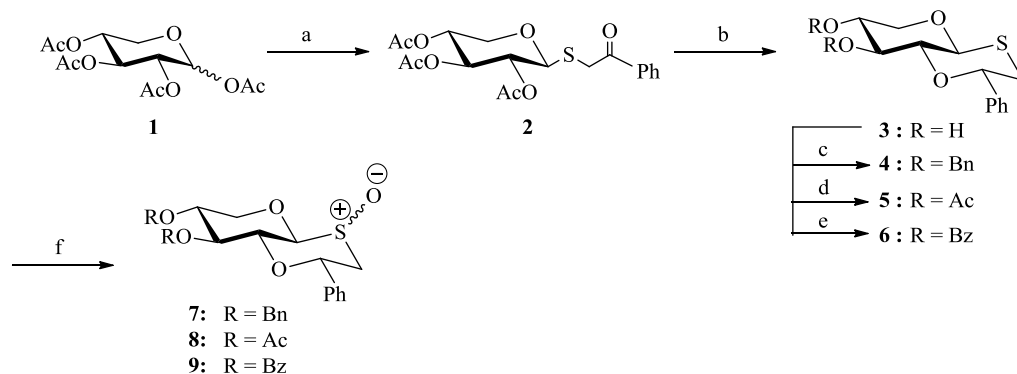
Scheme 2.3: a) i. S=C(NH$_2$)$_2$, BF$_3$.OEt$_2$, CH$_3$CN; ii. 2-bromoacetophenone, Et$_3$N (45%); b) i. NaOMe, MeOH; ii. TMS$_2$O, TMSOTf, 0 $^{\circ}$C, 30 min, then Et$_3$SiH, 3h (65 %, 2 steps); c) BnBr, NaH, DMF (81%); d) Ac$_2$O, Py (89%) ; e) BzCl, Py (77%); f) *m*CPBA, DCM, -78 $^{\circ}$C, 5 min (**7:** 92%; **8:** 90%; **9:** 82%).

In the next step, the 3,4-diol of **3** was protected as benzyl ether, acetyl and benzoyl ester using standard conditions to give fully protected **4**, **5**, and **6** respectively. The oxathianes **4**-**6** were then oxidized by *m*CPBA at -78 $^{\circ}$C to give the corresponding sulfoxides **7**-**9** as mixtures of diastereoisomers (Scheme **2.3**). The successful preparation of these compounds demonstrates that oxathianes can be subjected to a wide variety of protecting group manipulations and can withstand acidic and basic conditions. Having the sulfoxides **7**-**9** in hand, attention was focused on their conversion into anomeric sulfonium ions for subsequent glycosylation. In the first instance, oxathiane **7** was activated with Tf$_2$O in the presence of 1,3,5-trimethoxybenzene in DCE at -20 $^{\circ}$C. After completion of the electrophilic aromatic substitution and formation of the intermediate sulfonium ion, glucose acceptor **11** was added and the reaction was heated to 50 $^{\circ}$C and stirred for 18 h. The crude reaction mixture was then treated with 10% TFA in DCM at 0 $^{\circ}$C for 1 h to afford, after standard work-up and silica gel purification, disaccharide **16** in 79% yield and α/β ratio of 5:1 (Table **2.1**, entry **2**). Next, glycosylation of oxathiane **8** with acceptor **11**, following the same conditions gave disaccharide **17** in 69% and α/β ratio of 12:1 (Table **2.1**, entry **3**). The increase in α-selectivity can be attributed to the presence of electron-withdrawing acetyl groups on the C-3 and C-4 positions of the xylose. These observations are in agreement with previous findings that highly electron withdrawing protecting groups on the oxathiane donor disfavor glycosylation through an

**Table 2.1:** Stereoselective glycosylation using xylose oxathiane donors

7: R^1 = Bn
8: R^1 = Ac
9: R^1 = Bz

15: R^1 = Ac R^2 = Bn R^3 = **11**
16: R^1 = Bn R^2 = H R^3 = **11**
17: R^1 = Ac R^2 = H R^3 = **11**
18: R^1 = Bz R^2 = H R^3 = **11**
19: R^1 = Bz R^2 = H R^3 = **12**
20: R^1 = Bz R^2 = H R^3 = **13**
21: R^1 = Bz R^2 = H R^3 = **14**
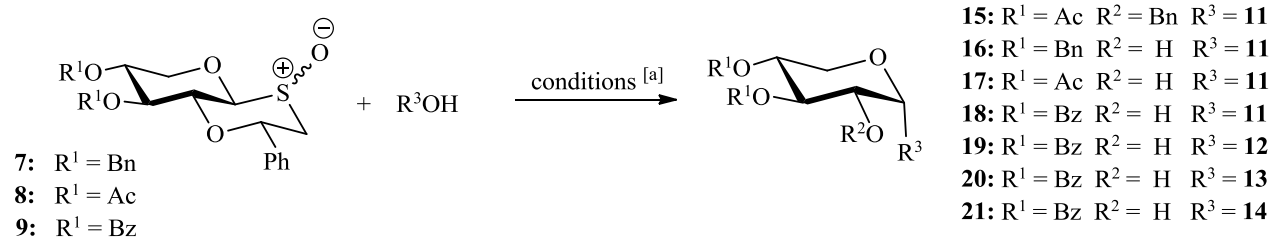
Entry	Donor	Acceptor (R³OH)	yield (%)	α/β
1[b]	**10**	**11**	74	1:1
2	**7**	**11**	79	5:1
3	**8**	**11**	69	12:1
4	**9**	**11**	69	15:1
5	**9**	**12**	77	15:1
6	**9**	**13**	67	15:1
7	**9**	**14**	65	12:1

[a] 1,3,5-Trimethoxybenzene, Tf$_2$O, DTBMP, molecular seives 4 A, -20 °C , 45 min, then add acceptor, -10 °C to 50 °C, 18 h, the crude reaction mixture was treated with 10% TFA in DCM, 0 °C, 1h; [b] TfOH, DCM, -78 °C.

oxacarbenium ion intermediate resulting in excellent α-stereoselectivity.[25] Encouraged by this result, xylosyl donor **9** was activated with Tf_2O in the presence of 1,3,5-trimethoxybenzene to give the intermediate sulfonium ion, which was glycosylated with acceptor **11** to give the corresponding disaccharide **18** in 69% yield and α/β ratio of 15:1 (Table **2.1**). The enhancement in α-selectivity prompted us to explore the versatility of xylosyl donor **9** with different acceptors. Glycosylation of donor **9** with acceptor **12** having a C-4 naphthyl ether and an azido propyl linker at the anomeric position proceeded to give the disaccharide **19** in 77% yields and α/β ratio of 15:1. Furthermore, it was also found that thioglycosides can be employed as glycosyl acceptors and glycosylation of **9** with acceptor **13** led to the formation of disaccharide **20** in 67% yield and α/β ratio of 15:1. This type of glycosylation is attractive because it is to be expected that the thioglycosyl products can be employed as glycosyl donors in subsequent glycosylations using an appropriate thiophilic reagent thereby offering a rapid strategy for oligosaccharide assembly. Finally glycosylation of donor **9** with a secondary alcohol acceptor **14** gave disaccharide **21** in 65% yield and α/β ratio of 12:1 (Table **2.1**). Thus, we have demonstrated that the present reaction methodology, using xylosyl 1,2-oxathiane donors having electron withdrawing protecting groups, give good 1,2-*cis* anomeric selectivity with a variety of glycosyl acceptors.

Synthesis of galactose donor 37: Galactosyl donor **37** (Scheme **2.4**) was synthesized by saponification of the acetyl ester of ethyl 2,3,4-tri-*O*-acetyl thiogalactoside **33** using Zemplén conditions followed by selective benzylation of the C-3 hydroxyl of the resulting compound by treatment with dibutyl tin oxide followed by reaction with benzyl bromide in the presence of CsF in DMF to give **34** in 55% yield.[26] In the next step, the 4, 6-diol of compound **34** is protected as a benzylidene acetal by treatment with $PhCH(OMe)_2$ and camphorsulfonic acid in DMF to afford compound **35** in 60% yield. The C-2 hydroxyl of compound **35** is protected as a levulinyl ester by reacting with levulinic acid, DIC and catalytic DMAP in DCM to afford compound **36** in 82% yield.[27] The anomeric thioethyl of compound **36** is hydrolysed using NIS/TFA to provide the corresponding hemiacetal which is reacted with *N*-(phenyl)-trifluoroacetimidoyl chloride and NaH in DCM to provide the galactosyl donor **37** in 55% yield over two steps.
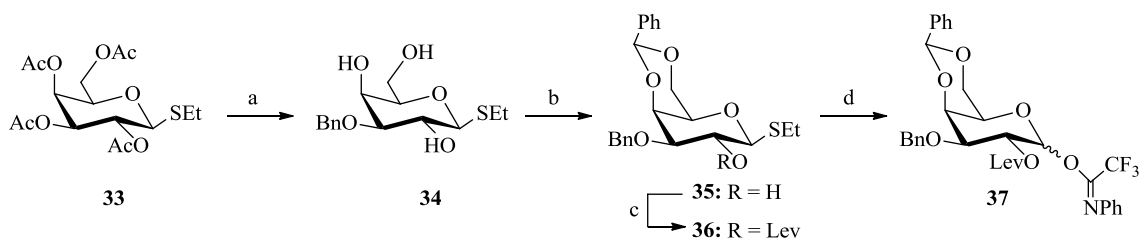
Scheme 2.4: a) i. NaOMe, MeOH; ii. (Bu$_2$Sn)O, MeOH, 80 $^\circ$C, BnBr, CsF, DMF, 16 h (55%, 2 steps); b) PhCH(OMe)$_2$, CSA, reduced pressure, DMF, 60 $^\circ$C, 18h (60%); c) Levulinic acid, DIC, DMAP, DCM (82%); d) i. NIS, TFA, 0 $^\circ$C- rt.; ii. *N*-(phenyl)-trifluoroacetimidoyl chloride, NaH, DCM (55%, 2 steps).

Synthesis of tetrasaccharide 25: With all the building blocks in hand attention was next focused on the synthesis of the target tetrasaccharide **25** (Scheme **2.5**). Thus, a TfOH-mediated glycosylation of galatosyl imidate donor **37** with disaccharide acceptor **19** in DCM followed by treatment of the crude trisaccharide with NH$_2$NH$_2$.HOAc[27] in EtOH/toluene (2/1) gave the trisaccharide **22** in 75% yield and α/β ratio of 1:2. The poor β-selectivity even with the presence of C-2 participating functionality was puzzling. In order to improve the β-selectivity, the glycosylation was carried out in a solvent mixture of CH$_3$CN/DCM (2:1). The β-directing effect of acetonitrile enhanced the β-selectivity and the corresponding trisaccharide **22** was obtained in 77% yield and α/β ratio of 1:4. In the next step, trisaccharide acceptor **22** was glycosylated with fucosylthioglycoside donor **26** using NIS/TfOH as the promoter in Et$_2$O/DCM (2:1) at -30 ^{o}C to give the fully protected tetrasaccharide **23** in a yield of 72% as only the α-anomer. The 4,6-benzylidene acetal on the galactose of tetrasaccharide **23** was removed by treating **23** with EtSH and *p*TSA in DCM for 1 h.[28] Oxidation of the primary hydroxyl of the corresponding diol using TEMPO/BAIB[29] in DCM/H$_2$O (2:1) provided the galacturonic acid tetrasaccharide **24** in 65% yield over two steps. In the final step, tetrasaccharide **24** was deprotected in a two-step procedure involving, saponification of acetyl esters with sodium methoxide in methanol followed by catalytic hydrogenolysis of the benzyl and naphthyl ethers as well as reduction of the azide using Pd(OH)$_2$/C. The structural identity of tetrasaccharide **25** was confirmed by ^{1}H NMR and coupled heteronuclear single quantum coherence (HSQC) experiments.[30] Finally, tetrasaccharide **25** was conjugated to *N*-hydroxy succinimide ester of biotin (biotin NHS) to give the biotinylated tetrasaccharide **27** in 89% yield.
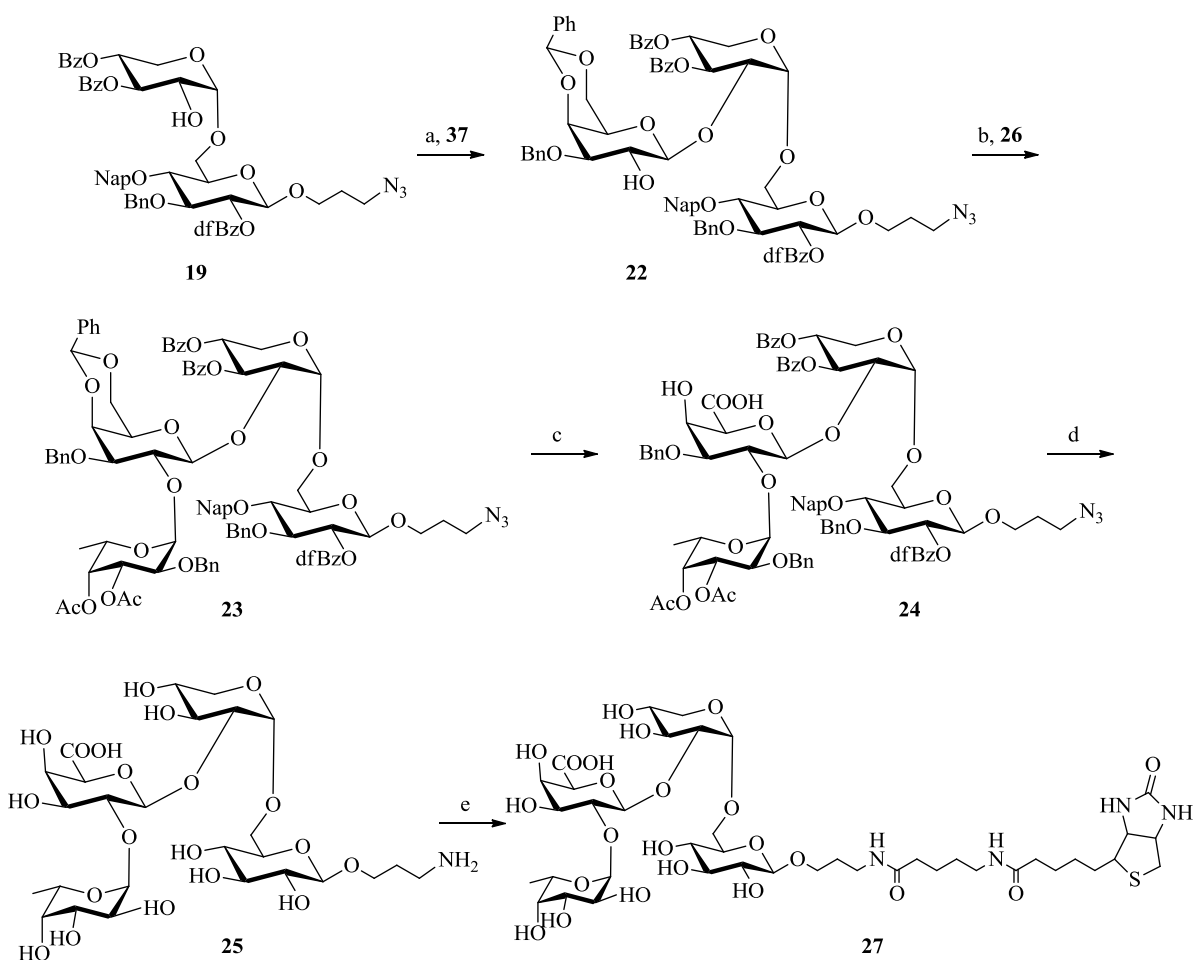
Scheme 2.5: a) i. TfOH, CH$_3$CN/DCM = 2:1, -20 $^{\circ}$C   ii. NH$_2$NH$_2$.HOAc, EtOH/toluene = 2:1 (77%, 2 steps, α/β = 1:4); b) NIS,TfOH, Et$_2$O/DCM = 2:1, -30 $^{\circ}$C (72%, α only); c) i. EtSH, *p*TsOH, DCM, 1h ; ii. TEMPO, BAIB, DCM/H$_2$O = 2:1 (65%, 2 steps); d) i. NaOMe, MeOH; ii. H$_2$, Pd(OH)$_2$/C, *t*BuOH/H$_2$O/AcOH = 40:1:1 (75%, 2 steps); e) Biotin-NHS, Et$_3$N, DMF/H$_2$O (9:1) (89%).

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA was performed by immobilizing the biotinylated tetrasaccharide **27** on to NeutrAvidin coated, pre-blocked clear polystyrene plates. The synthetic tetrasaccharide **Z** along with **Fg** oligosaccharide was screened against 32 monoclonal antibodies which are known to bind to xyloglucans. The ELISA results indicate that mAb CCRC-M102 binds to the **Z** sidechain with high affinity while CCRC-M1 binds with lower affinity. Both these antibodies bind to the **Fg** oligosaccharide with high affinity. While CCRC-M1 is able to distinguish the two

Table 2.2: Determination of monoclonal-antibody binding affinity using ELISA

Entry	mAb	Immunogen	Isoform	Fg	Z
1	M1	Syc RGI	G	0.298	0.034
2	M102	Syc XG	M	0.385	0.285
3	M39	4-MeGX	A	0.001	- 0.001
4	M106	Syc XG	G	0.001	- 0.002
5	M84	Syc PP	M	- 0.001	0.000
6	M87	Tom XG	G	- 0.004	0.004
7	M88	Tom XG	G	0.015	- 0.004
8	M93	Tom XG	G	- 0.003	- 0.002
9	M104	Tom XG	G	- 0.002	0.003
10	M108	Pt Xyl	G	0.010	0.006
11	M109	Pt Xyl	G	0.005	- 0.006
12	M95	Tom XG	G	0.003	- 0.005
13	M101	Tom XG	G	- 0.002	0.002
14	M89	Tom XG	G	0.003	0.000
15	M96	Tom XG	G	0.002	- 0.001
16	M50	Tam XG	G	0.000	0.011
17	M52	Tam XG	G	0.006	0.002
18	M53	Tam XG	G	0.001	0.001
19	M55	Tam XG	G	0.005	0.003
20	M48	Tam XG	G	0.000	0.004
21	M51	Tam XG	G	0.002	0.002
22	M58	Tam XG	G	0.003	0.003
23	M99	Tom XG	G	- 0.001	0.003
24	M111	Pt Xyl	G	0.001	0.000
25	M100	Syc XG	M	- 0.002	- 0.009
26	M103	Syc XG	M	0.000	0.001
27	M86	Tom XG	M	- 0.002	0.005
28	M49	Tam XG	G	0.001	0.009
29	M54	Tam XG	G	0.001	0.006
30	M57	Tam XG	G	0.001	0.005
31	LM15	XXXG	RAT G	0.004	- 0.006
32	LM24	X(XL)G	RAT G	0.015	N.A
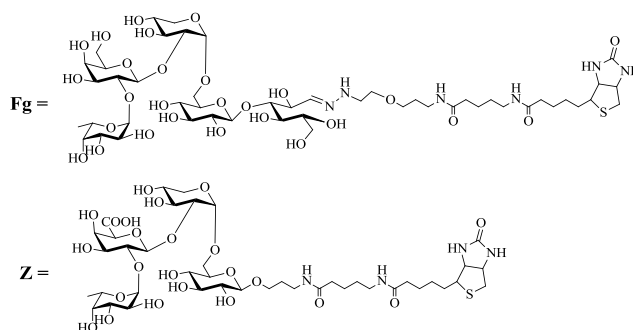
oligosaccharides, mAb CCRC-M102 is unable to distinguish between the Fuc-(1,2)-β-Gal and Fuc-(1,2)-β-GalA terminal residues (Table **2.2**, entry **1** & **2**).

2.4 Conclusion

We describe here, an efficient route for the synthesis of α-L-fucosyl-(1→2)-β-D-galacturonyl(1→2)-α-D-xylosyl-(1→6)-β-D-glucopyranoside which forms part of an unique acidic-xyloglucan side-chain present in the root hair tip of *Arabidopsis thaliana*. Xylose 1,2-oxathiane ether was employed for the stereoselective formation of 1,2-*cis* glycosidic linkage in the synthesis of α-xyloglucan. The 1,2-oxathiane ether was easily installed by a novel one-pot two-step procedure and it was found that the xylose 1,2-oxathiane ethers are stable to commonly employed protecting group manipulations making it possible to install groups such as benzyl ethers as well as acetyl and benzoyl esters. The 1,2-oxathianes could be easily converted to xylosyl donors by oxidation to sulfoxides followed by arylation using 1,3,5-trimethoxybenzene to give bicyclic anomeric sulfonium ions. These donors gave good 1,2-*cis* stereoselectivity in glycosylations with a variety of acceptors. The biotinylated tetrasaccharide **27** was screened against 32 monoclonal antibodies that bind to xyloglucans and their binding affinities were determined using ELISA. Only two monoclonal antibodies, CCRC-M1 and CCRC-M102 showed binding affinity toward tetrasaccharide **27**, but the ELISA study was unable to identify antibodies that bind specifically to the **Z** sidechain as these two antibodies also bind to the **Fg** oligosaccharide.

2.5 Experimental Section

General Procedure:

All reactions were carried out under argon with anhydrous solvents, unless otherwise stated. CH_2Cl_2 was distilled from CaH_2 prior to use in reactions. All the starting materials were kept *in vacuo* with P_2O_5 prior to use. Chemicals used were reagent grade as supplied except where noted. Column chromatography was performed on silica gel G60 (60-200 μm 60 Å). Reactions were monitored by TLC on Silicagel 60 F_{254}. The compounds were detected by examination under UV light and visualized by charring with 10% sulfuric acid in MeOH or cerium ammonium molybdate in 20% aq. sulfuric acid. Solvents were

removed under reduced pressure at ≤ 30 °C. ^{1}H-NMR and HSQC spectra were recorded in CDCl$_3$ at 500 MHz on a Varian Inova spectrometer with trimethylsilane as internal standard, unless otherwise stated. High resolution mass spectra were obtained by using MALDI-ToF with 2,5-dihydroxybenzoic acid as matrix.

2, 3, 4-Tri-*O*-acetyl-1-thio-β-D-xylopyranosyl acetophenone (2)

BF$_3$.OEt$_2$ (17 mL, 141 mmol) was added in portions (3 x 5.8 mL) every 15 min to a solution of thiourea (14 g, 188.4 mmol) and D-xylose-pentaacetate **1** (30g, 94.2 mmol) in acetonitrile (200 mL) at 80 °C. The reaction was heated under reflux for 1 h, and then allowed to cool to room temperature. Triethylamine (41 mL, 295 mmol), followed by 2-bromoacetophenone (37.5g, 188.4 mmol) in acetonitrile (50 mL) were then added to the reaction mixture, which was stirred for 18 h and then concentrated. The residue was dissolved in ethylacetate (100 mL) and washed with saturated NaHCO$_3$ (2 x 75 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (gradient elution 10%-25% EtOAc in hexane) to afford compound **2** (17.4 g, 45%); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.98 –7.44 (m, 5H, ArH), 5.17 (t, *J* = 8.4 Hz, 1H, H-3), 5.06 – 4.87 (m, 2H, H-2, H-4), 4.64 (d, *J* = 8.6 Hz, 1H, H-1), 4.18 (dd, *J* = 11.7,5.1 Hz, 1H, H-5a), 4.05 (s, 1H, SC*H*$_2$), 3.37 (dd, *J* = 11.7, 9.1 Hz, 1H, H-5b), 2.04 (s, 3H, OC*H*$_3$), 2.04 (s, 3H, OC*H*$_3$), 1.95 (s, 3H, OC*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.8, 128.9, 128.8, 82.9, 72.2, 69.6, 69.2, 65.7, 36.0, 20.9, 20.8; HR MALDI-TOF MS: m/z: calcd for C$_{19}$H$_{22}$O$_8$S [M+Na]$^+$: 433.0933; found 433.0964.

2-(*S*)-Phenyl-(1,2-dideoxy-β-D-xylopyranoso) [1,2-*e*]-1, 4-oxathiane (3)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of 2,3,4-Tri-*O*-acetyl-1-thio-β-D-xylopyranosyl acetophenone **2** (15 g, 36.5 mmol) in methanol (30 mL), and the reaction mixture was stirred for 8 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H$^+$ resin, filtered and concentrated to leave a crude solid which was dissolved in anhydrous CH$_3$CN (30 mL) and hexamethyldisiloxane (TMS$_2$O) (46 mL, 0.22 mol) and TMSOTf (3.3 mL, 18.3

mmol) were added at 0 °C. After 30 min, Et$_3$SiH (47 mL, 0.29 mol) was added and the reaction mixture was stirred for another 3 h before quenching by the addition of MeOH (50 mL) and Et$_3$N (10 mL). The solution was concentrated *in vacuo* and the crude was purified by flash chromatography over silica gel (40% EtOAc in hexane) to give **3** (6.4g, 65%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.41 − 7.28 (m, 5H, ArH), 4.68 (dd, *J* = 10.7, 2.0 Hz, 1H, SCH$_2$C*H*Ph), 4.34 (d, *J* = 8.4 Hz, 1H, H-1), 4.09 (dd, *J* = 11.3, 5.5 Hz, 1H, H5a), 3.91 − 3.74 (m, 1H, H-4), 3.66 − 3.43 (m, 2H, H-2, H-3), 3.36 (dd, *J* = 11.3,10.5 Hz, 1H, H-5b), 3.05 (dd, *J* = 14.0, 10.7 Hz, 1H, SCH*H*CHPh), 2.73 (dd, *J* = 14.1, 2.0 Hz, 1H, SC*H*HCHPh); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.9, 126.1, 83.6, 80.6, 76.2, 76.2, 70.4, 70.0, 35.5; HR MALDI-TOF MS: m/z: calcd for C$_{13}$H$_{16}$O$_4$S [M+Na]$^+$: 291.0667; found 291.0632.

2-(*S*)-Phenyl-(3,4-di-*O*-benzyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (4)

Benzyl bromide (0.4 mL, 3.5 mmol) and sodium hydride (160 mg, 4.2 mmol) were added to a stirred solution of **3** (377 mg, 1.4 mmol) in DMF (5 mL). After stirring for 16 h, the reaction mixture was quenched with MeOH (2 mL), diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (100 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (5%-10% EtOAc in hexane) to give **4** (777 mg, 81%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.42 − 7.15 (m, 15H, ArH), 4.89 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.82 − 4.71 (m, 3H, PhC*H*$_2$, SCH$_2$C*H*Ph), 4.65 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.34 (d, *J* = 8.5 Hz, 1H, H-1), 4.06 (dd, *J* = 11.3, 5.4 Hz, 1H, H-5a), 3.76 − 3.57 (m, 3H, H-2, H-3, H-4), 3.35 (t, *J* = 10.9 Hz, 1H, H-5b), 3.03 (dd, *J* = 13.9, 10.7 Hz, 1H, SCH*H*CHPh), 2.78 (dd, *J* = 13.9, 2.1 Hz, 1H, SC*H*HCHPh); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.4, 128.2, 84.6, 83, 80.1, 77.4, 76.5, 75.6, 75.2, 74.3, 74.0, 73.9, 69.6, 35.6; HR MALDI-TOF MS: m/z: calcd for C$_{27}$H$_{28}$O$_4$S [M+Na]$^+$: 471.1607; found 471.1689.

2-(*S*)-Phenyl-(3,4-di-*O*-acetyl-1, 2-dideoxy-β-D-xylopyranoso) [1,2-*e*]-1, 4-oxathiane (5)

Compound **3** (377 mg, 1.4 mmol) was dissolved in pyridine (5 mL) and acetic anhydride (2.5 mL) was added. After stirring for 8 h, the reaction mixture was diluted with DCM

(50 mL) and washed with saturated NaHCO$_3$ (2 x 20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (10% EtOAc in hexane) to give **5** (440 mg, 89%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.45 − 7.18 (m, 5H, ArH), 5.27 (t, *J* = 9.6 Hz, 1H, H-3), 5.16 – 5.01 (m, 1H, H-4), 4.69 (dd, *J* = 10.4, 2.1 Hz, 1H, SCHHC*H*Ph), 4.43 (d, *J* = 8.9 Hz, 1H, H-1), 4.21 (dd, *J* = 11.2, 5.7 Hz, 1H, H-5a), 3.69 (t, *J* = 9.3 Hz, 1H, H-2), 3.44 (t, *J* = 11.1, 1H, H-5b), 2.98 (dd, *J* = 14.0, 10.6 Hz, 1H, CH*H*CHPh), 2.80 (dd, *J* = 14.0, 2.2 Hz, 1H, C*H*HCHPh), 2.05 (s, 3H, COC*H*$_3$), 2.02 (s, 3H, COC*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.6, 125.5, 81.5, 79.8, 79.8, 76.7, 72.6, 69.5, 68, 35.8, 21, 20.9; HR MALDI-TOF MS: m/z: calcd for C$_{17}$H$_{20}$O$_6$S [M+Na]$^+$: 375.0878; found 375.0813.

2-(*S*)-Phenyl-(3,4-di-*O*-benzoyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (6)

Compound **3** (1.3 g, 4.8 mmol) was dissolved in pyridine (15 mL) and benzoyl chloride (5 mL) was added. After stirring for 16 h, the reaction mixture was diluted with DCM (50 mL) and washed with saturated NaHCO$_3$ (2 x 20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (10% EtOAc in hexane) to give **6** (1.7 g, 77%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.00 − 7.16 (m, 15 H, ArH), 5.75 (t, *J* = 9.6 Hz, 1H, H-3), 5.59 – 5.34 (m, 1H, H-4), 4.73 (dd, *J* = 10.6, 2.2 Hz, 1H, SCHHC*H*Ph), 4.58 (d, *J* = 8.8 Hz, 1H, H-1), 4.46 (dd, *J* = 11.2,5.7 Hz, 1H, H-5a), 3.95 (t, *J* = 9.3 Hz, 1H, H-2), 3.62 (t, *J* = 10.9 Hz, 1H, H-5b), 3.03 (dd, *J* = 14.0, 10.6 Hz, 1H, SCH*H*CHPh), 2.85 (dd, *J* = 14.0, 2.2 Hz, 1H, SC*H*HCHPh); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.6, 130.1, 130.0, 128.6, 128.5, 125.6, 125.4, 81.8, 79.7, 77, 73, 70.3, 68.4, 35.8; HR MALDI-TOF MS: m/z: calcd for C$_{27}$H$_{24}$O$_6$S [M+Na]$^+$: 499.1191; found 499.1154.

General procedure for the preparation of sulfoxide donors 7-9 from their corresponding oxathianes 4-6.

*m*CPBA (≤ 77%, 1.1 equiv) was dissolved in DCM and slowly injected into a cooled (-78 ^{o} C) solution of the oxathiane in DCM. The mixture was stirred at -78 ^{o} C for 5 min, diluted with DCM and poured into 10% Na$_2$S$_2$O$_3$ aqueous solution. The organic layer was

washed with saturated NaHCO$_3$, dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel.

2-(*S*)-Phenyl-(3,4-di-*O*-benzyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (*R, S*)-*S*-oxide (7)

Compound **7** (512 mg, 92%, d.r. = 1:1) was prepared as an inseparable mixture of diastereoisomers according to the general procedure for the preparation of sulfoxide donors starting from **4** (538 mg, 1.2 mmol) and using *m*CPBA (268 mg, ≤ 77%, 1.32 mmol); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.50 − 7.12 (m, 30H, ArH), 5.40 (dd, *J* = 11.3, 1.7 Hz, 1H, SCHHC*H*Ph(*R/S*)), 4.90 (d, *J* = 11.6 Hz, 1H, PhCH*H*), 4.87 − 4.73 (m, 6H, 3 x PhC*H$_2$*), 4.72 − 4.60 (m, 2H, PhC*H*H, SCHHC*H*Ph(*R/S*)), 4.49 (t, *J* = 9.1 Hz, 1H, H-2'), 4.26 − 4.08 (m, 3H, H-1, H5a, H-5a'), 4.00 (d, *J* = 9.6 Hz, 1H, H-1'), 3.84 − 3.58 (m, 5H, H-3, H-3', H-4, H-4', SC*H*HCHPh(*R/S*)), 3.53 (dd, *J* = 9.9, 8.4 Hz, 1H, H-2), 3.39 (m, 2H, H-5b, H-5b'), 3.18 (dd, *J* = 14.4, 1.7 Hz, 1H, SC*H*HCHPh(*R/S*)), 3.09 (t, *J* = 12.2 Hz, 1H, SC*H*HCHPh(*R/S*)), 2.76 (dd, *J* = 14.5, 11.3 Hz, 1H, SC*H*HCHPh(*R/S*)); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.9, 128.4, 128.3, 125.9, 95.8, 86, 83, 78.4, 76.7, 76, 75.5, 75.3, 74.2, 74.1, 72.3, 69.4, 68.9, 57.5, 52.9; HR MALDI-TOF MS: m/z: calcd for C$_{27}$H$_{28}$O$_5$S [M+Na]$^+$: 487.1550; found 487.1564.

2-(*S*)-Phenyl-(3,4-di-*O*-acetyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (*R, S*)-*S*-oxide (8)

Compound **8** (277mg, 90%, d.r. = 1:1) was prepared as an inseparable mixture of diastereoisomers according to the general procedure for the preparation of sulfoxide donors starting from **5** (295 mg, 0.83 mmol) and using *m*CPBA (187 mg, ≤ 77%, 0.913 mmol); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 − 7.22 (m, 10H, ArH), 5.53 − 5.30 (m, 3H, H-3, H-3', SCHHC*H*Ph(*R/S*)), 5.07 (m, 2H, H-4, H-4'), 4.72 (d, *J* = 11.8 Hz,1H, SCHHC*H*Ph(*R/S*)), 4.51 (t, *J* = 9.6 Hz, 1H, H-2'), 4.35 (m, 2H, H-5a, H-5a'), 4.26 (d, *J* = 10.0 Hz, 1H, H-1), 4.11 (d, *J* = 9.6 Hz, 1H, H-1'), 3.65 (dd, *J* = 12.8, 1.5 Hz, 1H, SC*H*HCHPh(*R/S*)), 3.59 − 3.44 (m, 3H, H-2, H-5b, H-5b'), 3.22 (dd, *J* = 14.5, 1.7 Hz, 1H, SC*H*HCHPh(*R/S*)), 3.07 (dd, *J* = 12.8, 11.6 Hz, 1H, SC*H*HCHPh(*R/S*)), 2.75 (dd, *J* = 14.5, 11.2 Hz, 1H, SC*H*HCHPh(*R/S*)), 2.06 (s, 3H, COC*H$_3$*), 2.05 (s, 3H, COC*H$_3$*), 2.05

(s, 3H, COCH_3), 2.02 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 129.0, 125.6, 95.6, 85.8, 75.8, 75.6, 72.6, 72.6, 69.5, 68.7, 68.7, 68, 57.7, 53, 52.9, 21, 20.9; HR MALDI-TOF MS: m/z: calcd for C$_{17}$H$_{20}$O$_7$S [M+Na]$^+$: 391.0827; found 391.0877.

2-(*S*)-Phenyl-(3,4-di-*O*-benzoyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (*R, S*)-*S*-oxide (9)

Compound **9** (957 mg, 82%, d.r. = 1.4:1) was prepared as an inseparable mixture of diastereoisomers according to the general procedure for the preparation of sulfoxide donors starting from **6** (1.13 g, 2.37 mmol) and using *m*CPBA (531 mg, ≤ 77%, 2.61 mmol); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.06 − 7.18 (m, 30H, ArH), 5.90 (m, 2H, H-3, H-3'), 5.51 − 5.37 (m, 3H, H-4, H-4', SCHHC*H*Ph(*R/S*)), 4.83 − 4.72 (m, 2H, H-2, SCHHC*H*Ph(*R/S*)), 4.59 (ddd, *J* = 11.1, 5.7, 2.3 Hz, 2H, H-5a, H-5a'), 4.41 (d, *J* = 9.9 Hz, 1H, H-1), 4.26 (d, *J* = 9.6 Hz, 1H, H-1'), 3.83 (dd, *J* = 10.0, 9.3 Hz, 1H, H-2), 3.74 − 3.60 (m, 3H, H-5b, H-5b', SC*H*HCHPh(*R/S*)), 3.26 (dd, *J* = 14.5, 1.7 Hz, 1H, SCH*H*CHPh(*R/S*)), 3.12 (dd, *J* = 12.8, 11.7 Hz, 1H, SCH*H*CHPh(*R/S*)), 2.79 (dd, *J* = 14.2, 11.6, 1H, SCH*H*CHPh(*R/S*)); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.6, 130, 128.9, 128.7, 125.5, 95.9, 86.1, 75.9, 75.7, 73.1, 69.7, 69.3, 68.4, 57.8, 53.0; HR MALDI-TOF MS: m/z: calcd for C$_{27}$H$_{24}$O$_7$S [M+Na]$^+$: 515.1140; found 515.1112.

General glycosylation procedure for oxathiane donors with various acceptors and general procedure for removal of the C-2 auxilliary

A mixture of sulfoxide donor (1.5 equiv), 1,3,5-trimethoxybenzene (2.5 equiv), 2,6-di-*tert*-butyl-4-methyl pyridine (5 equiv), and activated molecular sieves (4 Å) in DCE (adjusted donor concentration to 0.15 M) was stirred for 1 h under an atmosphere of argon. After cooling (-20 °C), trifluoromethanesulfonic anhydride (1.2 equiv) was added. After 45 min, the reaction mixture was cooled (-30 °C) and a solution of acceptor (1 equiv) in DCE (adjusted donor concentration to 0.1 M) was added. The temperature of the reaction mixture was allowed to warm to room temperature and then heated (50 °C) for 18 h, allowed to cool and diluted with DCM (10 mL), washed with 1M HCl (3 x 10 mL), aqueous NaHCO$_3$ (2 x 10 mL). The organic layer is concentrated and dissolved in DCM (10 mL) and trifluoroacetic acid was added dropwise at 0 °C adjusting the final concentration to 10% (v/v). The reaction mixture was stirred for 1 h, diluted with DCM

and poured into saturated NaHCO₃. The organic layer was dried (MgSO₄), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α,β-D-xylopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranoside (15)

Trichloroacetimidate xylosyl donor **10** (83 mg, 0.177 mmol) and glucose acceptor **11** (55 mg, 0.12 mmol) were dissolved in DCM (2 mL) followed by addition of activated molecular sieves (4Å) and stirred at room temperature for 30 min, after which the reaction mixture was cooled (-60 °C), followed by addition of TfOH (1.4 µL, 0.015 mmol). The reaction was stirred for 45 min and quenched with Et₃N (0.5 mL).). The crude was diluted with DCM (50 mL) and washed with saturated NaHCO₃ (25 mL). The organic layer was dried (MgSO₄), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (gradient elution 15%-30% EtOAc in hexane) to afford compound **15** (54 mg, 74%, α/β = 1:1); ^{1}H NMR (300 MHz, CDCl₃) δ 7.43 − 7.11 (m, 40H, ArH), 5.43 (t, *J* = 9.7 Hz, 1H, Xyl H-3), 5.13 (t, *J* = 9.1 Hz, 1H, Xyl' H-3), 5.00 − 4.75 (m, 11H, Xyl H-1, Xyl H-4, Xyl' H-4, 8xPhC*H*H), 4.74 − 4.46 (m, 10H, Glc H-1, Glc' H-1, 8xPhC*H*H), 4.37 (d, *J* = 7.2 Hz, 1H, Xyl' H-1), 4.14 − 3.92 (m, 4H, Glc H-3, Glc' H-3, Glc' H-6a, Xyl' H-5a), 3.85 − 3.56 (m, 8H, Xyl H-5a, Xyl H-5b, Glc H-6a, Glc H-6b, Glc' H-6b, Glc H-4, Glc H-5, Glc' H-5), 3.55 − 3.38 (m, 5H, Glc H-2, Glc' H-2, Glc' H-4, Xyl H-2, Xyl' H-2), 3.37 (s, 3H, OC*H*₃), 3.34 (s, 3H, OC*H*₃), 3.30 − 3.17 (m, 1H, Xyl' H-5b), 2.00 (s, 3H, COC*H*₃), 2.00 (s, 6H,2xCOC*H*₃), 1.90 (s, 3H, COC*H*₃); ^{13}C NMR (75 MHz, CDCl₃) δ 130.6, 128.3, 128.1, 126, 124.8, 103.9, 98.3, 98.2, 97.1, 82.3, 79.8, 78.3, 77.6, 75.7, 75.3, 75, 74.1, 73.6, 73.2, 73.1, 72.6, 71.3, 70.4, 69.7, 68.6, 66.4, 66.2, 62.6, 58.7, 58.6, 55.4, 21; HR MALDI-TOF MS: m/z: calcd for C₄₄H₅₀O₁₂ [M+Na]$^+$:793.3200; found 793.3179.

Methyl 3,4-di-*O*-benzyl-α-D-xylopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (16)

Compound **16** was prepared according to the general glycosylation procedure using xylosyl donor **7** (180 mg, 0.38 mmol) and glycosyl acceptor **11** (120 mg, 0.26 mmol).

Purification by flash chromatography over silica gel afforded compound **16** (158 mg, 79%, α/β = 5:1); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.46 – 7.15 (m, 25H, ArH), 4.99 (d, J = 10.8 Hz, 1H, PhC*H*H), 4.92 (d, J = 11.0 Hz, 1H, PhC*H*H), 4.86 – 4.76 (m, 5H, 4 x PhC*H*H, Xyl H-1), 4.70 – 4.56 (m, 5H, 4 x PhC*H*H, Glc H-1), 4.00 (t, J = 9.2 Hz, 1H, Glc H-3), 3.92 (dd, J = 11.2, 4.5 Hz, 1H, Glc H-6a), 3.77 (ddd, J = 10.1, 4.5, 1.9 Hz, 1H, Glc H-5), 3.69 – 3.45 (m, 6H, Xyl H-5a, Xyl H-5b, Xyl H-2, Glc H-2, Glc H-4, Glc H-6b), 3.36 (s, 3H, OC*H$_3$*), 2.26 (d, J = 7.7 Hz, 1H, O*H*); ^{13}C NMR (75 MHz, CDCl$_3$) δ 142.1, 136, 128.8, 124.3, 120.9, 117.9, 112.1, 106.1, 99.3, 98.3, 82.3, 81.4, 78.5, 76, 75.2, 75.1, 74.9, 74.1, 73.8, 73.5, 73.3, 72.4, 69.9, 67.2, 67.2, 60.5, 55.2; HR MALDI-TOF MS: m/z: calcd for C$_{47}$H$_{52}$O$_{10}$ [M+Na]$^+$:799.3458; found 799.3410.

Methyl 3,4-di-*O*-acetyl-α-D-xylopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17)

Compound **17** was prepared according to the general glycosylation procedure using xylosyl donor **8** (104 mg, 0.30 mmol) and glycosyl acceptor **11** (93 mg, 0.20 mmol). Purification by flash chromatography over silica gel afforded compound **17** (94 mg, 69%, α/β = 12:1); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.46 – 7.15 (m, 15H, Ar*H*), 5.18 (t, J = 9.7 Hz, 1H, Xyl H-3), 5.00 (d, J = 10.8 Hz, 1H, PhC*H*H), 4.94 (d, J = 11.3 Hz, 1H, PhC*H*H), 4.91 – 4.84 (m, 2H, Xyl H-1, Xyl H-4), 4.84 – 4.77 (m, 2H, 2 x PhC*H*H), 4.71 – 4.58 (m, 3H, 2 x PhC*H*H, Glc H-1), 4.00 (t, J = 9.2 Hz, 1H, Glc H-3), 3.91 (dd, J = 11.0, 4.7 Hz, 1H, Glc H-6a), 3.79 (ddd, J = 9.5, 4.8, 2.0 Hz, 1H, Glc H-5), 3.75 – 3.65 (m, 2H, Glc H-6b, Xyl H-5a), 3.63 – 3.44 (m, 4H, Xyl H-5b, Xyl H-2, Glc H-2, Glc H-4), 3.38 (s, 3H, OC*H$_3$*), 2.29 (d, J = 11.3 Hz, 1H, O*H*), 2.09 (s, 3H, COC*H$_3$*), 2.02 (s, 3H, COC*H$_3$*); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.4, 99.1, 98.2, 82.2, 80.4, 77.8, 76.1, 75.9, 75.2, 73.8, 72.9, 71.3, 69.9, 69, 67.7, 67.6, 59.3, 59.2, 55.5, 21.1, 21; HR MALDI-TOF MS: m/z: calcd for C$_{37}$H$_{44}$O$_{12}$ [M+Na]$^+$:703.2730; found 703.2749.

Methyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (18)

Compound **18** was prepared according to the general glycosylation procedure using xylosyl donor **9** (60 mg, 0.12 mmol) and glycosyl acceptor **11** (37 mg, 0.08 mmol).

Purification by flash chromatography over silica gel afforded compound **18** (45 mg, 69%, α/β = 15:1); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.03 − 7.22 (m, 25H, ArH), 5.64 (t, J = 9.7 Hz, 1H, Xyl H-3), 5.26 (td, J = 10.2, 5.7 Hz, 1H, Xyl H-4), 5.05 − 4.93 (m, 3H, 2 x PhC*H*H, Xyl H-1), 4.83 (d, J = 3.1 Hz, 1H, PhC*H*H), 4.79 (d, J = 4.2 Hz, 1H, PhC*H*H), 4.75 − 4.59 (m, 3H, 2 x PhC*H*H, Glc H-1), 4.07 − 3.86 (m, 3H, Glc H-3, Glc H-6a, Xyl H-5a), 3.87 − 3.69 (m, 4H, Xyl H-2, Xyl H-5b, Glc H-5, Glc H-6b), 3.64 − 3.48 (m, 2H, Glc H-2, Glc H-4), 3.42 (s, 3H, OC*H*$_3$), 2.42 (d, J = 11.1 Hz, 1H, O*H*);); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.5, 130.2, 130, 128.5, 128.3, 99.3, 98.3, 82.2, 80.6, 78, 76.1, 75.9, 75.7, 75.3, 73.8, 73.6, 73.4, 70.2, 70.1, 69.9, 67.7, 67.6, 59.4, 59.3, 55.6; HR MALDI-TOF MS: m/z: calcd for C$_{47}$H$_{48}$O$_{12}$ [M+Na]$^+$:827.3043; found 827.3086.

3-azidopropyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-2-methylnaphthyl-α-D-glucopyranoside (19)

Compound **19** was prepared according to the general glycosylation procedure using xylosyl donor **9** (93 mg, 0.18 mmol) and glycosyl acceptor **12** (80 mg, 0.13 mmol). Purification by flash chromatography over silica gel afforded compound **19** (95 mg, 77%, α/β = 15:1); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.16 − 6.96 (m, 25H, ArH), 5.71 (t, J = 9.7 Hz, 1H, Xyl H-3), 5.30 − 5.24 (m, 2H, Xyl H-4, Glc H-2), 5.09 (d, J = 11.3 Hz, 1H, NapCH*H*), 5.02 (d, J = 3.7 Hz, 1H, Xyl H-1), 4.84 (d, J = 9.7 Hz, 1H, PhCH*H*), 4.82 (d, J = 9.9 Hz, 1H, PhCH*H*), 4.72 (d, J = 11.3 Hz, 1H, NapCH*H*), 4.57 (d, J = 8.0 Hz, 1H, Glc H-1), 3.98 − 3.82 (m, 6H, Glc H-3, Glc H-6a, Glc H-6b, Xyl H-2, Xyl H-5a, C*H*H linker), 3.76 (t, J = 10.8 Hz, 1H, Xyl H-5b), 3.73 − 3.66 (m, 2H, Glc H-4, Glc H-5), 3.60 (ddd, J = 9.9, 7.8, 4.6 Hz, 1H, CH*H* linker), 3.31 − 3.16 (m, 2H, C*H*$_2$ linker), 2.51 (d, J = 11.0 Hz, 1H, O*H*), 1.79 − 1.62 (m, 2H, C*H*$_2$ linker); ^{13}C NMR (151 MHz, CDCl$_3$) δ 133.1, 129.8, 129.4, 128.6, 128, 127.8, 126.4, 126.2, 121.4, 118.3, 100.9, 98.7, 82.9, 78.1, 75.4, 75.3, 75.2, 74.3, 74.2, 73.3, 72, 71.2, 69.7, 67.1, 66.8, 66.4, 59.1, 47.9, 28.8; HR MALDI-TOF MS: m/z: calcd for C$_{53}$H$_{49}$F$_2$N$_3$O$_{13}$ [M+Na]$^+$: 996.3131; found 996.310.

Ethyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside (20)

Compound **20** was prepared according to the general glycosylation procedure using xylosyl donor **9** (56 mg, 0.11 mmol) and glycosyl acceptor **13** (44 mg, 0.07 mmol). Purification by flash chromatography over silica gel afforded compound **20** (47 mg, 67%, α/β = 15:1); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.05 − 6.92 (m, 25H, ArH), 5.62 (t, J = 9.7 Hz, 1H, Xyl H-3), 5.27 − 5.16 (m, 2H, Xyl H-4, Glc H-2), 5.01 (d, J = 11.3 Hz, 1H, NapC*H*H), 4.94 (d, J = 3.7 Hz, 1H, Xyl H-1), 4.78 (d, J = 9.0 Hz, 1H, PhC*H*H), 4.75 (d, J = 8.9 Hz, 1H, PhC*H*H), 4.66 (d, J = 11.2 Hz, 1H, NapC*H*H), 4.52 (d, J = 10.0 Hz, 1H, Glc H-1), 3.89 − 3.76 (m, 5H, Glc H-3, Glc H-6a, Glc H-6b, Xyl H-2, Xyl H-5a), 3.71 (t, J = 10.8 Hz, 1H, Xyl H-5b), 3.66 − 3.62 (m, 2H, Glc H-4, Glc H-5), 2.73 − 2.60 (m, 2H, SC*H*$_2$CH$_3$), 1.18 (t, J = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.5, 131.2, 130.1, 129.9, 128.4, 128.2, 128.1, 127.8, 127.7, 126.8, 126.3, 125.8, 125.7, 124, 121.4, 118.6, 118.5, 98.8, 84.5, 83.4, 78.2, 76, 75.4, 75.3, 75.2, 73.5, 73.3, 72.9, 71.3, 69.5, 67.3, 59.1, 59, 23.9, 15.2; HR MALDI-TOF MS: m/z: calcd for C$_{52}$H$_{48}$F$_2$O$_{12}$S [M+Na]$^+$: 957.2732; found 957.2774.

Methyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (21)

Compound **21** was prepared according to the general glycosylation procedure using xylosyl donor **9** (55 mg, 0.11 mmol) and glycosyl acceptor **13** (35 mg, 0.07 mmol). Purification by flash chromatography over silica gel afforded compound **21** (39mg, 65%, α/β = 12:1); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.98 − 6.98 (m, 25H, ArH), 5.63 (t, J = 9.8 Hz, 1H, Xyl H-3), 5.18 (d, J = 3.5 Hz, 1H, Xyl H-1), 5.12 (td, J = 10.1, 5.6 Hz, 1H, Xyl H-4), 5.05 (d, J = 10.5 Hz, 1H, PhC*H*H), 4.83 (d, J = 10.5 Hz, 1H, PhC*H*H), 4.65 (d, J = 12.1 Hz, 1H, PhC*H*H), 4.55 − 4.49 (m, 3H, 2 x PhC*H*H, Glc H-1), 4.47 (d, J = 11.8 Hz, 1H, PhC*H*H), 4.00 (t, J = 9.4 Hz, 1H, Glc H-3), 3.87 − 3.78 (m, 3H, Glc H-4, Glc H-6a, Xyl H-5a), 3.74 − 3.62 (m, 4H, Xyl H-2, Xyl H-5b, Glc H-5, Glc-H-6b), 3.54 (dd, J = 9.6, 3.5 Hz, 1H, Glc H-2), 3.32 (s, 3H, OC*H*$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.2, 130.5, 129.9, 128.2, 128, 100.9, 97.8, 80.5, 80.3, 77.8, 75.5, 73.4, 73.1, 73, 70.7, 70, 69.6,

68.5, 68.4, 60, 55.3; HR MALDI-TOF MS: m/z: calcd for $C_{40}H_{42}O_{12}$ $[M+Na]^+$: 827.3043; found 827.3023.

Ethyl 4,6-*O*-(2-naphthalidene)-1-thio-β-D-glucopyranoside (29)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **28** (15 g, 38.0 mmol) in methanol (30 mL), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H^+ resin, filtered and concentrated to leave crude solid which was dissolved in DMF (30 mL) and 2-dimethoxynaphthalene (15 g, 76 mmol) and camphorsulfonic acid (1.7 g, 7.6 mmol) were added. The reaction mixture was heated to 60 ^{o}C under reduced pressure for 16 h after which it was quenched by adding Et_3N (3 mL). The crude was diluted with DCM (300 mL) and washed with saturated $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (50% EtOAc in hexane) to afford compound **29** (8.35 g, 60%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.97- 7.44 (m, 7H, ArH), 5.71 (s, 1H, NapC*H*<), 4.49 (d, *J* = 9.8 Hz, 1H, H-1), 4.41 (dd, *J* = 10.5, 4.7 Hz, 1H, H-6a), 3.94 – 3.78 (m, 2H, H-3, H-6b), 3.72 – 3.49 (m, 3H, H-4, H-5, H-2), 2.95 – 2.69 (m, 2H, SC*H*$_2$CH$_3$, O*H*), 2.60 (d, *J* = 2.1 Hz, 1H, O*H*), 1.34 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.3, 126.6, 126.0, 123.9, 102.2, 87, 80.6, 74.9, 73.5, 71.1, 68.9, 25.0, 15.6; HR MALDI-TOF MS: m/z: calcd for $C_{19}H_{22}O_5S$ $[M+Na]^+$: 385.1086; found 385.1012.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-1-thio-β-D-glucopyranoside (30)

A solution of ethyl 4,6-*O*-(2-naphthalidene)-1-thio-β-D-glucopyranoside **29** (8 g, 22 mmol) and dibutyltin oxide (6.5g, 26.5 mmol) in methanol (80 mL) was refluxed for 4 h to produce a clear mixture. The solvent was then evaporated and the resultant residue was dissolved in DMF (80 mL) followed by the addition of BnBr (4 mL, 33 mmol) and CsF (4g, 26.5 mmol). The mixture was stirred at room temperature overnight, concentrated and the residue was purified by flash chromatography over silica gel (40 % EtOAc in toluene) to afford compound **30** (5.49 g, 55%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.97 − 7.26

(m, 12H, ArH), 5.73 (s,1H, NapCH<), 4.99 (d, J = 11.7 Hz,1H, PhCHH), 4.84 (d, J = 11.7 Hz, 1H, PhCHH), 4.48 (d, J = 9.6 Hz, 1H, H-1), 4.41 (dd, J = 10.5, 4.9 Hz, 1H, H-6a), 3.89 − 3.67 (m, 3H, H-6b, H-3, H-4), 3.66 − 3.50 (m, 2H, H-5, H-2), 2.76 (q, J = 7.4 Hz, 2H, SCH_2CH$_3$), 2.54 (d, J = 2.0 Hz, 1H, OH), 1.32 (t, J = 7.4 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.4, 128.3, 128.2, 126.6, 125.7, 123.9, 101.8, 87.0, 81.6, 75, 75.0, 73.3, 71.1, 69, 24.9, 15.3; HR MALDI-TOF MS: m/z: calcd for C$_{26}$H$_{28}$O$_5$S [M+Na]$^+$: 475.1555; found 475.1537.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (31)

2,5-difluorobenzoyl chloride (0.82 mL, 6.63 mmol) and 4-dimethylaminopyridine (DMAP) (108 mg, 0.88 mmol) were added to a stirred solution of **30** (2 g, 4.42 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in toluene) to afford compound **31** (2.06 g, 79%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.98 − 7.07 (m, 15H, ArH), 5.77 (s, 1H, NapCH<), 5.33 (dd, J = 10.1, 8.3 Hz, 1H, H-2), 4.89 (d, J = 11.9 Hz, 1H, PhCHH), 4.70 (d, J = 11.9 Hz, 1H, PhCHH), 4.63 (d, J = 10.1 Hz, 1H, H-1), 4.46 (dd, J = 10.5, 4.9 Hz, 1H, H-6a), 3.99 − 3.83 (m, 3H, H-6b,H-4,H-3), 3.68 − 3.55 (m, 1H, H-5), 2.74 (m, 2H, SCH_2CH$_3$), 1.25 (t, J = 7.5 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.3, 128.2, 126.7, 125.7, 123.9, 121.7, 118.7, 118.6, 101.7, 84.3, 81.9, 79.7, 74.6, 74.6, 72.7, 71, 68.9, 24.2, 15.0; HR MALDI-TOF MS: m/z: calcd for C$_{33}$H$_{30}$F$_2$O$_6$S [M+Na]$^+$: 615.1629; found 615.1699.

3-azidopropyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranoside (32)

Thioglycoside donor **31** (509 mg, 0.859 mmol) and 3-azidopropanol (175 mg, 1.72 mmol) were dissolved in DCM (5 mL), followed by addition of molecular sieves (4 Å) and stirring at room temperature for 30 min, after which the reaction was cooled (-20 ^{o}C) and *N*-iodosuccinimide (NIS) (289 mg, 1.28 mmol) was added followed by addition of

TMSOTf (31 µL, 0.17 mmol). The reaction mixture was stirred for 45 min and quenched with saturated $Na_2S_2O_3$ solution (2 mL), diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (15% EtOAc in hexane) to afford compound **32** (358 mg, 66%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.17 – 6.94 (m, 15H, ArH), 5.77 (s, 1H, NapC*H*<), 5.28 (ddd, *J* = 7.7, 6.2, 3.1 Hz, 1H, H-2), 4.89 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.71 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.62 (d, *J* = 7.9 Hz, 1H, H-1), 4.45 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6a), 4.03 – 3.82 (m, 4H, H-3, H-4, H-6a, C*H*H linker), 3.59 (m, 2H, H-5, CH*H* linker), 3.36 – 3.22 (m, 2H, C*H*$_2$ linker), 1.98-1.68 (m, 2H, C*H*$_2$ linker); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.4, 128.2, 128, 126.6, 125.7, 123.8, 121.8, 118.7, 118.2, 101.8, 101.8, 81.8, 78.4, 74.5, 74.1, 68.9, 66.7, 48, 29.0; HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{31}F_2N_3O_7$ [M+Na]$^+$: 654.2028; found 654.2065.

3-azidopropyl 4-*O*-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranoside (12)

Compound **32** (668 mg, 1.05 mmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 °C), triethylsilane (0.5 mL, 3.15 mmol) and dichlorophenylborane (0.47 mL, 3.59 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and Et$_3$N (0.5 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **12** (609 mg, 91%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.92-7.04 (m, 15 H, ArH), 5.22 (t, *J* = 8.6 Hz, 1H, H-2), 5.02 (d, *J* = 11.2 Hz, 1H, PhC*H*H), 4.86 (d, *J* = 5.2 Hz, 1H, PhC*H*H), 4.82 (d, *J* = 5.4 Hz, 1H, PhC*H*H), 4.70 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.00 – 3.88 (m, 2H, H-6a, CH*H* linker), 3.89 – 3.71 (m, 3H, H-3, H-4, H-6b), 3.64 – 3.53 (m, 1H, CH*H* linker), 3.49 (ddd, *J* = 9.3, 4.6, 2.7 Hz, 1H, H-5), 3.28 (m, 2H, C*H*$_2$ linker), 2.01 (t, *J* = 6.7 Hz, 1H, O*H*), 1.88 – 1.70 (m, 2H, C*H*$_2$ linker). ^{13}C NMR (126 MHz, CDCl$_3$) δ 136.1, 134.1, 133.8, 132.8, 128.4, 128.3, 128.3, 128.2, 127.2, 126.4, 126.2, 121.7, 118.6, 118.6, 101.3, 82.8, 77.9, 77.7, 75.7, 75.4,

74.4, 73.2, 72.2, 66.4, 62.5, 62.4, 62, 48, 29; HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{33}F_2N_3O_7$ [M+Na]$^+$: 656.2184; found 656.2198.

Ethyl 4-*O*-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (13)

Compound **31** (287 mg, 0.48 mmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (0.2 mL, 1.44 mmol) and dichlorophenylborane (0.2 mL, 1.63 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and Et$_3$N (0.5 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **13** (195 mg, 68%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.91 – 6.91 (m, 15H, ArH), 5.19 (t, *J* = 9.5 Hz, 1H, H-2), 4.94 (d, *J* = 11.1 Hz, 1H, NapC*H*H), 4.79 (d, *J* = 3.4 Hz, 1H, PhC*H*H), 4.75 (d, *J* = 3.6 Hz, 1H, PhC*H*H), 4.64 (d, *J* = 11.3 Hz, 1H, NapC*H*H), 4.49 (d, *J* = 10.0 Hz, 1H, H-1), 3.94 – 3.75 (m, 2H, H-3, H-6a), 3.75 – 3.64 (m, 2H, H-4, H-6b), 3.44 (ddd, *J* = 9.7, 4.7, 2.6 Hz, 1H, H-5), 2.72 – 2.55 (m, 2H, SC*H*$_2$CH$_3$), 1.17 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) 128.1, 128, 127, 126.2, 126, 125.8, 121.5, 118.5, 118.4, 84.4, 83.7, 79.9, 77.8, 75.5, 73.1, 62.2, 24.5, 15.3; HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{32}F_2O_6$ [M+Na]$^+$: 617.1785; found 617.1741.

Ethyl 3-*O*-benzyl-1-thio-β-D-galatcopyranoside (34)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **33** (10 g, 25 mmol) in methanol (30 mL), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H$^+$ resin, filtered and concentrated to leave crude solid which was dissolved in dry methanol (30 mL), dibutyltin oxide (7.6 g, 30.76 mmol) was added and the solution refluxed for 4 h to produce a clear mixture. The solvent was then evaporated and the resultant residue was dissolved in DMF (30 mL) followed by the addition of BnBr (3.65 mL, 30.7 mmol) and

CsF (4.6 g, 30.7 mmol). The mixture was stirred at room temperature overnight, concentrated and the residue was purified by flash chromatography over silica gel (75% EtOAc in toluene) to afford compound **34** (4.4 g, 55%, 2 steps); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 − 7.26 (m, 5H, ArH), 4.77 (s, 2H, PhC*H$_2$*), 4.31 (d, *J* = 9.8 Hz, 1H, H-1), 4.05 (d, *J* = 3.4 Hz, 1H, H-4), 3.95 (dd, *J* = 11.8, 6.6 Hz, 1H, H-6a), 3.87 − 3.75 (m, 2H, H-2, H-6b), 3.56 − 3.49 (m, 1H, H-5), 3.44 (dd, *J* = 8.9, 3.3 Hz, 1H, H-3), 2.87 − 2.67 (m, 2H, SC*H$_2$*CH$_3$), 1.31 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H$_3$*); ^{13}C NMR (75 MHz, CDCl$_3$) 128.5, 86.5, 81.3, 78.4, 72.4, 69.5, 67.7, 62.8, 62.8, 24.1, 15.2; HR MALDI-TOF MS: m/z: calcd for C$_{15}$H$_{22}$O$_5$S [M+Na]$^+$: 337.1086; found 337.1057.

Ethyl 4,6-*O*-benzylidene-3-*O*-benzyl-1-thio-β-D-galatcopyranoside (35)

Ethyl 3-*O*-benzyl-1-thio-β-D-galatcopyranoside **34** (4 g, 12.8 mmol) was dissolved in DMF (30 mL) and benzaldehyde dimethyl acetal (2.89 mL, 19.2 mmol) and camphorsulfonic acid (594 mg, 2.56 mmol) were added. The reaction mixture was heated (60 °C) under reduced pressure for 16 h after which it was quenched by adding Et$_3$N (5 mL). The crude was diluted with DCM (200 mL) and washed with saturated NaHCO$_3$ (100 mL). The organic layer was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (35 % EtOAc in toluene) to afford compound **35** (3.07 g, 60%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.70 − 7.18 (m, 10H, ArH), 5.45 (s, 1H, PhC*H*<), 4.77 (s, 2H, PhC*H$_2$*), 4.38 − 4.28 (m, 2H, H-1, H-6a), 4.18 (d, *J* = 3.3 Hz, 1H, H-4), 4.07 (td, *J* = 9.4, 1.6 Hz, 1H, H-2), 3.97 (dd, *J* = 12.5, 1.8 Hz, 1H, H-6b), 3.50 (dd, *J* = 9.2, 3.4 Hz, 1H, H-3), 3.41 (s, 1H, H-5), 3.01 − 2.63 (m, 2H, SC*H$_2$*CH$_3$), 2.55 (d, *J* = 1.6 Hz, 1H, O*H*), 1.33 (t, *J* = 7.5 Hz, 3H, SCH$_2$C*H$_3$*); ^{13}C NMR (75 MHz, CDCl$_3$) 128.8, 126.8, 101.6, 85.5, 80.6, 73.9, 71.9, 70.5, 69.8, 68.2, 23.3, 15.4; HR MALDI-TOF MS: m/z: calcd for C$_{22}$H$_{26}$O$_5$S [M+Na]$^+$: 425.1399; found 425.1326.

Ethyl 4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-levulinoyl-1-thio-β-D-galatcopyranoside (36)

Levulinic acid (0.82 mL, 6.63 mmol), *N,N′*-Diisopropylcarbodiimide (DIC) (1.15 mL,7.45 mmol) and 4-dimethylaminopyridine (DMAP) (60 mg, 0.5 mmol) were added to

a stirred solution of **35** (2 g, 4.97 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated $NaHCO_3$ (2 x 50 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (35% EtOAc in hexane) to afford compound **36** (2.03g, 82%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 – 7.17 (m, 10H, ArH), 5.56 – 5.36 (m, 2H, H-2, PhCH<), 4.68 (d, *J* = 1.3 Hz, 2H, PhC*H*$_2$), 4.37 (d, *J* = 9.9 Hz, 1H, H-1), 4.30 (dd, *J* = 12.4, 1.6 Hz, 1H, H-6a), 4.18 (d, *J* = 3.4 Hz, 1H, H-4), 3.97 (dd, *J* = 12.5, 1.8 Hz, 1H, H-6b), 3.60 (dd, *J* = 9.6, 3.4 Hz, 1H, H-3), 3.39 (d, *J* = 1.3 Hz, 1H, H-5), 2.98 – 2.53 (m, 6H, 2 x C*H*$_2$ Lev, SC*H*$_2$CH$_3$), 2.17 (s, 3H, C*H*$_3$ Lev), 1.27 (t, *J* = 7.5 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) 128.7, 127, 126.3, 124.7, 101.7, 83, 78.6, 73.8, 71.5, 70.3, 69.7, 69.6, 68.7, 38, 29.8, 28.1, 22.7, 22.6, 14.8; HR MALDI-TOF MS: m/z: calcd for $C_{27}H_{32}O_7S$ [M+Na]$^+$: 523.1766; found 523.1780.

3-azidopropyl [4,6-*O*-benzylidene-3-*O*-benzyl-β-D-galactopyranosyl]-(1→2)-[3,4-di-*O*-benzoyl-α-D-xylopyranosyl]-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-2-methylnaphthyl-β-D-glucopyranoside (22)

Trifluoroacetic acid (1.5 mL, 1.99 mmol) and *N*-iodosuccinimide (NIS) (537 mg, 2.39 mmol) were added to a cooled (0 ^{o}C) solution of **36** (1.0 g, 1.99 mmol) in DCM (5 mL), reaction mixture was stirred for 3 h. The mixture was diluted in DCM (50 ml) and washed with saturated $Na_2S_2O_8$ (30 ml) solution followed by H_2O (30 mL), the organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (70 % EtOAc in hexane) to afford the hemiacetal (684 mg, 75%). *N*-phenyltrifluoroacetimidoyl chloride (1.5 mL, 7.5 mL) and NaH (43 mg, 1.8 mmol) were added to a solution of the hemiacetal in DCM (5 ml). The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (30% EtOAc in hexane) to afford *N*-phenyltrifluoroacetimidoyl donor **37** (490 mg, 55%). Galactosyl donor **37** (113 mg, 0.18 mmol) and 3-azidopropyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-2-methylnaphthyl-β-D-glucopyranoside **19** (97 mg, 0.10 mmol) were dissolved in CH_3CN/DCM (1.5 mL, 2:1) followed by addition of activated molecular sieves (4Å) and stirring at room temperature was 1 h. The reaction was cooled (- 20 ^{o}C), followed by addition of TfOH (3.2 μL, 0.036

mmol). The reaction was stirred for 45 min, before quenching with Et$_3$N (1 mL). The reaction was washed with saturated NaHCO$_3$, the organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude trisaccharide was dissolved in EtOH/toluene (3 mL, 2:1) and hydrazine acetate (NH$_2$NH$_2$.HOAc) (46 mg, 0.9 mmol) was added and the reaction was stirred for 4 h. The reaction mixture was concentrated, dissolved in DCM (30 mL) and washed with saturated NaHCO$_3$. The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (35% EtOAc in hexane) to afford compound **22** (100 mg, 77%, α/β = 1:4, 2 steps); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.33 – 6.65 (m, 35H, ArH), 5.99 (t, *J* = 9.5 Hz, 1H, Xyl H-3), 5.45 (s, 1H, PhC*H*<), 5.31 (m, 1H, Xyl H-4), 5.29 – 5.18 (m, 2H, Xyl H-1, Glc H-2), 4.92 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.80 – 4.74 (m, 2H, NapC*H$_2$*), 4.68 (m, 3H, 3xPhC*H*H), 4.53 (d, *J* = 7.9 Hz, 1H, Glc H-1), 4.37 (d, *J* = 7.6 Hz, 1H, Gal H-1), 4.26 (d, *J* = 12.3 Hz, 1H, Gal H-6a), 4.08 – 3.72 (m, 11H, Glc H-3, Xyl H-2, Gal H-2, Gal H-4, Gal H-3,Glc H-6a, H-6b, Xyl H-5a,H-5b, Gal H-6b, C*H*H linker), 3.53 (m, 2H, Glc H-4, CH*H* linker), 3.32 (d, *J* = 7.8 Hz, 2H, Glc H-5, Gal H-5), 3.20 (t, *J* = 7.0 Hz, 2H, C*H$_2$* linker), 1.66 (m, 2H, C*H$_2$* linker); ^{13}C NMR (126 MHz, CDCl$_3$) 129.8, 128.4, 128, 127.9, 127.8, 126.4, 125.9, 104.6, 101, 100.6, 98.3, 82.8, 78.6, 78.3, 75.1, 74.4, 73.5, 71.7, 71.6, 70.2, 69, 68, 66.8, 66.4, 58.7, 47.9, 28.7; HR MALDI-TOF MS: m/z: calcd for C$_{79}$H$_{69}$F$_2$O$_{18}$N$_3$ [M+Na]$^+$: 1336.4442; found 1336.4468.

3-azidopropyl [3,4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl]-(1→2)-[4,6-*O*-benzylidene-3-*O*-benzyl-β-D-galactopyranosyl]-(1→2)-[3,4-di-*O*-benzoyl-α-D-xylopyranosyl]-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-β-D-glucopyranoside (23)

A mixture of fucosyl thioglycoside donor **26** (25 mg, 0.065 mmol) and trisaccharide acceptor **22** (43 mg, 0.032 mmol) was dissolved in Et$_2$O/DCM (1.5 mL, 2:1) and activated molecular sieves (4 Å) was added and solution was stirred at room temperature for 1 h, after which the reaction was cooled (-20 ^{o}C) and *N*-iodosuccinimide (NIS) (29 mg, 0.131 mmol) was added followed by addition of TfOH (1.7 μL, 0.019 mmol). The reaction mixture was stirred for 45 min and quenched with saturated Na$_2$S$_2$O$_3$ solution (1 mL), diluted with DCM (10 mL) and washed with saturated NaHCO$_3$ (5 mL). The

organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (gradient elution 20%-35% EtOAc in hexane) to afford compound **23** (38 mg, 72%, α only); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.40 − 6.97 (m, 40H, ArH), 5.93 (t, *J* = 9.7 Hz, 1H, Xyl H-3), 5.46 (d, *J* = 4.1 Hz, 1H, Fuc H-1), 5.37 (s, 2H, Fuc H-4, PhC*H*<), 5.25 − 5.15 (m, 3H, Fuc H-3, Xyl H-4, Glc H-2), 5.12 (d, *J* = 3.3 Hz, 1H, Xyl H-1), 4.89 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.78 − 4.69 (m, 2H, NapC*H*$_2$), 4.67 − 4.60 (m, 2H, Fuc H-5, PhC*H*H), 4.57 (d, *J* = 7.5 Hz, 1H, Gal H-1), 4.53 (d, *J* = 11.9 Hz, 1H, PhC*H*H), 4.50 (d, *J* = 8.0 Hz, 1H, Glc H-1), 4.44 (d, *J* = 12.5 Hz, 1H, PhC*H*H), 4.32 (t, *J* = 12.6 Hz, 2H, Gal H-6a, PhC*H*H), 4.21 (d, *J* = 12.4 Hz, 1H, PhC*H*H), 4.15 (dd, *J* = 10.1, 3.3 Hz, 1H, Xyl H-2), 4.09 − 4.01 (m, 2H, Gal H-2, Gal H-4), 4.01 − 3.95 (m, 2H, Gal H-6b, Glc H-6a), 3.93 − 3.82 (m, 4H, Xyl H-5a,H-5b, Glc H-3, 1xC*H*$_2$ linker), 3.72 (m, 2H, Glc H-6b, Glc H-5), 3.66 (dd, *J* = 10.5, 4.0 Hz, 1H, Fuc H-2), 3.52 (ddd, *J* = 10.6, 7.7, 4.5 Hz, 1H, 1xC*H*$_2$ linker), 3.48 − 3.41 (m, 2H, Gal H-3, Glc H-4), 3.29 (s, 1H, Gal H-5), 3.13 − 3.01 (m, 2H, C*H*$_2$ linker), 2.09 (s, 3H, COC*H*$_3$), 1.90 (s, 3H, COC*H*$_3$), 1.55 (m, 2H, C*H*$_2$ linker), 1.25 (d, *J* = 6.7 Hz, 3H, C*H*$_3$ Fuc); ^{13}C NMR (151 MHz, CDCl$_3$) 133.1, 130.3, 129.5, 128.5, 128.2, 128.2, 128.1, 128.1, 128, 127.5, 127.1, 126.7, 126.3, 126, 125.9, 125.8, 121.4, 118.3, 118.2, 101.3, 100.8, 100.7, 98.2, 97.5, 82.9, 80.7, 78.8, 75.3, 75.2, 75.1, 75, 74.5, 74.4, 74.3, 73.9, 73.7, 72.6, 72.3, 71.7, 71.6, 70.6, 70.5, 70.5, 69.9, 69.2, 69.1, 68.2, 67.8, 67.2, 66.6, 66.4, 66.3, 64.4, 58.3, 47.9, 28.7, 20.9, 20.8, 15.7; HR MALDI-TOF MS: m/z: calcd for C$_{90}$H$_{89}$F$_2$O$_{24}$N$_3$ [M+Na]$^+$: 1656.5702; found 1656.5715.

3-azidopropyl [3,4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl]-(1→2)-[3-*O*-benzyl-β-D-galactopyranosyluronic acid]-(1→2)-[3,4-di-*O*-benzoyl-α-D-xylopyranosyl]-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-β-D-glucopyranoside (24)

To a solution of the tetrasaccharide **23** (35 mg, 0.022 mmol) in DCM (1 mL) was added ethanethiol (9.4 µL, 0.13 mmol) and *p*TsOH (0.82 mg, 0.004 mmol), and the resulting solution was stirred at ambient temperature for 1 h.The reaction mixture was quenched by the addition of Et$_3$N (200 µL) and concentrated *in vacuo*. The crude was stirred vigorously in a mixture of DCM:H$_2$O (1.5 mL, 2:1) followed by addition of TEMPO (1.5 mg, 0.004 mmol) and BAIB (24 mg,0.108 mmol). After stirring at room temperature for

3 h, the mixture was quenched by the addition of saturated $NaHCO_3$ (5 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (60:40:1 EtOAc: hexane: AcOH) to afford compound **24** (22 mg, 65%).

3-aminopropyl-α-L-fucopyranosyl]-(1→2)-β-D-galactopyranosyluronic acid-(1→2)-α-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (25)

Freshly prepared NaOMe in a methanolic solution (0.5 mL, 1 M) was added to a stirred solution of **24** (18 mg, 0.012 mmol) in methanol (2 mL). The reaction mixture was stirred for 2 h and then neutralized by the addition of Dowex® 50W X8-200 H^+ resin. The resin was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in a mixture of *t*BuOH (4 mL), H_2O (0.1 mL), and AcOH (0.1 mL) and a catalytic amount of 20 wt% $Pd(OH)_2$/C was added. The reaction mixture was purged with H_2 gas for 2 min followed by stirring for 6 h under an atmosphere of H_2. The progress of the reaction was monitored by MALDI-TOF mass spectrometer. Upon completion, the reaction mixture was purged with Ar gas followed by filtration through a plug of celite. The filtrate was concentrated *in vacuo* and purified by C18 column (5%-20% MeOH in H_2O) to afford **25** (5 mg, 75% over two steps); ^{1}H NMR (600 MHz, CDCl$_3$) δ 5.12 (d, *J* = 3.9 Hz, 1H, Fuc H-1), 5.10 (d, *J* = 3.5 Hz, 1H, Xyl H-1), 4.48 (d, *J* = 8.1 Hz, 1H, Gal H-1), 4.42 (q, *J* = 6.8 Hz, 1H, Fuc H-5), 4.38 (m, 8.0 Hz, 1H, Glc H-1), 4.03 (m, 1H, Gal H-4), 3.89 (s, 1H, Gal H-5), 3.88 − 3.82 (m, 1H, 1xC*H*$_2$ linker), 3.79 (dd, *J* = 11.3, 4.3 Hz, 1H, Glc H-6a), 3.78 – 3.71 (m, 3H, Gal H-3, Fuc H-3, 1xC*H*$_2$ linker), 3.70 – 3.44 (m, 9H, Glc H-6b, Fuc H-4, Fuc H-2, Glc H-5, Gal H-2, Xyl H-4, Xyl H-3, Xyl H-2, Xyl H-5a), 3.43 – 3.30 (m, 2H, Glc H-4, Glc H-3), 3.13 (t, *J* = 8.6 Hz, 1H, Glc H-2), 3.01 (t, *J* = 6.6 Hz, 2H, C*H*$_2$ linker), 1.85 (m, 2H, C*H*$_2$ linker), 1.10 (d, *J* = 6.5 Hz, 3H, Fuc C*H*$_3$); ^{13}C NMR (151 MHz, D_2O) 102.9, 102.6, 99.1, 97.5, 80.1, 76, 75.7, 75.3, 74.1, 73.4, 72.9, 72.3, 71.9, 70.4, 70, 69.4, 69.2, 68.4, 68.3, 66.7, 66.2, 64.9, 61.3, 60.7, 60.6, 37.9, 26.7, 15; HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{45}O_{20}$ [M+Na]$^+$: 714.2433; found 714.2439.

2-(6-Biotinylamidohexanoyl)aminopropyl-α-L-fucopyranosyl]-(1→2)-β-D-galactopyranosyluronic acid-(1→2)-α-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (27)

3-aminopropyl-α-L-fucopyranosyl]-(1→2)-β-D-galactopyranosyluronic acid-(1→2)-α-D-xylopyranosyl-(1→6)-β-D-glucopyranoside **25** (2mg, 0.0029 mmol) was dissolved in a mixture of DMF/H_2O (0.7 mL, 9:1) and the pH was adjusted to 8-9 using Et_3N. Succinimidyl-6-(biotinamido)hexanoate (2mg, 0.0043 mmol) was added and the reaction was stirred at room temperature for 2 h.The solvent was evaporated and the crude was purified by C18 chromatography (5%-30% MeOH in H_2O) to afford **26** 2.65 mg. 89%); ^{1}H NMR (600 MHz, D_2O) δ 5.08 (t, J = 3.6 Hz, 2H, Fuc H-1, Xyl H-1), 4.49 (d, J = 7.9 Hz, 1H, Gal H-1), 4.46 (dd, J = 7.9, 4.9 Hz, 1H, SCH biotin), 4.38 (q, J = 6.6 Hz, 1H, Fuc H-5), 4.31 (d, J = 8.0 Hz, 1H, Glc H-1), 4.27 (dd, J = 8.0, 4.5 Hz, 1H, CH biotin), 4.02 (d, J = 3.5 Hz, 1H, Gal H-4), 3.87 (s, 1H, Gal H-5), 3.79 (m, 1H, CHH linker), 3.74 (ddd, J = 10.0, 7.6, 2.7 Hz, 3H, Glc H-6a, Fuc H-3, Gal H-3), 3.68 (dd, J = 11.0, 6.3 Hz, 1H, Glc H-6b), 3.66 – 3.44 (m, 10H, Fuc H-2, Fuc H-4, Gal H-2, Glc H-5, Xyl H-2, Xyl H-3, Xyl H-4, Xyl H-5a, Xyl H-5b, CHH linker), 3.35 (t, J = 9.3 Hz, 1H, Glc H-3), 3.24 (t, J = 9.5 Hz, 1H, Glc H-4), 3.21 – 3.16 (m, 1H, CH biotin), 3.16 – 3.08 (m, 3H, Glc H-2, 2xCH_2 linker), 3.05 – 3.00 (m, 2H, CH_2 biotin), 2.84 (dd, J = 13.1, 5.0 Hz, 1H, CHH biotin), 2.63 (d, J = 13.1 Hz, 1H, CHH biotin), 2.11 – 2.07 (m, 4H, 2xCH_2 biotin), 1.73 – 1.61 (m, 2H, CH_2 linker), 1.61 – 1.39 (m, 6H, 3xCH_2 biotin), 1.36 (p, J = 7.0 Hz, 2H, CH_2 biotin), 1.31 – 1.21 (m, 2H, CH_2 biotin), 1.16 (m, 2H, CH_2 biotin), 1.11 – 1.09 (m, 3H, Fuc CH_3); ^{13}C NMR (151 MHz, D_2O) δ 102.7, 102.1, 99.4, 97.6, 79.3, 76.4, 75.7, 75.3, 74.4, 73.5, 73, 72.4, 71.9, 70.5, 69.9, 69.4, 68.3, 67.8, 66.7, 66.2, 65.9, 62, 60.8, 60.2, 55.3, 39.7, 39, 36.2, 35.4, 28.5, 27.9, 27.6, 25.6, 24.9, 15.6; HR MALDI-TOF MS: m/z: calcd for $C_{41}H_{68}N_4O_{23}S$ $[M+Na]^+$: 1039.3893; found 1038.3981.

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CHAPTER III

SYNTHESIS OF HEXASACCHARIDE XYLOGLUCAN FRAGMENT

## CONTAINING A UNIQUE GALACTURONIC-ACID SIDE-CHAIN

3.1 Introduction

Arabidopsis thaliana root hairs contain xyloglucan that is composed of both neutral and acidic subunits.[1] The acidic xyloglucan in the root hair cell walls contains galacturonic acid (GalA) side chain composed of the disaccharide β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→) and the trisaccharide α-L-fucosyl-(1→2)-β-D-galactosyluronic acid (1→2)-α-D-xylosyl-(1→) in addition to neutral side chains. Moreover, xyloglucan containing glacturonic acid is present only in the root hairs and is absent in the cell walls of the leaf, stem and also walls of the other roots cells indicating that the acidic xyloglucan plays an important role in the growth of the root hair tip.[1] *Arabidopsis* produces XXXG-type xyloglucan in which three consecutive glucopyranosyl backbone residues are substituted at the O-6 with a glycosyl side chain.[2] MALDI-TOF MS and ^{1}H-NMR analysis of the oligosaccharide subunits, formed by fragmentation of the xyloglucan backbone with xyloglucan specific endoglucanase (XEG) revealed YXXG and XXZG to be the major acidic subunits in the xyloglucan along with small amounts of YXFG, XXZG, YXYG, and YXLG subunits.[1, 3] Together, the acidic subunits account for at least 30% of the total subunits present in root hair tip xyloglucan. Though, root hairs provide a model system to study plant cell growth, little is known about the role of these acidic xyloglucans in root tip expansion. In this respect, generation of monoclonal antibodies against acidic xyloglucan will not only help in the identification and localization of these structures in the root hairs of different plant species but will also provide fundamental information that will facilitate our understanding of the biological

role of xyloglucan in root hair cell walls.[4] But, due to the practical difficulties in isolation and purification of homogenous, well defined acidic xyloglucan fragments from natural sources, the chemical synthesis of these unique side chains will aid in the generation of well characterized monoclonal antibodies. Herein we report the synthesis of hexasaccharide **1** which contains terminal GalA subunit and will be used to screen monoclonal antibodies which are known to bind to xyloglucans using ELISA based screening assay. Hexasaccharide **1** is equipped with an anomeric aminopropyl spacer which can be conjugated to a carrier protein and used for the generation of monoclonal antibodies using hybridoma cell culture.

1
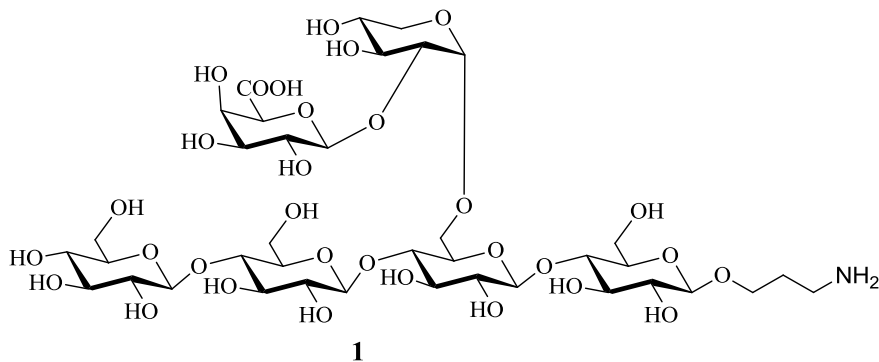
3.2 Results and Discussion

Retrosynthetic Analysis: The target hexasaccharide **1** could be synthesized from its fully protected precursor **2** by first orthogonally deprotecting the 4,6-benzylidene acetal on the galactose and regioselectively oxidizing the C-6 hydroxyl to form the galacturonic acid, followed by global deprotection of the ester and ether protecting groups. Hexasaccharide **2** can in turn be assembled from core disaccharide **6** by first glycosylating with glucose acceptor **7** using NIS/TfOH to give trisaccharide **3,** which has two orthogonal protecting groups, levulinoyl ester on C-2 of the xylose and napthyl ether on C-4 of the glucose on the non-reducing end. Removal of the levulinoyl ester and glycosylation with galactosyl donor **4**, followed by removal of the napthyl ether and
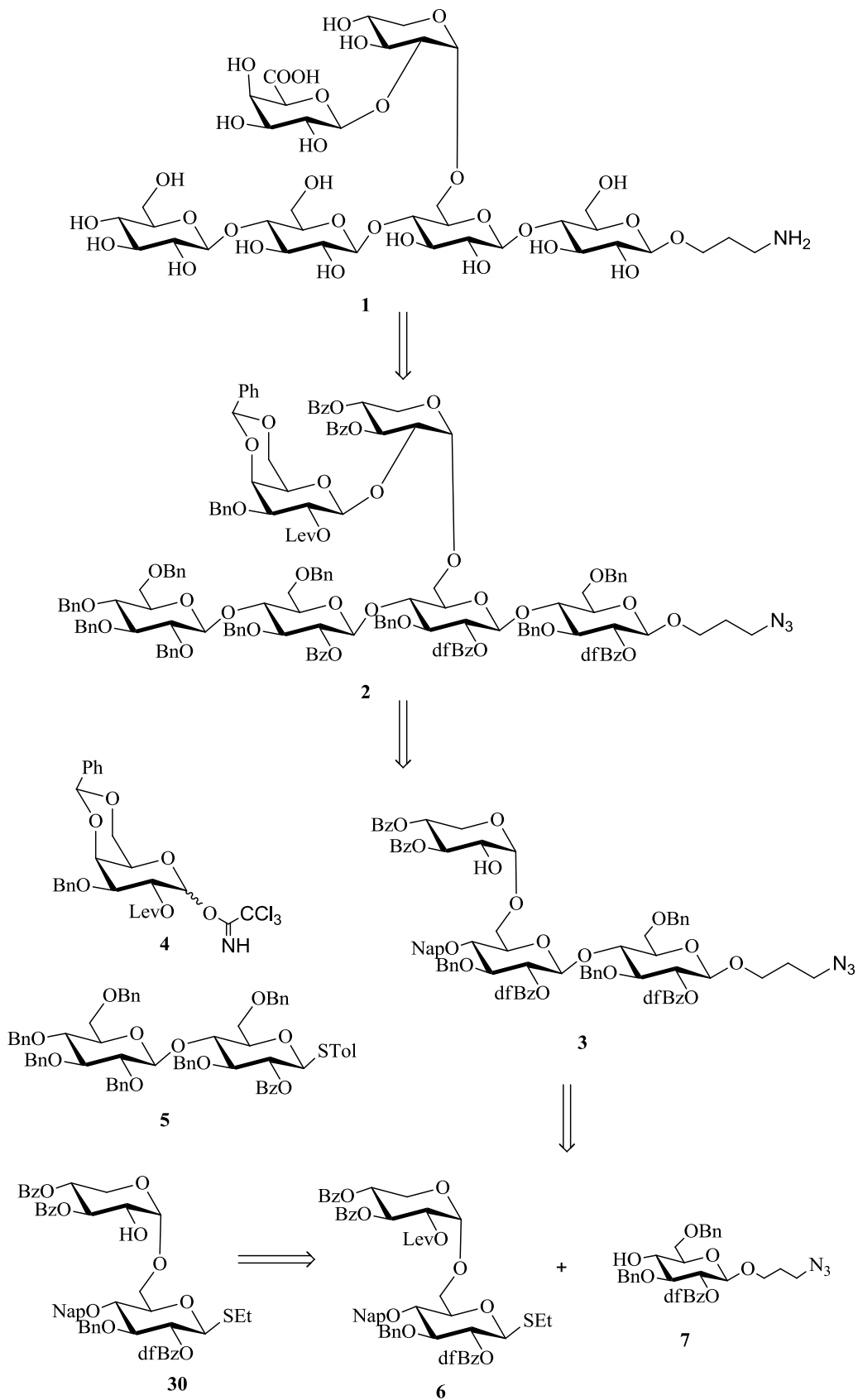
Scheme 3.1: Retrosynthetic analysis

glycosylation with cellobiose thioglycoside donor **5** will yield fully protected hexasaccharide **2**. The synthesis of the core disaccharide **6,** which contains a 1,2-*cis* glycosidic linkage, will be accomplished by using chiral auxiliary mediated glycosylation. Xylosyl 1,2-oxathiane sulfoxide **12**, can be converted to the aryl sulfonium donor, by reaction with Tf$_2$O and 1,3,5-trimethoxybenzene followed by glycosylation with glucosyl acceptor **17** to give disaccharide **6**.[5-6] Thus with a synthetic strategy in place, efforts were focused on the synthesis of the building blocks required for the assembly of the target hexasaccharide **1**.

Preparation of the required building blocks- Synthesis of disaccharide 6: Disaccharide **6** was synthesized by glycosylation of glucose acceptor **17** with xylose 1,2-oxathiane sulfoxide **12**. Compound **17** was readily obtained by saponification of the acetyl ester of ethyl 2,3,4-tri-*O*-acetyl thioglucoside **13** using standard conditions followed by selective protection of the 4,6-diol of the resulting compound as a naphthalidene acetal by treatment with 2-(dimethoxymethyl)-naphthalene[7] and camphorsulfonic acid in DMF to afford compound **14** in 60% yield over two steps. In the next step, the C-3 hydroxyl was regioselectively benzylated by first preparing a stannylidene acetal by treatment with dibutyl tin oxide followed by reaction with benzyl bromide in the presence of CsF in DMF to give **15** in 55% yield. Reacting compound **15** with 2,5-difluorobenzoylchloride with catalytic amount of DMAP in pyridine gave the fully protected compound **16** in 79% yield. The 4,6-naphthalidene acetal on compound **16** was reductively opened using dichlorophenylborane and Et$_3$SiH in DCM at -78 ^{o}C to give the glucose acceptor **17** having a C-6 hydoxyl and C-4 benzyl ether in 68% yield (Scheme **3.2**).[8]
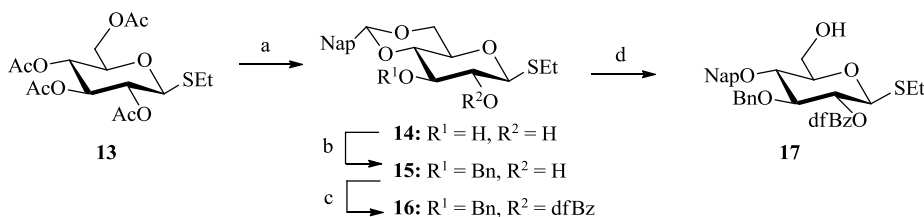
Scheme 3.2: a) i.) NaOMe, MeOH; ii.) 2-(Dimethoxymethyl)-napthalene, CSA, reduced pressure, DMF, 60 ^{o} C, 18h (60%, 2 steps); b) (Bu$_2$Sn)O, MeOH, 80 ^{o} C, BnBr, CsF, DMF, 16 h (55%); c) dfBzCl,DMAP, Py (79%); d) Et$_3$SiH, PhBCl$_2$, DCM, - 78 ^{o} C (68 %).

Xylose sulfoxide **12** was synthesized in four steps starting from peracetylated xylose **8**. Lewis acid activation of the anomeric acetate in the presence of thiourea provided a β-thiouronium salt which was treated with Et$_3$N and 2-bromoacetophenone *in situ*.[9] The resulting thioglycoside **9** was de-esterified under Zemplén conditions to give the corresponding triol which was subsequently converted into oxathiane **10** by a one-pot, two- step reaction involving the treatment of the xylosyl triol with TMS$_2$O in the
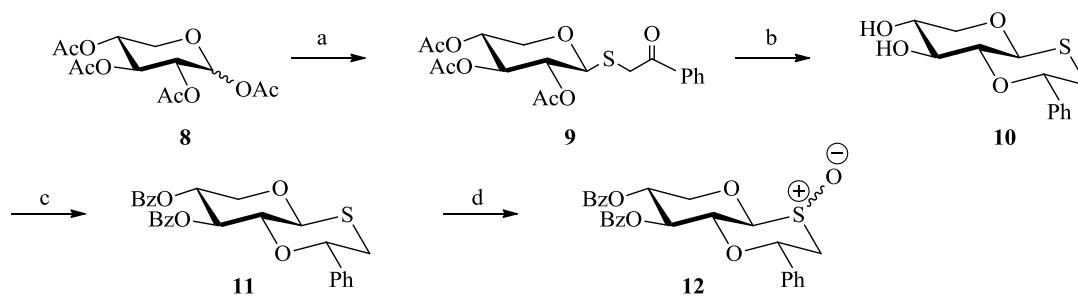
Scheme 3.3: a) i. S=C(NH$_2$)$_2$, BF$_3$.OEt$_2$, CH$_3$CN; ii. 2-bromoacetophenone, Et$_3$N (45%); b) i. NaOMe, MeOH; ii. TMS$_2$O, TMSOTf, 0 °C, 30 min, then Et$_3$SiH, 3h (65 %, 2 steps); c) BzCl, Py (77%); d) *m*CPBA, DCM, -78 °C, 5 min (82%).

presence of TMSOTf to give an intermediate trimethylsilyl acetal, which was reduced by the addition of Et$_3$SiH.[10] In the next step, the 3,4-diol of **10** was protected as benzoyl ester using benzoyl chloride and pyridine to give fully protected compound **11** in 77% yield. Finally, oxidation using *m*CPBA in DCM at -78 °C gave compound **12** as 1:1 mixture of diastereoisomers in 82% yield (Scheme **3.3**). In the next step, oxathiane **12** was activated with Tf$_2$O in the presence of 1,3,5-trimethoxybenzene in DCE at -20 °C. After completion of the electrophilic aromatic substitution and formation of the intermediate sulfonium ion, glucose acceptor **17** was added and the reaction was heated to 50 °C and stirred for 18 h. The crude reaction mixture was then treated with 10% TFA in DCM at 0 °C for 1 h to afford, after standard work-up and silica gel purification, disaccharide **30** in 67% yield and α/β ratio of 15:1. Subsequently, the C-2 hydroxyl of xylose is protected as the levulinoyl ester by reacting **30** with levulinic acid in the presence of DIC, DMAP to afford disaccharide **6** in 87% yield (Scheme **3.4**).[11]

Synthesis of trisaccharide 3: Trisaccharide was synthesized by coupling disaccharide donor **6** with glucosyl acceptor **7** using NIS/TfOH as promoter. Compound **7** was easily obtained starting from compound **24**[12]. Reaction of **24** with 2,5-difluorobenzoyl chloride and DIC with catalytic DMAP in pyridine afforded **25** in 83% yield which was further glycosylated with 3-azido propanol[13] with NIS/TMSOTf[14] as promoter in DCM to provide spacer-containing **26** as only the β-anomer. The 4,6-benzylidene acetal of **26** was reductively opened using Et$_3$SiH/TfOH in DCM at -78 ^{o}C to give glucose acceptor **7** having a C-6 benzyl ether and C-4 hydroxyl in 75% yield (Scheme **3.5**).[8] NIS/TfOH promoted glycosylation of glucose acceptor **7** with disaccharide donor **6**, yielded trisaccharide which was subjected to de-levulination conditions using NH$_2$NH$_2$.HOAc[15] and EtOH/toluene (2:1) to afford trisaccharide acceptor **3** in 57% yield over two steps as only the β anomer (Scheme **3.4**).
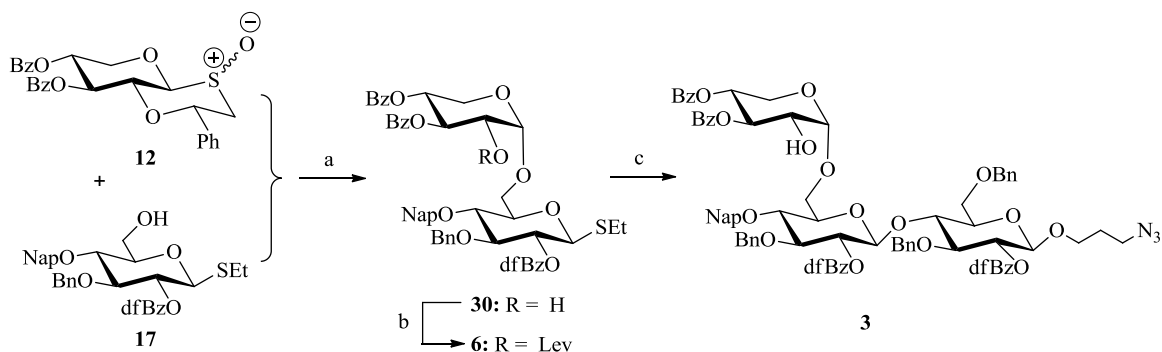

Scheme 3.4: a) 1,3,5-Trimethoxybenzene, Tf$_2$O, DTBMP, molecular seives 4 A, -20 ^{o}C , 45 min, then add acceptor **17**, -10 ^{o}C to 50 ^{o}C, 18 h, the crude reaction mixture was treated with 10% TFA in DCM, 0 ^{o}C, 1h (67%, α/β = 15:1); b) Levulinic acid, DIC, DMAP, DCM (87%); c) i. NIS, TfOH, DCM, 0 ^{o}C; ii. NH$_2$NH$_2$.HOAc, EtOH/toluene (2:1) (57%, 2 steps, β only).
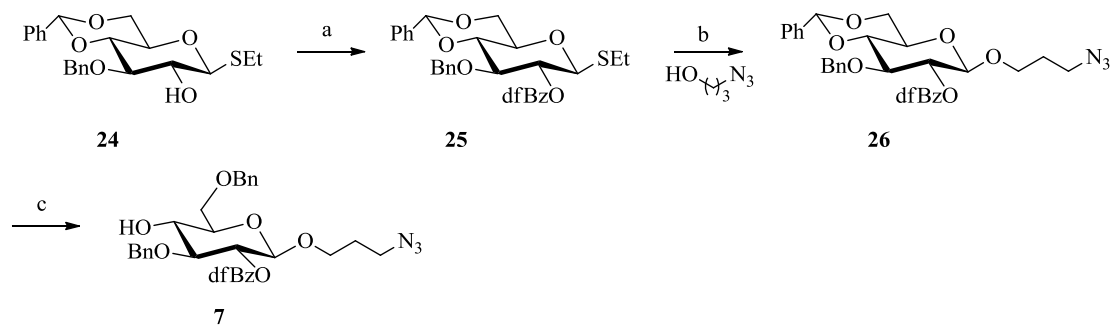
Scheme 3.5: a) dfBzCl, DIC, DMAP, Py (83%); b) NIS, TMSOTf, DCM, - 20 ^{o}C (71%); c) Et$_3$SiH, TfOH, DCM, - 78 ^{o}C (75%).

3.3 Future Work

With trisaccharide acceptor **3** in hand, steps towards the synthesis of hexasaccharide **1** are outlined in Scheme **3.6**. TfOH catalyzed glycosylation of trisaccharide acceptor **3** with galactose imidate donor **4** will afford tetrasaccharide **31**. The naphthyl ether on the C-4 of the glucose can be removed using DDQ in DCM/H_2O (9:1) to give the tetrasaccharide acceptor **32**.[16] NIS/TMSOTf promoted glycosylation of cellobiose thioglycoside donor **5** with **32** will yield the fully protected hexasaccharide **2**. The 4,6-benzylidene acetal on the galactose of hexasaccharide **2** can be removed by reaction with EtSH and *p*TSA in DCM for 1 h.[12] Oxidation of the primary hydroxyl of the corresponding diol using TEMPO/BAIB[7] in DCM/H_2O (2:1) will provide the galacturonic acid containing hexasaccharide. Finally, a two-step global deprotection involving, saponification of acetyl esters with sodium methoxide in methanol followed by catalytic hydrogenolysis of the benzyl and naphthyl ethers as well as reduction of the azide using $Pd(OH)_2/C$ will afford the final target hexassacharide **1**.
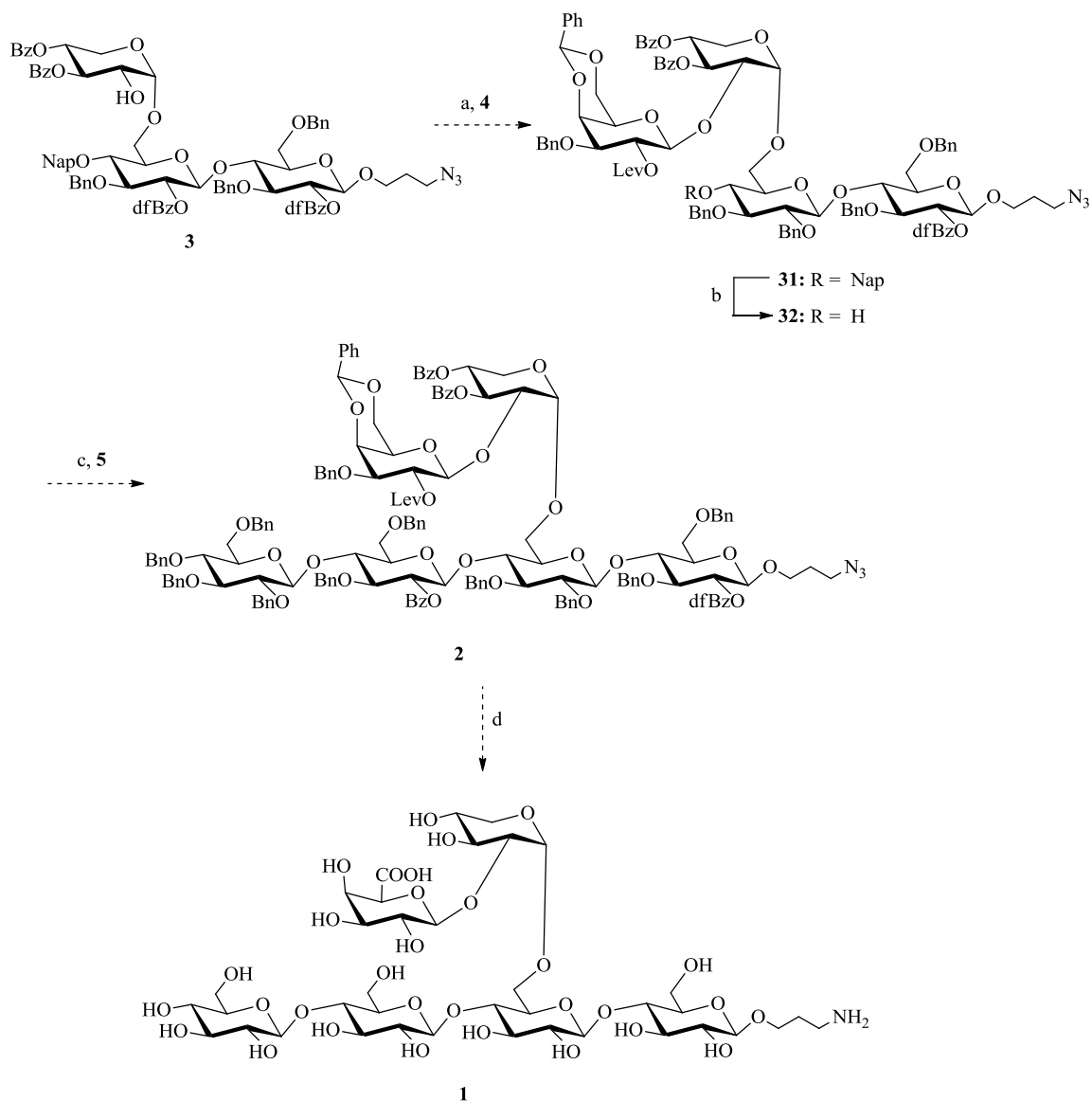
Scheme 3.6: a) i. TfOH, CH$_3$CN/DCM = 2:1, -20 °C; b) DDQ, DCM/H$_2$O (9:1) ; c) NIS/TMSOTf, DCM. 0 °C; d) i. EtSH, *p*TsOH, DCM, 1h ; ii. TEMPO, BAIB, DCM/H$_2$O = 2:1; iii. NaOMe, MeOH; iv. H$_2$, Pd(OH)$_2$/C, *t*BuOH/H$_2$O/AcOH = 40:1:1.

3.4 Experimental Section

General Procedure:

All reactions were carried out under argon with anhydrous solvents, unless otherwise stated. CH$_2$Cl$_2$ was distilled from CaH$_2$ prior to use in reactions. All the starting materials were kept *in vacuo* with P$_2$O$_5$ prior to use. Chemicals used were reagent grade as supplied except where noted. Column chromatography was performed on silica gel G60 (60-200 μm 60 Å). Reactions were monitored by TLC on Silicagel 60 F$_{254}$. The compounds were

detected by examination under UV light and visualized by charring with 10% sulfuric acid in MeOH or cerium ammonium molybdate in 20% aq. sulfuric acid. Solvents were removed under reduced pressure at $\leq$ 30 $^{\circ}$ C. ^{1}H-NMR and HSQC spectra were recorded in CDCl$_3$ at 500 MHz on a Varian Inova spectrometer with trimethylsilane as internal standard, unless otherwise stated. High resolution mass spectra were obtained by using MALDI-ToF with 2, 5-dihydroxybenzoic acid as matrix.

2,3,4-Tri-*O*-acetyl-1-thio-β-D-xylopyranosyl acetophenone (9)

BF$_3$.OEt$_2$ (17 mL, 141 mmol) was added in portions (3 x 5.8 mL) every 15 min to a solution of thiourea (14 g, 188.4 mmol) and D-xylose-pentaacetate **8** (30g, 94.2 mmol) in acetonitrile (200 mL) at 80 $^{\circ}$ C. The reaction was heated under reflux for 1 h, and then allowed to cool to room temperature. Triethylamine (41 mL, 295 mmol), followed by 2-bromoacetophenone (37.5g, 188.4 mmol) in acetonitrile (50 mL) were then added to the reaction mixture, which was stirred for 18 h and then concentrated. The residue was dissolved in ethylacetate (100 mL) and washed with saturated NaHCO$_3$ (2 x 75 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (gradient elution 10%-25% EtOAc in hexane) to afford compound **9** (17.4 g, 45%); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.98 –7.44 (m, 5H, Ar*H*), 5.17 (t, *J* = 8.4 Hz, 1H, H-3), 5.06 – 4.87 (m, 2H, H-2, H-4), 4.64 (d, *J* = 8.6 Hz, 1H, H-1), 4.18 (dd, *J* = 11.7, 5.1 Hz, 1H, H-5a), 4.05 (s, 1H, SC*H*$_2$), 3.37 (dd, *J* = 11.7, 9.1 Hz, 1H, H-5b), 2.04 (s, 3H, OC*H*$_3$), 2.04 (s, 3H, OC*H*$_3$), 1.95 (s, 3H, OC*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.8, 128.9, 128.8, 82.9, 72.2, 69.6, 69.2, 65.7, 36.0, 20.9, 20.8; HR MALDI-TOF MS: m/z: calcd for C$_{19}$H$_{22}$O$_8$S [M+Na]$^{+}$: 433.0933; found 433.0964.

2-(*S*)-Phenyl-(1, 2-dideoxy-β-D-xylopyranoso) [1, 2-*e*]-1, 4-oxathiane (10)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranosyl acetophenone **9** (15 g, 36.5 mmol) in methanol (30 mL), and the reaction mixture was stirred for 8 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H^{+} resin, filtered and

concentrated to leave a crude solid which was dissolved in anhydrous CH_3CN (30 mL) and hexamethyldisiloxane (TMS_2O) (46 mL, 0.22 mol) and TMSOTf (3.3 mL, 18.3 mmol) were added at 0 °C. After 30 min, Et_3SiH (47 mL, 0.29 mol) was added and the reaction mixture was stirred for another 3 h before quenching by the addition of MeOH (50 mL) and Et_3N (10 mL). The solution was concentrated *in vacuo* and the crude was purified by flash chromatography over silica gel (40% EtOAc in hexane) to give **10** (6.4g, 65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 − 7.28 (m, 5H, ArH), 4.68 (dd, *J* = 10.7, 2.0 Hz, 1H, SCH_2CHPh), 4.34 (d, *J* = 8.4 Hz, 1H, H-1), 4.09 (dd, *J* = 11.3, 5.5 Hz, 1H, H5a), 3.91 − 3.74 (m, 1H, H-4), 3.66 − 3.43 (m, 2H, H-2, H-3), 3.36 (dd, *J* = 11.3,10.5 Hz, 1H, H-5b), 3.05 (dd, *J* = 14.0, 10.7 Hz, 1H, SC*H*HCHPh), 2.73 (dd, *J* = 14.1, 2.0 Hz, 1H, SC*H*HCHPh); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.9, 126.1, 83.6, 80.6, 76.2, 76.2, 70.4, 70.0, 35.5; HR MALDI-TOF MS: m/z: calcd for $C_{13}H_{16}O_4S$ [M+Na]$^+$: 291.0667; found 291.0632.

2-(*S*)-Phenyl-(3,4-di-*O*-benzoyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (11)

Compound **10** (1.3 g, 4.8 mmol) was dissolved in pyridine (15 mL) and benzoyl chloride (5 mL) was added. After stirring for 16 h, the reaction mixture was diluted with DCM (50 mL) and washed with saturated $NaHCO_3$ (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (10% EtOAc in hexane) to give **11** (1.7 g, 77%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 − 7.16 (m, 15 H, ArH), 5.75 (t, *J* = 9.6 Hz, 1H, H-3), 5.59 − 5.34 (m, 1H, H-4), 4.73 (dd, *J* = 10.6, 2.2 Hz, 1H, SCHHC*H*Ph), 4.58 (d, *J* = 8.8 Hz, 1H, H-1), 4.46 (dd, *J* = 11.2,5.7 Hz, 1H, H-5a), 3.95 (t, *J* = 9.3 Hz, 1H, H-2), 3.62 (t, *J* = 10.9 Hz, 1H, H-5b), 3.03 (dd, *J* = 14.0, 10.6 Hz, 1H, SCH*H*CHPh), 2.85 (dd, *J* = 14.0, 2.2 Hz, 1H, SC*H*HCHPh); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.6, 130.1, 130.0, 128.6, 128.5, 125.6, 125.4, 81.8, 79.7, 77, 73, 70.3, 68.4, 35.8; HR MALDI-TOF MS: m/z: calcd for $C_{27}H_{24}O_6S$ [M+Na]$^+$: 499.1191; found 499.1154.

2-(*S*)-Phenyl-(3,4-di-*O*-benzoyl-1,2-dideoxy-β-D-xylopyranoso)[1,2-*e*]-1,4-oxathiane (*R, S*)-*S*-oxide (12)

*m*CPBA (531 mg, ≤ 77%, 2.61 mmol) was dissolved in DCM (5 ml) and slowly injected into a cooled (-78 ^{o}C) solution of **11** (1.13 g, 2.37 mmol) in DCM (10 mL). The mixture was stirred at -78 ^{o}C for 5 min, diluted with DCM and poured into 10% $Na_2S_2O_3$ aqueous solution. The organic layer was washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (35% EtOAc in hexane) to afford **12** (957 mg, 82%, d.r. = 1.4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 – 7.18 (m, 30H, ArH), 5.90 (m, 2H, H-3, H-3'), 5.51 – 5.37 (m, 3H, H-4, H-4', SCHHC*H*Ph(*R/S*)), 4.83 – 4.72 (m, 2H, H-2, SCHHC*H*Ph(*R/S*)), 4.59 (ddd, *J* = 11.1, 5.7, 2.3 Hz, 2H, H-5a, H-5a'), 4.41 (d, *J* = 9.9 Hz, 1H, H-1), 4.26 (d, *J* = 9.6 Hz, 1H, H-1'), 3.83 (dd, *J* = 10.0, 9.3 Hz, 1H, H-2), 3.74 – 3.60 (m, 3H, H-5b, H-5b', SC*H*HCHPh(*R/S*)), 3.26 (dd, *J* = 14.5, 1.7 Hz, 1H, SCH*H*CHPh(*R/S*)), 3.12 (dd, *J* = 12.8, 11.7 Hz, 1H, SCH*H*CHPh(*R/S*)), 2.79 (dd, *J* = 14.2, 11.6, 1H, SCH*H*CHPh(*R/S*)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.6, 130, 128.9, 128.7, 125.5, 95.9, 86.1, 75.9, 75.7, 73.1, 69.7, 69.3, 68.4, 57.8, 53.0; HR MALDI-TOF MS: m/z: calcd for $C_{27}H_{24}O_7S$ [M+Na]$^+$: 515.1140; found 515.1112.

Ethyl 4,6-*O*-(2-naphthalidene)-1-thio-β-D-glucopyranoside (14)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **13** (15 g, 38.0 mmol) in methanol (30 mL), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H$^+$ resin, filtered and concentrated to leave crude solid which was dissolved in DMF (30 mL) and 2-dimethoxynaphthaldehyde (15 g, 76 mmol) and camphorsulfonic acid (1.7 g, 7.6 mmol) were added. The reaction mixture was heated (60 ^{o}C) under reduced pressure for 16 h after which it was quenched by adding Et_3N (3 mL). The crude was diluted with DCM (300 mL) and washed with saturated $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (50% EtOAc in hexane) to afford compound **14** (8.35 g, 60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97- 7.44 (m, 7H, ArH), 5.71 (s, 1H,

NapC*H*<), 4.49 (d, *J* = 9.8 Hz, 1H, H-1), 4.41 (dd, *J* = 10.5, 4.7 Hz, 1H, H-6a), 3.94 – 3.78 (m, 2H, H-3, H-6b), 3.72 – 3.49 (m, 3H, H-4, H-5, H-2), 2.95 – 2.69 (m, 2H, SC*H*$_2$CH$_3$, O*H*), 2.60 (d, *J* = 2.1 Hz, 1H, O*H*), 1.34 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.3, 126.6, 126.0, 123.9, 102.2, 87, 80.6, 74.9, 73.5, 71.1, 68.9, 25.0, 15.6; HR MALDI-TOF MS: m/z: calcd for C$_{19}$H$_{22}$O$_5$S [M+Na]$^+$: 385.1086; found 385.1012.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-1-thio-β-D-glucopyranoside (15)

A solution of ethyl 4, 6-*O*-(2-napthalidene)-1-thio-β-D-glucopyranoside **14** (8 g, 22 mmol) and dibutyltin oxide (6.5g, 26.5 mmol) in methanol (80 mL) was refluxed for 4 h to produce a clear mixture. The solvent was then evaporated and the resultant residue was dissolved in DMF (80 mL) followed by the addition of BnBr (4 mL, 33 mmol) and CsF (4g, 26.5 mmol). The mixture was stirred at room temperature overnight, concentrated and the residue was purified by flash chromatography over silica gel (40 % EtOAc in toluene) to afford compound **15** (5.49 g, 55%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.97 – 7.26 (m, 12H, ArH), 5.73 (s,1H, NapC*H*<), 4.99 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.84 (d, *J* = 11.7 Hz, 1H, PhCH*H*), 4.48 (d, *J* = 9.6 Hz, 1H, H-1), 4.41 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.89 – 3.67 (m, 3H, H-6b, H-3, H-4), 3.66 – 3.50 (m, 2H, H-5, H-2), 2.76 (q, *J* = 7.4 Hz, 2H, SC*H*$_2$CH$_3$), 2.54 (d, *J* = 2.0 Hz, 1H, O*H*), 1.32 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.4, 128.3, 128.2, 126.6, 125.7, 123.9, 101.8, 87.0, 81.6, 75, 75.0, 73.3, 71.1, 69, 24.9, 15.3; HR MALDI-TOF MS: m/z: calcd for C$_{26}$H$_{28}$O$_5$S [M+Na]$^+$: 475.1555; found 475.1537.

Ethyl 4,6-*O*-(2-naphthalidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (16)

2,5-difluorobenzoyl chloride (0.82 mL, 6.63 mmol) and 4-dimethylaminopyridine (DMAP) (108 mg, 0.88 mmol) were added to a stirred solution of **15** (2 g, 4.42 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in toluene) to afford compound **16**

(2.06 g, 79%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.98 – 7.07 (m, 15H, ArH), 5.77 (s, 1H, NapC*H*<), 5.33 (dd, J = 10.1, 8.3 Hz, 1H, H-2), 4.89 (d, J = 11.9 Hz, 1H, PhC*H*H), 4.70 (d, J = 11.9 Hz, 1H, PhCH*H*), 4.63 (d, J = 10.1 Hz, 1H, H-1), 4.46 (dd, J = 10.5, 4.9 Hz, 1H, H-6a), 3.99 – 3.83 (m, 3H, H-6b, H-4, H-3), 3.68 – 3.55 (m, 1H, H-5), 2.74 (m, 2H, SC*H*$_2$CH$_3$), 1.25 (t, J = 7.5 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.3, 128.2, 126.7, 125.7, 123.9, 121.7, 118.7, 118.6, 101.7, 84.3, 81.9, 79.7, 74.6, 74.6, 72.7, 71, 68.9, 24.2, 15.0; HR MALDI-TOF MS: m/z: calcd for C$_{33}$H$_{30}$F$_2$O$_6$S [M+Na]$^+$: 615.1629; found 615.1699.

Ethyl 4-*O*-(2-methylnaphthyl)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (17)

Compound **16** (287 mg, 0.48 mmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (0.2 mL, 1.44 mmol) and dichlorophenylborane (0.2 mL, 1.63 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and Et$_3$N (0.5 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **17** (195 mg, 68%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.91 – 6.91 (m, 15H, ArH), 5.19 (t, J = 9.5 Hz, 1H, H-2), 4.94 (d, J = 11.1 Hz, 1H, NapC*H*H), 4.79 (d, J = 3.4 Hz, 1H, PhC*H*H), 4.75 (d, J = 3.6 Hz, 1H, PhC*H*H), 4.64 (d, J = 11.3 Hz, 1H, NapC*H*H), 4.49 (d, J = 10.0 Hz, 1H, H-1), 3.94 – 3.75 (m, 2H, H-3, H-6a), 3.75 – 3.64 (m, 2H, H-4, H-6b), 3.44 (ddd, J = 9.7, 4.7, 2.6 Hz, 1H, H-5), 2.72 – 2.55 (m, 2H, SC*H*$_2$CH$_3$), 1.17 (t, J = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) 128.1, 128, 127, 126.2, 126, 125.8, 121.5, 118.5, 118.4, 84.4, 83.7, 79.9, 77.8, 75.5, 73.1, 62.2, 24.5, 15.3; HR MALDI-TOF MS: m/z: calcd for C$_{33}$H$_{32}$F$_2$O$_6$ [M+Na]$^+$: 617.1785; found 617.1741.

Ethyl 3-*O*-benzyl-1-thio-β-D-galatcopyranoside (19)

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **18** (10 g, 25 mmol) in methanol (30 mL), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H⁺ resin, filtered and concentrated to leave crude solid which was dissolved in methanol (30 mL), dibutyltin oxide (7.6 g, 30.76 mmol) was added and the solution refluxed for 4 h to produce a clear mixture. The solvent was then evaporated and the resultant residue was dissolved in dry DMF (30 mL) followed by the addition of BnBr (3.65 mL, 30.7 mmol) and CsF (4.6 g, 30.7 mmol). The mixture was stirred at room temperature overnight, concentrated and the residue was purified by flash chromatography over silica gel (75% EtOAc in toluene) to afford compound **19** (4.4 g, 55%, 2 steps); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 – 7.26 (m, 5H, ArH), 4.77 (s, 2H, PhC*H*$_2$), 4.31 (d, J = 9.8 Hz, 1H, H-1), 4.05 (d, J = 3.4 Hz, 1H, H-4), 3.95 (dd, J = 11.8, 6.6 Hz, 1H, H-6a), 3.87 – 3.75 (m, 2H, H-2, H-6b), 3.56 – 3.49 (m, 1H, H-5), 3.44 (dd, J = 8.9, 3.3 Hz, 1H, H-3), 2.87 – 2.67 (m, 2H, SC*H*$_2$CH$_3$), 1.31 (t, J = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) 128.5, 86.5, 81.3, 78.4, 72.4, 69.5, 67.7, 62.8, 62.8, 24.1, 15.2; HR MALDI-TOF MS: m/z: calcd for C$_{15}$H$_{22}$O$_5$S [M+Na]⁺ : 337.1086; found 337.1057.

Ethyl 4,6-*O*-benzylidene-3-*O*-benzyl-1-thio-β-D-galatcopyranoside (20)

Ethyl 3-*O*-benzyl-1-thio-β-D-galatcopyranoside **19** (4 g, 12.8 mmol) was dissolved in DMF (30 mL) and benzaldehyde dimethyl acetal (2.89 mL, 19.2 mmol) and camphorsulfonic acid (594 mg, 2.56 mmol) were added. The reaction mixture was heated (60 °C) under reduced pressure for 16 h after which it was quenched by adding Et$_3$N (5 mL). The crude was diluted with DCM (200 mL) and washed with saturated NaHCO$_3$ (100 mL). The organic layer was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (35% EtOAc in toluene) to afford compound **20** (3.07 g, 60%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.70 – 7.18 (m, 10H, ArH), 5.45 (s, 1H, PhC*H*<), 4.77 (s, 2H, PhC*H*$_2$), 4.38 – 4.28 (m, 2H, H-1, H-6a), 4.18 (d, J = 3.3 Hz, 1H, H-4), 4.07 (td, J = 9.4, 1.6 Hz, 1H, H-2), 3.97 (dd, J = 12.5, 1.8 Hz, 1H, H-6b), 3.50 (dd, J = 9.2, 3.4 Hz, 1H, H-3), 3.41 (s, 1H, H-5), 3.01 –

2.63 (m, 2H, SCH_2CH$_3$), 2.55 (d, J = 1.6 Hz, 1H, OH), 1.33 (t, J = 7.5 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (75 MHz, CDCl$_3$) 128.8, 126.8, 101.6, 85.5, 80.6, 73.9, 71.9, 70.5, 69.8, 68.2, 23.3, 15.4; HR MALDI-TOF MS: m/z: calcd for C$_{22}$H$_{26}$O$_5$S [M+Na]$^+$: 425.1399; found 425.1326.

Ethyl 4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-levulinoyl-1-thio-β-D-galatcopyranoside (21)

Levulinic acid (0.82 mL, 6.63 mmol), *N,N′*-Diisopropylcarbodiimide (DIC) (1.15 mL,7.45 mmol) and 4-dimethylaminopyridine (DMAP) (60 mg, 0.5 mmol) were added to a stirred solution of **20** (2 g, 4.97 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (35% EtOAc in hexane) to afford compound **21** (2.03g, 82%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.67 − 7.17 (m, 10H, ArH), 5.56 − 5.36 (m, 2H, H-2, PhCH<), 4.68 (d, J = 1.3 Hz, 2H, PhCH_2), 4.37 (d, J = 9.9 Hz, 1H, H-1), 4.30 (dd, J = 12.4, 1.6 Hz, 1H, H-6a), 4.18 (d, J = 3.4 Hz, 1H, H-4), 3.97 (dd, J = 12.5, 1.8 Hz, 1H, H-6b), 3.60 (dd, J = 9.6, 3.4 Hz, 1H, H-3), 3.39 (d, J = 1.3 Hz, 1H, H-5), 2.98 − 2.53 (m, 6H, 2 x CH_2 Lev, SCH_2CH$_3$), 2.17 (s, 3H, CH_3 Lev), 1.27 (t, J = 7.5 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (75 MHz, CDCl$_3$) 128.7, 127, 126.3, 124.7, 101.7, 83, 78.6, 73.8, 71.5, 70.3, 69.7, 69.6, 68.7, 38, 29.8, 28.1, 22.7, 22.6, 14.8; HR MALDI-TOF MS: m/z: calcd for C$_{27}$H$_{32}$O$_7$S [M+Na]$^+$: 523.1766; found 523.1780.

Ethyl 6-*O*-acetyl-3, 4-*O*-benzyl-2-*O*-levulinoyl-1-thio-β-D-galatcopyranoside (22)

Compound **21** (295 mg, 0.58 mmol) was dissolved in dry DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (0.28 mL, 1.44 mmol) and dichlorophenylborane (0.26 mL, 1.63 mmol) were added and the reaction stirred for 2 min followed by the addition of MeOH (1 mL) and Et$_3$N (0.5 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in pyridine (2 mL) and acetic anhydride (1 mL) was added. After stirring for 4 h, the reaction mixture was diluted with DCM (50 mL) and

washed with saturated NaHCO$_3$ (2 x 20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude was purified by flash chromatography over silica gel (20% EtOAc in hexane) to give **22** (140 mg, 45%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.64 − 7.19 (m, 10H, ArH), 5.45 (t, *J* = 9.8 Hz, 1H, H-2), 4.99 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.73 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.65 (dd, *J* = 14.7, 11.9 Hz, 2H, 2xPhC*H*H), 4.36 (d, *J* = 9.9 Hz, 1H, H-1), 4.23 (dd, *J* = 11.3, 6.7 Hz, 1H, H-6a), 4.09 (dd, *J* = 11.4, 5.8 Hz, 1H, H-6b), 3.87 (d, *J* = 2.7 Hz, 1H, H-4), 3.65 − 3.56 (m, 2H, H-3, H-5), 2.98 − 2.45 (m, 6H, 2xC*H*$_2$ Lev, SC*H*$_2$CH$_3$), 2.20 (s, 3H, COC*H*$_3$ Lev), 2.00 (s, 3H, OC*H*$_3$), 1.26 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 130.4, 128.2, 128.1, 125.7, 83.7, 81.5, 76.3, 74.3, 74.2, 74, 72.7, 72.7, 72.6, 70.5, 70, 63.3, 37.8, 30, 28.2, 26, 23.9, 21, 15; HR MALDI-TOF MS: m/z: calcd for C$_{29}$H$_{36}$O$_8$S [M+Na]$^+$: 567.2029; found 567.2067.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate-6-*O*-acetyl-3,4-*O*-benzyl-2-*O*-levulinoyl-α-D-galatcopyranoside (23)

Trifluoroacetic acid (20 µL, 0.257 mmol) and *N*-iodosuccinimide (NIS) (69 mg, 0.308 mmol) were added to a cooled (0 °C) solution of **22** (140 g, 0.257 mmol) in DCM (3 mL), reaction mixture was stirred for 3 h. The mixture was diluted in DCM (30 ml) and washed with saturated Na$_2$S$_2$O$_8$ (20 ml) solution followed by H$_2$O (20 mL), the organic layer was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (70 % EtOAc in hexane) to afford the hemiacetal (90 mg, 70%). *N*-phenyltrifluoroacetimidoyl chloride (0.18 mL, 0.9 mmol) and NaH (25 mg, 1.08 mmol) were added to a solution of the hemiacetal in DCM (5 ml). The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The resulting residue was purified by flash chromatography over silica gel (30% EtOAc in hexane) to afford compound **23** (82 mg, 68%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.50 − 7.06 (m, 15H, ArH), 5.76 − 5.54 (m, 2H, H-1, H-2), 4.99 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.75 (d, *J* = 12.2 Hz, 1H, PhCH*H*), 4.67 (dd, *J* = 11.9, 6.7 Hz, 2H, PhC*H*$_2$), 4.28 (dd, *J* = 11.4, 7.1 Hz, 1H, H-6a), 4.16 (dd, *J* = 11.5, 5.5 Hz, 1H, H-6b), 3.92 − 3.84 (m, 1H, H-4), 3.76 − 3.61 (m, 2H, H-5, H-3), 2.88 − 2.68 (m, 2H, C*H*$_2$ Lev), 2.66 − 2.51 (m, 2H, C*H*$_2$ Lev), 2.19 (s, 3H, COC*H*$_3$ Lev), 1.97 (s, 3H, COC*H*$_3$); ^{13}C NMR

(126 MHz, CDCl$_3$) δ 128.7, 128.4, 128.2, 126.1, 125.9, 124.4, 119.2, 94.8, 79.5, 74.3, 74.2, 73.4, 72.8, 72.1, 70.3, 62.8, 37.8, 29.9, 27.9, 20.9; HR MALDI-TOF MS: m/z: calcd for C$_{35}$H$_{36}$O$_9$F$_3$N [M+Na]$^+$: 694.2240; found 694.2137.

Ethyl   4,6-*O*-(benzylidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-1-thio-β-D-glucopyranoside (25)

2,5-difluorobenzoyl chloride (0.92 mL, 7.45 mmol) and 4-dimethylaminopyridine (DMAP) (60 mg, 0.49 mmol) were added to a stirred solution of **24** (2 g, 4.97 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in toluene) to afford compound **25** (2.23 g, 83%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.89 – 6.88 (m, 13H, Ar*H*), 5.61 (s, 1H, PhC*H*<), 5.31 (dd, *J* = 10.1, 8.3 Hz, 1H, H-2), 4.87 (d, *J* = 11.9 Hz, 1H, PhC*H*H), 4.68 (d, *J* = 11.9 Hz, 1H, PhCH*H*), 4.61 (d, *J* = 10.1 Hz, 1H, H-1), 4.41 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.99 – 3.73 (m, 3H, H-3, H-4, H-6b), 3.56 (td, *J* = 9.5, 4.9 Hz, 1H, H-5), 2.73 (m, 2H, SC*H*$_2$CH$_3$), 1.24 (t, *J* = 7.5 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 138.1, 137.4, 129.3, 128.5, 128.4, 128.2, 127.8, 126.2, 121.8, 121.7, 121.5, 121.4, 118.8, 118.7, 118.5, 118.4, 101.5, 84.3, 81.8, 79.7, 74.7, 72.6, 71, 68.8, 24.2, 15; HR MALDI-TOF MS: m/z: calcd for C$_{29}$H$_{28}$F$_2$O$_6$S [M+Na]$^+$: 565.1472; found 565.1489.

3-azidopropyl-4,6-*O*-(benzylidene)-3-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranoside (26)

Thioglycoside donor **25** (2.0 g, 3.69 mmol) and 3-azidopropanol (563 mg, 5.53 mmol) were dissolved in DCM (8 mL), followed by addition of molecular sieves (4 Å) and stirring at room temperature for 30 min, after which the reaction was cooled (-20 ^{o}C) and *N*-iodosuccinimide (NIS) (1.24 g, 5.53 mmol) was added followed by addition of TMSOTf (0.13 mL, 0.74 mmol). The reaction mixture was stirred for 45 min and quenched with saturated Na$_2$S$_2$O$_3$ solution (5 mL), diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography

over silica gel (15% EtOAc in hexane) to afford compound **26** (1.58 g, 71%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.69 – 6.86 (m, 13H, ArH), 5.61 (s, 1H, PhC*H*<), 5.25 (t, *J* = 8.4 Hz, 1H, H-2), 4.87 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.68 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.59 (d, *J* = 8.0 Hz, 1H, H-1), 4.39 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 4.00 – 3.89 (m, 1H, C*H*$_2$ linker), 3.89 – 3.79 (m, 3H, H-3, H-4, H-6a), 3.62 – 3.46 (m, 2H, H-5, C*H*$_2$ linker), 3.29 (m, 2H, C*H*$_2$ linker), 1.95 – 1.64 (m, 2H, C*H*$_2$ linker); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.8, 128.2, 126.4, 126.3, 101.8, 101.6, 81.8, 78.5, 74.6, 74.1, 68.9, 66.7, 48.1, 29; HR MALDI-TOF MS: m/z: calcd for C$_{30}$H$_{29}$F$_2$N$_3$O$_7$ [M+Na]$^+$: 604.1871; found 604.1843.

3-azidopropyl 3, 6-*O*-benzyl-2-*O*-difluorobenzoyl-β-D-glucopyranoside (7)

Compound **26** (198 mg, 0.34 mmol) was dissolved in DCM (5 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o}C), triethylsilane (163 μL, 1.02 mmol) and TfOH (102 μL, 1.15 mmol) were added. After 30 min, the reaction was quenched by the addition of MeOH (1 mL) and Et$_3$N (0.5 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **7** (148 mg, 758%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.80 – 6.79 (m, 13H, ArH), 5.20 (dd, *J* = 9.5, 8.0 Hz, 1H, H-2), 4.75 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.72 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.63 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.58 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.50 (d, *J* = 8.0 Hz, 1H, H-1), 3.92 (dt, *J* = 10.4, 5.5 Hz, 1H, C*H*$_2$ linker), 3.83 – 3.74 (m, 3H, H-4, H-6a, H-6b), 3.66 (t, *J* = 9.2 Hz, 1H, H-3), 3.58 – 3.50 (m, 2H, H-5, C*H*$_2$ linker), 3.26 (m, 2H, C*H*$_2$ linker), 1.87 – 1.67 (m, 2H, C*H*$_2$ linker); ^{13}C NMR (126 MHz, CDCl$_3$) δ 128.3, 128.2, 128.1, 121.4, 121.3, 118.5, 118.3, 118.2, 101, 82.2, 74.6, 73.9, 73.8, 72.4, 70.3, 66.2, 47.8, 29; HR MALDI-TOF MS: m/z: calcd for C$_{30}$H$_{31}$F$_2$N$_3$O$_7$ [M+Na]$^+$: 606.2028; found 606.2039.

Ethyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside (30)

Compound **12** (56 mg, 0.11 mmol) was dissolved in DCE (2 mL) and stirred with activated molecular sieves (4 Å) for 1 h. The reaction mixture was cooled (-20 ^{o}C), Tf$_2$O

(60 μL, 0.35 mmol), DTBMP (303 mg, 1.47 mmol) and 1,3,5-trimethoxybenzene (124 mg, 0.739 mmol) was added. After stirring for 45 min, glycosyl acceptor **17** (44 mg, 0.07 mmol) was added and the reaction mixture was heated (50 $^{\circ}$C) for 18 h. The reaction mixture was allowed to cool and diluted with DCM (20 mL), washed with 1 M HCl (10 mL) followed by aqueous $NaHCO_3$ (10 mL). The organic phase was concentrated and dissolved in DCM (10 mL) and cooled (0 $^{\circ}$C), followed by addition of TFA (1 mL). After stirring for 1 h, the reaction mixture was diluted with DCM (20 mL) and washed with aqueous $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **30** (47 mg, 67%, α/β = 15:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 – 6.92 (m, 25H, ArH), 5.62 (t, *J* = 9.7 Hz, 1H, Xyl H-3), 5.27 – 5.16 (m, 2H, Xyl H-4, Glc H-2), 5.01 (d, *J* = 11.3 Hz, 1H, NapC*H*H), 4.94 (d, *J* = 3.7 Hz, 1H, Xyl H-1), 4.78 (d, *J* = 9.0 Hz, 1H, PhC*H*H), 4.75 (d, *J* = 8.9 Hz, 1H, PhC*H*H), 4.66 (d, *J* = 11.2 Hz, 1H, NapC*H*H), 4.52 (d, *J* = 10.0 Hz, 1H, Glc H-1), 3.89 – 3.76 (m, 5H, Glc H-3, Glc H-6a, Glc H-6b, Xyl H-2, Xyl H-5a), 3.71 (t, *J* = 10.8 Hz, 1H, Xyl H-5b), 3.66 – 3.62 (m, 2H, Glc H-4, Glc H-5), 2.73 – 2.60 (m, 2H, SC*H*$_2$CH$_3$), 1.18 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.5, 131.2, 130.1, 129.9, 128.4, 128.2, 128.1, 127.8, 127.7, 126.8, 126.3, 125.8, 125.7, 124, 121.4, 118.6, 118.5, 98.8, 84.5, 83.4, 78.2, 76, 75.4, 75.3, 75.2, 73.5, 73.3, 72.9, 71.3, 69.5, 67.3, 59.1, 59, 23.9, 15.2; HR MALDI-TOF MS: m/z: calcd for $C_{52}H_{48}F_2O_{12}S$ [M+Na][+] : 957.2732; found 957.2774.

Ethyl 3,4-di-*O*-benzoyl-α-D-xylopyranosyl-(1→6)-2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside (6)

Levulinic acid (79 mg, 0.683 mmol), *N,N′*-Diisopropylcarbodiimide (DIC) (52 μL, 0.342 mmol) and 4-dimethylaminopyridine (DMAP) (4.2 mg, 0.034 mmol) were added to a stirred solution of **30** (320 mg, 0.342 mmol) in DCM (5 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (50 mL) and washed with saturated $NaHCO_3$ (2 x 25 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in hexane) to afford compound **6** (295mg, 83%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 – 6.91

(m, 25H, ArH), 6.01 (t, J = 9.9 Hz, 1H, Xyl H-3), 5.29 (m, 2H, Xyl H-1, Xyl H-4), 5.22 (t, J = 9.6 Hz, 1H, Glc H-2), 5.14 – 5.08 (m, 2H, Xyl H-2, PhCHH), 4.94 (d, J = 11.3 Hz, 1H, PhCHH), 4.82 (d, J = 11.3 Hz, 1H, PhCHH), 4.70 (d, J = 11.2 Hz, 1H, PhCHH), 4.56 (d, J = 10.0 Hz, 1H, Glc H-1), 4.01 (dd, J = 10.9, 5.9 Hz, 1H, Xyl H-5a), 3.97 – 3.86 (m, 3H, Glc H-3, Glc H-6a, Glc H-6b), 3.82 (m, 2H, Glc H-4, Xyl H-5b), 3.67 (m, 1H, Glc H-5), 2.79 – 2.48 (m, 6H, 2xCH_2 Lev, SCH_2CH$_3$), 2.01 (s, 3H, OCH_3), 1.26 (t, J = 7.4 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.4, 130, 129.9, 128.4, 128.3, 128, 127.9, 127.7, 126.9, 126, 125.7, 121.3, 118.4, 96.1, 84.2, 83.3, 79, 78.1, 75.4, 75.3, 72.9, 71.1, 70.3, 69.7, 66.1, 58.7, 37.7, 29.4, 28.1, 23.9, 15.2; HR MALDI-TOF MS: m/z: calcd for $C_{57}H_{54}F_2O_{14}S$ [M+Na]$^+$: 1055.3100; found 1055.2978.

Bromo 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (28)

Compound **27** (5.0 g, 7.36 mmol) was dissolved in HBr in acetic acid (15 mL, 33% w/w, 51 mmol), cooled (0 ^{o}C) and stirred for 6 h. After 6 h, the mixture was diluted with DCM (200 mL) and poured into crushed ice and neutralized with saturated NaHCO$_3$. The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo* to afford compound **28** (4.63 g, 90%) which was used without further purification in the synthesis of compound **29**; ^{1}H NMR (300 MHz, CDCl$_3$) δ 6.53 (d, J = 4.0 Hz, 1H, Glc H-1), 5.54 (t, J = 9.6 Hz, 1H, Glc H-3), 5.27 – 5.00 (m, 2H, Glc' H-2, Glc' H-4), 5.00 – 4.89 (t, J = 8.6 Hz, 1H, Glc' H-3), 4.77 (dd, J = 10.0, 4.1 Hz, 1H, Glc H-2), 4.59 – 4.43 (m, 2H, Glc H-6a, Glc' H-1), 4.37 (dd, J = 12.5, 4.4 Hz, 1H, Glc' H-6a), 4.19 (m, 2H, Glc H-5, Glc H-6b), 4.06 (dd, J = 12.5, 2.3 Hz, 1H, Glc' H-6b), 3.84 (t, J = 9.8 Hz, 1H, Glc H-4), 3.68 (m, 1H, Glc' H-5), 2.14 (s, 3H, COCH_3), 2.09 (s, 6H, 2x COCH_3), 2.05 (s, 6H, 2x COCH_3), 2.02 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 100.8, 86.7, 75.4, 73.2, 72.3, 71.9, 70.9, 69.7, 68, 61.9, 61.1, 20.9, 20.8; HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{35}BrO_{17}$ [M+Na]$^+$: 721.0955; found 721.

***p*-Tolyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-acetyl-1-thio-β-D-glucopyranoside (29)**

Compound **28** (4.63 g, 6.62 mmol) was dissolved in CH_3CN (15 mL) and Me_4NI (388 mg, 3.35 mmol), triethyl amine (3.75 mL, 26 mmol) and MeOH (1.6 mL, 40 mmol) was added and the reaction was stirred at room temperature for 6 h. After 6 h, the reaction mixture was concentrated and dissolved in methanol (15 mL) and freshly prepared sodium methoxide in methanol (10 mL, 1 M) was added, and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H⁺ resin, filtered and concentrated to leave crude solid which was dissolved in DMF (15 mL) and cooled (0 °C). NaH (1.92 g, 80 mmol) was added in portions, followed by addition of BnBr (7.2 mL, 60 mmol).The reaction mixture was stirred for 4 h at room temperature and carefully quenched by addition of methanol (5 mL). The crude mixture was diluted with DCM (50 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The resulting residue (5.5 g), *p*-toluenethiol (635 mg, 5.11 mmol), and $HgBr_2$ (61 mg, 0.17 mmol) was dissolved in CH_3CN (25 mL) and refluxed for 12 h. The solvent was evaporated and the residue dissolved in DCM (50 mL), and washed with saturated $NaHCO_3$ (2 x 25 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (15% EtOAc in hexane) to afford compound **29** (2.56g, 40%, 4 steps); HR MALDI-TOF MS: m/z: calcd for $C_{63}H_{66}O_{11}S$ $[M+Na]^+$: 1053.4224; found 1053.4276.

***p*-Tolyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-benzoyl-1-thio-β-D-glucopyranoside (5)**

Freshly prepared sodium methoxide in methanol (20 mL, 1 M) was added to a solution of **29** (2 g, 1.94 mmol) in methanol (15 mL) and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was then neutralized with Dowex® 50W X8-200 H⁺ resin, filtered and concentrated to leave crude solid which was dissolved in DCM (10 mL), cooled (0 °C), followed by the addition of benzoylchloride (0.34 mL, 2.91 mmol) and pyridine (3 mL). The reaction was stirred for 4 h, diluted with DCM (30 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered

and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (15% EtOAc in hexane) to afford compound **5** (1.78 g, 84%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.21 − 6.87 (m, 35H, ArH), 5.22 (t, *J* = 9.5 Hz, 1H, Glc H-2), 4.96 − 4.86 (m, 2H, 2xPhC*H*H), 4.83 (t, *J* = 10.8 Hz, 3H, 3xPhC*H*H), 4.79 − 4.74 (m, 2H, 2xPhC*H*H), 4.69 (d, *J* = 10.1 Hz, 1H, Glc H-1), 4.66 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.59 − 4.51 (m, 3H, Glc' H-1, 2xPhC*H*H), 4.46 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.38 (s, 1H, PhC*H*H), 4.10 (t, *J* = 9.3 Hz, 1H, Glc H-4), 3.87 (dd, *J* = 11.1, 4.2 Hz, 1H, Glc H-6a), 3.81 − 3.73 (m, 2H, Glc H-3, Glc H-6b), 3.72 − 3.63 (m, 2H, Glc' H-4, Glc' H-6a), 3.61 − 3.53 (m, 2H, Glc' H-3, Glc' H-6b), 3.49 (dd, *J* = 10.3, 3.9 Hz, 1H, Glc H-5), 3.42 (t, *J* = 8.4 Hz, 1H, Glc' H-2), 3.31 (dd, *J* = 10.0, 3.7 Hz, 1H, Glc' H-5), 2.28 (s, 3H, C*H*$_3$ STol); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.6, 133.6, 132.9, 130.1, 130, 129.5, 128.5, 128.1, 128, 127.9, 127.8, 127.8, 127.8, 127, 102.6, 86.2, 84.8, 82.8, 81.6, 79.8, 77.9, 76.5, 75.3, 75, 75, 74.6, 74.5, 74.3, 73.8, 73.3, 73.3, 73.3, 71.9, 68.7, 68.2, 20.9; HR MALDI-TOF MS: m/z: calcd for C$_{68}$H$_{68}$O$_{11}$S [M+Na]$^+$: 1115.4380; found 1115.4364.

3-azidopropyl [3,4-di-*O*-benzoyl-α-D-xylopyranosyl]-(1→6)-[2-*O*-difluorobenzoyl-3-*O*-benzyl-4-*O*-(2-methylnaphthyl)-β-D-glucopyranosyl]-(1→4)-2-*O*-difluorobenzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (3)

Thioglycoside donor **6** (315 mg, 0.304 mmol) and glucose acceptor **7** (148 mg, 0.254 mmol) were dissolved in DCM (3 mL), followed by addition of molecular sieves (4 Å) and stirring at room temperature for 30 min, after which the reaction was cooled (-20°C) and *N*-iodosuccinimide (NIS) (103 mg, 0.456 mmol) was added, followed by addition of TfOH (5.3 µL, 0.061 mmol). The reaction mixture was stirred for 45 min and quenched with saturated Na$_2$S$_2$O$_3$ solution (2 mL), diluted with DCM (20 mL) and washed with saturated NaHCO$_3$ (10 mL). The organic phase was concentrated *in vacuo* and the trisaccharide dissolved in EtOH/toluene (5 mL, 2:1) and hydrazine acetate (NH$_2$NH$_2$.HOAc) (75 mg, 0.81 mmol) was added and the reaction was stirred for 4 h. The reaction mixture was concentrated, dissolved in DCM (15 mL) and washed with saturated NaHCO$_3$ (10 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in hexane) to afford compound **3** (211 mg, 57%, β only, 2 steps);

^{1}H NMR (500 MHz, CDCl$_3$) δ 8.16 – 6.88 (m, 42H, ArH), 5.62 (t, J = 9.7 Hz, 1H. Xyl H-3), 5.27 (t, J = 8.7 Hz, 1H, Glc' H-2), 5.19 (td, J = 10.0, 5.8 Hz, 1H, Xyl H-4), 5.15 – 5.11 (m, 1H, glc H-2), 5.04 (d, J = 11.5 Hz, 1H, PhCHH), 4.94 (d, J = 3.6 Hz, 1H, Xyl H-1), 4.90 (d, J = 13.3, 1H, PhCHH), 4.82 (dd, J = 16.5, 11.4 Hz, 2H, 2x PhCHH), 4.69 – 4.61 (m, 4H, 3x PhCHH, Glc' H-1), 4.40 (d, J = 12.0 Hz, 1H, PhCHH), 4.35 (d, J = 8.0 Hz, 1H, Glc H-1), 4.15 (t, J = 9.3 Hz, 1H, Glc H-4), 3.81 (m, 3H, Xyl H-5a, Glc' H-6a, 1xCH_2 linker), 3.75 – 3.59 (m, 6H, Glc H-6a, Glc H-6b, Xyl H-2, Glc H-3, Glc' H-3, Glc' H-4), 3.54 (t, J = 10.8 Hz, 1H, Xyl H-5b), 3.51 – 3.41 (m, 2H, Glc' H-6b, 1xCH_2 linker), 3.38 (m, 1H, Glc' H-5), 3.34 – 3.29 (m, 1H, Glc H-5), 3.29 – 3.14 (m, 2H, CH_2 linker), 1.89 – 1.60 (m, 2H, CH_2 linker); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.1, 130, 129.9, 128.2, 128, 128, 127.9, 127.8, 126.6, 126.1, 125.7, 121.6, 118.7, 118.5, 118.5, 118.1, 100.9, 100.1, 98.1, 83.1, 80, 78.3, 76.4, 75.1, 74.9, 74.8, 74.8, 74.6, 74.6, 73.6, 73.4, 73.3, 73.2, 70.9, 69.9, 67.7, 66.4, 66.4, 65.9, 59.1, 47.9, 28.9; HR MALDI-TOF MS: m/z: calcd for C$_{80}$H$_{73}$F$_4$N$_3$O$_{19}$ [M+Na]$^+$: 1478.4672; found 1478.4564.

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CHAPTER IV

NOVEL PROTECTING GROUP FOR THE ONE-POT SYNTHESIS OF

BRANCHED OLIGOSACCHARIDES*

4.1 Abstract

To streamline the process of oligosaccharide synthesis, several groups are pursuing one-pot multi-step glycosylation strategies based on chemoselective, orthogonal or pre-activation protocols. But, the synthesis of branched oligosaccharides, using these methods cannot be readily accomplished. In order to address this issue, we report here the synthesis of a novel protecting group that can be deprotected *in situ* in combination with triflic acid promoted glycosylations of trichloroacetimidate donors, paving the way for the synthesis of branched oligosaccharides in an one-pot procedure.

4.2 Introduction

A major obstacle to advances in glycobiology is the lack of pure and structurally well-defined carbohydrates and glycoconjugates. Although methods for oligosaccharide synthesis have seen a huge amount of progress, their synthesis remains a significant challenge due to the demands of glycosyl donor and acceptor preparations as well as requirements of regio- and stereoselectivity in glycoside bond formation. To streamline the synthesis of complex oligosaccharides, one-pot multi-step glycosylation protocols are being developed, that does not require intermediate work-up and purification steps and can expedite the process of chemical synthesis considerably.[1-2] Chemoselective, orthogonal and iterative glycosylation strategies have been developed that exploit the difference in reactivity between glycosyl donors, allowing multi-step glycosylations to occur in one-pot, resulting in a single oligosaccharide product.[3-6] Synthesis of branched oligosaccharides, which cannot readily be accomplished by chemoselective, orthogonal or iterative glycosylation strategies, are synthesized by exploiting the difference in reactivity between the hydroxyl groups of an acceptor molecule.[7-10] But, the scope of this approach is limited because of the need for high regioselectivities. In this respect, an one-pot strategy that combines regioselective opening of benzylidene acetals and glycosylation has been developed for the synthesis of branched oligosaccharides.[11] In this method, a 4, 6-benzylidene acetal protected acceptor with one free hydroxyl group is first glycosylated with a trichloroacetimidate donor in the presence of catalytic amount of triflic acid. After glycosylation is complete, the benzylidene acetal is reductively opened using triflic acid and triethylsilane *in situ* to reveal a new hydroxyl group. Finally,

addition of the second trichloroacetimidate donor afforded the final branched trisacccharide in good yields and excellent regioselectivity. The utility of this one-pot benzylidene opening and glycosylation protocol was demonstrated in the synthesis of biologically important 3,4-branched Le^x trisaccharide and more recently by Ohtsuka and co-workers in the synthesis of a novel marine glycosphingolipid containing β-D-Gal$p$(1→4)[α-D-Fuc$p$-(1→3)]β-D-Glc$p$-(1→)Cer motif in 47% yield.[11-12] Although, this procedure has proved efficient for the one-pot synthesis of oligosaccharides branched at the C-4 hydroxyl, the branching at C-6 hydroxyl remains elusive. The conditions required for the reductive opening of benzylidene acetal to afford the free C-6 hydoxyl[13] are not compatible with the final glycosylation step, resulting in poor yields.[11] To address this problem, we report here the synthesis of a novel protecting group 3-((2S, 4R)-2-phenyl-1, 3-dioxan-4-yl)propanoic acid **4** (PDP) which can be coupled to any of the sugar hydroxyl groups and can be deprotected *in situ* under reaction conditions which are compatible with glycosylation with trichloracetimidate donors, lending itself to the synthesis of branched oligosaccharides in an one-pot multi-step glycosylation strategy.

4.3 Results and Discussion

Efforts to develop a protecting group that can be used in one-pot multi-step glycosylations need to fulfill two requirements: 1) the protecting group must have a handle that can be coupled to sugar hydroxyl groups easily and efficiently using known coupling chemistry, 2) we must be able to remove the protecting group, *in situ* under glycosylation conditions in high yields. To this end, it was decided that synthesis of compound **4** would fit both these criteria quite well. Compound **4** (Scheme **4.1**) can be viewed as a 4, 6-benzylidene acetal protected dihydroxy hexanoic acid. The carboxylic group can be coupled to the sugar alcohol using DIC/DMAP coupling chemistry and it was envisaged that the deprotection could be achieved under the same conditions required for reductive opening of benzylidene acetals using TfOH/Et$_3$SiH.[14-15] The driving force for the deprotection is the intramolecular cyclization and release of the five-membered lactone, upon opening of the 6-membered cyclic acetal. Previous studies have

shown that conditions for the reductive opening of benzylidene acetals are compatible with TfOH mediated glycosylations of trichloroaceimidates.[11-12]
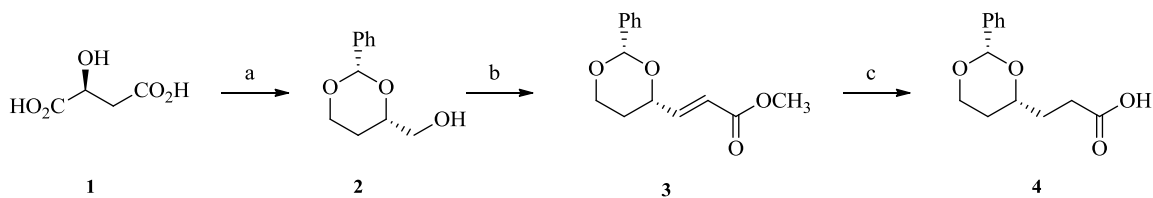

Scheme 4.1: a) i. BH$_3$.SMe$_2$, B(OMe)$_3$, THF, 0 ^{o}C-rt; ii. PhCH(OMe)$_2$, CSA, DCM (62%, 2 steps); b) i. (COCl)$_2$,DMSO,Et$_3$N,DCM, -78 ^{o}C-0 ^{o}C; ii. Ph$_3$P=CHCO$_2$CH$_3$, DCM, rt (75%, 2 steps); c) i. H$_2$, Pd(OH)$_2$, MeOH; ii. NaOMe, MeOH (85%, 2 steps).

The synthesis of compound **4** can be achieved in six steps starting from the commercially available (*S*)-malic acid (Scheme **4.1**). Reduction of (*S*)-malic acid with borane-dimethyl complex afforded the chiral 1, 2, 4-butanetriol in quantitative yields.[16] The crude triol was reacted with benzaldehyde dimethylacetal and catalytic camphorsulfonic acid under reduced pressure to give compound **2** in 62% yield as the major product, along with trace amount of its epimer (the *trans*-dioxane).[17] Alcohol **2** was then subjected to a one-pot sequential Swern oxidation and Wittig olefination to afford the *trans*-ester *E*-**3** in 75% overall yield.[18] Next, the double bond is reduced by hydrogenating *E*-**3** with catalytic Pd(OH)$_2$ in methanol followed by saponification of the methyl ester with sodium methoxide in methanol to give the target compound **4** in 85% yield (Scheme **4.1**).[19]

With compound **4** in hand, attention was next focused on establishing conditions for the coupling of **4** to sugar hydroxyl and subsequent deprotection under conditions for reductive opening of benzylidene acetals. Compound **4** was coupled to the C-6 hydroxyl group of α-methylglucoside **5**[20] using DIC and catalytic DMAP in DCM at room temperature to give compound **6** in 76% yield (Scheme **4.2**).[21] Deprotection was achieved by treating **6** with TfOH (1.8 equiv) and Et$_3$SiH (2.0 equiv) in DCM at -78 ^{o}C and warming it gradually to 0 ^{o}C (Scheme **4.2**).[11] Fortunately, the deprotection proceeded smoothly to give **5** in 88% yield. Thus, we were able to deprotect **6** efficiently and in high yields using the same conditions required for the reductive opening of 4, 6-benzylidene acetals. Based on previous work in this lab, it has been established that conditions required for 4, 6-benzylidene acetal opening are also compatible with glycosylations

using trichloroacetimidate as donors.[11] So, in the next step we wanted to explore if compound **6** can be deprotected and glycosylated in a one-pot two-step procedure to give
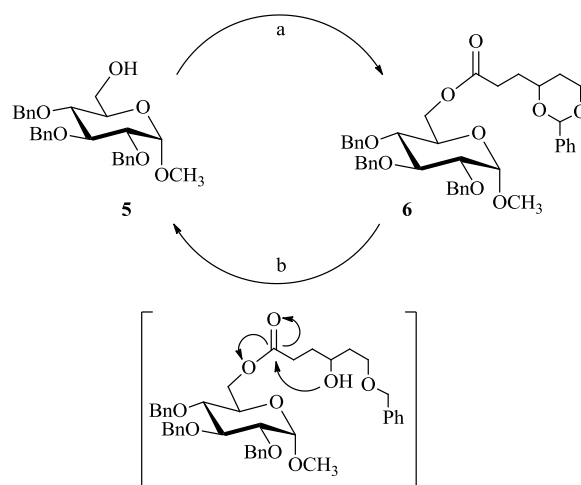

Scheme 4.2: a) **4**, DIC, DMAP, DCM (76%); b) TfOH,Et$_3$SiH, DCM, -78 ^{o}C-0 ^{o}C (88%).

the corresponding disaccharide. Thus, compound **6** (1.0 equiv) in DCM at − 78 ^{o}C was treated with TfOH (1.8 equiv) and Et$_3$SiH (2.0 equiv) and the reaction mixture was gradually warmed to 0 ^{o}C. After TLC and MALDI-MS analysis indicated complete deprotection and formation of **5**, the reaction mixture was cooled to -30 ^{o}C and trichloroacetimidate **12**[22](1.5 equiv) was added. After being stirred for 30 min, the reaction was quenched by addition of triethylamine and methanol, and purification by silica gel column chromatography gave the disaccharide **16** in 64% yield. As expected only the β-galactoside was formed due to the presence of 2, 5-difluorobenzoyl group[23] at C-2 of the galactosyl donor **12**. This protecting group is an excellent neighboring group participant and can be cleaved under mild conditions using only catalytic amount of sodium methoxide in methanol. In the next set of experiments, the reaction sequence was repeated using donors **13**, **14**[24], **15**[25] to get the corresponding disaccharides **17**, **18**, **19** as a single regio- and stereoisomer in 68%, 65% and 72% yields respectively (Scheme **4.3**).

Scheme 4.3: One-pot synthesis by *in situ* deprotection and glycosylation.

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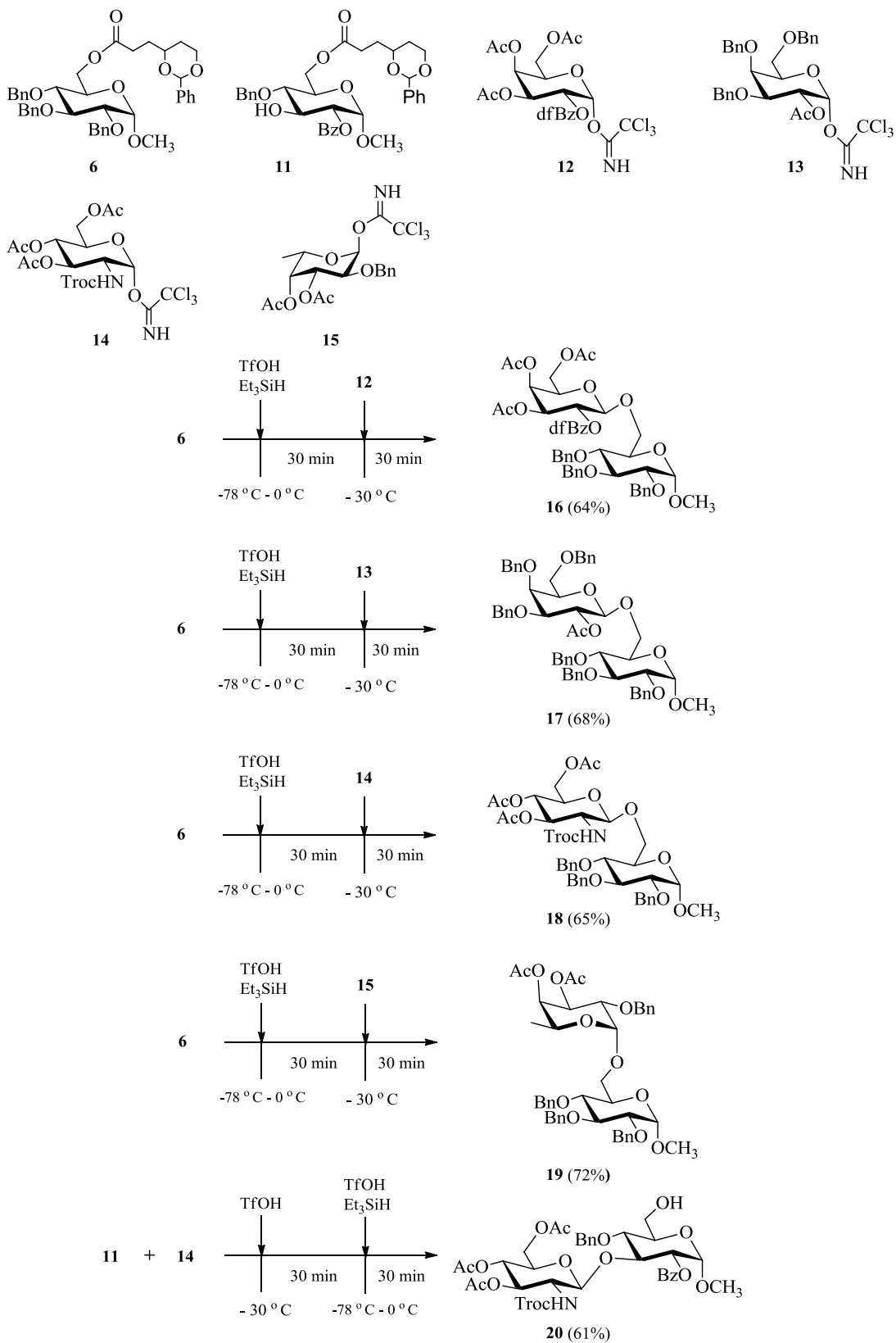
Next, we wanted to focus attention on a reaction sequence, whereby glycosylation is followed by deprotection of PDP to give the disaccharide acceptor. In this respect, glycosyl acceptor **11** was synthesized starting from known methyl 4, 6-*O*-benzylidene-α-D-glucopyranoside **7**[26] (Scheme **4.4**). The hydroxyl group at C-2 of the diol **7** was benzoylated by reacting with 1-(benzoyloxy)benzotriazole (1 equiv) and Et$_3$N in DCM to get compound **8** in 81% yield along with 4% of the 3-*O*-benzoate and 2% of the 2,3-di-*O*-benzoate.[27] Treatment of **8** with levulinic acid in the presence of DIC, DMAP afforded the fully protected sugar **9** in 87% yield.[21] In the next step, 4, 6-benzylidene acetal is reductively opened by reaction with dichlorophenylborane[15] and Et$_3$SiH at -78 °C for 2 min after which the reaction is immediately quenched with methanol and triethylamine to give compound **10** with C-4 benzyl ether and a free hydroxyl group at C-6 in 56% yield. Longer reaction times will not only result in reductive opening of the benzylidene acetal but also complete reduction of the ketone of the levulinyl group to the alcohol. Coupling of compound **10** with **4** in the presence of DIC/DMAP and subsequent delevulination using hydrazine acetate[28] in EtOH/Toluene (2:1) afforded the glycosyl acceptor **11** in 66% yield in two steps (Scheme **4.4**).
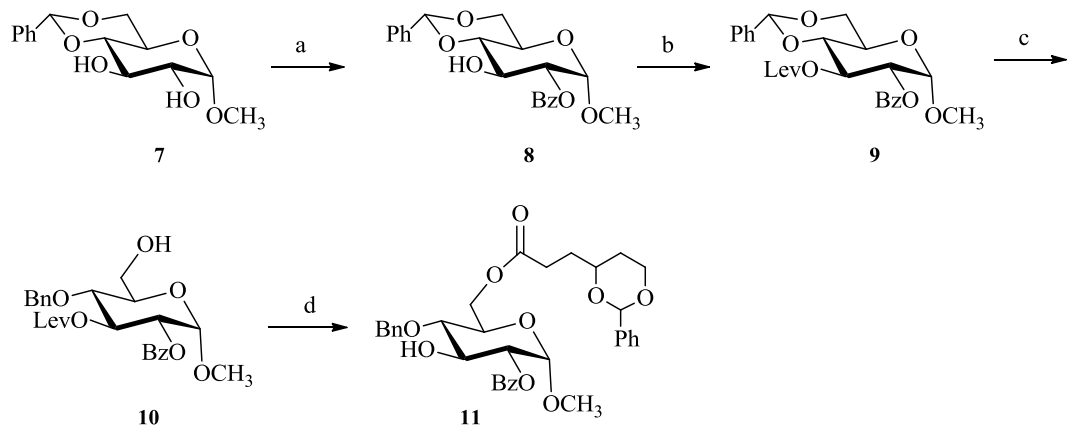
Scheme 4.4: a) 1-(benzoyloxy)benzotriazole, Et$_3$N, DCM (81%); b) Levulinic acid, DIC, DMAP, DCM (87%); c) Et$_3$SiH, PhBCl$_2$, DCM, - 78 ° C, 2 min (56%); d) i. **4**, DIC, DMAP, DCM; ii. NH$_2$NH$_2$.HOAc, EtOH/toluene = 2:1 (66%, 2 steps).

Thus, a mixture of trichloroacetimidate donor **14** (1.5 equiv) and glycosyl acceptor **11** (1.0 equiv) in dichloromethane at -30 °C was treated with catalytic amount of TfOH.

After a reaction time of 30 min, the formation of the expected disaccharide was confirmed by TLC and MALDI-MS. The reaction mixture was then cooled to -78 ^{o}C, followed by addition of TfOH (1.8 equiv) and Et$_3$SiH (2.0 equiv). After stirring for 30 min and gradually warming to 0 ^{o}C, the reaction was quenched by addition of triethylamine and methanol. Purification by silica gel column chromatography afforded the disaccharide **20** as a single regio- and stereoisomer in a yield of 61% (Scheme **4.3**). In the next set of experiments, we explored the possibility of synthesizing branched trisaccharides by a reaction sequence involving glycosylation, removal of PDP, followed by a second glycosylation. Thus, a mixture of trichloroacetimidate donor **12** (1.5 equiv) and glycosyl acceptor **11** (1.0 equiv) in dichloromethane at -30 ^{o}C was treated with catalytic amount of TfOH and stirred for 30 min. The reaction mixture was then cooled to -78 ^{o}C followed by addition of TfOH (1.8 equiv) and Et$_3$SiH (2.0 equiv), and stirred for another 30 min. The reaction mixture was then warmed up to -30 ^{o}C followed by addition of galactosyl donor **13** (1.8 equiv). The reaction was allowed to stir for a period of 30 min to give, after standard workup and purification by silica gel column chromatography, trisaccharide **21** in a yield of 58%. Similarly, trisaccharide **22** was obtained by glycosylation of **11** with **12**, followed by deprotection and glycosylation of the resulting disaccharide acceptor with glucosamine donor **14**. Finally, glycosylation of **11** with **14** followed by TfOH/Et$_3$SiH mediated deprotection, and addition of fucosyl donor **15** gave the corresponding trisaccharide **24** in an overall yield of 60% (Scheme **4.5**).

4.4 Conclusion

In conclusion, we have demonstrated that the novel protecting group (PDP) can be used in combination with triflic acid promoted glycosylations for the synthesis of branched trisaccharides using our one-pot procedure. It is expected that the protecting group can be coupled to any hydroxyl of the sugar to yield a variety of branched oligosaccharides. The utility of this methodology can be further extended by the use of sugars other than glucose at the core or using a thioglycoside as the initial acceptor, to synthesize more complex structures by employing the resulting thioglycosyl trisaccharide as a glycosyl donor in the concomitant step.
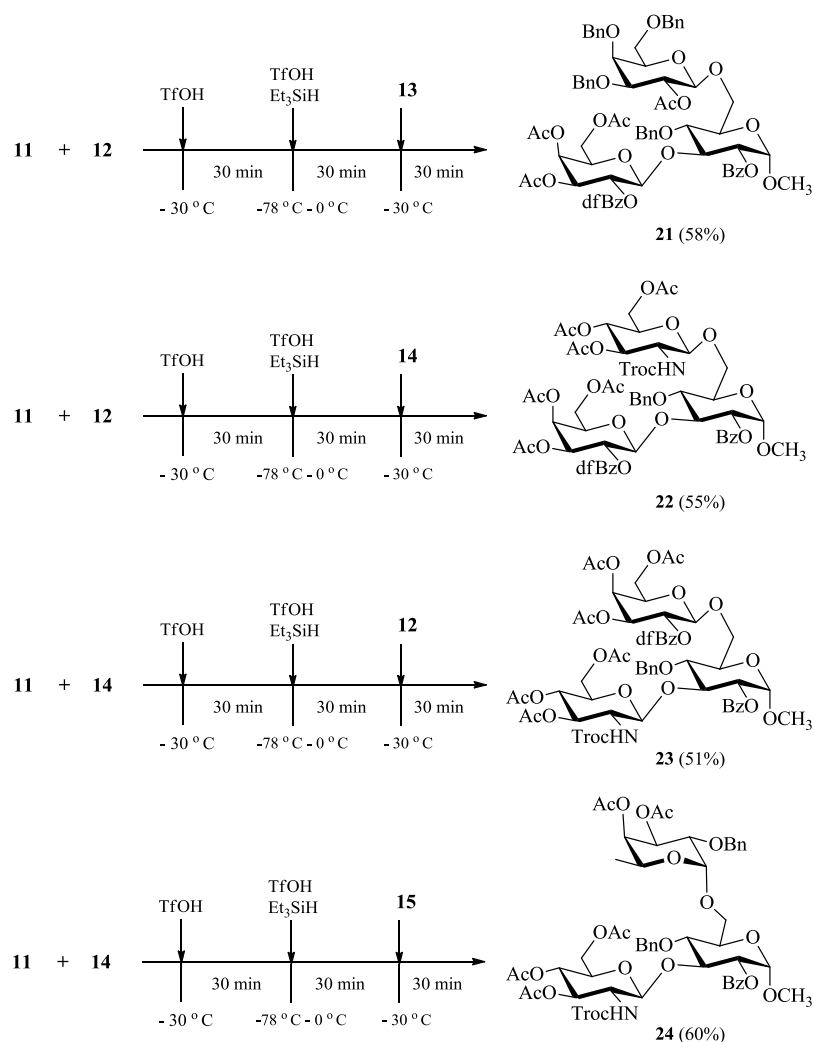
Scheme 4.5: One-pot synthesis of trisaccharides by glycosylation and *in situ* deprotection.

4.5 Experimental Section

General Procedure:

All reactions were carried out under argon with anhydrous solvents, unless otherwise stated. CH_2Cl_2 was distilled from CaH_2 prior to use in reactions. All the starting materials were kept *in vacuo* with P_2O_5 prior to use. Chemicals used were reagent grade as supplied except where noted. Column chromatography was performed on silica gel G60 (60-200 µm 60 Å). Reactions were monitored by TLC on Silicagel 60 F_{254}. The compounds were detected by examination under UV light and visualized by charring with 10% sulfuric acid in MeOH or cerium ammonium molybdate in 20% aq. sulfuric acid. Solvents were

removed under reduced pressure at ≤ 30 °C. ^{1}H and ^{13}C (data from HSQC) NMR spectra were recorded in CDCl$_3$ on Varian Mercury 300 MHz, Varian INOVA 500 MHz or 600 MHz spectrometers with trimethylsilane as internal standard. High resolution mass spectra were obtained by using MALDI-TOF with 2, 5-dihydroxybenzoic acid as matrix.

(2S, 4S)-4-(Hydroxymethyl)-2-phenyl-1, 3-dioxane (2)

(S)-Malic acid (2 g, 14.9 mmol) in THF (10 mL) was added dropwise (0 °C) to a stirred solution of borane-dimethylsulfide complex (24 mL, 48.0 mmol) and trimethylborate (5 mL, 43.8 mmol). After the mixture had been stirred for 15 min (0 °C), the cooling bath was removed. After the reaction mixture had been stirred for 16 h, methanol (12 mL) was carefully added and the resulting solution was concentrated *in vacuo* to afford the crude (S)-1, 2, 4-butanetriol as a colorless oil (1.53 g, quant).The crude triol (1.58g, 14.9 mmol) was dissolved in dry DCM (20 mL) and benzaldehyde dimethylacetal (2.6 mL, 15.9 mmol) was added followed by addition of camphorsulfonic acid (174 mg, 0.75 mmol) and the reaction was stirred at room temperature. After 16 h, triethylamine was added and the solvents were removed *in vacuo* and the residue was purified by flash chromatography over silica gel (25% EtOAc in hexane) to afford compound **2** (1.8 g, 62%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.52 − 7.32 (m, 5H, ArH), 5.56 (s, 1H, H-2), 4.31 (dd, J = 11.3, 5.1 Hz, 1H, H-6a), 4.08 − 3.94 (m, 2H, H-6b, H-4), 3.76 − 3.60 (m, 2H, CH_2OH), 1.94 (qd, J = 12.4, 5.1 Hz, 1H, H-5a), 1.46 (dd, J = 13.3, 2.8 Hz, 1H, H-5b); ^{13}C NMR (126 MHz, CDCl$_3$) δ 128.4, 126.1, 101.3, 77.5, 66.6, 65.7, 26.9; HR MALDI-TOF MS: m/z: calcd for C$_{11}$H$_{14}$O$_3$ [M+Na]$^+$:217.0841; found 217.0857.

(E)-2-(Methoxycarbonyl)ethenyl-(2S, 4S)-2-phenyl-1,3-dioxane (3)

A solution of oxalyl chloride (0.43 mL, 4.9 mmol) in DCM (20 mL) was mixed with dimethyl sulfoxide (0.70 mL, 9.95 mmol) at -78 °C with stirring and then a solution of (2S, 4S)-4-(hydroxymethyl)-2-phenyl-1, 3-dioxane **2** (0.8g, 4.1 mmol) in DCM (10 mL) was added dropwise over 10 min. After 10 min, triethylamine (2 mL) was added and the reaction was allowed to warm (0 °C). A solution of methyl (triphenylphosphoranylidene) acetate (4 g, 2.9 mmol) in DCM (20 mL) was added to the reaction mixture and the

solution was stirred at room temperature for 2 h. The crude was diluted with DCM (300 mL) and washed with saturated NaCl (100 mL). The organic layer was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (15 % EtOAc/hexanes) to afford compound **3** (0.76g, 75% over two steps); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.57 – 7.23 (m, 5H, ArH), 6.90 (dd, J = 15.7, 4.0 Hz, 1H, C*H*=), 6.08 (dd, J = 15.7, 1.8 Hz, 1H, C*H*=), 5.53 (s, 1H, H-2), 4.49 (ddt, J = 11.5, 4.2, 2.3 Hz, 1H, H-4), 4.25 (ddd, J = 11.5, 5.0, 1.4 Hz, 1H, H-6a), 3.96 (td, J = 11.9, 2.6 Hz, 1H, H-6b), 3.68 (s, 3H, OC*H*$_3$), 1.96 – 1.74 (m, 1H, H-5a), 1.62 (dq, J = 13.3, 2.4 Hz, 1H, H-5b); ^{13}C NMR (75 MHz, CDCl$_3$) δ 146.4, 128.8, 126.3, 120.5, 101.4, 75.5, 67, 51.9, 30.9; HR MALDI-TOF MS: m/z: calcd for C$_{14}$H$_{16}$O$_4$ [M+Na]$^+$: 271.0946; found 279.0912.

3-((2*S*, 4*S*)-2-phenyl-1, 3-dioxan-4-yl) propanoic acid (4)

(*E*)-2-(Methoxycarbonyl)ethenyl-(2*S*,4S)-2-phenyl-1,3-dioxane **3** (0.5g, 2 mmol) was dissolved in dry MeOH (5 mL) , flushed with argon, and catalytic amount of 20 wt% Pd(OH)$_2$/C was added. The mixture was flushed with argon and then with H$_2$ gas and stirred at room temperature for 4 h. Upon completion of the reaction, the reaction mixture was purged with argon followed by filtration through a plug of Celite. The filtrate was concentrated *in vacuo* to afford the crude ester which was dissolved in MeOH (5 mL) and freshly prepared NaOMe in MeOH (0.5 mL, 1 M) was added to the stirred solution. The reaction was stirred for 1 h and then neutralized with Dowex® 50W X8-200 H$^+$ resin. The resin was filtered and the filtrate was concentrated *in vacuo* to afford compound **4** (0.4g, 85%, over 2 steps) which was used without further purification; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.50 – 7.07 (m, 5H, ArH), 5.43 (s, 1H, H-2), 4.20 (dd, J = 11.5, 4.9 Hz, 1H, H-6a), 3.99 – 3.70 (m, 2H, H-6b, H-4), 2.50 (t, J = 7.3 Hz, 2H, C*H*$_2$CO), 1.87 (q, J = 7.1 Hz, 2H, C*H*$_2$), 1.75 (td, J = 12.1, 5.2 Hz, 1H, H-5a), 1.47 (d, J = 13.2 Hz, 1H, H-5b); ^{13}C NMR (75 MHz, CDCl$_3$) δ 128.7, 126.2, 101.3, 76.1, 67.1, 31.4, 30.9; HR MALDI-TOF MS: m/z: calcd for C$_{13}$H$_{16}$O$_4$ [M+Na]$^+$:259.0946; found 259.0978.

Methyl 6-*O*-(2-phenyl-1,3-dioxan-4-propanoate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (6)

3-((2*S*,4*S*)-2-phenyl-1, 3-dioxan-4-yl) propanoic acid **4** (880 mg, 3.76 mmol), *N,N′*-Diisopropylcarbodiimide (DIC) (0.87 mL, 5.64 mmol) and 4-dimethylaminopyridine (DMAP) (46 mg, 0.37 mmol) were added to a stirred solution of methyl 2,3,4-*O*-benzyl-α-D-glucopyranoside (1.75 g, 3.76 mmol) in DCM (20 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (200 mL) and washed with saturated NaHCO$_3$ (2 x 100 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **6** (1.95 g, 76%); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.59 – 7.09 (m, 20H, ArH), 5.43 (s, 1H, PhC*H*<), 4.99 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.85 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.81 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.78 (d, *J* = 12.2 Hz, 1H, PhC*H*H), 4.65 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.57 (d, *J* = 3.6 Hz, 1H, H-1), 4.53 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.31 – 4.20 (m, 3H, H-6a, H-6b, OC*H*H), 3.99 (t, *J* = 9.3 Hz, 1H, H-3), 3.96 – 3.76 (m, 3H, OCH*H*, OC*H*, H-5), 3.51 (dd, *J* = 9.6, 3.5 Hz, 1H, H-2), 3.46 (t, *J* = 9.5 Hz, 1H, H-4), 3.35 (s, 3H, OC*H*$_3$), 2.50 (m, 2H, COC*H*$_2$), 1.90 (q, *J* = 7.1 Hz, 2H, C*H*$_2$, C*H*H), 1.80 (qd, *J* = 12.4, 5.1 Hz, 1H, CH*H*); ^{13}C NMR (126 MHz, CDCl$_3$) δ 128.1, 127.8, 126, 125.9, 101, 98, 82, 79.9, 77.5, 75.8, 75.1, 75, 73.4, 68.6, 66.8, 62.9, 55.2, 31, 30.7, 29.6; HR MALDI-TOF MS: m/z: calcd for C$_{41}$H$_{46}$O$_9$ [M+Na]$^+$: 705.3040; found 705.3046.

Methyl 4,6-*O*-benzylidene-2-*O*-benzoyl-α-D-glucopyranoside (8)

To a stirred solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **7** (1g, 3.54 mmol) and 1-(benzoyloxy)benzotriazole (858 mg, 3.54 mmol) in DCM (15 mL) at room temperature was added triethylamine (0.5 mL, 3.8 mmol). The reaction mixture was stirred at room temperature for 5 h, diluted with DCM (200 mL) and washed with saturated NaHCO$_3$ (100 mL). The organic layer was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (15 % EtOAc in toluene) to afford compound **8** (1.2 g, 81%); NMR (300 MHz, CDCl$_3$) δ 8.13 – 7.24 (m, 10H, ArH), 5.52 (s, 1H, PhC*H*<), 5.11 – 4.88 (m, 2H, H-1, H-

2), 4.28 (m, 2H, H-3, H-6a), 3.85 (td, J = 9.7, 4.5 Hz, 1H, H-5), 3.74 (t, J = 10.1 Hz, 1H, H-6b), 3.58 (t, J = 9.3 Hz, 1H, H-4), 3.34 (s, 3H, OCH_3), 2.39 (d, J = 3.0 Hz, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ 130.1, 126.6, 129.1, 102.3, 98, 74.2, 69.2, 62.6, 69.3, 81.7, 55.7; HR MALDI-TOF MS: m/z: calcd for C$_{21}$H$_{22}$O$_7$ [M+Na]$^+$: 409.1263; found 409.1231.

Methyl 4,6-*O*-benzylidene-3-*O*-levulinoyl 2-*O*-benzoyl-*α*-D-glucopyranoside (9)

Levulinic acid (360 mg, 3.10 mmol), *N,N′*-Diisopropylcarbodiimide (DIC) (0.6 mL, 3.88 mmol) and 4-dimethylaminopyridine (DMAP) (32 mg, 0.26 mmol) were added to a stirred solution of **8** (1 g, 2.59 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated NaHCO$_3$ (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **9** (1.09g, 87%); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.12 − 7.09 (m, 10H, ArH), 5.78 (t, J = 9.8 Hz, 1H, H-3), 5.55 (s, 1H, PhCH<), 5.12 (d, J = 3.7 Hz, 1H, H-1), 5.07 (dd, J = 9.8, 3.7 Hz, 1H, H-2), 4.34 (dd, J = 10.2, 4.7 Hz, 1H, H-6a), 4.05 − 3.92 (m, 1H, H-5), 3.82 (t, J = 10.2 Hz, 1H, H-6b), 3.74 (t, J = 9.6 Hz, 1H, H-4), 3.40 (s, 3H, OCH_3), 2.68 − 2.58 (m, 2H, CH_2 Lev), 2.56 − 2.47 (m, 2H, CH_2 Lev), 2.00 (s, 3H, OCH_3 Lev); ^{13}C NMR (75 MHz, CDCl$_3$) δ 133.7, 130.4, 130.3, 128.8, 126.4, 101.8, 97.9, 79.4, 72.6, 69.4, 69.1, 62.7, 55.7, 38.2, 29.8, 28.3; HR MALDI-TOF MS: m/z: calcd for C$_{26}$H$_{28}$O$_9$ [M+Na]$^+$: 507.1631; found 507.1649.

Methyl 4-*O*-benzyl-3-*O*-levulinoyl 2-*O*-benzoyl-*α*-D-glucopyranoside (10)

Compound **9** (1.0 g, 2.06 mmol) was dissolved in DCM (15 mL) and stirred with activated molecular sieves (4Å) for 1 h. After cooling (-78 ^{o} C), triethylsilane (0.9 mL, 6.19 mmol) and dichlorophenylborane (1 mL, 7.85 mmol) were added. After 2 min, the reaction was quenched by the addition of MeOH (5 mL) and Et$_3$N (1 mL). The resulting mixture was diluted with DCM (30 mL) and washed with saturated NaHCO$_3$ (20 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (20% EtOAc in hexane) to afford compound **10** (0.562 mg, 56%).

Methyl 6-*O*-(2-phenyl-1,3-dioxan-4-propanoate)-4-*O*-benzyl-2-*O*-benzoyl-*α*-D-glucopyranoside (11)

3-((2*S*,4*S*)-2-phenyl-1, 3-dioxan-4-yl) propanoic acid **4** (558 mg, 2.38mmol), *N,N'*-Diisopropylcarbodiimide (DIC) (0.5 mL, 3.57 mmol) and 4-dimethylaminopyridine (DMAP) (29 mg, 0.24 mmol) were added to a stirred solution of **10** (1.16 g, 2.38 mmol) in DCM (10 mL). After stirring for 16 h, the reaction mixture was diluted with DCM (100 mL) and washed with saturated $NaHCO_3$ (2 x 50 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The resulting crude compound was dissolved in EtOH/toluene (10 mL, 2:1) and hydrazine acetate (NH_2NH_2.HOAc) (1.1 g, 11.9 mmol) was added and the reaction was stirred for 4 h. The reaction mixture was concentrated, dissolved in DCM (200 mL) and washed with saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (25% EtOAc in hexane) to afford compound **11** (954 mg, 66%, 2 steps); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.06 − 7.15 (m, 15H, Ar*H*), 5.41 (s, 1H, PhC*H*<), 4.92 (d, *J* = 3.7 Hz, 1H, H-1), 4.86 (dd, *J* = 9.9, 3.7 Hz, 1H, H-2), 4.78 (d, *J* = 11.2 Hz, 1H, PhC*H*H), 4.63 (d, *J* = 11.2 Hz, 1H, PhC*H*H), 4.27 (m, 4H, H-6a, H-6b, H-3, OC*H*H), 3.93 − 3.77 (m, 3H, H-5, OCH*H*, OC*H*), 3.46 (t, *J* = 9.4 Hz, 1H, H-4), 3.28 (s, 3H, OC*H*$_3$), 2.49 (q, *J* = 7.7 Hz, 2H, C*H*$_2$CO), 2.21 (d, *J* = 3.8 Hz, 1H, O*H*), 1.88 (q, *J* = 7.2 Hz, 2H, C*H*$_2$), 1.75 (td, *J* = 12.0, 5.0 Hz, 1H, C*H*H), 1.53 − 1.43 (m, 1H, CH*H*); ^{13}C NMR (75 MHz, CDCl$_3$) δ 130.2, 128.8, 128.6, 101.3, 97.3, 78, 76.2, 75.2, 74.2, 68.6, 67.1, 63.1, 55.5, 31, 29.7; HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{38}O_{10}$ [M+Na]$^+$: 629.2363; found 629.2394.

General procedure for the synthesis of 16, 17, 18 and 19

A solution of methyl 6-*O*-(2-phenyl-1,3-dioxan-4-propanoate)-2,3,4-tri-*O*-benzyl-*α*-D-glucopyranoside **6** (0.15 mmol) in DCM (1 mL) was stirred with activated molecular sieves (4Å) for 1 h under an atmosphere of argon at room temperature. The reaction mixture was cooled (-78 ^{o}C) and TfOH (0.27 mmol, 1.8 eq) was added followed by addition of Et$_3$SiH (0.3 mmol, 2 eq). The reaction mixture was allowed to warm (0 ^{o}C) over 30 min. The reaction mixture was again cooled (-30 ^{o}C) and a solution of trichloroacetimidate donor (0.22 mmol, 1.5 eq) in DCM (1 mL) was added. The progress

of the reaction was monitored by TLC and MALDI-TOF MS. The reaction was quenched by the addition of triethylamine (50 µL) and methanol (0.2 mL), diluted with DCM (20 mL) and washed with saturated NaHCO$_3$ (10 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel.

Methyl 3,4,6-tri-*O*-acetyl-2-*O*-difluorobenzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (16)

Compound **16** was obtained from **6** and **12**, and purified by flash chromatography over silica gel (20% EtOAc in hexane) in 64% yield; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.56 − 6.77 (m, 18H, ArH), 5.51 (dd, *J* = 10.5, 8.0 Hz, 1H, Gal H-2), 5.42 (d, *J* = 3.4 Hz, 1H, Gal H-4), 5.16 (dd, *J* = 10.5, 3.4 Hz, 1H, Gal H-3), 4.90 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.76 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.70 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.64 − 4.54 (m, 3H, Gal H-1, 2 x PhC*H*H), 4.50 (d, *J* = 3.5 Hz, 1H, Glc H-1), 4.34 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.23 − 4.08 (m, 3H, Glc H-6a, Gal H-6a, Gal H6b), 3.95 − 3.86 (m, 2H, Gal H-5, Glc H-3), 3.73 (ddd, *J* = 10.1, 4.6, 1.8 Hz, 1H, Glc H-5), 3.66 (dd, *J* = 10.7, 4.6 Hz, 1H, Glc H-6b), 3.42 (dd, *J* = 9.6, 3.5 Hz, 1H, Glc H-2), 3.37 − 3.28 (m, 1H, Glc H-4), 3.26 (s, 3H, OC*H*$_3$), 2.15 (s, 3H, COC*H*$_3$), 2.04 (s, 3H, COC*H*$_3$), 1.94 (s, 3H, COC*H*$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 128.2, 128.1, 127.6, 121.5, 118.5, 117.9, 101.4, 98, 81.8, 79.9, 77.6, 77.1, 75.6, 74.7, 73.4, 70.8, 70.7, 70.1, 69.4, 68.6, 67, 61.1, 55.1, 20.8, 20.8, 20.5; HR MALDI-TOF MS: m/z: calcd for C$_{47}$H$_{50}$F$_2$O$_{15}$ [M+Na]$^+$: 915.3015; found 915.3134.

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17)

Compound **17** was obtained from **6** and **13**, and purified by flash chromatography over silica gel (10% EtOAc in hexane) in 68% yield; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 − 7.05 (m, 30H, ArH), 5.35 (t, *J* = 10.0 Hz, 1H, Gal H-2), 4.88 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.84 (d, *J* = 11.5 Hz, 1H, PhC*H*H), 4.76 − 4.66 (m, 3H, 3 x PhC*H*H), 4.57 (t, *J* = 12.7 Hz, 2H, 2 x PhC*H*H), 4.51 − 4.45 (m, 3H, Glc H-1, PhC*H*H), 4.42 (d, *J* = 12.2 Hz, 1H, PhC*H*H), 4.36 − 4.26 (m, 3H, Gal H-1, 3 x PhC*H*H), 3.97 (d, *J* = 10.7 Hz, 1H, Glc H-6a), 3.91 − 3.84 (m, 2H, Glc H-3, Gal H-4), 3.66 (dd, *J* = 10.4, 4.5 Hz, 1H, Glc H-5),

3.62 – 3.38 (m, 6H, Glc H-6b, Gal H-6a,H-6b, Glc H-2, Gal H-3, Gal H-5), 3.35 (t, J = 9.5 Hz, 1H, Glc H-4), 3.25 (s, 3H, OCH_3), 1.87 (s, 3H, COCH_3);); ^{13}C NMR (126 MHz, CDCl$_3$) δ 135.4, 133.8, 132.2, 130.8, 128.4, 128.3, 128.2, 125.7, 124.2, 122.7, 101.6, 98.3, 82.4, 80.6, 80.2, 78.1, 75.9, 75.7, 75.5, 74.9, 74.8, 74, 73.9, 73.8, 73.6, 73, 72.9, 72.2, 72.1, 71.4, 70.1, 69.3, 68.8, 68.7, 67.6, 67.5, 55.4, 21.5; HR MALDI-TOF MS: m/z: calcd for C$_{57}$H$_{62}$O$_{12}$ [M+Na]$^+$: 961.4139; found 961.4156.

Methyl 3,4,6-tri-*O*-acetyl-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-glucopyranosyl-(1→6)-2, 3, 4-tri-*O*-benzyl-α-D-glucopyranoside (18)

Compound **18**, was obtained from **6** and **14**, and purified by flash chromatography over silica gel (25% EtOAc in hexane) in 65% yield; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.62 – 6.97 (m, 15H, ArH), 5.18 (t, J = 10.0 Hz, 1H, GlcN H-3), 5.08 – 4.95 (m, 2H, GlcN H-4, PhC*H*H), 4.92 – 4.84 (m, 2H, N*H* , PhC*H*H), 4.79 (dd, J = 11.5, 5.9 Hz, 2H, 2 x PhC*H*H), 4.65 (d, J = 12.1 Hz, 1H, PhC*H*H), 4.62 – 4.56 (m, 2H, Glc H-1, PhC*H*H), 4.53 (s, 2H, CH_2 NHTroc), 4.40 (d, J = 8.3 Hz, 1H, GlcN H-1), 4.22 (dd, J = 12.3, 4.6 Hz, 1H, GlcN H-6a), 4.11 (d, J = 11.9 Hz, 1H, GlcN H-6b), 4.07 (d, J = 10.6 Hz, 1H, Glc H-6a), 3.98 (t, J = 9.3 Hz, 1H, Glc H-3), 3.75 (d, J = 10.0 Hz, 1H, Glc H-5), 3.68 (dd, J = 10.5, 4.0 Hz, 1H, Glc H-6b), 3.65 – 3.58 (m, 2H, GlcN H-2, GlcN H-5), 3.51 – 3.43 (m, 2H, Glc H-2, Glc H-4), 3.36 (s, 3H, OCH_3), 2.04 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3); ^{13}C NMR (126 MHz, CDCl$_3$) δ 128.2, 127.9, 100.8, 98.1, 82.1, 79.8, 77, 75.7, 74.5, 74.4, 73.4, 71.9, 71.8, 69.5, 68.6, 68.1, 62, 57.5, 56, 55.2, 20.9, 20.7; HR MALDI-TOF MS: m/z: calcd for C$_{43}$H$_{50}$ Cl$_3$NO$_{15}$ [M+Na]$^+$: 948.2144; found 948.2163.

Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (19)

Compound **19**, was obtained from **6** and **15**, and purified by flash chromatography over silica gel (20% EtOAc in hexane) in 72% yield; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.58 – 7.00 (m, 20H, ArH), 5.34 (dd, J = 10.5, 3.4 Hz, 1H, Fuc H-3), 5.29 (d, J = 3.4 Hz, 1H, Fuc H-4), 4.96 (d, J = 11.0 Hz, 1H, PhC*H*H), 4.90 (d, J = 3.5 Hz, 1H, Fuc H-1), 4.84 – 4.77 (m, 3H, 3 x PhC*H*H), 4.68 (d, J = 12.0 Hz, 1H, PhC*H*H), 4.63 (d, J = 11.1 Hz, 1H, PhC*H*H), 4.61 – 4.57 (m, 2H, Glc H-1, PhC*H*H), 4.56 (d, J = 12.0 Hz, 1H, PhC*H*H), 4.17

(q, *J* = 6.5 Hz, 1H, Fuc H-5), 3.97 (t, *J* = 9.3 Hz, 1H, Glc H-3), 3.84 (dd, *J* = 10.5, 2.9 Hz, 2H, Fuc H-2, Glc H-6a), 3.77 (dd, *J* = 10.1, 4.2 Hz, 1H, Glc H-5), 3.67 (dd, *J* = 10.8, 4.3 Hz, 1H, Glc H-6b), 3.62 (t, *J* = 9.5 Hz, 1H, Glc H-4), 3.57 (dd, *J* = 9.6, 3.6 Hz, 1H, Glc H-2), 3.33 (s, 3H, OC*H₃*), 2.13 (s, 3H, COC*H₃*), 1.97 (s, 3H, COC*H₃*), 1.08 (d, *J* = 6.5 Hz, 3H, Fuc C*H₃*); ^{13}C NMR (126 MHz, CDCl₃) δ 128.1, 128, 127.9, 127.8, 127.7, 125.5, 98.1, 97.8, 82.1, 80.3, 77.6, 75.7, 75.1, 74.7, 73.5, 72.9, 71.6, 70.4, 69.8, 66.8, 66.7, 64.3, 55.1, 21.1, 20.8, 15.8; HR MALDI-TOF MS: m/z: calcd for $C_{45}H_{52}O_{12}$ [M+Na]⁺ : 807.3356; found 807.3367.

Methyl 3,4,6-tri-*O*-acetyl-2-(2,2,2 trichloroethoxy)carbonylamino-β-D-glucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-benzoyl-α-D-glucopyranoside (20)

Premixed glucosyl donor **14** (110 mg, 0.18 mmol) and glucosyl acceptor **11** (73 mg, 0.12 mmol) were dissolved in DCM (2 mL) and stirred with activated molecular sieves (4Å) under an atmosphere of argon for 1 h at room temperature. The reaction mixture was cooled (-30 °C) and TfOH (3.2 μL, 0.036 mmol) was added. After 30 min. the reaction was further cooled (- 78 °C) and TfOH (19 μL, 0.22 mmol) was added followed by Et₃SiH (38 μL, 0.24 mmol) and the reaction was allowed to warm (0 °C). The progress of the reaction was monitored by TLC and MALDI-TOF MS. The reaction was quenched by addition of Et₃N (50 μL) and MeOH (0.2 mL), diluted with DCM (20 mL) and washed with saturated NaHCO₃ (10 mL). The organic phase was dried (MgSO₄), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (30% EtOAc in hexane) to obtain compound **20** (62.4 mg, 61%); ^{1}H NMR (500 MHz, CDCl₃) δ 8.19 − 7.28 (m, 10H, ArH), 5.10 (d, *J* = 11.1 Hz, 1H, PhC*H*H), 5.05 (t, *J* = 9.7 Hz, 1H, GlcN H-4), 5.02 − 4.97 (m, 2H, Glc H-1, Glc H-2), 4.90 (t, *J* = 10.1 Hz, 1H, GlcN H-3), 4.82 − 4.75 (m, 2H, GlcN H-1, 1 x C*H₂* NHTroc), 4.60 (d, *J* = 11.1 Hz, 1H, PhC*H*H), 4.49 − 4.43 (m, 1H, Glc H-3), 4.39 (d, *J* = 12.1 Hz, 1H, 1 x C*H₂* NHTroc), 4.35 (d, *J* = 9.4 Hz, 1H, N*H*), 4.29 (dd, *J* = 12.3, 4.4 Hz, 1H, Glc H-6a), 4.05 (dd, *J* = 12.3, 2.4 Hz, 1H, Glc H-6b), 3.83 − 3.77 (m, 1H, GlcN H-6a), 3.76 − 3.62 (m, 5H, GlcN H-6b, GlcN H-5, GlcN H-2, Glc H-4, Glc H-5), 3.35 (s, 3H, OC*H₃*), 2.00 (s, 3H, COC*H₃*), 1.97 (s, 3H, COC*H₃*), 1.91 (s, 3H, COC*H₃*); ^{13}C NMR (126 MHz, CDCl₃) δ 133.9, 129.8, 129, 128.3, 102, 96.9, 79.1, 75.7, 75, 74.4, 74.3, 72, 71.7, 70.9, 68.4, 62,

61.8, 56.6, 55.3, 20.8, 20.7, 20.5; HR MALDI-TOF MS: m/z: calcd for $C_{36}H_{42}Cl_3NO_{16}$ $[M+Na]^+$: 872.1467; found 872.1459.

General procedure for the synthesis of 21, 22, 23 and 24

A mixture of glycosyl acceptor (0.16 mmol) and trichloroacetimidate donor (0.24 mmol) in DCM (1.5 mL) was stirred with activated molecular sieves (4Å) for 1 h under an atmosphere of argon at room temperature. The reaction mixture was then cooled (-30 ^{o}C) and TfOH (0.05 mmol, 0.2 eq) was added. After 30 min the reaction was cooled (-78 ^{o}C) followed by the addition of TfOH (0.28 mmol, 1.8 eq) and Et_3SiH (0.32, 2eq). The reaction was warmed (0 ^{o}C) over 30 min after which it is again cooled (-30 ^{o}C) and a solution of the second trichloroacetimidate donor (0.28 mmol) in DCM (1 mL) was added. The progress of the reaction was monitored by TLC and MALDI-TOF MS. The reaction was quenched by the addition of triethylamine (50 µL) and methanol (0.2 mL), diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel.

Methyl 3,4,6-tri-*O*-acetyl-2-*O*-difluorobenzoyl-β-D-galactopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-α-D-glucopyranoside (21)

Compound **21** was obtained, from **11**, **12** and **13,** and purified by flash chromatography over silica gel (30% EtOAc in hexane) in 58% yield; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.97 − 6.67 (m, 28H, ArH), 5.45 − 5.34 (m, 3H, Gal H-2, Gal H-4, Gal' H-2), 5.08 (d, J = 10.4 Hz, 1H, PhC*H*H), 4.98 (dd, J = 10.5, 3.5 Hz, 1H, Gal H-3), 4.95 − 4.91 (m, 2H, Gal H-1, PhC*H*H), 4.88 (dd, J = 9.8, 3.9 Hz, 1H, Glc H-2), 4.84 (d, J = 3.7 Hz, 1H, Glc H-1), 4.66 (d, J = 12.3 Hz, 1H, PhC*H*H), 4.56 (dd, J = 11.2, 3.4 Hz, 2H, 2 x PhC*H*H), 4.50 (d, J = 12.2 Hz, 1H, PhC*H*H), 4.45 (t, J = 9.2 Hz, 1H, Glc H-3), 4.43 − 4.35 (m, 3H, Gal' H-1, 2 x PhC*H*H), 4.09 − 3.98 (m, 3H, Glc H-6a, Gal H-6a, Gal H-6b), 3.96 − 3.91 (m, 2H, Gal H-5, Gal' H-4), 3.79 (dd, J = 10.6, 5.2 Hz, 1H, Glc H-5), 3.66 (dd, J = 10.8, 5.3 Hz, 1H, Glc H-6b), 3.62 (t, J = 8.2 Hz, 1H, Gal' H-6a), 3.59 − 3.45 (m, 4H, Gal' H-6b, Gal' H-5, Gal' H-3, Glc H-4), 3.25 (s, 3H, OC*H*$_3$), 2.09 (s, 3H, COC*H*$_3$), 2.02 (s, 3H, COC*H*$_3$),

2.01 (s, 3H, COCH3), 1.85 (s, 3H, COCH3); ^{13}C NMR (151 MHz, CDCl3) δ 133.4, 129.6, 128.6, 128, 127.8, 126.3, 125.7, 121, 118.1, 117.5, 101.6, 101, 96.3, 80.3, 78.1, 75.7, 75.6, 74.7, 74.6, 74.5, 74.1, 73.6, 73.5, 73.4, 72.5, 71.9, 71.3, 70.9, 70.8, 70.6, 70.5, 70.3, 69.7, 68.4, 67.6, 67.4, 66.9, 60.7, 54.9, 20.7, 20.5, 20.4; HR MALDI-TOF MS: m/z: calcd for $C_{69}H_{72}O_{22}F_2$ [M+Na]$^+$: 1313.4381; found 1313.4372.

Methyl 3,4,6-tri-*O*-acetyl-2-*O*-difluorobenzoyl-β-D-galactopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-(2,2,2 trichloroethoxy)carbonyl amino-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-α-D-glucopyranoside (22)

Compound **22**, was obtained, from **11**, **12** and **14**, and purified by flash chromatography over silica gel (40% EtOAc in hexane) in 55% yield; ^{1}H NMR (600 MHz, CDCl3) δ 7.89 – 6.96 (m, 13H, ArH), 5.40 – 5.35 (m, 2H, Gal H-2, Gal H-4), 5.28 (t, *J* = 10.0 Hz, 1H, GlcN H-4), 5.10 (d, *J* = 10.7 Hz, 1H, PhC*H*H), 5.05 (t, *J* = 9.5 Hz, 2H, GlcN H-3, N*H* Troc), 4.99 (dd, *J* = 10.6, 3.4 Hz, 1H, Gal H-3), 4.94 (d, *J* = 7.9 Hz, 1H, Gal H-1), 4.92 – 4.86 (m, 2H, Glc H-1, Glc H-2), 4.67 – 4.55 (m, 4H, GlcN H-1, 2 x C*H*2 NHTroc, PhC*H*H), 4.46 (t, *J* = 9.1 Hz, 1H, Glc H-3), 4.25 (dd, *J* = 12.4, 4.6 Hz, 1H, GlcN H-6a), 4.16 – 4.08 (m, 2H, GlcN H-6b, Glc H-6a), 4.06 (d, *J* = 6.9 Hz, 2H, Gal H-6a, Gal H-6b), 3.95 (t, *J* = 6.9 Hz, 1H, Gal H-5), 3.83 (dd, *J* = 10.3, 4.8 Hz, 1H, Glc H-5), 3.76 (dd, *J* = 10.9, 4.8 Hz, 1H, Glc H-6b), 3.72 – 3.62 (m, 2H, GlcN H-2, GlcN H-5), 3.56 (t, *J* = 9.4 Hz, 1H, Glc H-4), 3.28 (s, 3H, OC*H*3), 2.10 (s, 3H, COC*H*3), 2.04 (s, 3H, COC*H*3), 2.02 (s, 3H, COC*H*3), 2.01 (s, 9H, 3 x COC*H*3), 1.86 (s, 3H, COC*H*3); ^{13}C NMR (151 MHz, CDCl3) δ 133.3, 129.6, 128.5, 128.3, 127.8, 121, 118.1, 117.5, 101.1, 100.8, 96.6, 78.1, 75.5, 74.8, 74.7, 74.4, 73.9, 71.9, 71.7, 70.8, 70.6, 70.3, 69.6, 68.6, 68.1, 66.8, 62, 60.6, 56.1, 55.2, 20.5, 20.4; HR MALDI-TOF MS: m/z: calcd for $C_{55}H_{60}Cl_3F_2NO_{25}$ [M+Na]$^+$: 1300.2386; found 1300.2397.

Methyl 3,4,6-tri-*O*-acetyl-2-(2,2,2 trichloroethoxy)carbonyl amino-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-*O*-difluorobenzoyl-β-D-galactopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-α-D-glucopyranoside (23)

Compound **23**, was obtained, from **11**, **14** and **12**, and purified by flash chromatography over silica gel (35% EtOAc in hexane) in 51% yield; ^{1}H NMR (500 MHz, CDCl3) δ 8.37 – 7.03 (m, 13H, ArH), 5.50 (dd, *J* = 10.5, 7.9 Hz, 1H, Gal H-2), 5.44 (d, *J* = 3.4 Hz, 1H,

Gal H-4), 5.18 (dd, J = 10.5, 3.4 Hz, 1H, Gal H-3), 5.00 (t, J = 9.6 Hz, 1H, GlcN H-4), 4.95 (d, J = 11.1 Hz, 1H, PhCHH), 4.93 – 4.86 (m, 2H, Glc H-2, GlcN H-3), 4.81 (d, J = 3.6 Hz, 1H, Glc H-1), 4.74 (dd, J = 12.6, 10.2 Hz, 2H, GlcN H-1, 1 x CH_2NHTroc), 4.62 (d, J = 8.1 Hz, 1H, Gal H-1), 4.42 – 4.29 (m, 4H, Glc H-3, 1 x CH_2NHTroc, 1 x PhCHH, NH), 4.22 – 4.11 (m, 4H, Glc H-6a, GlcN H-6a, Gal H-6a, Gal H-6b), 4.00 – 3.89 (m, 2H, GlcN H-6b, Gal H-5), 3.83 (dd, J = 10.2, 5.9 Hz, 1H, Glc H-5), 3.72 – 3.59 (m, 3H, GlcN H-2, Glc H-6b,GlcN H-5), 3.42 (t, J = 9.3 Hz, 1H, Glc H-4), 3.19 (s, 3H, OCH_3), 2.17 (s, 0H), 2.04 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3), 1.92 (s, 3H, COCH_3), 1.90 (s, 3H, COCH_3); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.8, 129.7, 129, 127.9, 127.7, 125.4, 121.6, 118.5, 118, 101.8, 101.5, 96.4, 79.2, 76.2, 74.6, 74.2, 74.2, 72, 71.6, 70.8, 70.7, 70.1, 69.7, 69, 68.9, 68.4, 67.1, 62, 61.4, 56.3, 55, 20.8, 20.8, 20.6, 20.5, 20.5; HR MALDI-TOF MS: m/z: calcd for C$_{55}$H$_{60}$Cl$_3$F$_2$NO$_{25}$ [M+Na]$^+$: 1300.2386; found 1300.2392.

Methyl 3,4,6-tri-*O*-acetyl-2-(2,2,2 trichloroethoxy)carbonyl amino-β-D-glucopyranosyl-(1→3)-[3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-α-D-glucopyranoside (24)

Compound **24**, was obtained, from **11**, **14** and **15,** and purified by flash chromatography over silica gel (30% EtOAc in hexane) in 60% yield; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.25 – 7.13 (m, 15H, ArH), 5.36 (dd, J = 10.5, 3.4 Hz, 1H, Fuc H-3), 5.30 (d, J = 3.2 Hz, 1H, Fuc H-4), 5.06 (d, J = 11.2 Hz, 1H, PhCHH), 5.04 – 4.99 (m, 2H, GlcN H-4, Glc H-2), 4.98 – 4.90 (m, 3H, Glc H-1, GlcN H-3, Fuc H-1), 4.81 (d, J = 8.4 Hz, 1H, GlcN H-1), 4.73 (d, J = 12.1 Hz, 1H, 1 x CH_2NHTroc), 4.65 (d, J = 12.2 Hz, 1H, PhCHH), 4.60 (d, J = 12.1 Hz, 1H, PhCHH), 4.53 (d, J = 11.2 Hz, 1H, PhCHH), 4.46 – 4.39 (m, 1H, Glc H-3), 4.37 (t, J = 9.7 Hz, 2H, 1 x CH_2NHTroc, NH Troc), 4.22 (dd, J = 12.3, 4.5 Hz, 1H, GlcN H-6a), 4.15 (q, J = 6.7 Hz, 1H, Fuc H-5), 3.99 (dd, J = 12.3, 2.4 Hz, 1H, GlcN H-6b), 3.91 – 3.81 (m, 3H, Glc H-6a, Fuc H-2, Glc H-5), 3.71 – 3.59 (m, 4H, GlcN H-2, Glc H-6b, GlcN H-5, Glc H-4), 3.28 (s, 3H, OCH_3), 2.13 (s, 3H, COCH_3), 1.99 (s, 6H, 2 x COCH_3), 1.93 (s, 3H, COCH_3), 1.91 (s, 3H, COCH_3), 1.08 (d, J = 6.5 Hz, 3H, Fuc CH_3); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.7, 129.7, 129, 127.9, 125.5, 101.7, 97.9, 96.6, 79.3, 76, 74.7, 74.4, 74.3, 73.5, 72.8, 71.7, 71.6, 70.3, 70, 68.4, 67, 64.3, 62, 56.4, 55.1, 20.9,

20.8, 20.7, 20.4, 15.8; HR MALDI-TOF MS: m/z: calcd for $C_{53}H_{62}Cl_3NO_{22}$ $[M+Na]^+$: 1192.2727; found 1192.2739.

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CHAPTER V

CONCLUSION

Xyloglucans are a major class of plant cell wall polysaccharides with structural and regulatory functions in the cell wall of plants. The amounts, localization as well as the structures of xyloglucans are known to vary among different plant species, tissue and cell type, and according to the developmental stage of the cell. The structural diversity of xyloglucan and their biological significance in the plant cell wall is not clearly understood. In this respect, immunofluoresence techniques have emerged as powerful tools to determine the fine structure and localization of xyloglucans in the plant cell walls. But, the lack of well defined xyloglucan structures is an impediment in generating well characterized antibodies which in turn makes structural interpretation using these methods uncertain. There is a need for chemical synthesis of xyloglucan oligosaccharides, which can be used to determine the minimum epitope requirements of these antibodies. To this end we have demostarated the synthesis of unique xyloglucan oligosaccharide which contains an acidic galacturonic acid subunit. These acidic xyloglucans have been found to be localized only in the root hair tips and are not found in the leaf or stem cell walls. The key step in the synthesis of tetrasaccharide xyloglucan sidechain α-L-fucosyl-(1→2)-β-D-galacturonyl(1→2)-α-D-xylosyl-(1→6)-β-D-glucopyranoside was the chiral auxilliary mediated synthesis of α-D-xylosyl-(1→6)-β-D-glucopyranoside disaccharide. Xylose 1,2-oxathiane donor was employed for the stereoselective formation of the 1,2-*cis* linkage. The 1,2-oxathiane ether was installed by a novel one-pot two-step procedure and was found to be stable to commonly employed protecting group manipulations. The 1,2-oxathaine could easily be converted into glycosyl donors by oxidation to sulfoxides followed by arylation using 1,3,5-trimethoxybenzene to give bicyclic sulfonium ions. The presence of electron withdrawing

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protecting groups on xylose 1,2-oxathiane donors significantly improved the α-stereoselectivity by disfavoring oxacarbenium ion formation. Due to the low-reactivity of uronic acid donors, the galcturonic acid was introduced by a late-stage post glycosylation oxidation strategy. A 4,6-benzylidene acetal protected galactose donor was used during the initial assembly of the tetrasaccharide. After the tetrasaccharide was assembled, the 4,6-benzylidene acetal was removed and the C-6 hydroxyl of the galactose was oxidized to the galacturonic acid using TEMPO/BAIB. Furthermore, the tetrasaccharide was equipped with an anomeric aminopropyl linker, which was conjugated to biotin and the biotinylated tetrasaccharide was used in epitope characterization of thirty two xyloglucan-binding monoclonal antibodies.

To further streamline the process of oligosaccharide synthesis we have developed a protecting group (PDP) which can be used in the one-pot synthesis of branched oligosaccharides. The protecting group can easily be coupled to any hydroxyl group of the sugar molecule using DIC/DMAP coupling chemistry and can be deprotected *in situ* in combination with triflic acid promoted glycosylations of trichloroacetimidate donors, paving the way for the synthesis of branched oligosaccharides in an one-pot procedure. The utility of this methodology can be further extended by the use of sugars other than glucose at the core or using a thioglycoside as the initial acceptor, to synthesize more complex structures by employing the resulting thioglycosyl trisaccharide as a glycosyl donor in the concomitant step.