



Synthesis of S-linked oligoxylans

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Synthesis of *S*-linked oligoxylans

Ph.D. Thesis

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February 2016



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PREFACE

The work described in this PhD thesis represents three years of research conducted at DTU Chemistry, under the supervision of Prof. Mads H. Clausen. The project is part of a larger project called Sustainable Enzyme Technologies for Future Bioenergy (SET4FUTURE) that involves different partners from industry and academia. The key outcomes of this project ultimately relate to improve the conversion of biomass to fuel through the interplay of research in different areas, like oligosaccharide synthesis, carbohydrate microarray technology, enzymology and bio-resource management.

SET4FUTURE project is carried out in collaboration between researchers in Denmark, USA and the UK. As part of the project, three months' work of the PhD program had been carried out at the Joint BioEnergy Institute (JBEI) part of Lawrence Berkeley Laboratories under the supervision of Prof. Henrik V. Scheller.

Manuscripts describing the work presented in Chapter 5 of the thesis are currently in preparation.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Mads Hartvig Clausen for selecting me as PhD student in this project. By choosing me, he gave me not only the chance of experiencing a different scientific environment but also the possibility to grow as researcher and as a person. In these three years and four months of PhD he taught me to be more independent and professional in my research but also to relax outside the lab, always creating a pleasant and cheerful environment around me and my colleagues.

Thanks also to former and present members of the Clausen group (Alexandra Zakharova, Asger Bjørn Petersen, Brian Brylle Dideriksen, Carlos Azevedo, Christine Kinneart, Enzo Mancuso, Faranak Nami, Gauthier Mike Luc Scavée, Geanna Min, Henrik Schaarup-Jensen, Hélène Viart, Ignacio Martínez San Segundo, Irene Boos, Jorge Peiró, Mathias Christian Franch Andersen, Mathilde Daugaard, Martin Jæger Pedersen, Peter Hammershøj, Peng Wu, Philip Charlie Johansen, Shahid Iqbal Awan, Thomas Flagstad) for the creative environment and social atmosphere. I would like to give a very special thanks to my labmate, flatmate, dinner advisor, most of all great friend, Henrik. You know how much your company means to me.

I would like to thank all the members of the Carbohydrate Journal Club, especially the people that helped me proof-reading my thesis. I wish you all the best for your project. A special thanks for Irene Boos for agreeing on taking over my project and for being always so kind to me.

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Brian Ekman-Gregersen, Paul Erik Wibe and Tina Gustafsson and the sweet Anne Hector, thanks for providing superb technical support.

I would like to thank Professor Henrik Vibe Scheller for giving me the opportunity to work within an interesting field of research in an interdisciplinary and highly

dynamic group. I had a great time in Berkeley during my external stay, also thanks to the company of my friend and colleague Christine.

I would like to express my deepest gratitude to my family in Italy and to my family here in Denmark, meaning my dearest friend, but also flatmate, colleague, life mentor, and much more Clotilde d'Errico. Words cannot express how grateful I am for our friendship. You truly are family to me.

Last but not least, I would like to thank my boyfriend Jens for the support, love and unbelievable patience he shows me every day. The realization of the present thesis would have not been possible without your encouragement and help.

ABSTRACT

The transition from a petroleum-dependent economy to one based on sustainable bio-resources will largely be founded on plant cell walls, as these are the largest source of biomass on earth. However, the development of lignocellulosic biomass conversion to fine chemicals and polymers still remains a big challenge for the biofuel industry. In particular, the enzymatic hydrolysis of lignocellulosic polysaccharides is one of the limiting steps of the entire procedure and therefore the enzymes involved in the degradation process must ideally be characterized and understood. This requires a detailed understanding of cell wall polysaccharide composition and architecture. Hemicelluloses are the second most abundant polymers in lignocellulosic biomass. They include different types of polysaccharides like xyloglucans, xylans, mannans, glucomannans and β -(1 \rightarrow 3,1 \rightarrow 4)-glucans. Xylans are heteropolymers possessing a β -(1 \rightarrow 4)-D-xylopyranose backbone, which is branched by short carbohydrate chains. The branches include D-glucuronic acid and its methyl ether, L-arabinose and/or various oligosaccharides like D-xylose, L-arabinose, D- or L-galactose and D-glucose. The hydrolysis of these polysaccharides is catalyzed by several families of enzymes, collected under the name of Glycosyl Hydrolases (GHs). Among other methods, the use of enzyme inhibitors like thio-linked oligosaccharides has for a long time been a common tool to analyze and characterize these enzymes.

In the present work the chemical synthesis of thio-analogs of xylo- and arabinoxyloglycans is presented. Furthermore, the selection of a reliable method for the incorporation of thiolinkages in the synthesis of oligoxylans is also investigated.

Therefore, different strategies for assembling *S*-linked-disaccharides have been approached both involving 1-thioglycoside donors and thioacceptors. Advantages and disadvantages concerning the different methods are described and evaluated in relation to the synthesis of linear and branched oligoxylans.

RESUMÉ

Overgangen fra en olie-afhængig økonomi til én baseret på bæredygtige bioressourcer vil overvejende være underbygget af plantecellevægge, da disse er den største kilde til biomasse på jorden. Dog er udviklingen af biomasse konvertering, til finkemikalier og polymerer en af de større udfordringer for biobrændstof industrien. Især den enzymatiske hydrolyse af lignocellulose polysaccharider er begrænsende for hele processen, derfor er karakterisering og forståelse af enzymer involveret i nedbrydningsprocessen ideel. Dette kræver en detaljeret forståelse af sammensætningen og arkitekturen af polysacchariderne i plantecellevæggen. Hemicellulose er den næstmest udbredte polymer i lignocellulose biomasse. De omfatter forskellige typer af polysaccharider såsom; xyloglucaner, xylaner, mannaner, glucomannaner og β -(1 \rightarrow 3,1 \rightarrow 4) glucaner. Xylaner er en heteropolymer med et backbone bestående af β -(1 \rightarrow 4)-D-xylopyranose, forgrenet med korte kulhydratkæder. Forgreningerne omfatter D-glucuronsyre og dens methylether, L-arabinose og/eller forskellige oligosaccharider såsom; D-xylose, L-arabinose, D- eller L-galactose og D-glucose. Hydrolysen af disse polysaccharider katalyseres af flere familier af enzymer, klassificeret som glycosylhydrolaser (GHS). Blandt andre fremgangsmåder har anvendelsen af enzyminhibitorer, som thioether i oligosaccharider, i langtid været et velkendt værktøj til at analysere og karakterisere disse enzymer.

I det foreliggende arbejde præsenteres den kemiske syntese af thio-analoger af xylo- og arabinoxyloglycaner. Endvidere er udvalget af en pålidelig metode til inkorporering af thiobindinger i syntesen af oligoxylans også undersøgt.

Derfor er forskellige strategier til at koble *S*-forbundne disaccharider blevet tilgået, involverende både - thioglycosid donorer og acceptorer. Fordele og ulemper for de forskellige fremgangsmåder er beskrevet og evalueret i relation til syntesen af lineære og forgrenede oligoxylaner.

ABBREVIATIONS

Ac	acetyl
AGA	apiogalacturonan
All	allyl, prop-2-en-1-yl
Ara	arabinose
AX	arabinoxylan
BDA	butane diacetal
Bn	benzyl
BSP	1-benzenesulfinyl piperidine
BTMSS	bis(trimethylsilyl) disulfide
Bu	butyl
Bz	benzoyl
COSY	correlation spectroscopy
CSA	camphor-10-sulfonic acid
CSL	cellulose synthase-like
DAST	(diethylamino) sulfur trifluoride
DBU	1,8-diazabicycloundec-7-ene
DDQ	2,3-dichloro-5,6-dicyano- <i>p</i> -benzoquinone
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DMTST	dimethylthiomethylsulfonium triflate
DTE	1,4-dithioerythritol
DTT	dithiothreitol
Et	ethyl
Gal	galactose
GalA	galacturonic acid
GH	glycosyl hydrolases
Glc	glucose

HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMBC	heteronuclear multiple bond correlation spectroscopy
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HG	homogalacturonan
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
IDCP	iodonium di- <i>sym</i> -collidine perchlorate
Im	imidazole
IR	infrared spectroscopy
LC-MS	liquid chromatography-mass spectrometry
<i>m</i> -CPBA	<i>meta</i> -chloroperoxybenzoic acid
Me	methyl
MES	2-(<i>N</i> -morpholino) ethanesulfonic acid
MS	molecular sieves
NBS	<i>N</i> -bromosuccinimide
NIS	<i>N</i> -iodosuccinimide
NMR	nuclear magnetic resonance
PG	protecting group
Ph	phenyl
PMB	<i>p</i> -methoxybenzyl group
Py	pyridine
R_f	<i>ratio frontis</i>
RG	rhamnogalacturonan
Rha	rhamnose
TAPS	3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid
TBDMS	<i>tert</i> -butyldimethylsilyl
TES	triethylsilyl
Tf	triflate, trifluoromethanesulfonyl

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	tosyl, <i>p</i> -toluenesulfonyl
UDP	uracil diphosphate
XGA	xylogalacturonan

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“It always seems impossible until it’s done”

Nelson Mandela

1 Biomass as an alternative energy source

Biomass is a general term that indicates biological material derived from both animals and plants. The future energy economy will likely be based on a broad range of alternative energy sources like wind, water, sun, nuclear fission and fusion, as well as biomass^{1,2}. Therefore, the exploitation and utilization of biomass energy have attracted much interest around the world, not only for most second generation biofuel production but also for a vast range of other products such as paper, food ingredients, dietary and industrial fibers, pharmaceuticals and nutraceuticals³. Biofuels are classified into two different categories depending on the source of biomass⁴. First generation biofuels are obtained from sugar or starch, which is easily extracted and fermented to ethanol. The second generation of biofuel is derived from lignocellulosic non-food plants, or from municipal or agricultural waste. While production of first generation biofuel often competes with food crops for land and therefore can only meet a limited fraction of the global fuel requirement, lignocellulosic feedstocks do not represent a food source for humans and therefore could represent a sustainable alternative to fossil-derived liquid fuels^{5,6}.

2 Plant cell wall

Plant cell walls are the largest source of biomass on earth. The plant cell wall is a highly organized composite of many different polysaccharides, proteins and aromatic substances. The polysaccharides are constructed of some of the most complex carbohydrates that not only play an important role in growth and development of the plant, but also form its main structural framework⁷ (Figure 1). The molecular composition and arrangements of the wall polymers differ among species, individual cells, and even among regions of the wall⁸. The study of the cell wall polysaccharides across the plant kingdom is necessary for developing our understanding of cell wall plant evolution, and for optimizing its utilization⁹. The

cell wall provides support and shape for the plant, allowing it to stand upright. It also offers a barrier against the environment and potentially pathogenic organisms, but at the same time, it is metabolically active, meaning that it allows the exchange of material and signals between cells.

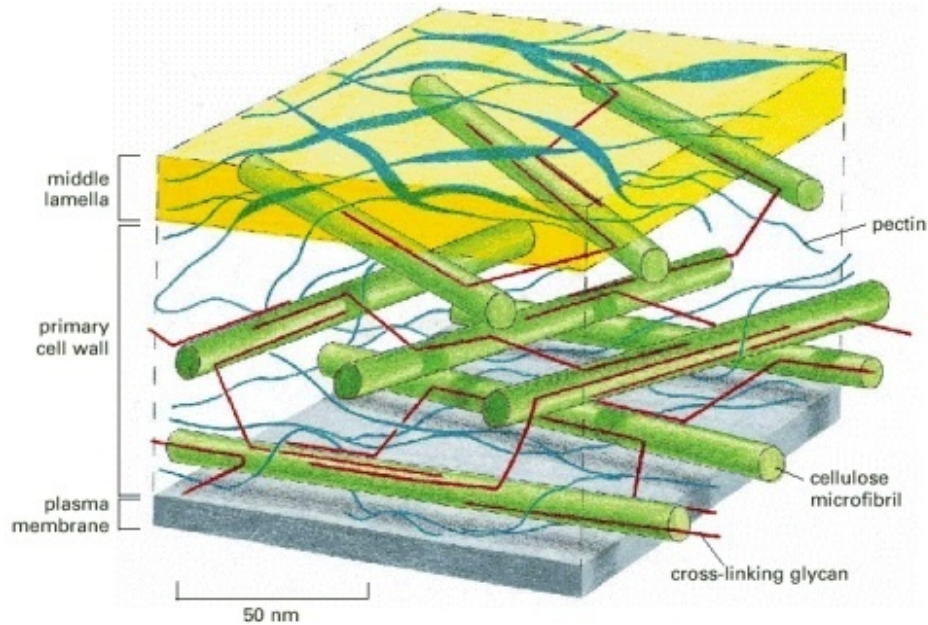


Figure 1: segment of the primary cell wall. Hemicellulose is represented here as cross-linking glycans¹⁰.

The primary cell wall develops into the cell during cell division and rapidly increases in surface area during cell expansion. When cells stop growing some cells develop a secondary wall, which gives additional strength. The main components of the plant cell wall are different polymers including polysaccharides, proteins, aromatic substances and also water and minerals. Lignocellulosic biomass is composed of polysaccharides, lignins and a small amount of proteins and minerals. The relative amount and interactions of these components define the different biomechanical properties of the plant cell wall¹¹. Cellulose and hemicelluloses polymers bring rigidity to the wall and pectin provides fluidity through the gelatinous polysaccharides matrix. Cellulose and hemicelluloses are embedded in the amorphous pectin polymers and are stabilized by proteins and phenolic

compounds¹². Cellulose is the main cell wall polymer that brings support to the plant and consists of a linear insoluble unbranched polymer of β -(1 \rightarrow 4)-D-glucose residues associated with other cellulose chains by hydrogen bonding and Van der Waals forces. Cellulose chains aggregate together to form microfibrils, which represent the core of the plant cell wall and about one third of the total mass of the wall¹³. The physical properties of cellulose are complex, depending on the degree of polymerization, the number of chains in a single cellulose fibril, and the inter- and intra-chain hydrogen bonds and interactions. Hence, cellulose can form different allomorphs. Hemicelluloses and pectins are commonly called “matrix polysaccharides” because of their highly branched and amorphous structure. Major hemicelluloses in biomass include polymers of pentoses, hexoses and sugar acids, while pectins are the widest complex family of polysaccharides in nature. They are comprised of α -1,4-linked-galacturonic acid polysaccharides, including homogalacturonans, rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and substituted galacturonans^{14,15}. On the other hand, lignins are a group of hydrophobic polymers that contain three major units, called guaiacyl, syringyl and *p*-hydroxyphenyl. The biological functions of lignin are to provide mechanical support for the cell walls and to restrict water transport through vascular tissue to ensure efficient water transport in the plant¹⁶.

2.1 Hemicellulose

Hemicelluloses are low-molecular-weight polysaccharides in plant cell walls that have β -(1 \rightarrow 4)-linked backbones with an equatorial configuration¹⁷. The most important biological role of hemicelluloses is to strengthen the cell wall as a result of their interaction with cellulose and, in some walls, with lignin¹⁸. Hemicelluloses are synthesized by glycosyltransferases, which are located in the membrane of the Golgi apparatus and include different types of polysaccharides like xyloglucans, xylans, mannans, glucomannans and β -(1 \rightarrow 3,1 \rightarrow 4)-glucans (Figure 2). The complex structure of the hemicelluloses and their distribution vary significantly between different species and cell types¹⁸. The polysaccharide chains of

hemicellulose are very different from each other in structure and physicochemical properties, except for β -(1 \rightarrow 3,1 \rightarrow 4)-glucans, which are very rare and mostly restricted to Poales.

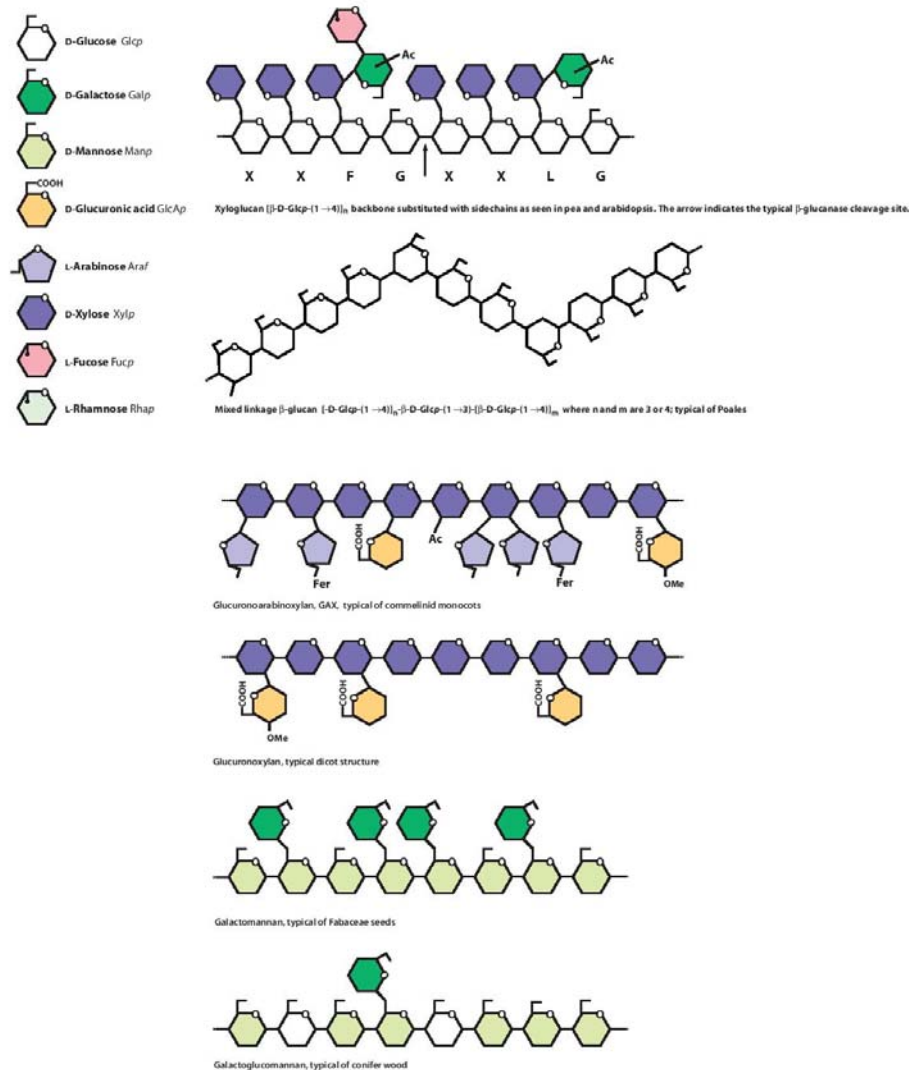


Figure 2: Schematic illustration of the types of hemicelluloses found in plant cell walls. The letters written under the xyloglucan molecule represent the symbols used for the common side chains. “Fer” represents esterification with 3-methoxy-4-hydroxycinnamic acid (ferulic acid), which is characteristic of xylans in monocots¹⁸.

Hemicelluloses are characterized by having β -(1 \rightarrow 4)-linked backbones of glucose, mannose or xylose. These glycans have the same equatorial configuration at C1 and C4, therefore the backbones of these polysaccharides have significant structural similarities¹⁸. In the next section only D-xyloglycans will be discussed, in view of the relevance to the present work.

2.1.1 D-Xyloglycans

Xylan-type polysaccharides are the main hemicellulose components of secondary cell walls and they constitute about 20-30% of the biomass of hardwoods and herbaceous plants and up to 50% in grasses and cereals¹⁹. Xylans are heteropolymers possessing a β -(1 \rightarrow 4)-D-xylopyranose backbone, which is branched by short carbohydrate chains. These branches include D-glucuronic acid and its methyl ether, L-arabinose and/or various monosaccharides like D-xylose, L-arabinose, D- or L-galactose and D-glucose²⁰. One of the most frequent modifications of xylans is the substitution with α -(1 \rightarrow 2)-linked glucuronosyl and 4-*O*-methyl glucuronosyl residues (Figure 2). These types of oligosaccharides are often known as glucuronoxylans and they are the dominating non-cellulosic polysaccharide in the secondary walls of dicots (hardwoods and herbaceous plants). In contrast, in monocots, which include grasses and related species, arabinoxylans (AXs) are the major non-cellulosic polysaccharide in the primary wall¹⁸, even though the frequency and composition of the branches are dependent on the source of xylan²¹

2.1.2 Arabinoxylans

Arabinoxylan has been identified in many of the main commercial cereals like wheat, rye or barley as well as in other plants such as rye grass and bamboo shoots. They represent the major hemicellulose component of cell walls of flour and bran of cereal grain²². AX has a linear backbone that is partially substituted by α -L-arabinofuranose residues, attached to the backbone. The substitutions in grass cell walls are mostly at the O-3 of the backbone xylose residues while in grass endosperm it is very common to observe double substitution with

α -L-arabinofuranose at both the O-2 and O-3 of the xylopyranose monomer units²³ (Figure 3).

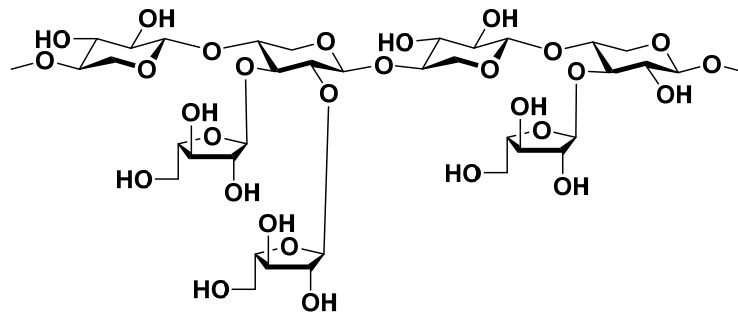


Figure 3: Primary structure of L-arabino-D-xylan represented with the commonly observed branches

In addition, most xylans are acetylated to various degrees at the O-3 of xylose residues and to a lesser extent at O-2¹⁸. Another important characteristic of grass xylans is the presence of ferulic acid esters attached to the O-5 of some of the branching arabinofuranosyl residues (Figure 2). Esters of *p*-coumaric acid are also abundant in grass cell walls though it is not clear if they are attached directly to the xylan backbone²⁴.

2.2 Biosynthesis and hydrolysis of xylans

Unlike cellulose, which is synthesized by large enzyme complexes at the plasma membrane, xylans are synthesized by enzymes in the Golgi apparatus²⁵. Therefore, xylan synthesis requires the coordinated involvement and regulation of the synthetic enzymes, such as synthases and transferases, as well as other enzymes that synthesize and transport substrates into the Golgi apparatus. Moreover, because of the structural similarities of xylan to the β -(1 \rightarrow 4)-linked backbones of the other hemicelluloses, it has been widely assumed that the biosynthesis would involve members of the same cellulose synthase-like (CSL) glycosyltransferase families¹⁸. However, it is unclear how many glycosyltransferases are needed to make the entire oligosaccharide. The reducing end of the polysaccharide is probably synthesized by a different glycosyltransferase since synthesis of the cell

wall polysaccharides is generally assumed to take place by transfer to the non-reducing end of the growing chain. Importantly, this theory is still widely discussed and irrefutable evidences have not been reported yet²⁶. However, evidences show that the most important side chains of xylans (glucuronic acid and arabinofuranose) are formed by α -glucuronosyltransferases and α -arabinosyltransferases respectively and both activities have been detected in vitro^{27,28,29}. As mentioned earlier, arabinosyl residues may be linked to the 2- or 3-position or both positions of the xylosyl residues in the backbone. Since a monosubstituted xylosyl residue is expected to be a very different acceptor compared with an unsubstituted residue, at least three arabinosyltransferases are expected. These are an α -(1 \rightarrow 2), an α -(1 \rightarrow 3) arabinosyltransferase and at least a third for adding the second residue to the monosubstituted xylose¹⁸.

Xylans can be hydrolyzed into mono- and oligosaccharides by xylanases. Generally xylanases refer to a large group of enzymes responsible for hydrolysis of xylan. The main enzymes involved are endo-1,4- β -xylanases and β -xylosidases¹⁷. Endo-xylanases have shown to catalyze the random internal cleavage of the main chain of xylan, producing in this way a mixture of xylooligosaccharides³⁰, while β -xylosidases release xylose by removing terminal monosaccharides from the non-reducing end of short oligosaccharides³¹.

In addition, the debranching activities have been proposed to be performed by means of α -D-arabinofuranosidases that hydrolyze the L-arabinose residues substituted at position 2 and/or 3 of the xylan backbone³².

In the same fashion, α -D-glucuronidases have been shown to be responsible for the cleavage of the α -1,2-bonds between the glucuronic acid residues and the xylan backbone³³. Furthermore, acetylxylan esterase and ferulic and coumaroyl esterases hydrolyze the *O*-acetyl of acetylxylan and the phenolic acids linked to position 5 of the arabinofuranoside residues respectively^{34,35}.

The enzymes involved in the degradation of xylan and their mode of action are listed in Table 1 while a schematic view is shown in Figure 4.

Table 1 Enzymes involved in the hydrolysis of complex heteroarabinoxylans (adopted from Saha B.C., 2002³⁶).

Enzyme	Mode of action
Endo-xylanase	Hydrolyzes mainly internal β -1,4-xylose linkages of the xylan backbone
Exo-xylanase	Hydrolyzes β -1,4-xylose linkages releasing xylobiose
β -Xylosidase	Releases xylose from xylobiose and short chain xylooligosaccharides
α -Arabinofuranosidase	Hydrolyzes α -arabinofuranose from arabinoxylans
α -Glucuronidase	Releases glucuronic acid from glucuronoxylans
Acetylxylan esterase	Hydrolyzes acetyl ester bonds in acetyl xylans
Ferulic acid esterase	Hydrolyzes feruloyl ester bonds in xylans
<i>p</i> -Coumaric acid esterase	Hydrolyzes <i>p</i> -coumaryl ester bonds in xylans

The xylan backbone is hydrolyzed primarily by the endo- β -1,4-xylanases belonging to the enzyme families GH 10 and GH 11³⁷. They hydrolyze the glycosidic bonds by acid base-assisted catalysis via a double displacement mechanism leading to retention of anomeric configuration at the newly formed reducing end³⁸.

Consistent with their “endo” mode of action, the substrate binding cleft of xylanases extends along the entire length of the proteins and can accommodate from four to seven xylose residues^{39,40}. Four xylose residues is thus the minimum length a substrate needs to have to be hydrolyzed by these families. One of the fundamental differences between these two major families of xylanases is that GH 11 enzymes hydrolyze unsubstituted regions of xylan, while GH 10 enzymes can hydrolyze the corresponding substituted chains^{41,30}.

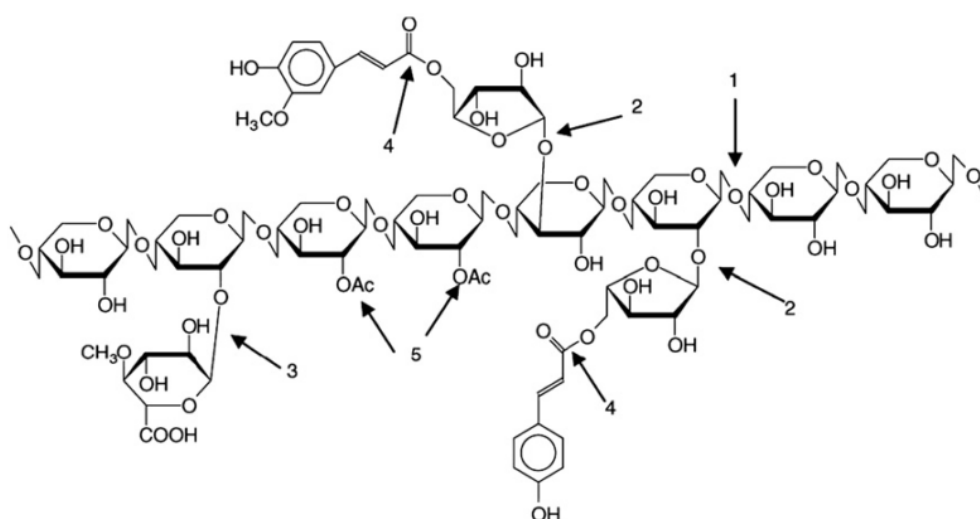


Figure 4: The structure of xylan and sites of enzymatic hydrolysis of the xylanase complexes. 1: endoxylanases; 2: α -L-arabinofuranosidases; 3: glucuronidases; 4: feruloyl and coumaroyl esterases; 5: acetyl xylan esterases¹⁷.

2.3 β -xylosidases and α -arabinofuranosidases in degradation of arabinoxylans

As mentioned before, the search for biofuels has been focused on sustainable biomass feedstock, such as waste biomass and biomass grown on degraded and abandoned agricultural lands^{42,43}. AX is one of the major components of feedstocks that are currently being investigated as a source for advanced biofuels^{36,44}. As mentioned in the previous section, many enzymes are required for its degradation and among them we find endoxylanases, α -L-arabinofuranosidases and to complete the process, β -xylosidases. Endoxylanases and β -xylosidases act in synergy, since endoxylanases generate more non-reducing ends for β -xylosidases to act on, and β -xylosidases remove the end product (short chain oligoxylans) that inhibit endoxylanases^{45,46}. Although several comprehensive reviews are available on xylanases^{47,48}, there is a need for more accurate information on AX degrading enzymes that can be used in the production of biofuels, especially regarding

“accessory enzymes”, like α -L-arabinofuranosidases and β -xylosidases. These not only play a key role but also perform, in the case of α -L-arabinofuranosidases, a rate-limiting reaction in the degradation process^{49,50}. The properties of these enzymes, such as their substrate specificity, activity and other biochemical properties need thus to be studied in order to evaluate their possible applications. α -L-arabinofuranosidases are found in enzyme families GH 3, 43, 51, 54 and 62, while xylosidases are present in GH 3, 39, 43, 52, 54, 116 and 120. Except for GH 43, which is an inverting GH, all these families perform hydrolysis with retention of configuration⁴⁹. Enzyme family GH 43 and GH 3 also have another important uniqueness. These families are known for their bifunctional enzymes, capable of acting both as α -L-arabinofuranosidases and β -xylosidases⁵¹. However, more experimental evidence is required to firmly state xylosidase activity linked to the GH 43 family⁴⁹. In an industrial process, such as the production of biofuel, where an intensive enzymatic degradation of arabinoxylans is needed, it is clear that enzymes presenting dual activity have an important advantage and need to be studied and characterized in more detail. This could, for example, be obtained through X-ray crystal structures, which would greatly aid in explaining why these enzymes, and not others, can release both arabinose and xylose from natural substrates⁴⁹. The investigation of enzyme activity with a variety of methods results in synergy in understanding the characteristics of an enzyme. Application of different techniques, such as crystallography, enzymatic activity measurement or genetic engineering can validate data obtained by one method by the results of the others, yield more specific information on a particular aspect of a system, and provide results in a range of assay conditions ordinarily not available to a single method^{52,53}.

2.4 The use of thio-glycosides as inhibitors in the study of enzymes

The use of inhibitors has for a long time been a common tool for investigating the mechanism of enzyme action⁵⁴. The binding of the glycosyl part of the substrate can be studied by designing inhibitors of the same structural type. These substrate analogs enable detection of conformational changes during the catalysis. It has been speculated that the adaptation of the binding site to the substrate could optimize the orientation of the catalytic groups in accordance with the bond that has to be cleaved⁵⁵. In this way it would be possible to obtain evidence, like X-ray crystallographic structure, for conformational changes by the use of inhibitors. When the glycosidic oxygen of glycosides is replaced by sulfur, the rate of the catalytic reaction is usually decreased for acid catalyzed hydrolysis. The first step of the catalytic reaction is considered to be the protonation of the anomeric oxygen by an AH group of the enzyme⁵⁴. Sulfur is less basic than oxygen, which means that the concentration of *S*-protonated conjugate acid is lower than *O*-protonated conjugate acids, and hence, the rate of the hydrolysis decreases by 10^2 - 10^3 fold⁵⁴. However, when thioglycosides are used as substrates with generic glycosidases even larger differences in the rates of hydrolysis has been detected⁵⁴. Additionally, the size of the electron density of the sulfur atom is more dispersed; consequently it is less electronegative than oxygen. The C-S bond (ca 1.8 Å) is also longer than C-O (ca 1.4 Å) and the C-S-C angle (ca 95-100°) is smaller than the corresponding oxygen-containing structure⁵⁶. Despite all these differences, which are quite discrete, *S*-linked oligosaccharides are generally recognized by enzymes in the similar manner as the corresponding *O*-linked substrates, since they are also capable of acting as hydrogen bond acceptors, which usually play a significant role in binding of the substrate⁵⁷. This property of thioglycosides allows for the study of the formation of the first complex (Michaelis complex) and the kinetic parameters for the binding and dissociation of this complex easier than the natural counterpart that has a much higher rate of hydrolysis⁵⁴. Because of these differences between

O- and S-linked oligosaccharides, the use of thioglycosides as strictly competitive inhibitors has been widely used^{58,59,60,61}, also in relation to hydrolyses^{57,62}.

2.5 The targets

In order to study and collect more information on β -xylosidases and endo- β -1,4-xylanases, a suitable target, recognizable by the enzymes but also inhibitory to them, was designed. Since both enzymes, as explained before, act on four-units-substrates, a tetraxylan was suggested. In addition, to inhibit their capacity to cleave interglycosidic bonds at the non-reducing end, a thio-linkage on that position was inserted into the target. Furthermore, the structure was maintained unbranched to have selectivity for the GH 11 family enzymes, since GH 11 have demonstrated to act mostly on unsubstituted xylan chains³⁰. With these considerations in mind, the tetrasaccharide **1** was chosen as the first target of the present work (Figure 5).

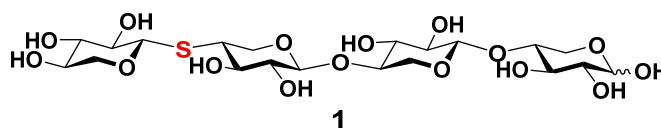


Figure 5: Tetraxylan with a thiolinkage-Target **1**

The length of the structure is four units as this is the minimal length recognized by endo- β -1,4-xylanases. Four units is also a suitable length for studying β -xylosidases, since they catalyze the hydrolysis of short xylan chains (1 to 5 units) starting from the non-reducing end⁴⁹. The second target was designed as a substrate of α -arabinofuranosidases (Target **2**). Here, the main goal is the characterization of arabinofuranosidases that, based on current knowledge, are either difficult to purify or present a dual activity⁴⁹. In this way, the β -xylosidase and the residual endo- β -1,4-xylanase activity would be reduced, while α -arabinofuranosidase catalysis would still be active. Moreover, since most of the common arabinoxylans

bear the arabinofuranoside at the C-3¹⁸, the target was designed with α -L-arabinofuranose positioned at this position (Figure 6).

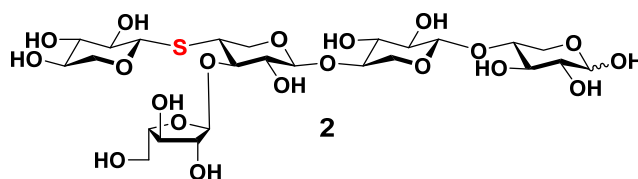


Figure 6: Branched tetraoxylan-Target 2

Arabinofuranosidases can be divided in two separate classes depending on their substrate specificities towards specific arabinose substitutions: those that are able to hydrolyze arabinoses from monosubstituted xyloses, both C-2 and C-3 linked, and those that cleave the arabinose from the C-3 position of disubstituted xyloses⁴⁹. The two classes act in a synergistic fashion and with the β -xylosidases⁶³. Therefore, we envision that a disubstituted S-linked tetraoxylan can also be a relevant substrate. Hence, compound 3 was also chosen as a suitable target (Figure 7).

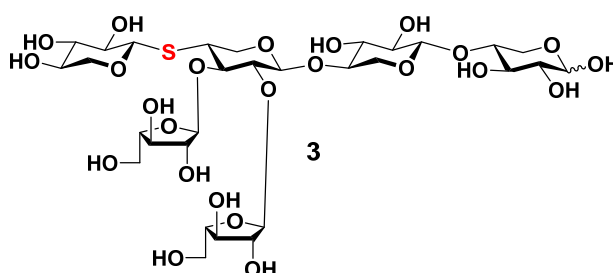


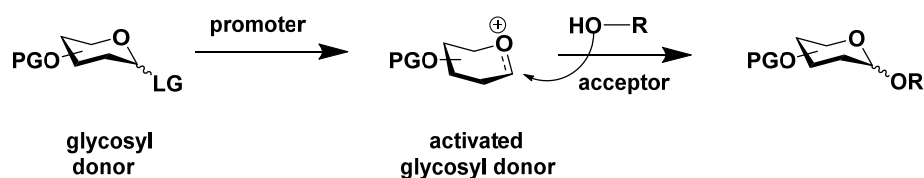
Figure 7: Branched tetraoxylan-Target 3

Compound 3 should be active as a substrate on the class of arabinofuranosidases that cleave α -arabinofuranosides in C-3 while being resistant to xylanase and xylosidase activity.

In the following chapter some general aspects of the glycosidic bond formation and in more details, thio-linkage formation will be discussed.

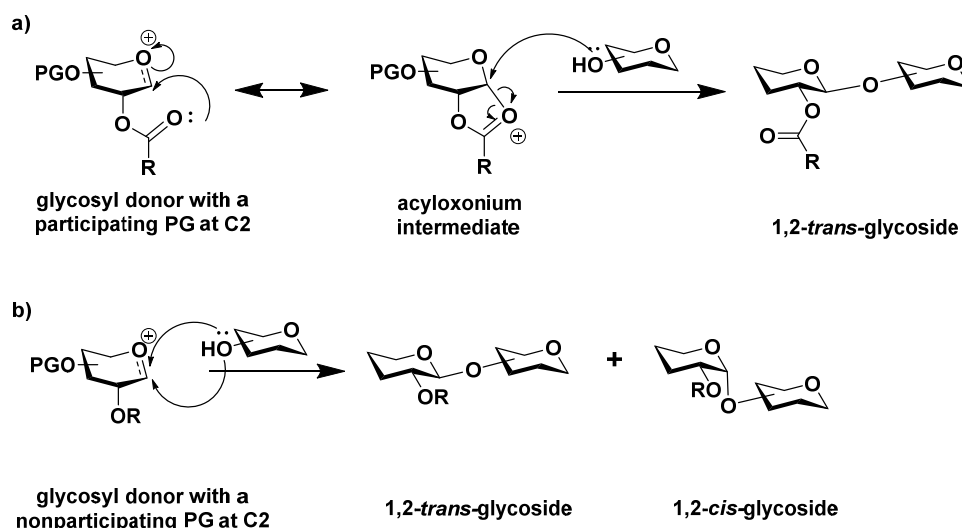
3 General aspect of glycosidic bond formation

The biological activity of glycoconjugates has stimulated much activity in glycoside synthesis in the past⁶⁴⁻⁶⁹. The discovery of the first controlled, general glycosylation procedure is credited to Koenigs and Knorr⁷⁰ and since then much knowledge has been accumulated and systematized on the topic⁷¹⁻⁷³. Detailed glycosylation mechanism has not been elucidated yet, but it has been commonly assumed that the glycosylation reaction involves nucleophilic displacement at the anomeric center⁷³. Since the reaction involves a secondary carbon (the oxocarbenium ion) and a weak nucleophile (the sugar acceptor), the unimolecular S_N1 mechanism is generally followed⁷⁴ (Scheme 1).



Scheme 1: Unimolecular S_N1 glycosylation mechanism

As a result of the S_N1-type character of the glycosylation reaction, the control of the stereochemistry is the most challenging aspect of oligosaccharide synthesis. The nature of the protecting group (PG) on the 2-position of the donor has a significant impact on the stereoselectivity of glycosylations. When employing participating neighboring groups (i.e. esters), the reaction is usually stereospecific and the glycosidic outcome is a 1,2-*trans* linkage (for non-mannoside-like D-sugars). On the other hand, in case of PGs in C2 which are not capable of offering anchimeric assistance (i.e. ether), the nucleophilic attack of the oxocarbenium ion is possible from both sides of the sugar ring producing both possible anomeric products⁷³ (Scheme 2).



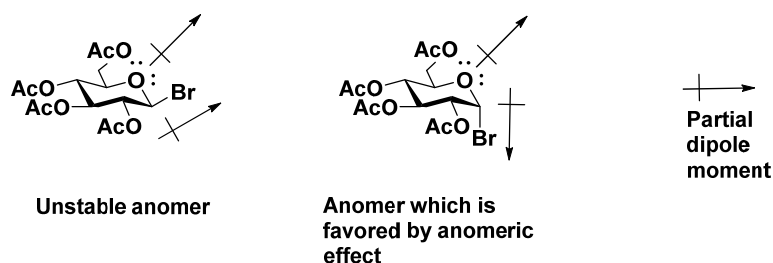
Scheme 2: Glycoside synthesis with a) neighbouring group participation b) no neighbouring group participation

Many variables like PGs, glycosylation promoters and scavengers, and also reaction conditions like temperature and solvent can influence the glycosylation outcome⁷³. It illustrates that one of the biggest challenges for carbohydrate chemists is the formation of regio- and stereoselective glycosidic linkages. Even though several factors dictate the stereochemical outcome of a glycosylation, the most commonly applied strategy through the years has been PG manipulation. For a particular target oligosaccharide, the PGs are chosen based on their selectivity in protecting hydroxyl groups, compatibility with other PGs present on the molecule and liability towards the transformation reactions that the molecule undergoes⁷⁵. To insure a better selectivity, the glycosyl acceptor usually bears only one free hydroxyl group while the remaining hydroxyl groups are protected with suitable PGs. However, in certain cases, differences among the numerous hydroxyl groups, like their relative position, axial rather than equatorial, or other characteristics, like being a secondary alcohol rather than primary, can highly affect their reactivity, and thus lead to a certain degree of regioselectivity, even in absence of PGs⁷⁶. The general rule states that primary hydroxyl groups are more reactive than equatorial

secondary hydroxyl groups that are then more reactive than axial secondary hydroxyl groups⁷⁷.

3.1 The anomeric effect

In pyranosides, groups with an electronegative charge tend to be more stable when bound in the axial position at the anomeric center. This is known as “anomeric effect” and it can be explained by electronic forces that in this case overrule the steric hindrance⁷⁸. The phenomenon involves partly a dipole-dipole effect based on intramolecular electrostatic interactions of two dipoles adjacent to the anomeric center. There is indeed a partial dipole along the polarized bond between the anomeric carbon atom and its bound atom and the other partial dipole is caused by the two lone pair of the oxygen in the carbohydrate ring (Scheme 3). In the axial configuration the two dipoles point out in two different directions almost opposite to each other, while in the equatorial configuration the two dipoles point almost in the same direction. Following this line of thoughts, in the axial configuration the dipole moments partially neutralize each other while the equatorial configuration leads to a partial intramolecular addition of the two dipole moments, resulting in a less stable molecule.



Scheme 3: Directions of the partial dipole moment in α and β anomers

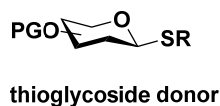
Moreover, the anomeric effect is also a stereoelectronic effect, which can be explained by the molecular orbital theory. Only when the anomeric substituent is axially positioned can the electrons of the lone pair belonging to the endocyclic carbohydrate ring oxygen and the antibonding orbital of the anomeric substituent

effectively overlap. The anomeric effect also varies according to the nature and the position of the substituent in C2, to the electronegativity of the anomericly bound atom and the polarity of the solvent used during the glycosylation. Equatorial substituents in C2 tend to weaken the influence of the anomeric effect, together with polar solvents. However, the electronegativity of the anomericly bound atom is proportional to the influence of the effect.

3.2 Glycosyl donors

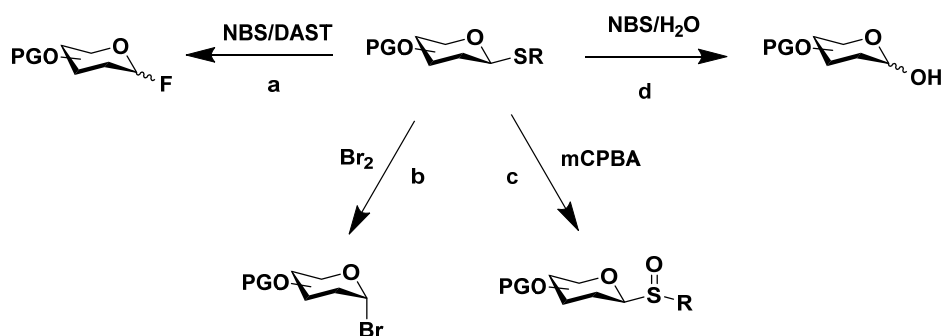
An enormous selection of glycosyl donor has been developed over the years, which includes anomeric halides^{70,79,80}, selenoglycosides⁸¹, glycols⁸², sulfoxides⁸³, vinyl glycosides⁸⁴, xanthases⁸⁵, pentenyl glycosides^{86,87}, orthoesters⁸⁸, thioglycosides⁸⁹, imidates^{90,91} and many others. For each type of glycosyl donor, different conditions are available for their activation⁷³. The following paragraphs will emphasize the last two of these glycosyl donors (thioglycosides and glycosyl imidates) since these will be employed in the present work.

3.2.1 Thioglycoside donors



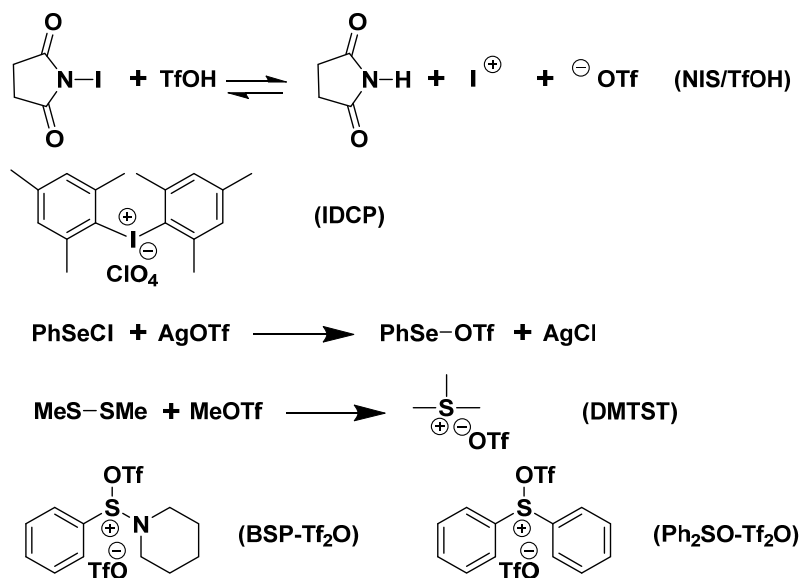
Thioglycosides were first introduced in 1973 by Ferrier and co-workers⁹² and by now they are some of the most widely used donors employed in carbohydrate chemistry. This popularity is not only due to their easy preparation, long shelf-life and stability under a variety of reaction conditions, but also to their facile conversion into the activated intermediates or other glycosyl donors⁹³ (Scheme 4). For example, a glycosyl fluoride can be achieved as an anomeric mixture by the combined treatment with *N*-bromosuccinimide (NBS) and diethylaminosulfur trifluoride (DAST)⁹⁴ (Scheme 4, a), while treatment with bromine yields exclusively the α -bromo-derivative⁹⁵ (Scheme 4, b). To afford the glycosyl

sulfoxide donor, oxidants such as *m*-chloroperoxybenzoic acid (*m*-CPBA) need to be employed⁸³ (Scheme 4, c), while to transform the thioglycoside into the correspondent hemiacetal, which can be applied in the conversion to other donors, a hydrolysis in the presence of NBS can be performed⁹⁶ (Scheme 4, d).



Scheme 4: Transformation of thioglycosides into other types of glycosyl donors

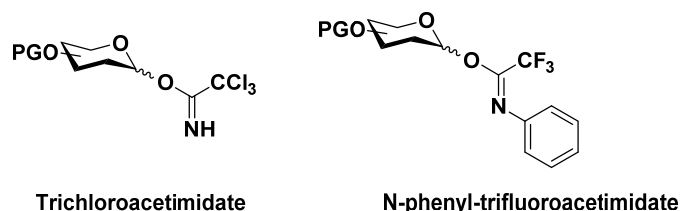
For thioglycosides activation, a variety of electrophilic promoters can be employed.



Scheme 5: The most commonly used activators for thioglycosides

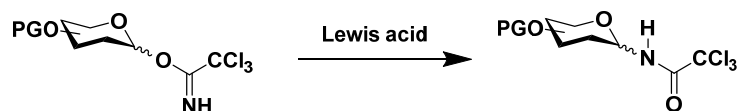
The most commonly used are the combination of N-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) or its trimethylsilyl derivative (TMSOTf)⁹⁷, iodonium di-*sym*-collidine perchlorate (IDCP)⁹⁸, methyl trifluoromethanesulfonate (MeOTf)⁹⁹, dimethylthiomethylsulfonium triflate (DMTST)¹⁰⁰, phenylselenyltriflate (PhSeOTf)¹⁰¹ and many others of more recent introduction like the combination of 1-benzenesulfinyl piperidine/triflic anhydride (BSP/Tf₂O)¹⁰² or diphenyl sulfoxide/Tf₂O (Ph₂SO/Tf₂O)¹⁰³ (Scheme 5). All promoters are typically added in stoichiometric amounts.

3.2.2 Glycosyl trichloroacetimidates and *N*-phenyl trifluoroacetimidates



Trichloroacetimidates were introduced by Schmidt and co-workers in 1980¹⁰⁴. They are generally sufficiently stable for purification and can be stored at low temperature for months. Trichloroacetimidates can be activated by a catalytic amount of Brønsted or Lewis acid, like trimethyl triflate (TMSOTf) or triethyl triflate (TESOTf)¹⁰⁵. They can be prepared by a base catalyzed reaction of trichloroacetonitrile and the glycosyl hemiacetal. The strength of the base employed determines the stereochemical outcome¹⁰⁶. The high reactivity of trichloroacetimidates can lead to side reactions or even to decomposition of the donor during the glycosylation. For example, if the acceptor is not reactive enough, rearrangement of the trichloroacetimidate donor into the corresponding unreactive glycosyl trichloroacetamide has been observed (Scheme 6)¹⁰⁷. In some cases pre-mixing the acceptor with an acidic promoter before the addition of the

trichloroacetimidate donor, can be advantageous and diminish their decomposition¹⁰⁸.



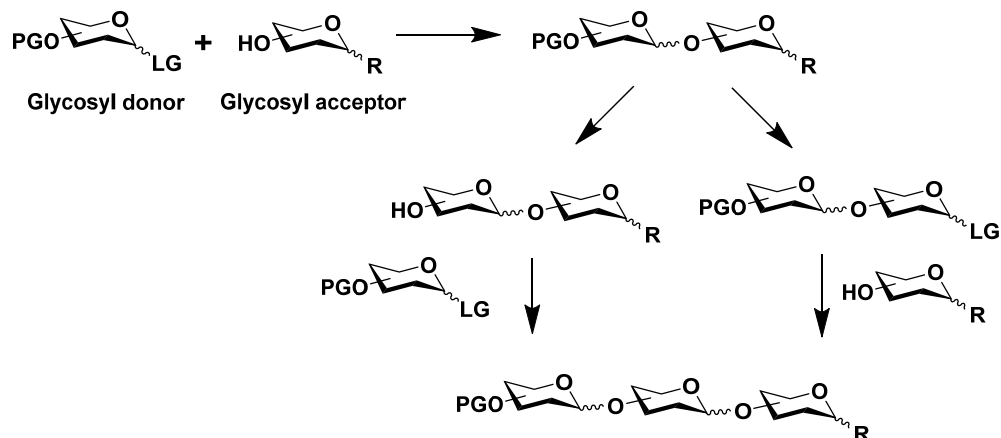
Scheme 6: Rearrangement of trichloroacetimidate into the corresponding trichloroacetamide

In 1983, Schmidt reported another type of glycosyl imidates, trifluoroacetimidates¹⁰⁹, as glycosyl donors. Differently from the trichloroacetimidate formation, the use of K₂CO₃ as the base generally favors α -*N*-phenyl-trifluoroacetimidates, whereas the use of NaH or DIPEA mainly yields β -products^{110–112}. Trifluoroacetimidates are generally less reactive than the corresponding trichloroacetimidate donors presumably because of the lower *N*-basicity or the presence of an *N*-substituent. Trifluoroacetimidate donors have shown advantages over trichloroacetimidates in the synthesis of β -mannosides because of their lower propensity to undergo side reactions and rearrangements to acetamide during glycosidations¹¹³. Particularly, this rearrangement takes place when the acceptor is of low nucleophilicity or sterically hindered, whereas it is diminished in trifluoroacetimidates glycosidation because of the increased steric hindrance of the *N*-phenyl group. Thus, the use of *N*-phenyl trifluoroacetimidates donors have widely spread in oligosaccharide synthesis in the last years.

3.3 Synthetic strategies for oligosaccharide formation

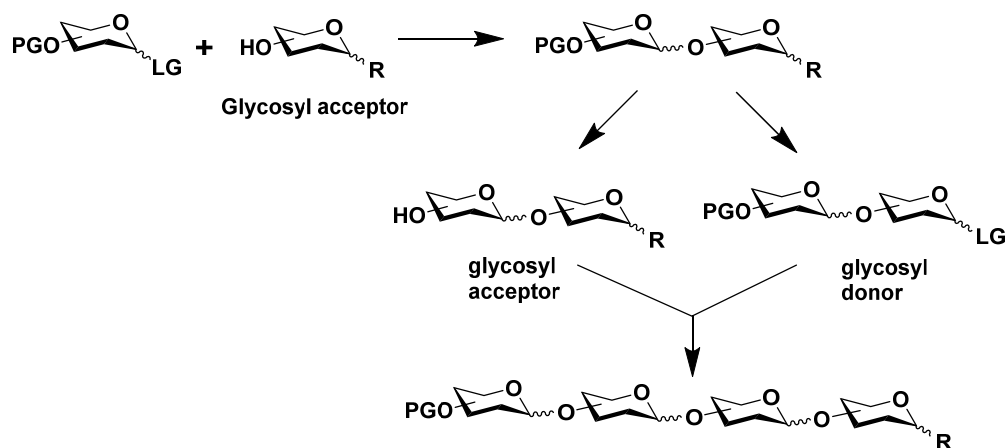
Two different approaches are generally used for the assembly of oligosaccharides with the final aim of reducing the number of linear synthetic steps. The two approaches are called linear (or stepwise) and convergent (or block) synthesis¹¹⁴. A traditional linear approach requires subsequent conversion of the disaccharide product, yielded by the first glycosylation, into a glycosyl acceptor or donor in

order to be coupled with a monosaccharide into a trisaccharide. The consequent trisaccharide, in turn, is also converted into acceptor or donor and coupled again with a monosaccharide until the length of the desired oligosaccharide is reached (Scheme 7).



Scheme 7: Linear (Stepwise) synthesis

On the contrary, in block synthesis a disaccharide building block is first assembled and transformed into the corresponding glycosyl donor and acceptor, which are then coupled together to yield a tetrasaccharide directly (Scheme 8).



Scheme 8: Convergent block synthesis

When applied to the synthesis of large oligosaccharides, the stepwise approach can be too tedious and inefficient. The block synthesis is thus preferred especially if two or more sequential repeating units are employed. Another advantage of the convergent strategy is that it allows for performing the most challenging glycosylation steps in an early stage, and thereby also facilitates the chromatographic purification of potential diastereoisomers.

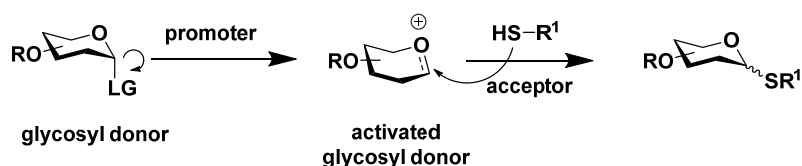
4 Thioglycosides in Glycobiology

Carbohydrate-protein recognition has been known for a long time to be essential for the biotransformation of natural polysaccharides. Furthermore, in recent years, it has been demonstrated as an important process for biological transfer of information in living organisms⁶⁰. Therefore, to promote new concepts in glycotherapy and for the engineering of enzymes, an accurate investigation of carbohydrate-protein interactions is required. As discussed earlier, enzyme-substrate interactions can be difficult to analyze by the introduction of specific substrate modifications since these interactions involve recognition, binding and ultimately the catalytic process. However, in some cases, it is possible to obtain some information about the geometry of the catalytic site or determine the mechanism of action of these enzymes¹¹⁵. On the other hand, a more versatile approach to study the active site of glycosyl hydrolases is the utilization of enzyme-resistant substrates, which act as competitive inhibitors. In these enzyme-resistant oligosaccharides the enzyme-labile oxygen connecting the individual saccharide units can be replaced with e.g. a methylene group, nitrogen or sulfur atom⁵⁴. In this section the synthetic strategies to accomplish thiolinkages are going to be discussed. There are two general approaches to synthesize thiooligosaccharides. The first approach is “normal” glycosylation (see chapter 3 on *O*-glycosylation) but with a saccharide thiol as glycosyl acceptor. The second approach is “inverse” glycosylation, by means of an anomeric thio function, which is introduced first to yield the anomeric thiol or thiolate and then reacted with a saccharide electrophile, to form the inter-*S*-glycosidic linkage through the S_N2 displacement reaction (Scheme 9). Both approaches are commonly used, but perhaps the latter approach, where the anomeric conformation of the product is more easily controlled since it is constructed during the formation of the anomeric thiol function and not during the coupling reaction. This approach has also been the most investigated. Furthermore, replacement of the oxygen with a sulfur to create *S*-thioglycosides presents other synthetic challenges than the mere formation of the thio-linkage itself due to the difference in chemical properties of the two elements.

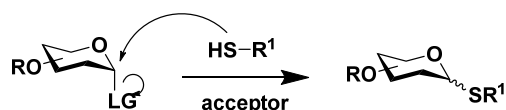
Firstly, sulfur functions are incompatible with catalytic hydrogenolysis¹¹⁶, which complicates the use of benzyl ethers as protective groups, and secondly thiols easily form disulfides, both as donor or acceptors¹¹⁷.

O-glycosylation-like coupling reactions:

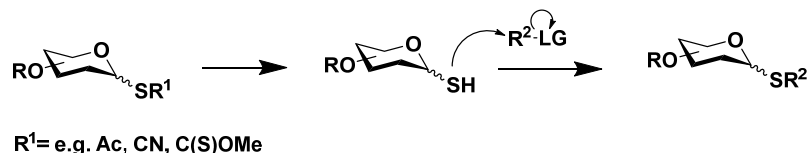
S_N1-type



S_N2-type



"Inverse" glycosylation reactions:



Scheme 9: General representation of glycosylation methods

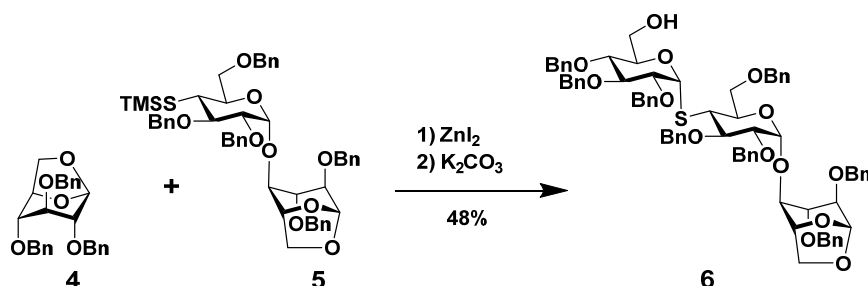
4.1 Glycosylation with thiol acceptors

Using thiol as acceptors facilitates the utilization of most of the known *O*-glycosylation methods and varies the nature of the donor in order to find the right "match" for the thiol acceptor we had. However, only glycosylation methods using promoters that are not thiophilic can obviously be used, which disqualifies some of the *O*-glycosylation methods (i.e. thiophenols). In the past, mostly

anhydrosugars and glycols were used as donors and more recently trichloroacetimidates.

4.1.1 Anhydrosugars as donors

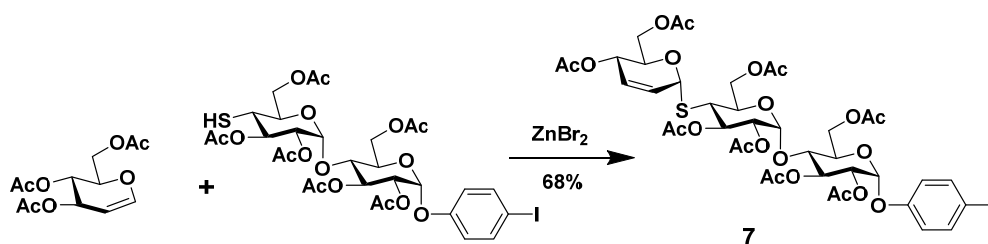
One example of anhydrosugars used as donors in thiolinkages formations was provided by Wang L. and co-workers in 1990. He utilized the benzylated 1,6-anhydroglucose **4** as a donor in the construction of maltose and maltotriose structures¹¹⁸. The condensation afforded the α -linked thio-trisaccharide **6** in moderate yield (Scheme 10). Interestingly, 1,6-anhydrodisaccharides (i.e. compound **5**) were found to be unreactive as donors with 4-thiosaccharide acceptors, and could consequently be used as alternative acceptors.



Scheme 10: Example of anhydrosugar used as donor

4.1.2 Glycols as donors

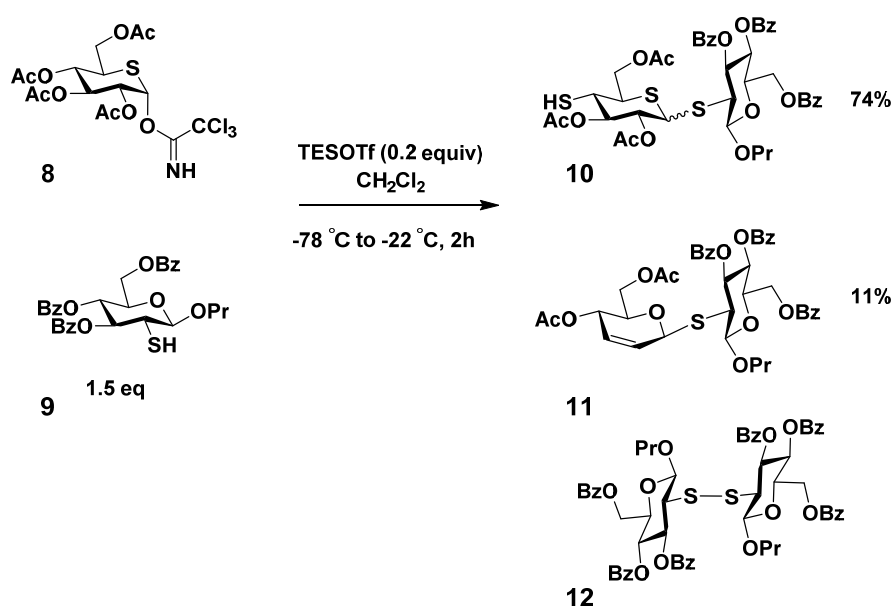
Glycols had also been employed in addition reactions of saccharide thiols. A peracetylated glycol was used in 1988 by Driguez and co-workers to obtain the α -linked thio-linked trisaccharide **7** in 68% yield¹¹⁹. The β -anomer and the product resulting from displacement of the 3-*O*-acetate were also isolated (Scheme 10).



Scheme 11: Example of glycols used as donor

4.1.3 Trichloroacetimidates as donors

This method has been extensively used for making *O*-glycosidic linkages⁶⁴ and in 1998 Pinto M. and co-workers synthesized a dithio analogue of n-propyl kojibioside and heteroanalogues of dimannosides using this coupling method^{120,121}. For the kojibioside derivatives, they used the acetylated trichloroacetimidate **8** as a donor and the benzoylated 2-thiol glycopyranoside **9** as acceptor affording the expected disaccharide **10** as an α/β mixture (Scheme 12).



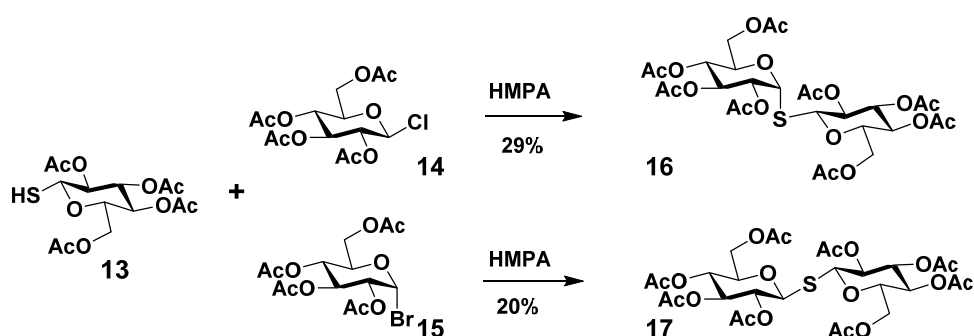
Scheme 12: Example of glycosyl trichloroacetimidate used as donor

One of the major byproducts was the unsaturated disaccharide **11**. The glycosylation reaction was optimized by reducing the quantity of molecular sieves, which were slowing down the reaction, in addition to increasing the amount of thiol acceptor to a donor–acceptor ratio of 1:2.25. Reducing the amount of promoter, triethylsilyl triflate, from 0.25 equiv. to 0.2 was also found to be helpful in decreasing the formation of the unsaturated byproduct. Another byproduct found in the reaction mixture in the presence of oxygen was the disulfide dimer **12**, and even though degassed solvent was used in the reactions to avoid oxidation of the

thiol, a small amount was always detected. Another important factor to take into consideration was the reaction temperature. The reaction was performed between -78 and -22 °C since lower temperature resulted in thioorthoester formation and at higher temperatures degradation and elimination products of the imidate were observed.

4.1.4 Glycosyl halides as donor

In addition to the three examples mentioned above, the S_N2 -displacement of an anomeric halogen by saccharide thiolates has been shown to be feasible⁷³. One of the first attempts to use a bromide glycoside as a donor in a glycosylation with a thiol acceptor was performed in 1978 by Driguez H. and co-workers¹²². In their work, the thio-analogues of maltoside **16**, and cellobioside **17**, were obtained, respectively, from the coupling reaction of the 1-thiopyranoside **13** with tetra-*O*-acetyl-galactopyranosyl chloride **14** or bromide **15**, respectively, in hexamethylphosphoric triamide (HMPA) in 29% and 20% yield (Scheme 13). Comparable results were obtained in the synthesis of a thiolactose by Goodman and co-workers in 1981¹²³.



Scheme 13: Example of halides used as donors

According to literature, the use of HMPA seems to enhance the coupling reaction in comparison with other solvents like DMF, THF, CH_3CN , 1,2-dimethoxyethane and MeOH, but it also has disadvantages such as toxicity, high boiling point, and

potential methylation of the thiol acceptor. Some optimizations were made in 1996 by Magnusson and co-workers by adding a base such as diethylamine or Cs_2CO_3 to the reaction in the synthesis of thiogalabiose derivatives, yielding the desired product in 85% yield¹²⁴. The same procedure has also been used successfully by other research groups for the synthesis of methyl 4-thio- β -cellobioside¹²⁵ and an *S*-linked heparin sulfate trisaccharide¹²⁶.

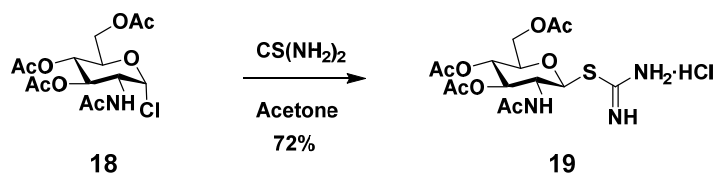
4.2 Displacement reactions with 1-thioglycosides

The second approach for making thiooligosaccharides has been favored in recent years because it allows better control of the stereochemistry of the glycosylation product. In this approach an anomeric thiol (or thiolate) is first synthesized by $\text{S}_{\text{N}}2$ displacement of an anomeric halogen or acetate with a thionucleophile, which is then reacted with a glycosyl electrophile to yield the thiooligosaccharide by a second $\text{S}_{\text{N}}2$ displacement reaction. The challenges of this method are the stereospecific introduction of the anomeric sulfur group and its activation into the corresponding thiolate, in addition to the nature of the electrophile. The most encountered side reactions in these types of couplings are the elimination of the electrophile and the anomerization of the thiol⁷³. Because they can be selectively cleaved into the thiol in the presence of ester protecting groups, almost only thiourea salts and thioacetates have been used as intermediates in the synthesis of anomeric thiols.

4.2.1 Thiourea salts as donor

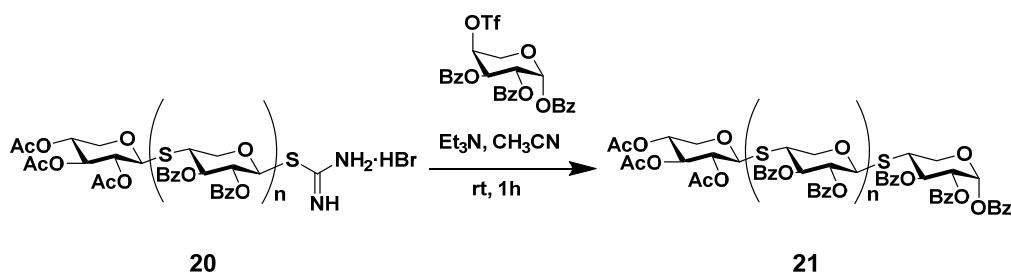
S-glycosyl isothiourea derivatives are known as very suitable precursors for the stereoselective synthesis of thioglycosides. This is due to several factors: the fixed 1,2-*trans*-configuration of the thioglycosidic bond, the simplicity of their preparation from corresponding glycoside halides and thiourea, and their ability to readily be converted into the target compound¹²⁷. For example, the synthesis of a

glucopyranosyl thiourea hydrochloride was reported in 1962 by Horton and co-workers, affording the crystalline salt **19** in high purity¹²⁷ (Scheme 14).



Scheme 14: Example of thiourea salt synthesis

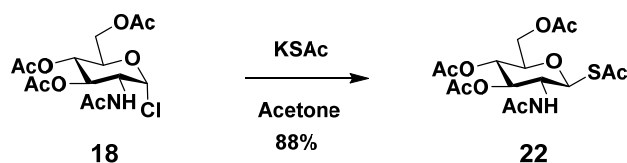
Analogous procedures have later used hydrobromide salts as electrophiles¹²⁸. The higher nucleophilicity of sulfur, as compared to the nitrogen, allows for the chemoselective formation of only 2-glycosyl-2-isothiurea salts, which can often be isolated by simple crystallization. Thiourea salts are easily converted to free thiol under basic treatment in water or with sodium thiosulphate¹²⁹. In 2001, an efficient procedure for the preparation, activation, and direct coupling of thiourea salts was reported by Ibatullin F. M. *et al.* for the synthesis of thioxylooligosaccharides¹³⁰. Using a one-pot protocol with Et₃N in CH₃CN, the thiourea salt **20** was converted into thiol and then reacted with the appropriate acceptor to yield the desired product **21** in high yield (Scheme 15).



Scheme 15: Thioliinkage formation using thiourea salts as donors

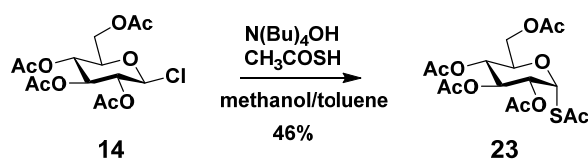
4.2.2 Thioacetate as donor

Glycosyl thioacetates are prepared from glycopyranosyl halides or acetates employing either thioacetic acid or potassium thioacetate (KSAc) as reagent under various conditions. One of the first procedures for synthesizing thioacetates was found in a patent from 1950, and the procedure reported by Horton *et al.* in an article published in 1961¹²⁷. The procedure was employed for the synthesis of the β -thioacetates **22** from α -bromo and α -chloro-glycopyranoses and KSAc in dry acetone (Scheme 16).



Scheme 16: Synthesis of thioacetate **22**

The reaction proceeds through S_N2 displacement. Therefore, in principle, α -products can be obtained from β -halides, and vice versa. In the article, HMPA was also widely described for being a solvent that allows for complete anomeric stereocontrol in the displacement of 1,2-*cis* and 1,2-*trans*-related acylglycosyl halides with sulfur nucleophiles^{122,131,132}. The procedure was optimized by Driguez and co-workers in a later article, where they reported the formation of the *S*-acetyl-1-thio- α -D-glucopyranose **23** from β -glucopyranose chloride **14** with tetrabutylammonium thioacetate as a promoter. The salt was generated *in situ* from thioacetic acid and tetrabutylammonium hydroxide in a mixture of toluene and methanol in 46% yield¹³¹ (Scheme 17).



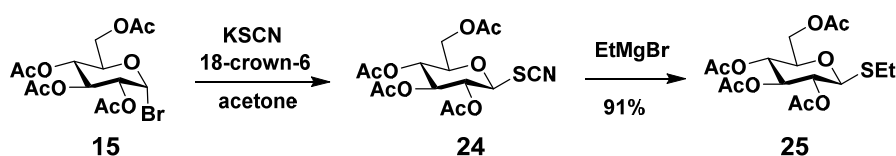
Scheme 17: Synthesis of thioacetate **23**

Glycosyl thioacetates can also be prepared efficiently from the 2-acetoxyglycal derivative by using either photochemical initiation and thiols, or peroxides (*t*-butyl or cumene) and thioacetic acid^{133,134}.

Several methods have described the selective conversion of thioacetates into thiols in the presence of other ester groups. Sodium methoxide in methanol at low temperature (below -20 °C) was reported to afford mainly the de-*S*-acetylation compound¹³⁵ or exclusively when quenching the excessive reactant by the addition of H⁺ resin at low temperature¹³⁶. Other methods have also been used for the chemoselective cleavage of anomeric thioacetates to the thioaldose by treatment with phenylmercury acetate followed by demercuration¹³⁷, cysteamine¹³⁸ and hydrazinium acetate¹³⁹.

4.2.3 Other types of thio precursors as donors

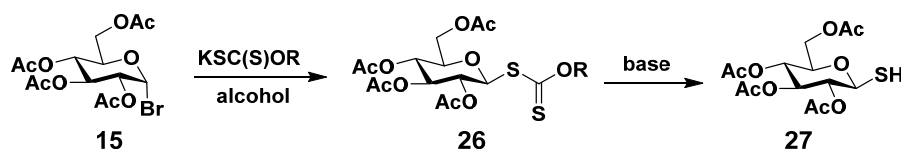
Beside thiourea salts and thioacetates, popular 1-thio precursors are thiocyanates, xanthases, 1,2-thiazoline derivatives, glycosyl thioimidocarbonates and diglycosyl disulfides. The glycosyl thiocyanate **24**, for example, is prepared from the corresponding bromo-sugar **15** through an S_N2 displacement reaction using thiocyanates as nucleophiles^{140,141} (Scheme 18). Notably, the formation of the 1-isothiocyanate isomer (not shown) is a competing reaction in this approach. When formed the glycosyl thiocyanate **24** can be transformed into thioglycosides through cleavage to the thioaldose by mild Zémpfen conditions followed by alkylation, or by a Grignard reaction which directly gives the thioglycoside **25**¹⁴².



Scheme 18: Example of synthesis of thioglycosides via thiocyanates

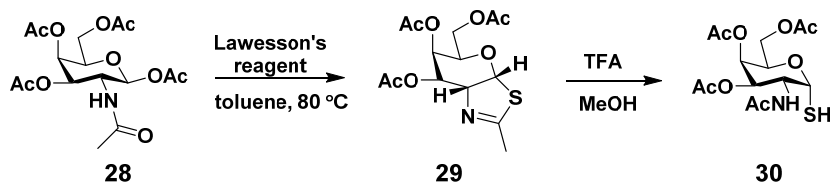
Glycosyl dithiocarbonates (xanthates) **26** are synthesized by the reaction of an acylated glycopyranosyl halide **15** with a potassium alkyl or benzyl xanthate in an

alcoholic solvent¹⁴³ or using phase transfer conditions^{144,145}. These compounds are converted to 1-thioaldoses such as **27** by treatment with a base, generally ammonia or sodium methoxide, to allow later alkylation with the acceptor to afford the desired thioglycoside (Scheme 19).



Scheme 19: Example of synthesis of thioglycosides via xanthates

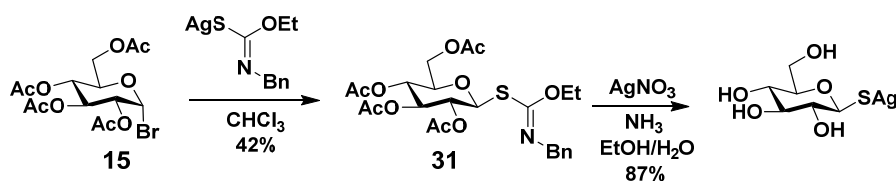
1,2-Thioazoline derivatives (e.g. **29**) are produced by treatment of peracetylated 2-acetamido- β -D-gluco- or galactopyranose **28** with Lawesson's reagent in quantitative yield^{146–148} (Scheme 20). Conditions have also been optimized later by Davis and co-workers with shorter reaction time and lower amount of reagent¹⁴⁹. Subsequent hydrolysis using TFA in methanol with a catalytic amount of water afforded the corresponding anomeric α -thioaldose **30**. However, it is to be noted that the direct formation of glycosylthiols from 1-*O*-unprotected sugars with Lawesson's reagent often leads to anomeric glycosylthiol mixtures.



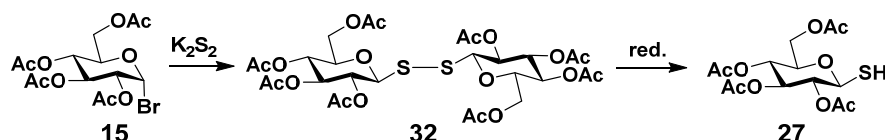
Scheme 20: Example of synthesis of thioglycosides via 1,2-thazolines

Thioimidocarbonates **31** and diglycosyl disulfides **32** are also synthesized by displacement reactions of an acylated glycopyranosyl halide and a thionucleophile, like the *O*-ethyl *N*-benzyl thioimidocarbonate or potassium disulfide.

Via Glycosyl Thioimidocarbonates



Via Diglycosyl Disulfides



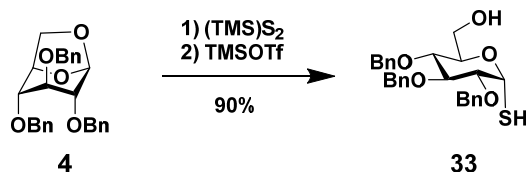
Scheme 21: Examples of synthesis of thiolglycoside via thioimidocarbonates and diglycosyl disulfides

Thioimidocarbonates can be converted to thioaldoses by saponification, and the disulfide can be reduced with a suitable reducing agent to give the free thiol **27**¹⁵⁰ (Scheme 21). However, the use of two equivalents of silver has made the employment of thioimidocarbonates less popular during the years.

4.2.4 New approaches to the synthesis of 1-thioglycosides

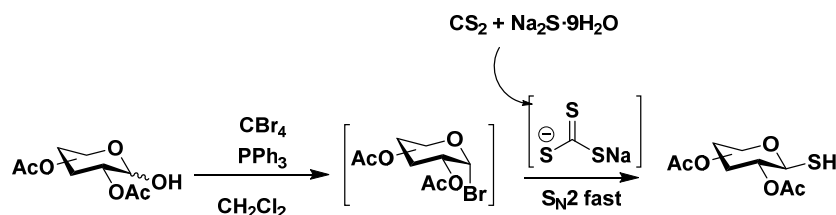
As glycosylthiols with the thiol group in axial position are not as easily available as their equatorials analogues, most of the efforts in the last years have focused on developing a more straightforward strategy for their synthesis. In 2008 Dere *et al.* reported a procedure for the synthesis of axial glycosylthiols that did not involve the utilization of unstable β -glycosylchlorides or neighboring groups assistance¹⁵¹. They described the stereospecific formation of α -glycosylthiols **33** by opening 1,6-anhydrosugars **4** with bis(trimethylsilyl) disulfide (BTMSS) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst (Scheme 22). A few years later, the method was extended to trichloroacetimidates yielding the α -glycosylthiol in comparable yields and selectivity¹⁵². However, the presence of

powerful participating groups at C2 has shown to override the selectivity of the anomeric outcome.



Scheme 22: Example of thioglycoside obtained from 1,6-anhydrosugars

New procedures to afford β -glycosylthiols have also been published recently. For example, a new method for achieving glycosylthiols with a reduced number of steps and intermediates was reported in 2013 by Misra A. K. and co-workers¹⁵³. In their work they reported the one-pot synthesis of β -glycosylthiol, directly from hemiacetals. This approach involves treatment of a hemiacetal under Appel reaction conditions followed by sodium carbonotrithiolate, which is generated *in situ* from CS_2 and $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (Scheme 23).



Scheme 23: Example of synthesis of thioglycosides from hemiacetals one-pot

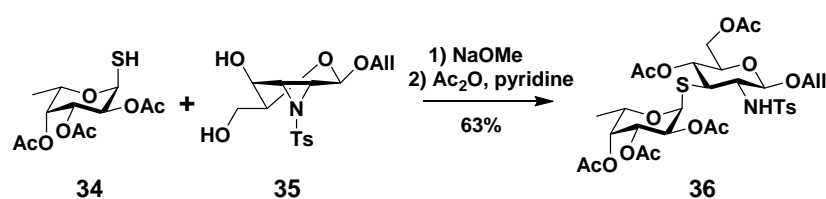
4.2.5 The stereochemical outcome

The stereochemical outcome of thio-glycosylation reaction between 1-thioglycoside donors and a glycosyl acceptor can be easily controlled, since the configuration of the anomeric center involved in the glycosylation process is already determined. Therefore, starting from 1,2-*trans* halogeno-sugars and employing conditions favoring $\text{S}_{\text{N}}2$ -type reactions, 1,2-*cis* thioglycosides are generally yielded^{60,154}. In addition, the synthesis of the 1,2-*trans*-1-thio derivatives is usually easier than the corresponding 1,2-*cis*-thioglycosides, due to the more

efficient synthesis of the 1,2-*cis*-halogeno-sugar precursor. Anchimeric effect of the C2 carbon can be also used to determine the stereochemical outcome of the reaction⁷³.

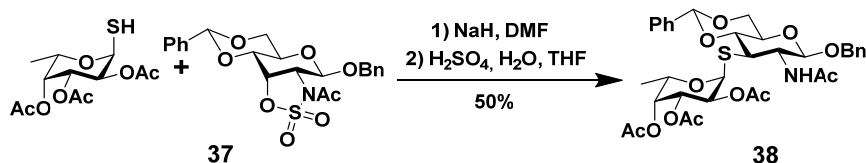
4.2.6 The nature of the electrophile

For making thiolinkages with 1-thiosugars, the hydroxyl group of an electrophile, which is usually the acceptor of the glycosylation reaction, needs to be converted to a leaving group to promote the reaction. Bromides, iodides, tosylates and triflates have been employed with primary leaving groups, in the synthesis of (1-6)-linked structures. However, at the secondary position only triflate is a good enough leaving group in S_N2-type reactions for the formation of the thiooligosaccharides⁷³. The concerted reaction mechanism leads to inverted configurations on the electrophile reactive center, which could in principle present a problem if the required electrophile is an “uncommon” sugar. For example, (1-4)-linked galactose- and glucose-like derivatives can be converted into each other because they are epimers at C4, and in the same fashion (1-2)-linked glucose- and mannose-like derivatives can be interconverted since they are C2-epimers. However, for other oligosaccharides like the (1-3)-linked, it is sometimes necessary to proceed through a more laborious route, like a double inversion pathway. In cases where triflate substrates have failed to give the desired product, various other electrophiles and solutions have been used. For example, Horito S. and co-workers achieved high regioselectivity in the synthesis of the (1-3)-linked disaccharide **36** by ring opening of the 2,3-*allo*-tosylaziridine **35**, obtained from *N*-acetylglucosamine precursor, with the 1-thiofucoopyranosyl derivative **34**¹⁵⁵ (Scheme 24).



Scheme 24: Example of 1,3-thiolinkage formation through ring opening

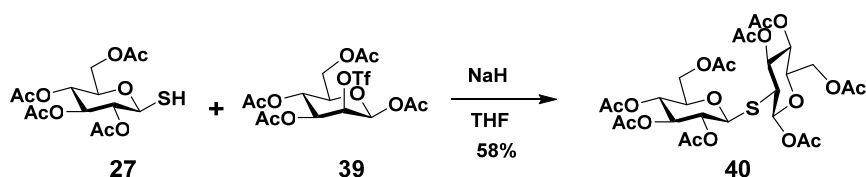
In the same fashion, Aguilera *et al.* synthesized the desired (1-3)-linked thiodisaccharide **38** by using the correspondent cyclic sulfamidate **37**¹⁵⁶ (Scheme 25).



Scheme 25: Example of 1,3 thiolinkage formation via through a sulfamidate

4.2.7 Reaction conditions

The reaction conditions vary considerably depending on whether a 1-thioglycoside is employed in the coupling reaction or one of its precursors. 1-Thioglycosides can either be isolated as thioaldoses and activated *in situ* as a thiolate or cleaved to anomeric thiols and then activated *in situ*. In the first case, an anomeric thiol such as **27** is converted to the thiolate during the coupling with the electrophile by treatment with a strong base like sodium hydride. The resulting sodium salt of the thio-sugar derivative is then coupled with the acceptor **39**, which has been pre-activated with a suitable leaving group, to yield compound **40**. One of the first attempts was performed by Driguez and co-workers in 1982 with HMPA as solvent¹³¹. However, problems of disulfide formation were encountered. Better yields were obtained by subjecting the thio salt to high vacuum in the presence of phosphorus pentoxide prior to usage. Later, other procedures favored the use of DMF or THF as a solvent for the coupling reaction to give higher yields^{138,157} (Scheme 26).

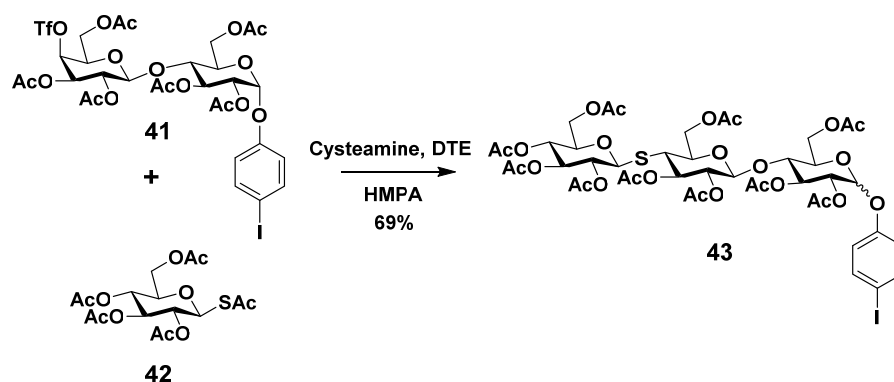


Scheme 26: Example of glycosylation condition with 1-thioglycosides

The displacement reaction is usually performed in aprotic polar or non-polar solvents. Phase-transfer conditions are also applicable for sugar triflates in the reaction with the sodium salt of thio sugar derivatives, using cryptands or crown ethers as catalysts¹⁵⁸. Again, better yields were obtained by subjecting the thio salt to high vacuum in presence of phosphorus pentoxide prior to use.

Another method relies on the activation of a thioacetate like **42** directly in the glycosylation reaction mixture¹⁵⁹. This procedure holds the advantage of using a thiodonor, which is stable enough for storage. Furthermore, the procedure minimizes the formation of the elimination byproduct from the triflate acceptor **41**, which is usually the major byproduct of reactions with thioaldoses^{138,157}. Another significant advantage is the limited formation of the disulfide by-product.

In 1988 Driguez and co-workers developed a new method involving an *in situ* selective *S*-deacetylation and activation of a 1-thioacetate with the nucleophile cysteamine¹⁵⁹ in HMPA in the presence of 1,4-dithioerythritol (DTE)^{119,160} (Scheme 27).



Scheme 27: Synthesis of oligosaccharide **43** using thioacetates

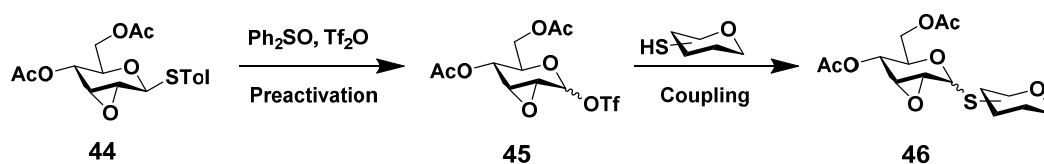
The activation of thioacetate **40** was proved by an experiment performed by Horton and co-workers in 1963¹⁶¹. The presence of dithioerythritol stabilizes the generated sulfide anion in the reduced form and does not interfere as additional nucleophile⁶¹. Improvement of this procedure was later investigated by other research groups. The

first was given by Yuan C. Lee and co-workers in 1996, where they suggested the utilization of DMF as a solvent for the reaction, obtaining higher yields and easier work-up. Further investigations performed on thioglycosides of *N*-acetylneuraminic acid, gave good results using a secondary alkyl amine like diethylamine instead of cysteamine¹⁶⁰. The procedure yielded a high percentage of product formation and very small amount of the elimination product of the triflate derivative, but unfortunately formation of small amount of the corresponding anomeric product was also observed. Moreover, it is mostly limited to secondary tosylate or bromide substrates. However, the positive results reported on the subject encouraged many chemists of the field to improve the procedure further. In 2000 John T. Ku and co-workers reported high yields with the use of highly hindered iminophosphorane bases (Schwesinger bases) in piperidine as suitable non-nucleophilic bases for this reaction. They obtained almost no elimination side-products and no α -linked thiodisaccharide. However, the method requires a high amount of iminophosphorane bases, which could sometimes be impractical due to their cost. Thus, the use of cysteamine and dithioerythritol is still generally favored, as they have demonstrated to be better applicable to a larger variety of substrates.

4.3 Recent approaches to the synthesis of thio-disaccharides

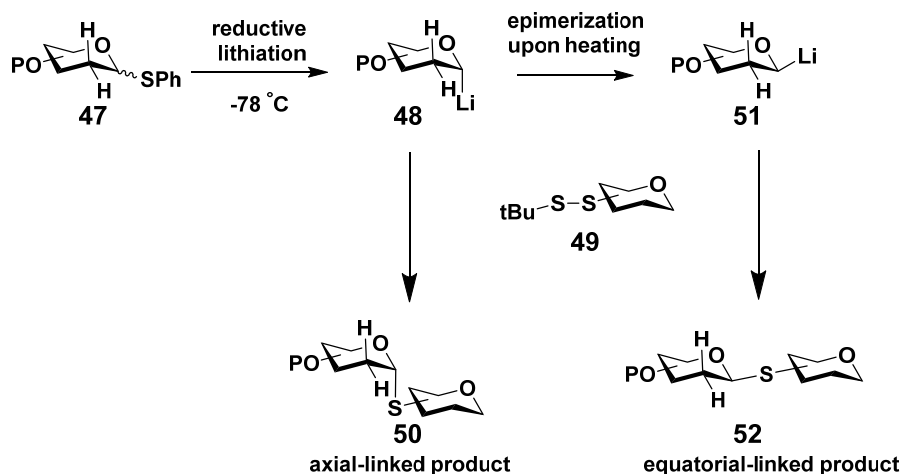
More recently, efforts for the assembling of thiooligosaccharides have focused on reducing the number of the synthetic steps and developing new precursors. New methods for the formation of thio-linkages have been employed for the synthesis of α -*S*-sialosides using phosphates of either anomeric configuration under Lewis acidic conditions in good yields¹⁶². Another interesting approach employs the pre-activation of thioglycosides (**44**) to triflates (**45**) by treatment with diphenyl sulfoxide and triflic anhydride, followed by coupling with a glycosylthiol. The method affords very high yields and good compatibility with many protecting

groups and functionalities, but the anomeric stereochemical outcome is hard to predict (Scheme 28)¹⁶³.



Scheme 28: Thioliinkage formation via triflate donors

More recent methods have employed an umpolung reactivity-based *S*-glycosylation for the stereoselective preparation of *S*-linked 2-deoxy glycosides¹⁶⁴. According to this approach, the reductive lithiation of an anomeric mixture of phenylthiolglycosides **45** affords predominantly the axial-lithium analogue **46**, which can react with a sugar derived asymmetric disulfide **47** to yield the desired *S*-linked oligosaccharide **48**. Furthermore, the axial lithium intermediate **46** can isomerize to the more thermodynamically stable equatorial anomer **49** and then react with the disulfide **47** to afford the opposite anomeric configuration of the *S*-linked oligosaccharide **50** (Scheme 29).



Scheme 29: Thioliinkage formation using reductive lithiation and disulfides

However, the method involves the use of lithiating reagents that can be incompatible with some functionalities or protecting groups. In addition, the preparation of the asymmetric disulfide can be time-consuming and not always straightforward. In conclusion, a general and stereospecific method for the thiooligosaccharide assembly has not been developed yet and therefore, it remains an open challenge for chemists.

5 Synthesis of the oligosaccharides

The aim of this work is to synthesize thio-analogs of xylo- and arabinoxyloglycans in order to analyze and characterize enzymes acting on their natural substrates. The targets **1-3** are depicted in Figure 8.

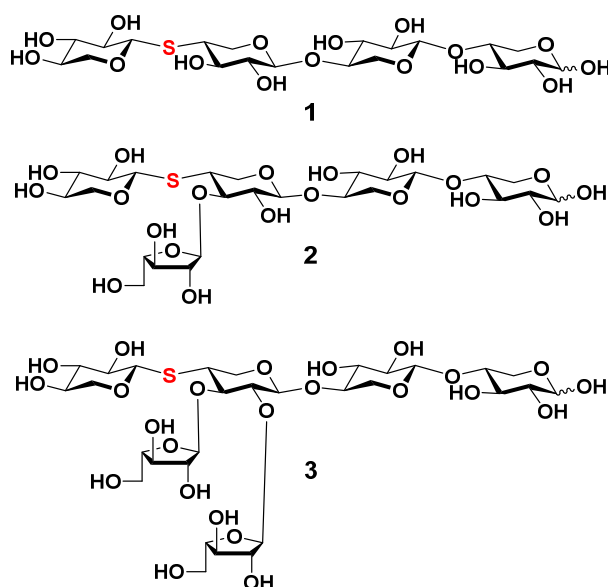


Figure 8: Target molecules **1-3**

The first challenge is selecting a method for formation of thiolinkages that is not only straightforward and flexible but also easily integrable into the synthesis of *O*-linked glycosides. The early stage of project was hence focused on the thiolinkage formation, selection of the building blocks thereto and their PGs.

5.1 Retrosynthetic analysis

The first synthetic strategy is based on employing the same building blocks for all the target compounds. In order to achieve this, different PGs are needed on the second sugar counting from the non-reducing end. We envision that target **2** is representing the biggest challenge since the arabinofuranose branch is exclusively on the 3-position of the second xylan from the non-reducing end. In order to discriminate that position from the corresponding 2-position, we decide to insert two orthogonal PGs. In this way it will be possible to use the same building blocks to obtain not only the three different target compounds, simply by removing the intended PGs, but also assembly of the fourth possible target **53**, bearing an arabinoxylan in the 2-position (Figure 9). Notably, the corresponding natural *O*-linked substrate of **53** has shown to be relevant, as it is widely present among hemicelluloses, even though less abundant¹⁸.

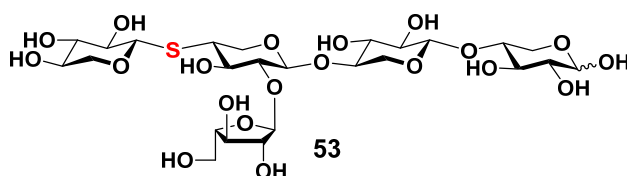
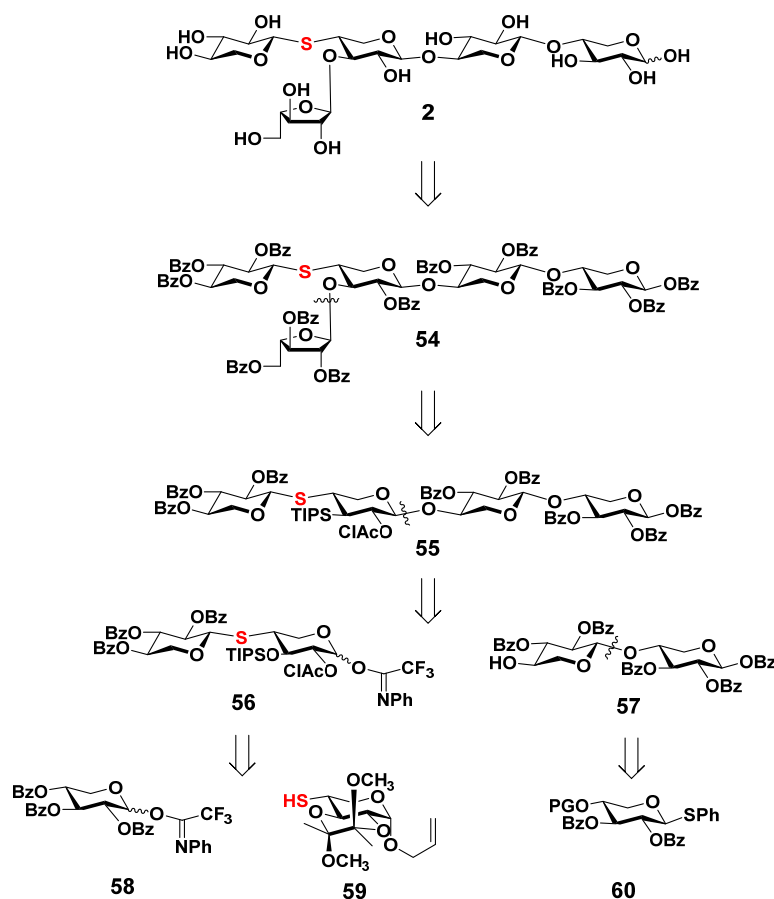


Figure 9: The fourth possible target, compound **53**

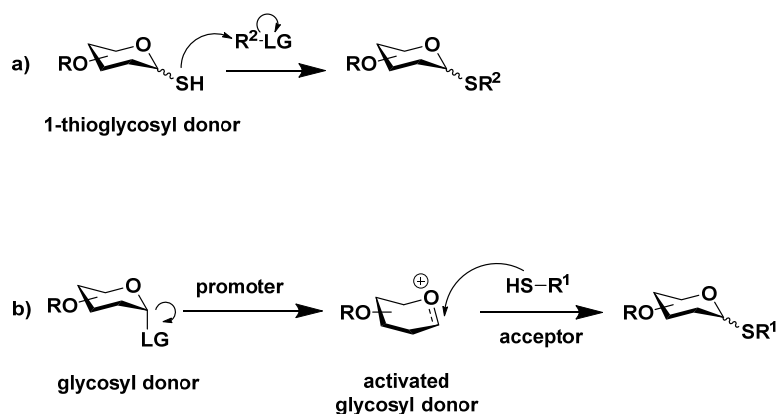
The retrosynthetic analysis of target **2** is presented in Scheme 30. First, we envisioned that target **2** could be obtained from the fully protected xyloarabinoglycan **54** by deprotection under Zémpfen conditions. Benzoyl groups were chosen as permanent PGs in both the *S*- and the *O*-disaccharide (respectively **56** and **57**), since they offer many advantages from a synthetic point-of-view. Benzoyl groups are stable under multiple conditions, easy removable and they can be used as neighbouring participating group in glycosylation reactions. Hence, they are also able to give high selectivity for 1,2-*trans* linkages in most cases⁷⁸. Next, orthogonal PGs to introduce branching on the 2- and 3-position of the second xylan were needed, and the selected PGs are shown in compound **56**.



Scheme 30: Retrosynthetic strategy of target compound **2**

The triisopropylsilane group is selected based on previous reports, which demonstrate its selectivity for the third position of partially protected pyranosides¹⁶⁵. In addition, the chloroacetyl group is selected, not only due to its orthogonality with the other groups, but also because of the ester functionality, which, like the benzoyl group, offers neighbouring group participation in glycosylation reactions. The tetrasaccharide **53** can be obtained by glycosylation between the *S*-linked disaccharide donor **54** and the *O*-linked-disaccharide **55**. It is envisioned to obtain the thiolinkage by mean of the imidate donor **56** and the thioacceptor **57**, in a similar fashion as described by Pinto and co-workers in the

early 1990s^{16681,120,121}. In the first chapter many methods to achieve *S*-linked disaccharides have been described. In spite of this, all of the methods have shown to have scope limitations and thus are not universally applicable for the synthesis of a larger series of substrates. In addition, most of them require the synthesis of 1-thioglycoside donors (Scheme 31, Path a), which cannot be used in the sequential assembly of oligosaccharides containing both *S*- and *O*-glycosidic linkages.



Scheme 31: a) Thiolinkage formation through 1-thioglycoside; b) Use of thio-acceptors for thioglycosides formation

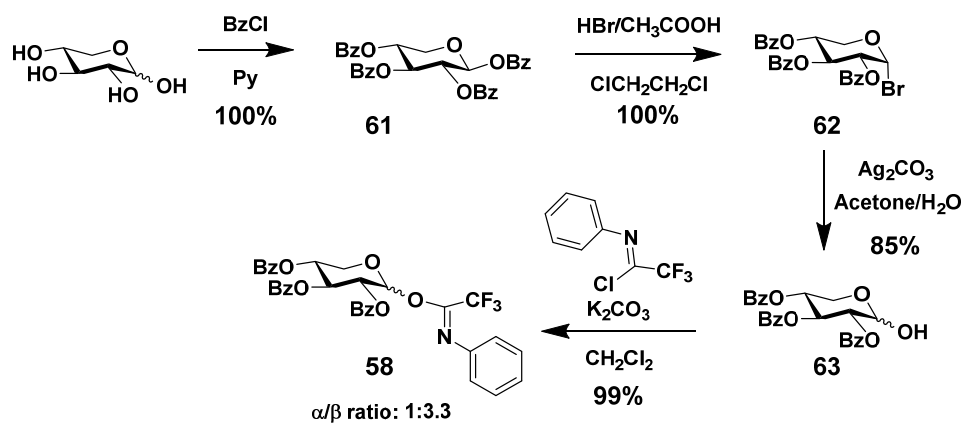
Since the targets chosen for the present work include both *S*- and *O*-glycosidic linkages, the synthetic strategy which does not involve 1-thioglycoside donors has initially been selected (Scheme 31, Path b). The glycosylation strategy presented in Path b utilizes a generic glycosyl donor and a specific acceptor, which already bears the sulfur on the position to be glycosylated. That combination can easily be integrated in a structure including both *S*- and *O*-glycosidic linkages, in addition to the option of employing different type of glycosyl donors. With this method it is, in theory, possible to use the same type of glycosyl donor for the formation of the *S*-linkage in disaccharide **56** and for the *O*-linkage required in tetrasaccharide **55**. The donor type selected for the formation of *S*-disaccharide **56** and *O*-linked tetrasaccharide **55** is the *N*-phenyl-trifluoroacetimidate (**58** and **56** respectively), since it has been shown to be more stable than the trichloro analogue, and therefore

less prone to decompose or give the corresponding acetamide¹⁰⁷. To synthesize tetrasaccharide **55**, a temporary PG is needed on donor **56**. The allyl group is selected as temporary anomeric protection for acceptor **59** due to its stability and orthogonality with the other PGs. The removal of the allyl group is usually performed by Pd catalysis, which could represent a challenge due to the presence of sulfur, which has shown to have catalyst poisoning abilities. However, in practice, the combination has shown to be possible^{116,167}. The functionalization at the 4-position of acceptor **59** is intended to be achieved by a temporary diacetalic protection of the two remaining 2- and 3- position (Scheme 30). Ultimately, the *O*-disaccharide **57** is designed from the common building block **60**, which conveniently can be employed for the synthesis of both acceptor and donor in the glycosylation step. In more detail, **60** can be used as a donor directly, but by means of two further steps an appropriate acceptor can also be acquired.

5.2 Synthesis of the donor for the *S*-linked disaccharide

The synthesis of the *S*-disaccharide started from the preparation of building blocks **58** and **59**. Donor **58** was obtained from commercially available D-xylose in 4 steps and the synthesis is illustrated in Scheme 32. The first step was the perbenzoylation of the unprotected sugar with benzoyl chloride in pyridine, which afforded β -xyloside **61** in quantitative yield. Using the standard conditions, benzoyl chloride (BzCl) in pyridine¹⁶⁸, usually only the β anomer is formed due to the anchimeric assistance of the ester in the 2-position and the bulkiness of the benzoyl group⁷⁸. Conversion of the hydroxyl group at the anomeric position into an acyl group is particularly useful since acyl groups like acetate or benzoate can readily act as leaving groups under the appropriate reaction conditions, and therefore, many other anomeric substituents may be introduced by nucleophilic substitution reactions⁷⁸. Thus, perbenzoylated xyloside **61** was converted into the corresponding halide **62** in quantitative yield. Glycosyl halides, such as bromides, are easily formed by

treatment of sugar acetates or benzoylates with a solution of HBr in acetic acid. Under these conditions, bromides are formed exclusively as the α -anomers, which are thermodynamically favoured by the anomeric effect, even though, for example, in the gluco- series the possibility of neighbouring group participation might lead to the formation of the β -product under other conditions⁷⁸.



Scheme 32: Synthesis of donor 58

Bromide **60** was subjected to basic hydrolysis¹⁶⁹ in the presence of silver carbonate (AgCO_3) to afford hemiacetal **63** in 85% yield. Compound **63** was partly obtained as the orthoester **64** (Figure 10), which was readily converted into desired hemiacetal **63** under slightly acidic conditions (Amberlite H⁺ in EtOAc).

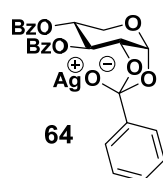
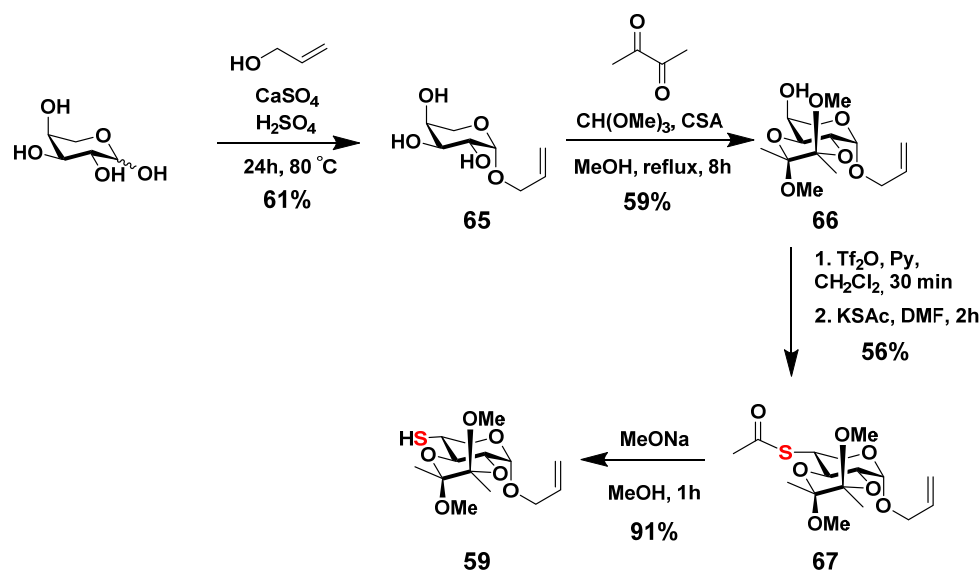


Figure 10: Orthoester **64** of the hemiacetal **63**

The last step of the synthesis was the conversion of the anomeric hydroxyl group to the *N*-phenyl trifluoroacetimidate donor **58**, which was easily obtained under basic conditions in 99% yield.

5.3 Synthesis of the acceptor for the *S*-linked disaccharide

The thio-acceptor **59** was obtained from commercially available L-arabinose in five steps (Scheme 33).



Scheme 33: Synthesis of thioacceptor **59**

The non-protected monosaccharide was functionalized on the anomeric position by Fisher glycosylation of allylic alcohol under acidic conditions. The glycosylation gave the desired compound **65** as the main product but also led to other byproducts, such as the β -anomer and the α - and β -furanosides in small percentages. This observation have previously also been reported in literature¹⁷⁰ and can be explained by the following: upon exposure of a sugar to Fisher glycosylation conditions a series of reactions, all of which are in equilibria, occur¹⁷¹. It is known that higher temperatures and longer reaction times lead, among the possible products, to the formation of the thermodynamic product, which is the pyranose form. In addition, the major anomer formed in the reaction is the 1,2-*cis*-linked product because of

the anomeric effect⁷⁸. The acid-catalyzed reaction of **65** with 2,3-butanedione allowed for protection of the *trans*-diequatorial hydroxyl groups on the 2- and 3-position with a cyclic butane diacetal (BDA) to afford compound **68** in 59% yield. The PG, introduced by Ley in 1996^{172,173}, demonstrated in this case only moderate selectivity. This could be explained by the fact the cyclic butane diacetal can also form the corresponding regioisomer **68**, although to a minor extent (Figure 11). Compound **66** is probably favoured due to the equatorial position of the hydroxyl group which are more reactive than the axial in the 4-position.

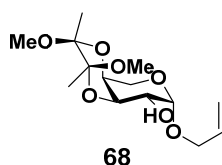
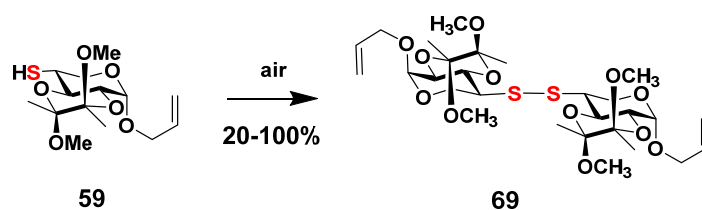


Figure 11: Isomer of compound **66**

To obtain the desired xylan acceptor **59**, the conversion of the axial hydroxyl group in the 4-position into a leaving group and a nucleophilic substitution with consequent inversion of configuration were needed. Nucleophilic substitution reactions in sugars are retarded by the presence of electrons withdrawing oxygen atoms (like butane diacetal BDA), which are in β -position to the carbon atom where the displacement is taking place⁷³. These types of nucleophilic substitutions invariably occur by an S_N2 mechanism, since this electron-withdrawing effect would greatly destabilise any carbenium ion involved in an S_N1 pathway. Therefore, according to previous procedures^{174,163}, to convert the hydroxyl group on the 4-position into an appropriate leaving group, conversion with a triflate (trifluoromethanesulfonate) was performed. Nucleophilic attack on the obtained triflate compound by sodium thioacetate afforded compound **67** in 56% yield over two steps (Scheme 33). The acetate was then fully converted to the corresponding thiol **59** under Zémlen conditions in 91% yield. Thiol **59** shows stability problems since it dimerizes if exposed to air to the corresponding disulfide **69** (Scheme 34), which has shown to be quite stable and thus difficult to reduce back to the thiol, as

will be further explained along the present work. The general stability of the disulfide has been one of the main challenges of the entire work.

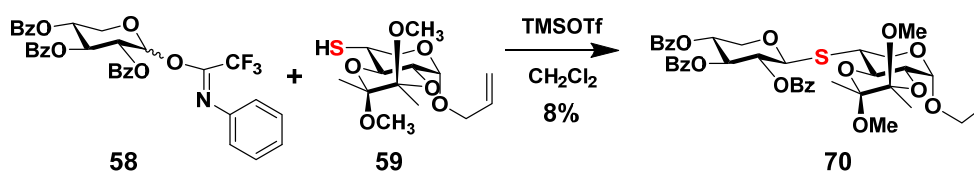


Scheme 34: Dimerization of thiol **59**

5.4 Thiolinkage formation

The main part of the present work has been focused on the selection and the development of an easy and straightforward method for assembling *S*-linked oligosaccharides.

As discussed in the previous chapter, Pinto and co-workers showed successful results in making the thiolinkage in CH_2Cl_2 in presence of a Lewis acid as catalytic promoter¹²⁰. Therefore, the first attempt to synthesize the *S*-linked disaccharide **70** was performed in CH_2Cl_2 using the acidic promoter TMSOTf (Scheme 35). First, the temperature was varied in a range between - 78 to 0 °C. Regrettably, the amount of the desired product was never afforded in higher yield than 8%, which was not useful for synthetic purposes on larger scale.



Scheme 35: Attempt of *S*-glycosylation reaction

Due to the insufficient yield, different reaction conditions were investigated in order to optimize the coupling reaction. The temperature was decided to be

maintained at -35 °C since at lower temperatures no consumption of the starting material was detected by TLC and at higher temperatures the multiple byproducts formed in the reaction mixture were challenging to separate. Different parameters like donor/acceptor ratio, reaction time, presence of molecular sieves (MS) and also employment of a co-solvent and amount and type of the promoter were screened unfortunately with limited results as listed in Table 2.

Table 2: Overview of the different reaction conditions used for the coupling; MS are molecular sieves 4 Å; All the reported attempts were performed -35 °C and in inert dry atmosphere.

Entry	Donor/acceptor Equiv. ratio	MS	Solvent Conc(M)	t (min)	Promoter	Yield
1	1.5/1.0	No	CH ₂ Cl ₂ (0.01 M)	20	TMSOTf (0.2 equiv.)	8%
2	1.5/1.0	Yes	CH ₂ Cl ₂ (0.01 M)	20	TMSOTf (0.2 equiv.)	-
3	1.5/1.0	No	CH ₂ Cl ₂ :Et ₂ O (0.01 M)	20	TMSOTf (0.2 equiv.)	8%
4	1.5/1.0	No	CH ₂ Cl ₂ (0.01 M)	60	TMSOTf (0.2 equiv.)	7%
5	1.5/1.0	No	CH ₂ Cl ₂ (0.03 M)	20	TMSOTf (up to 1 equiv.)	-
6	1.5/1.0	No	CH ₂ Cl ₂ (0.01 M)	20	TESOTf (0.2 equiv.)	6%
7	1.5/1.0	No	CH ₂ Cl ₂ (0.01 M)	20	TBDMSOTf (0.2 equiv.)	7%
8	1.5/1.0	No	CH ₂ Cl ₂ (0.01 M)	20	BF ₃ .Et ₂ O (1.5 eq)	-
9	0.75/1.0	No	CH ₂ Cl ₂ (0.01 M)	20	TMSOTf (0.2 eq)	20%

To understand the reasons for such poor yields, it was important to identify the byproducts formed during the reaction since the substrates were completely

consumed. In this way we envisioned to have a better understanding of the reaction and thus, to earn the opportunity to improve the yield of the desired product **70**. The main byproducts found in the reaction mixture are illustrated in Figure 12.

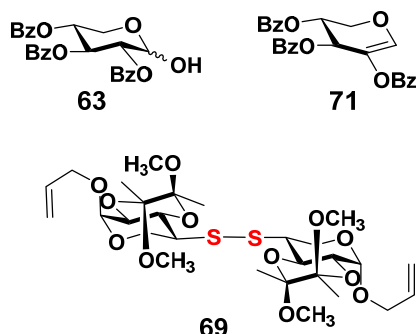
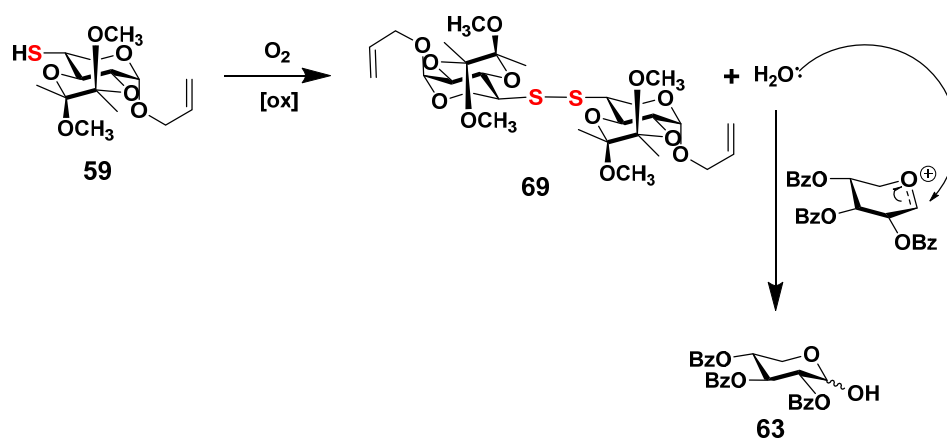


Figure 12: Major byproducts isolated from the glycosylation reaction

Hemiacetal **63** was found to be one of the most abundant byproducts (40 – 50%). It is known that the hydrolysis of the donor is one of the most common side reactions to occur during a glycosylation as reported by H. H. Jensen in a recent review on common side reactions of the glycosyl donor¹⁷⁵. One way to avoid their formation is to carefully perform the reaction under anhydrous conditions by flame drying the reaction vessel and using dry solvent. It is worth mentioning that both conditions have been applied during the reported attempts with no significant improvement. The azeotropic evaporation with toluene prior to the activation of the donor/acceptor mixture to avoid the presence of water in the reaction mixture is another important suggestion reported. However, this procedure could not be applied due to the facile dimerization of the acceptor **59** into byproduct **69** during concentration *in vacuo*.

Disulfide **69** was also found as one of the main byproducts of the glycosylation reaction. Therefore, we envisioned the acceptor dimerization as the major reason for the hydrolysis of the donor **58**. We hypothesized that the dimerization of two molecules of thioacceptor **59** by means of molecular oxygen is producing water *in*

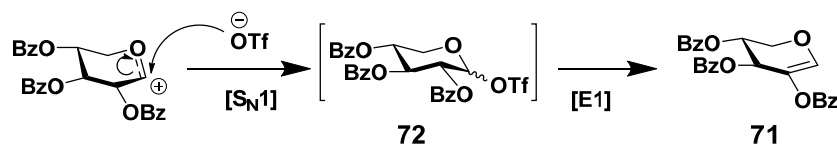
situ, which then is able to attack the oxocarbenium ion yielding the resulting hydrolyzed donor **63** (Scheme 36).



Scheme 36: Proposed mechanism for the formation of hemiacetal byproduct **63**

The employment of zeolite molecular sieves (MS) 4 Å could be a solution to this problem. However, their utilization actually resulted in no product formation (Table 2, Entry 2). A possible reason could be the alkaline nature of MS^{176,177}, capable of neutralizing the acidic promoter TMSOTf. Hence, it could be envisioned that a higher amount of the promoter could solve the problem, but only an increased amount of byproduct **71** (20 – 30%) was obtained in this attempt (Table 2, Entry 5).

Finding an explanation for this observation is not trivial. However, it has been demonstrated by D. Crich *et al.* that the presence of triflates in the reaction mixture at low temperature can lead to glycosyl triflates (i. e. intermediate **72**), which can easily eliminate in presence of unreactive acceptors, generating glycals^{178–180} (Scheme 37).

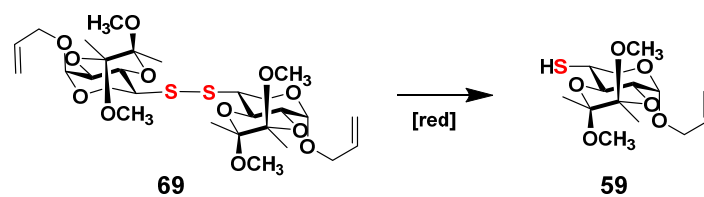


Scheme 37: Proposed mechanism for the formation of the elimination byproduct **71**

However, glycals are also known to be common byproducts of glycosylation reactions, especially in the presence of unreactive acceptor¹⁷⁵. Greater amounts of acidic promoter was also found detrimental in other works applied to thiolinkage formation¹⁶⁶. In the same paper Pinto B. M. and co-workers reported successful results when the donor/acceptor ratio was changed from 1.5:1.0 to 0.75:1.0. In fact, an excess of acceptor seems to slightly improve the yield of the glycosylation reaction (Table 2, Entry 8), yet not enough to be feasible in such an early stage of our synthesis. A reason for this final result could once again be the formation of disulfide **69**.

With these considerations in mind, we envisioned that avoiding the formation of disulfide **69** would possibly improve the desired outcome of the glycosylation reaction. If it turns out that this formation cannot be avoided, reducing the disulfide **69** to its corresponding thioacceptor **59** *in situ* could be an alternative solution to investigate.

Therefore, the attention was turned to avoiding this byproduct formation. This was done not only by addition of a degassing procedure to prior the activation of the donor/acceptor mixture, but also investigating the selection of a suitable reducing agent for reduction of the disulfide **69** back to the thioacceptor **59**. In this way, it would in theory also be possible to recover the thioacceptor from the disulfide that was formed, not only during the reaction but also by exposure to air. The screening of the reducing agents is summarized in Table 3. Although both sodium borohydride (NaBH_4) and triphenylphosphine ($\text{P}(\text{Ph})_3$) showed effective as reducing agents, only the latter was compatible with the reaction conditions due to the solubility properties.



Entry	Reducing agent	Solvent	Conversion
1	NaCNBH ₃	CH ₂ Cl ₂	-
2	NaBH ₄	MeOH	+
3	NaBH ₄	CH ₂ Cl ₂	-
4	P(OCH ₃) ₃	CH ₂ Cl ₂	-
5	D/L-DTT	CH ₂ Cl ₂	-
6	P(<i>n</i> -Bu) ₃	CH ₂ Cl ₂	-
7	P(Ph) ₃	CH ₂ Cl ₂	+

Table 3: Screening of the reducing agents; DTT: Dithioerytrytol; All the listed reactions were monitored by LC-MS. NB: + indicates sign of conversion while – indicates none.

With the identification of P(Ph)₃ as suitable reducing agent, different reaction conditions for the glycosylation were tested with three constant parameters: the donor/acceptor ratio, which was set to 1:1, second, the temperature, which was maintained around - 35 °C, and the degassing procedure prior to the addition of the promoter. The degassing procedure, called freeze pump thaw cycle, consisted of three rounds of vacuum/argon-filling applied to the reaction mixture, which was preventively frozen in liquid N₂.

Table 4: Overview of the different reaction conditions employed in the coupling reaction presented in Scheme 35. Equivalents and concentrations expressed in the table is relative to the amount of acceptor employed in the reaction.

Entry	P(Ph) ₃ (1 equiv)	MS	Solvent (0.01 M)	TMSOTf (equiv)	Yield
1	Yes	Yes	CH ₂ Cl ₂	0.2	9%
2	Yes	Yes	CH ₂ Cl ₂	0.6	23%
3	Yes	Yes	CH ₂ Cl ₂	1	-
4	Yes	No	Et ₂ O	0.2	18%
5	No	Yes	Et ₂ O	0.2	5%

From Table 4 a moderate improvement can be noticed when both the degassing procedure and reducing agent were employed, though the overall yield was not satisfying yet (Entry 2). Moreover, the major byproducts (Figure 12) were still present in the reaction mixture. Changing the solvent to ethyl ether did not improve the yield significantly either (Entry 4 and 5).

Based on these results, we decided to move on to a slightly different strategy by developing a precursor of the thio-acceptor, which could not dimerize but could be generated *in situ* under mild acidic conditions with a catalytic amount of TMSOTf. The selected precursor is a silyl ether derivative, which could be either a trimethylsilylether (TMS) **73** or the more stable analogue triisopropylsilylether (TIPS) derivative **74** of the thioacceptor **59** (Figure 13).

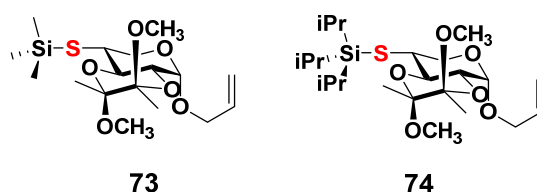
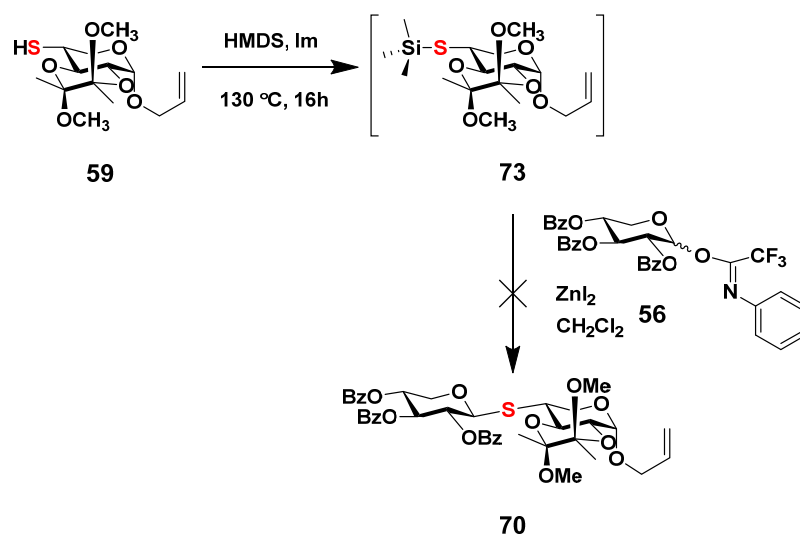


Figure 13: TMS- and TIPS-derivative of the thioacceptor **59**

The idea of using a TMS derivative was based on a paper published by Wang L. and co-workers in 1990, where they reported the successful formation of an *S*-linked disaccharide employing a silyl ether protected thioacceptor in combination with anhydrodonors¹⁸¹ (Scheme 10, Chapter 1).

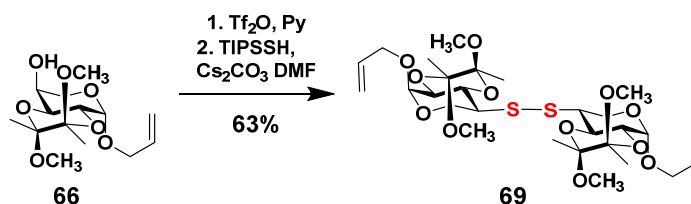
According to the described procedure, thioacceptor **59** was treated with hexamethyldisilazane (HMDS) and imidazole (Im) yielding intermediate **73**, which was used in the coupling reaction as a crude.

However, the glycosylation reaction did not give the desired product but a complex mixture of byproducts, which was not further investigated (Scheme 38).



Scheme 38: Attempt for thiolinkage formation by means of TMS-thioether intermediate **73**.

We envisioned that employing a more stable silylthioether would be a better choice for reducing the amount of byproducts in the glycosylation reaction. Moreover, the commercial availability of the triisopropylsilylthiol (TIPSSH) allowed for a much wider selection of synthetic opportunities^{182,183}. For example, the S_N2 displacement of the corresponding triflate with TIPSSH or its salts has shown to be possible on aromatic substrates. To the best of our knowledge though, the reaction had never been tried out on carbohydrate substrates. To promote the S_N2 displacement, DMF was selected as solvent, and a non-nucleophilic base like Cs_2CO_3 was added to the reaction mixture. First, compound **66** was activated with triflic anhydride in pyridine and then reacted with TIPSSH in presence of Cs_2CO_3 . The reaction was followed by TLC, which quickly showed a spot-to-spot conversion. However, subsequent analysis of the product by NMR and LC-MS, showed disulfide **69**, indicating that the displacement probably succeeded, but unfortunately compound **74** was potentially not stable to the reaction conditions (Scheme 39).



Scheme 39: Attempt for synthesizing compound 74

Consequently, we concluded that compound 74 does not have the stability and the characteristics that are needed for our purpose and therefore we abandoned the idea of employing a silylthioether in the present work.

5.4.1 Further investigation

When analyzing the reaction mixture depicted in Scheme 35 by LC-MS, besides the main byproducts depicted in Figure 12, other minor byproducts were found. Interestingly, a peak was observed, which could correspond to all the different isomers represented in Figure 14, plus the mass of Na^+ . We envisioned that compounds 76 and 77 were less probable due to the higher nucleophilicity of sulfur compared to oxygen. However, in order to support the hypothesis, we decided to investigate the result by isolating the product. This result was particularly interesting because it suggests that the butane diacetal (BDA) protecting group is not stable enough for moderate acidic environments. This could possibly explain the *in situ* removal of BDA prior to the coupling reaction and the formation of the byproducts pictured below.

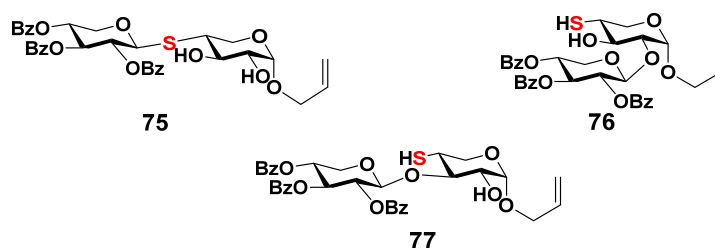
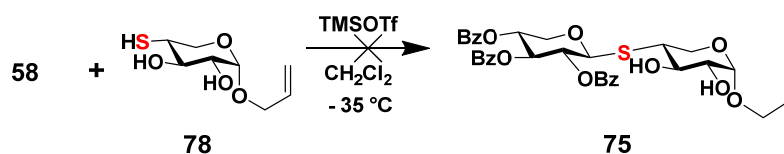


Figure 14: Minor byproducts found in the reaction mixture

To isolate the product we planned to perform the reaction with the partially protected acceptor **78** (Scheme 40). This should verify the hypothesis of the sulfur overruling the nucleophilicity of the oxygen and thus eliminate the possibility of the presence of compound **76** and **77** in the reaction mixture.



Scheme 40: Glycosylation coupling employing partially protected thioacceptor **78**

The reaction failed in the intended purpose since the resulting outcome turned out to be an even more complex mixture of byproducts, which was challenging to separate. This clearly showed the difficult selectivity of the reaction, when thiol and hydroxyl groups are competing as nucleophiles in this type of reactions, which also is in accordance with literature data^{125,174}. Therefore, instead of modifying the reaction conditions any further we decided to simplify the structure of the thioacceptor **59** into the more stable molecule **79** (Figure 15).

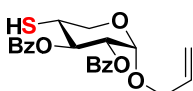
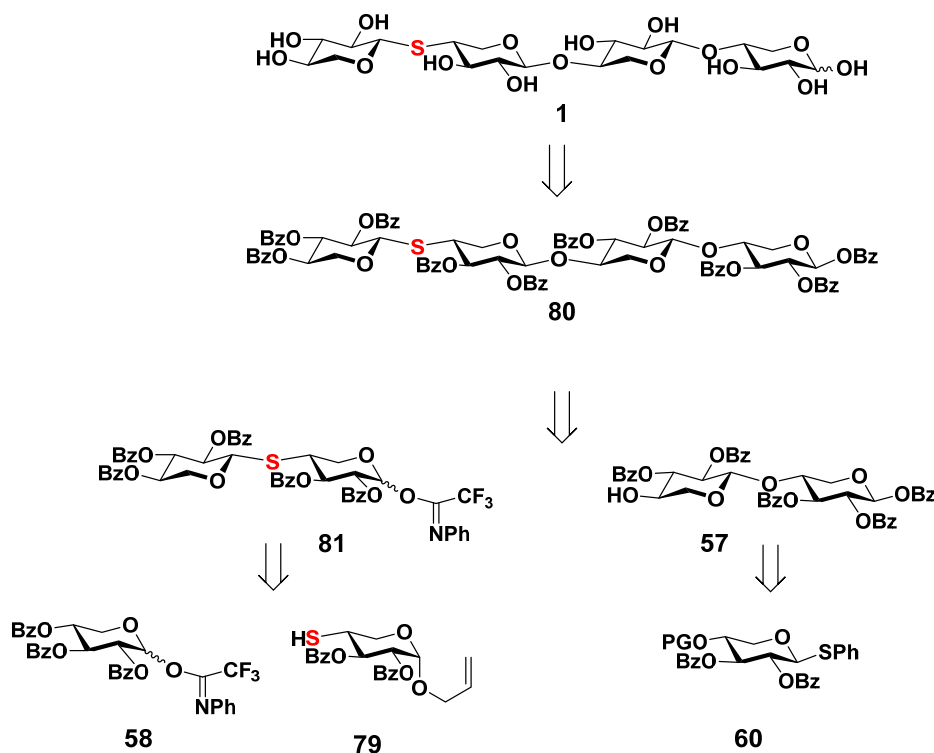


Figure 15: Simplified thioacceptor **79**

We envisioned that employing a more stable thioacceptor would make it easier to understand the coupling reaction, and in the same time provide a stable building block for the synthesis of target **1**.

5.4.2 A new retrosynthesis for the linear tetraoxylan

The idea behind changing the acceptor to **79**, was based on proving the overall strategy to be feasible and minimizing the variables related to unstable PGs. The new retrosynthetic analysis of target **1** is represented in Scheme 41.



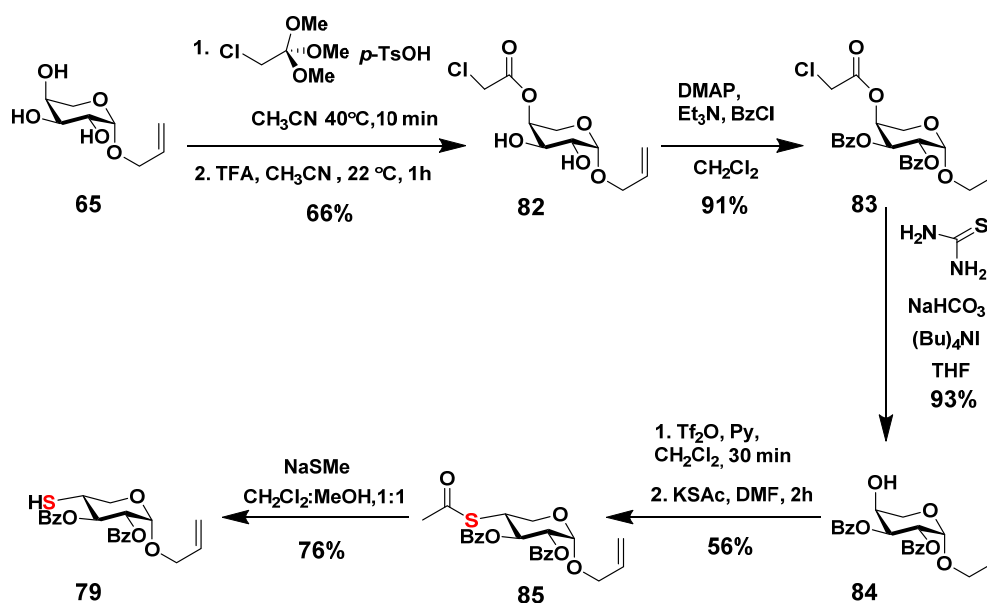
Scheme 41: Retrosynthetic strategy of target **1** employing thioacceptor **79**

Compound **79** was synthesized via a strategy based on different PG manipulations (Scheme 42). The planned strategy requires a selective protection of the hydroxyl group on C4 followed by benzylation of the other positions, and then deprotection of O-4 again to yield compound **84**.

The procedure for synthesizing compound **84** started with the treatment of α -allyl-xyloside **65** with chlorinated trimethylorthoformate in CH_3CN in the presence of catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) to form the acetal

intermediate **86**, which is subsequently regioselectively opened with trifluoroacetic acid (TFA) liberating the 3-position and yielding diol **82**.

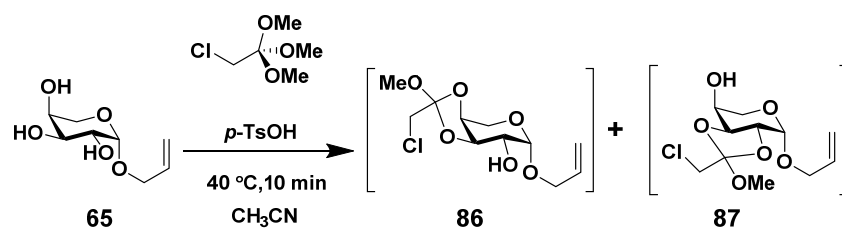
The regioselective opening on the 3-position could be explained with the higher reactivity of the equatorial 3-position if compared to the axial in 4-position.



Scheme 42: Synthesis of thioacceptor **79**

In the original procedure the reaction is performed at room temperature in CH_3CN ¹⁸⁴, but under those reaction conditions the solubility of **65** was low and the yield has never above 25%. The result was mainly due to the formation of the other possible regioisomer **87**, but also to the low solubility of compound **65** in CH_3CN . Therefore, the reaction has been tested in different solvents and at different temperatures to improve selectivity and yield (Table 5).

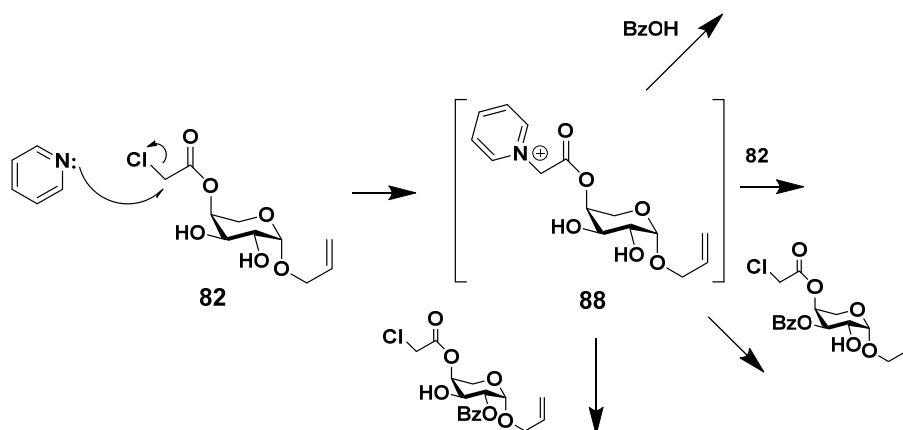
DMF showed to be an appropriate solvent, giving an overall yield of 80%. However, it failed in selectivity since the reaction yielded the two regioisomers **84** and **85** in a 1:1 ratio.



Entry	Solvent	Temp. (°C)	Overall yield	Ratio 86/87
1	acetonitrile	20	25%	9:1
2	DMF	20	80%	1:1
3	acetonitrile	40	66%	9:1

Table 5: Selected examples of different conditions utilized for synthesizing intermediate **86**. **NB:** Ratio and yield reported in the table are calculated after the partial opening of the diacetal PG, on isolated compounds.

Although raising the temperature of a reaction generally diminishes the selectivity, in this case heating the reaction in acetonitrile to 40 °C resulted in a better solubility, but it also maintained the selectivity in favor of intermediate **86**. The partial opening of the acetal PG provided compound **82** in 66% yield. Following benzylation with benzoyl chloride (BzCl) in pyridine only yielded compound **83** in 27% yield. Based on previous experience in our research group we could hypothesize the involvement of the chloroacetyl group in the degradation of compound **86**. A possible scenario could be the basic hydrolysis of the ester, but also the nucleophilic attack of pyridine on the chloromethylene, since the chloromethylene is to some extent electrophilic (Scheme 43). The resulting unstable pyridyl intermediate **88** could be subjected to the nucleophilic attack of the hydroxyl group of compound **82** (and its partly benzyolated derivatives), but also from the benzoyl alcohol, which is probably present in the reaction mixture. This process could lead to many different byproducts, which could explain the complex outcome of the reaction.



Scheme 43: Possible pathways for byproduct formation

Therefore, other reaction conditions were investigated in order to increase the obtained amount of **84**. The employment of triethylamine and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 proved to be beneficial for the reaction, affording the desired compound **83** in 91% yield. Removal of the chloroacetyl PG was performed by treatment of **83** with thiourea in the presence of catalytic amounts of $(\text{Bu})_4\text{NI}$ and NaHCO_3 in 93% yield¹⁸⁵.

However, all these unexpected challenges made us wonder whether there was an easier and more straightforward way to obtain compound **84**. L-arabinose has a very analogous structure to D-galactose (Figure 16), and therefore they should in theory share many chemical characteristics, like for example the relative reactivity of the different hydroxyl groups. Hence, reported literature on D-galactose was researched to find a procedure that could possibly be applied on L-arabinose.

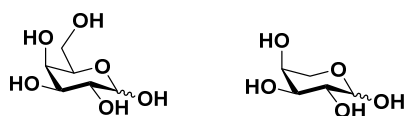
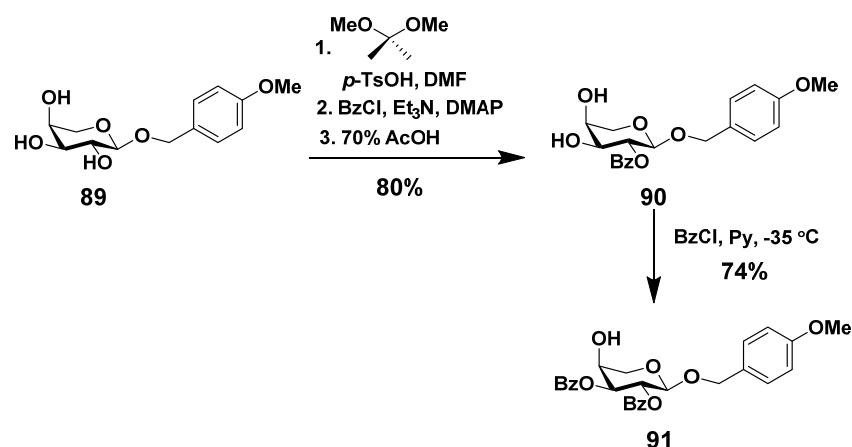


Figure 16: On the right, pyranose form of D-galactose; on the left, pyranose form of L-arabinose

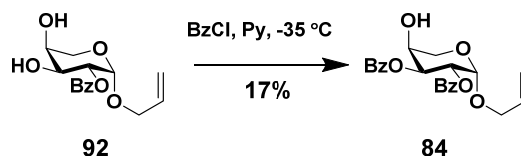
It has previously been reported that selective benzylation of D-galactose of all positions but the O-4 is possible¹⁸⁶. However, it has also been shown to be inefficient when applied to L-arabinose, suggesting after all an unexpected substantial difference in reactivity between the two monosaccharides¹⁸⁷⁻¹⁸⁹. In the papers, they obtain a mixture of different products in all examples, ranging from mono- to tri-substituted isomers in different ratios.

However, in 2004 Plé *et al.* reported the synthesis of the 2,3-di-O-benzoylated L-arabinose derivative **91** by means of a temporary PG in 59%¹⁸⁷ (Scheme 44).



Scheme 44: Reported procedure for selective benzylation of O-2 and O-3 in L-arabinose derivatives

Therefore, we decided to test the procedure on allyl-L-arabinoside to evaluate the efficiency compared to the synthesis that was earlier presented. Regrettably, the selective benzylation of the 3-position of compound **92** afforded the desired dibenzoylated product **84** in low yield (Scheme 45).

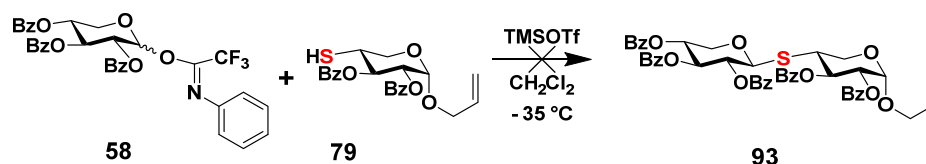


Scheme 45: Alternative synthesis of alcohol **84**

Compound **84** was converted into the thioacetate derivative **85** in the same fashion as earlier described for the analogue thioacetate **67**, while the removal of the acetate to obtain thiol **79** could not follow the same procedure used to yield **59**. This is due to the benzoyl groups, which are sensitive to Zémpfen conditions. Many procedures for the selective removal of acetyl groups in presence of other esters have been reported, generally by employment of Zémpfen conditions at low temperature^{135,136,190}, but the selectivity was not high when the procedures were performed on compound **85**, yielding compound **79** in poor to moderate yield. Therefore, a different approach was employed. In 1998 Wallace O.B. *et al.* reported the selective deprotection of thioacetates using sodium thiomethoxide (NaSCH₃) in MeOH and in 2011 the same procedure was optimized by introduction of a co-solvent by Murphy and co-workers^{191,192}. When the procedure was performed on compound **85**, the reaction proceeded smoothly to thiol **79**, in a satisfying yield of 76% (Scheme 42).

5.4.3 New attempts for thiolinkage formation

The coupling reaction was performed following the best guide lines we had collected at this point, meaning a higher ratio of acceptor **79** compared to the donor **58**, together with degassing procedures prior to addition of the promoter (Scheme 46).



Scheme 46: Attempt for making the thiolinkage with acceptor **79**

Unfortunately, no *S*-linked disaccharide **93** was detected at all in the reaction mixture. The main products isolated from the reaction were the disulfide **94**, the hemiacetal **63** and the elimination product **66** (Figure 17).

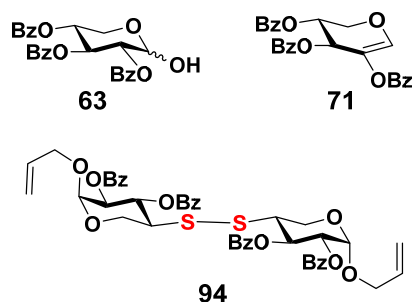
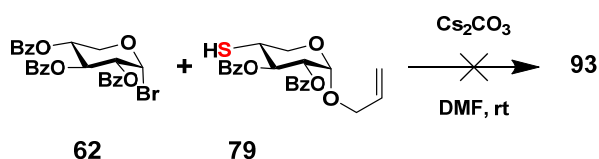


Figure 17: Major products from coupling reaction between **58** and **79**

Based on these discouraging results, we speculated whether we should change direction to a different approach at this point or at least investigate the reaction from another point of view. The chosen glycosylation strategy allowed for the employment of other donors, thus, we decided to move our attention from the acceptor to the donor **56**, performing the glycosylation reaction with different donors in order to find a better match for acceptor **77**.

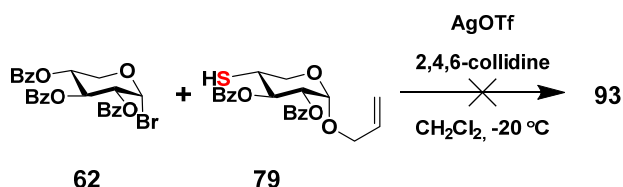
5.4.4 Employment of bromide donor in the coupling reaction

As illustrated in the previous chapter glycosylation reactions can also occur via S_N2 -type displacements when halides are utilized as donors^{122,123,131} (see Chapter 3). In 2005 Cao *et al.* also reported the successful synthesis of an *S*-linked heparan sulfate trisaccharide employing halides as donor, where acetimidate donors failed¹²⁶. Different conditions and promoters, also in the mentioned paper have been employed to promote the coupling between the imidate and the thioacceptor without improved results. However, they also reported that the employment of a bromide donor was successful, leading to product formation in moderate yield (67%). Same results were obtained by Driguez and co-workers by employing Cs_2CO_3 as a base to promote S_N2 -type reactions¹²⁵. Inspired by these results, glycosylation reaction between bromide **62**, which was conveniently one of the intermediates for the synthesis of donor **58**, and thioacceptor **79** was performed in DMF in the presence of Cs_2CO_3 (Scheme 47).



Scheme 47: First attempt for the formation of the thiolinkage with bromide donor **62**

The glycosylation reaction between bromide **62** and 4-thiol derivative **79** unfortunately failed, only providing primarily the byproducts pictured in Figure 17 in different percentages. A standard procedure for glycosylation reaction employing bromides donor and S_N2-type displacements was tested. This procedure has been shown to be successful in other works¹⁹³, however, failed to provide the desired product **93** (Scheme 48).



Scheme 48: Second attempt for formation of the thiolinkage with bromide donor **62**

5.4.5 Employment of trichloroacetimidate donor in the coupling reaction

In the beginning of our synthetic design, the trichloroacetimidate donor **95** was excluded from the selection of the possible donors not only because of the high reactivity and tendency to form the unreactive acetamide **96** shown in Figure 18, but also due to the chemical and structural similarities with the unsuccessful donor **58**.

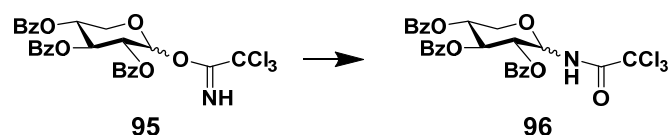
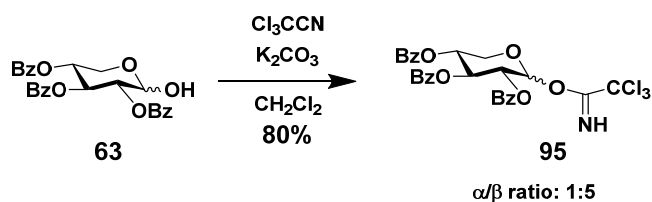


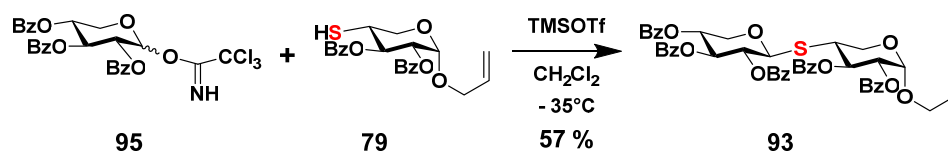
Figure 18: Conversion of the acetimidate **95** into the corresponding acetamide **96**

However, since it has been shown to be successful in other cases^{81,120,121,166}, we envisioned that investigating the reactivity in the coupling was important to better understand the dynamics of the reaction. Trichloroacetimidate donor **95** was synthesized from the hemiacetal **63** in one step by treatment with trichloroacetonitrile (Cl_3CCN) and anhydrous K_2CO_3 in dry CH_2Cl_2 according to the general procedure¹⁹⁴, as represented in Scheme 49.



Scheme 49: Synthesis of compound **95**

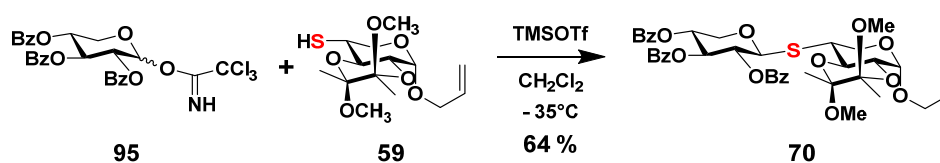
Interestingly, condensation of donor **95** with the acceptor **79** in presence of TMSOTf in dry CH_2Cl_2 unexpectedly afforded the *S*-linked disaccharide **93** in 57% yield (Scheme 50).



Scheme 50: Attempt for the formation of the thiolinkage by employment of trichloroacetimidate **95**

Other products were also found in the crude mixture, including disulfide **94** in 14% yield and acetamide **96**. Even though the reaction was performed without the employment of reducing agents and degassing procedures, the amount of disulfide

was significantly low compared with the previous results presented herein. Moreover no glycal 71 or hemiacetal 63 were detected by TLC. It could be speculated that this is a case of poor match/mis-match in the glycosylation reaction, supporting the popular theory that each glycosylation is a different case^{195–197}. However, the positive results obtained with the trichloroacetimidate 95 and the thioacceptor 79 (Scheme 50) invited us to try the coupling also with thioacceptor 59 to see whether the rigidity of the molecular conformation in 59 due to the BDA group would influence the outcome of the reaction. The coupling between 95 and 59 was also successful, affording *S*-disaccharide 70 in 64% yield (Scheme 51).



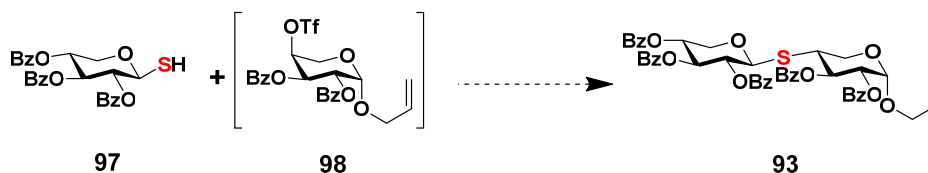
Scheme 51: Glycosylation reaction between 95 and 59

Thus gratifyingly, the reaction demonstrated to be reliable also in the case of thioacceptor 59. We were very pleased to verify that the original strategy we choose was actually a suitable method once we changed the donor to the trichloroacetimidate. Moreover, the study conducted on the byproducts provided us a deeper knowledge of the glycosylation reaction involving thioacceptors. Identification of the main byproducts, together with knowledge of the reaction conditions can indeed be difficult to find since only the successful reactions are generally reported. However, all these informations are very important for the optimization of a glycosylation reaction. The glycosylation method employing thioacceptor has surely been one of the first to be used for the synthesis of thiolinkages but it has not been reported as much as the other one involving 1-thioglycosyl donors. Literature on the topic is not abundant and therefore it can be difficult to decide in advance which method should be employed in a thiooligosaccharide synthesis. With these considerations in mind, we decide that it

was worth trying to perform the thiolinkage also by employing 1-thioglycosides to compare the two strategies and evaluate them.

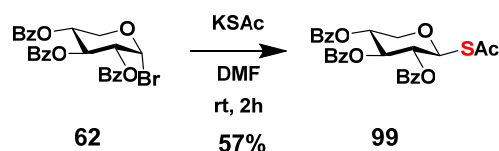
5.5 Evaluation of the method

In order to develop a robust method for synthesizing thiolinkages in oligoxylans, we decided to compare the results we obtained with the common method for synthesizing *S*-linked-oligosaccharide that involves the employment of 1-thioglycoside (see Chapter 1). For the synthesis of the *S*-disaccharide **93** we planned to employ the 1-thioxylan **97** with acceptor **84**, after conversion of the latter to the activated triflate derivative (intermediate **98**), as represented in Scheme 52.



Scheme 52: Planned synthesis of **93** employing donor **97**

Many methods for synthesizing 1-thioglycosyl donors have been published (see Chapter 4) and the most common ones employ thiourea salts and thioacetates, mostly from the corresponding halides. First, we intended to yield compound **97** from thiourea salts, according to successful procedures found in literature^{198,129}. Thiourea salts have the characteristic of a long shelf-life and being solid and easily storable. Following both procedures unfortunately failed to yield compound **97** and therefore we decided to move to thioacetates. The first attempt for synthesizing thioacetate **99** by S_N2 displacement with KSAc from the corresponding bromide **62** gave the desired product in a yield comparable to literature¹⁹² (Scheme 53).



Scheme 53: Synthesis of thioacetate **99** from bromide **62**

We also tried to look for a way to optimize the reaction by means of different solvents. It was found that acetone can be successfully used, providing same range of yields and easier removal by concentration *in vacuo*¹²⁷. The moderate yield intrigued us to analyze the reaction mixture in order to identify the byproducts. The main byproducts of the reaction proved to be the starting material (bromide **62**), which has a very similar R_f to the product **99** on TLC, and the corresponding α -anomer. Examination of the $^1\text{H-NMR}$ spectrum showed a duplet at 6.24, which indeed could represent the H-1 signal of the α -anomer of thioacetate **99** (Figure 19). Although the reaction should proceed through a $\text{S}_{\text{N}}2$ -type displacement and thus provide only the β -anomer, the H-1 coupling constant of the byproduct is also corresponding to the α -D-1-thio-glycoside, according to literature¹³¹. Moreover, the integrals of the present spectrum showed a possible correlation with the CH_3 -singlet of the thioacetate group (not shown).

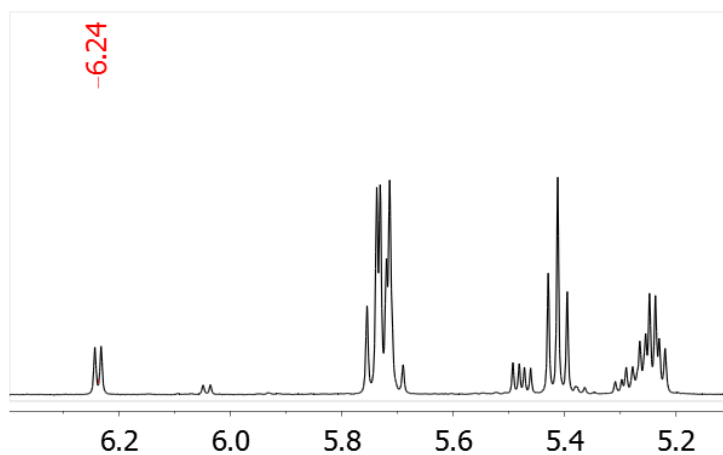
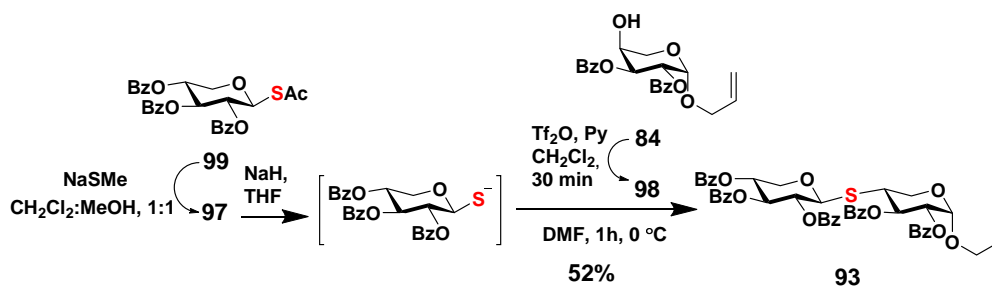


Figure 19: α -anomer of thioacetate **89** present in the mixture

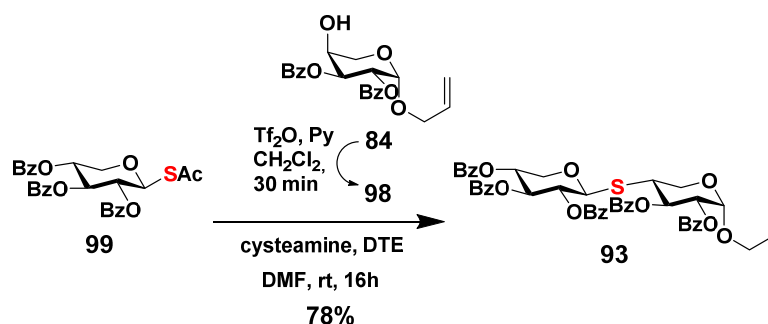
The β -anomer **99** was then converted to thiol **97** by means of sodium thiomethoxide (NaSCH₃) and reacted with freshly synthesized triflate **98** without further purification to yield *S*-disaccharide **93** in 52% yield (Scheme 54).



Scheme 54: Coupling between thiol **97** and triflate **98**

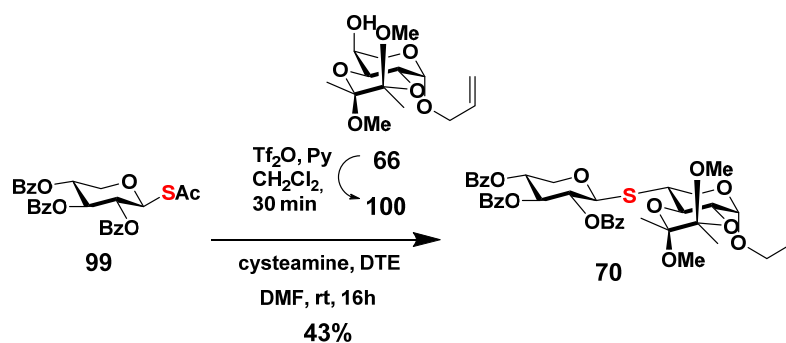
So far the inverse strategy that involves thioacceptor and trichloroacetimidate showed slightly higher yield compared to the traditional method reported by many research groups^{138,157,158,199} and represented in Scheme 54. In addition, the latter method employs two reaction crudes that are too unstable for prior isolation. That gives a certain degree of uncertainty about effective amount of reactant used in the reaction and hence about the effective yield.

Another option for the synthesis of *S*-disaccharide **93** using 1-thioglycosides employs thioacetate **99** as donor, directly in the reaction mixture. As explained in Chapter 4, the method involves the employment of cysteamine for the removal of the acetate *in situ* and dithioerythritol (DTT) as reducing agent and scavenger^{61,125,190,199}. The reaction between **99** and intermediate **98** was performed at room temperature for 16h and yielded the *S*-disaccharide **93** in 78% (Scheme 55).



Scheme 55: Coupling reaction between triflate **98** and thioacetate **99**

The unexpected high yield convinced us to perform an up-scaling process through this synthetic route and also to extend the method to the less stable and more strained acceptor **66**. We envisioned that it would be of interest to investigate not only the possible influence that different PGs have on the reaction but also the possibility of extending the reaction to different substrates (Scheme 56). This time the yield of the reaction was not as high as it was for the analogue compound **84**. We can hypothesize that compound **66**, which has a more strained structure due to the BDA protecting group, could in theory inhibit the transition state of the coupling reaction. Also, other byproducts were found in the reaction mixture, which derived both from triflate **100** and thioacetate **99**.



Scheme 56: Coupling reaction between thioacetate **99** and triflate **100**

One of the most common byproduct from this type of reaction, is the elimination products of triflate **100** (not shown)¹²⁵. Other byproducts found in the reaction

mixture derived from the donor **99** and could be due to the long reaction time. One hypothesis might be the formation of the corresponding disulfide **101**. The structure includes all the possible stereoisomers meaning β - β and α - α homodisulfide but also the α - β heterodisulfide (Figure 20)^{174,190}. However, further investigations were not performed in this case.

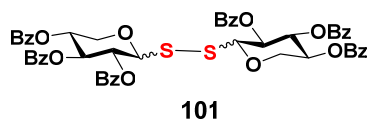


Figure 20: Possible byproducts of the coupling reaction between **99** and triflate **100**

The S_N2 -type glycosylation reaction with thioacetates, seems to be more influenced by the PGs present on the acceptor, resulting thus in significantly different yields, 43% and 78%. However, although the structure of the acceptor may play a role in governing the stereochemistry of these glycosylations, the results show that, in general terms, the relative influence of these factors is difficult to evaluate. For a given set of experimental conditions, the stereochemical course of these glycosylations depends on structural features of both glycosyl donor and glycosyl acceptor. It is a balance of these factors, where the structure of the glycosyl donor and acceptor always plays a major role, which determines the stereochemistry of the coupling reaction¹⁹⁷.

5.5.1 Concluding remarks

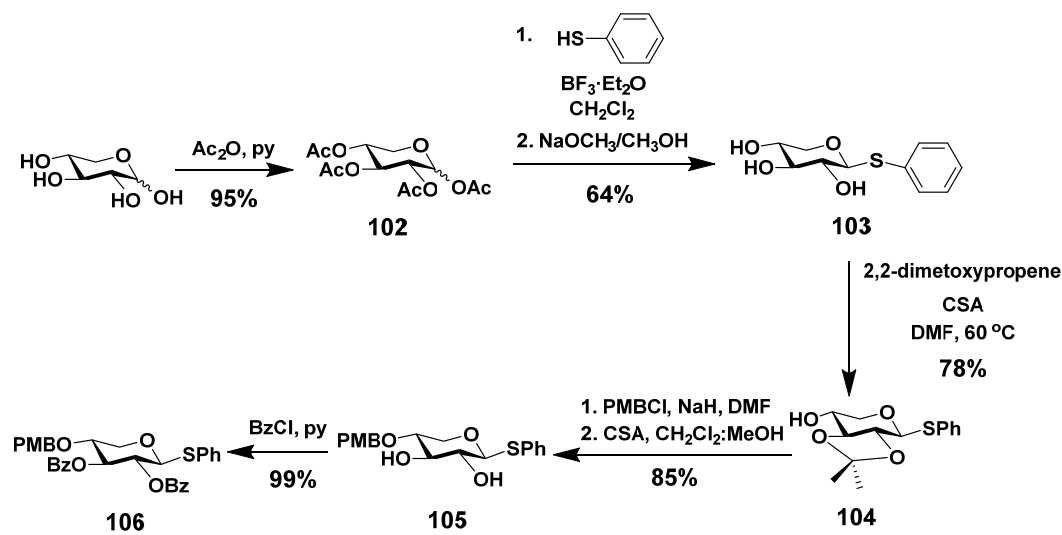
Different coupling methods have been investigated during the synthesis of *S*-disaccharide **68** and **93**. The overall result deduced from the performed investigations is that a general method for the synthesis of thiolinkages cannot be identified. Every method described in the present work has shown advantages and drawbacks. Synthetic methods employing thioacceptors (S_N1 -type glycosylation reactions) have shown to be particularly influenced by the reactivity of the donor and also by the tendency of the acceptor to dimerize. On the other hand, methods employing 1-thioglycosides (S_N2 -type glycosylation) have shown to some extents

to be more reliable yet partly influenced by the stability of the acceptor and the corresponding triflate intermediate. Also, the stability of the triflate seems to be influenced by the PGs present of the molecule, which determine the tendency of the triflate to eliminate. The different reaction mechanisms involved in the two reaction types (i.e. S_N2-type or S_N1-type) have different transition states and therefore they are influenced by different conformations and reactivity of the molecules involved in the coupling. However, the concept could be related to a general case of match/mis-match between donor-acceptor pairs. All these results conclude that it is difficult to predict which synthetic route could be more beneficial in advance and thus both synthetic methods are worth considering when planning a synthetic strategy.

5.6 Synthesis of the common building block for the *O*-disaccharide

The other moiety needed for the synthesis of tetraaxylan **1** is constituted by the *O*-disaccharide. The molecule is envisioned to be obtained using a common building block, which is bearing a thiophenol on the anomeric position and orthogonal PGs respectively on the 4-position and on the remaining two hydroxyl groups. One of the advantages of using thiophenol as anomeric protecting group is the fact that it can be activated during the glycosylation reaction but it can also easily be converted to several functional groups. Therefore compound **60** can be used as donor and also, as shown later, converted to the acceptor in few steps. PGs were selected according to previous experience in our research group. Benzoyl groups were chosen as long term PGs in accordance to the general synthetic strategy to be removed together with the others benzoyl groups present on the molecule. On the 4-position the *p*-methoxybenzyl group (PMB) was selected. PMB is a PG that offers several advantages since it possess the benefits of benzyl ethers but it can also be cleaved selectively and effectively by oxidative reagents. PMB is also stable to many different conditions and it is orthogonal to ester PGs in addition

to be removable in presence of other benzyl groups⁷⁸. The *O*-disaccharide synthesis starts with the preparation of building block **106**, which is prepared in 7 steps from D-xylose as represented in Scheme 57.



Scheme 57: Synthetic strategy for building block **106**

The entire strategic route was inspired by results reported by Yang B. and co-workers in 2012²⁰⁰. Following the procedure described in literature, D-xylose was peracetylated under standard conditions (compound **102**) and then glycosylated with thiophenol in presence of a catalytic amount of Lewis acid^{198,201}. The stereochemical outcome of the reaction is generally represented by the β -product for two principal reasons: first, as mentioned before, the anchimeric assistance of ester PGs on the 2-position (in this case the acetate) and second, the bulkiness of the aryl makes the equatorial conformation on the anomeric position more stable over the axial conformation. The acetylated intermediate (not shown) is subsequently deacetylated under Zémpfen conditions and crystallized to remove the small amount of the corresponding α -anomer and other impurities. Resultant thiosugar **103** is treated with 2,2-dimethoxypropane in the presence of catalytic amounts of camphorsulfonic acid (CSA), affording compound **104** in 78% yield.

Noteworthy, compound **104** was not the only product found in the reaction mixture, also the corresponding regioisomer **107** and compound **108** were found in the mixture in different quantities (Figure 21). This observation is not completely surprising, as similar results have previously been reported in literature²⁰².

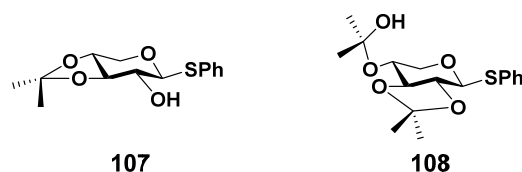
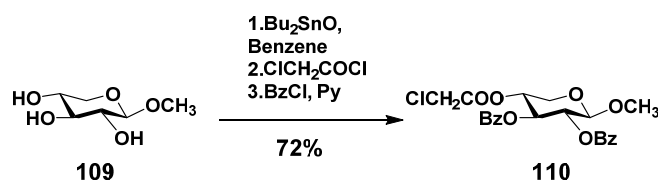


Figure 21: Byproducts found in the reaction mixture together with **103**

According to the reference, compound **104** is the kinetic product of the reaction and therefore the major product under the utilized reaction conditions. Even though we obtain a satisfying yield of **104**, the separation of the three products is not trivial, since it generally requires several chromatographic purifications with eluents composed of three different solvents. Hence, this step in the synthesis is very time consuming, especially on large scale. Treatment of compound **104** with *p*-methoxybenzyl chloride (PMBCl) and sodium hydride (NaH) and subsequent removal of the isopropylidene acetal group under mild acidic conditions afforded compound **105** in a high yield. Last step to afford building block **106** is the benzylation of the 2- and 3-position, which was successfully achieved in pyridine in quantitative yield.

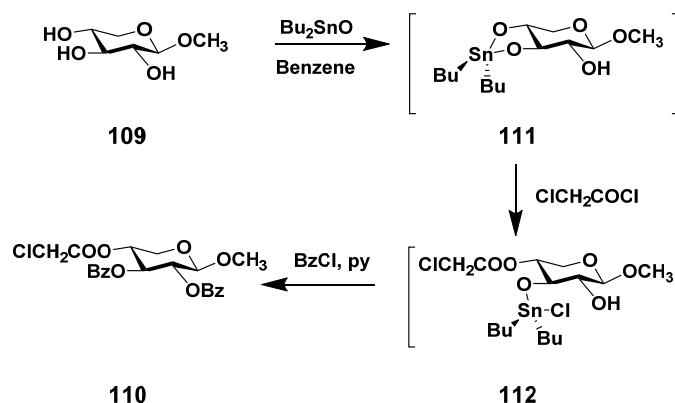
The synthesis of **106** proceeded with no particular complications and in very good yield but it is highly time-demanding. This motivated us to find alternative synthetic route to achieve compound **106** or a similar analogue from thiophenyl xyloside **103**, which was a shorter and more time efficient. Xylosides are known for having a general reactivity trend, where the 4-position is the most reactive, followed by the 3-position and the 2-position as the least reactive. However, our experience with the selective protection of the 4-position over the others, did not show a significant preference also when employing particular bulky PGs like

naphthalene. Therefore, other strategies that require fewer steps than the one employed and described in Scheme 57 were investigated. A widely used procedure is the temporary stannylidene acetal activation of O-3 and O-4, to enhance the reactivity of the 4- over the 3-position²⁰³. In 2002 Chen L. *et al* described the one-pot synthesis of compound **110** from the β -methyl xyloside **109** as represented in Scheme 58²⁰⁴.



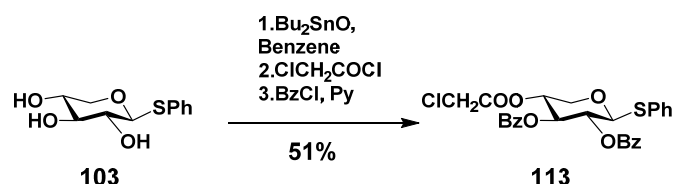
Scheme 58: Reported one-pot synthesis of compound **110**

The proposed pathway for the acylation of xyloside **109** is shown in Scheme 59. The presence of the methylene group at C5 favors the formation of the Sn-O linkage at the 4-position which leads to the formation of a 3,4-stannylidene acetal (intermediate **111**). Acylation of O-4 with chloroacetyl group creates at 3-dibutylchlorostannyl ether (intermediate **112**), which undergoes a benzylation with benzoyl chloride on the 3-position, followed by a second benzylation on the least reactive 2-position (intermediate **110**) yielding in this way the fully protected xyloside **110** in moderate yield²⁰⁵.



Scheme 59: Proposed intermediates of the one-pot synthesis of compound **110**

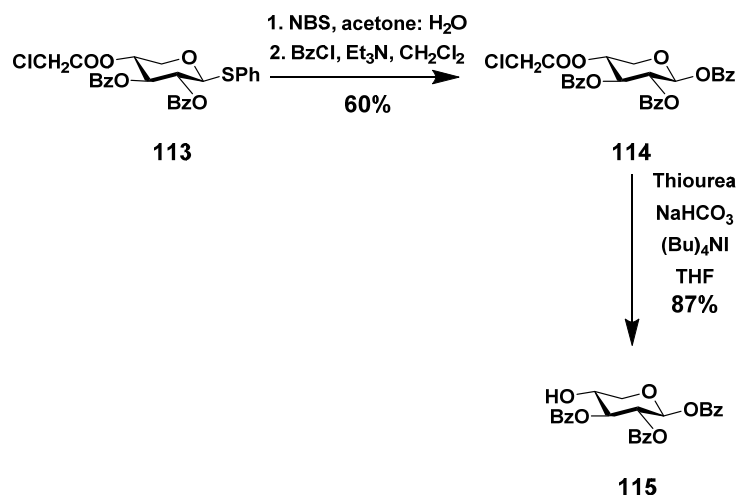
The procedure was tried on thioxyloside **103**, which successfully yielded compound **113** in 51% yield (Scheme 60).



Scheme 60: One-pot synthesis of compound **113**

Although, the one-pot synthesis only affords compound **113** in a moderate yield, it is significantly shorter than the other strategy used to yield compound **106**. Hence, we proceed with the synthesis of the acceptor from the donor **113**. Following literature procedures^{96,206–208}, building block **113** was hydrolysed in a mixture of acetone/water by means of a pre-activation with *N*-bromosuccinimide (NBS) and then benzoylated to yield compound **114**. The benzoylation employing Et₃N in dichloromethane affords primarily the β -anomer, where a benzoylation in standard conditions (BzCl in pyridine) usually yield the α/β mixture. Moreover, the employment of pyridine as solvent for the benzoylation reaction has shown in our experience to be problematic in combination with chloroacetyl groups, as earlier

explained. According to the literature, the high selectivity in providing the β -product of the employed reaction with Et_3N is probably due to the reaction kinetics²⁰⁸. By increasing the mutarotational rate with a strong base like Et_3N and diminishing the rate of acylation by adding BzCl in small portions over 10 minutes, the kinetic product (β -anomer in this case) seems to be prevalent. The preparation of acceptor **115** was achieved by subsequent deprotection of the chloroacetyl group in high yield (Scheme 61)¹⁸⁵.

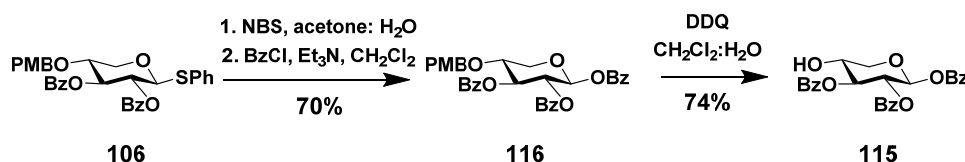


Scheme 61: Synthesis of acceptor **115** starting from **113**

However, when the procedure was scaled up the selectivity of the one-pot reaction significantly dropped, providing only 20-25% of the desired product **113**. This was probably mainly due to the different concentration of the test and up-scale reactions. The one-pot procedure is performed in dilute benzene, which during the up-scaling process is difficult to maintain due to the toxicity and availability of the solvent. Ultimately, looking at the results of the up-scaled reactions we can conclude that the selectivity is significantly influenced by the change of concentration. The procedure was also tested in toluene under the dilute concentration conditions, however failed to afford the same selectivity outcome as in benzene. Therefore, since we have already established an effective synthetic

procedure, yet more time-consuming, this strategy was not pursued any further at this point.

Building block **106**, can act as donor itself by carrying a thiophenol on the anomeric carbon, but it can also be converted into the required acceptor **115** in three steps (Scheme 62).



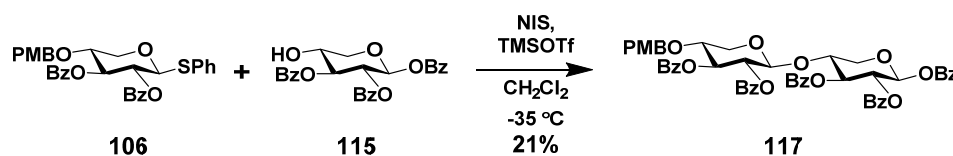
Scheme 62: Synthesis of acceptor **115** from donor **106**

The synthesis of compound **116** was afforded in the same fashion as earlier described for the chloroacetylated analogue **114** in high yield. Removal of PMB was accomplished by employing 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in a mixture of CH₂Cl₂:H₂O in 74% yield²⁰⁹. It is worth mentioning that the obtained yield was consistently lower when work-up involving ascorbic acid and citric acid in aqueous solution of NaOH was employed²¹⁰. However, this was solved by filtering the reaction mixture through a pad of Celite to remove the main amounts of DDQ residues and derivatives instead of the aqueous work-up. Acceptor **115** was hence obtained from donor **106** in 52% yield over 3 steps.

5.7 The *O*-glycosylation reaction

PMB has been shown to be incompatible with N-iodosuccinimide (NIS) and silver triflate (AgOTf) as combination in promoting glycosylations²¹¹. This is due to the fact that the aromatic ring is iodinated at the 3-position and the resulting 3-iodo-PMB is more resistant to acidic deprotection, however, it can still be cleaved with 10% TFA in CH₂Cl₂²¹². However, the incompatible employment of PMB with NIS/AgOTf has shown to be case-dependent since there are examples in literature

where they successfully reported their combined use in glycosylation reactions²¹³. We decided to try with a combination of NIS and TMSOTf to see whether changing the Lewis acid would make a difference. The first attempt of the coupling reaction between donor **106** and acceptor **115** was performed at -35 °C in CH₂Cl₂ and it afforded the desired product **117** in 21% yield (Scheme 63).



Scheme 63: First attempt for synthesis of compound **115**

TLC showed apparent signs of unreacted acceptor and donor, which convinced us to optimize the reaction. In Table 6 are listed all the different conditions and promoters that were tested in the optimization. From the results listed in Table 6 we observe a tendency for the reaction yield to increase when larger amount of NIS are used (Entries 1 to 4), in addition to, when the reaction temperature is allowed to rise from -35 to -20 °C during the coupling. Higher temperature seems to enhance the hydrolysis of the activated donor (Entry 1), while lower temperatures do not allow the reaction to proceed to completion. It is still unclear why higher amount of NIS are necessary. Also, the amount of Lewis acid (TMSOTf) was found to be crucial for the completion of the reaction. Lower amount of TMSOTf (< 0.4 equiv.) did not allow the reaction to consume all the acceptor. In that case lower yields and difficult separation of the product **117** due to co-elution with the acceptor **115** in the selected eluting system were experienced. Another promoter that has been tried out in the coupling reaction, was the combination of dimethyldisulfide (Me₂S₂) and triflic anhydride (Tf₂O). However, employment of the latter promoter (Entries 5 to 7) only afforded low to moderate yields of the desired product **117**.

Table 6: Optimization table of the coupling reaction between **106** and **115**;

NB: Method A. TMSOTf/NIS used as promoter ; Method B. Me₂S₂, Tf₂O used as promoter

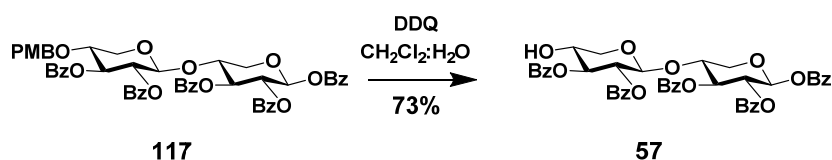
Entry	Donor/Acceptor ratio	Method	Amount of promoter	Temp. (°C)	Yield
1	1.6/1.0	A	0.2 (acid) +2.0 (NIS)	-5 °C → 0 °C	-
2	1.5/1.0	A	1.0 (acid) +2.4 (NIS)	-30 °C → -10 °C	-
3	1.5/1.0	A	0.2 + 0.2 (acid) + 2.0(NIS)	-45 °C → -10 °C	82 %
4	1.5/1.0	A	0.4 (acid) + 2.4 (NIS)	-30°C → -20°C	92 %
5	1.6/1.0	B	2.0	-30 °C → -10 °C	-
6	2.1/1.0	B	2.1	-50 °C → -35 °C	60 %
7	2.0/1.0	B	0.5 + 0.5 0.5 + 0.5	-50 °C → -20 °C	36 %

It is also worth mentioning that many of the reported reaction conditions have also been performed with the chloroacetyl protected donor **113** with no significant differences in yields.

The optimization of the coupling was achieved by employing an excess of NIS in the presence of a catalytic amount of TMSOTf and by stirring the reaction at a temperature range of - 30 to - 20 °C (Entry 7). In these conditions *O*-disaccharide **117** was formed in 92% yield.

5.8 Final steps for the synthesis of tetrasaccharide 1

The disaccharide **117** was treated with DDQ to remove the PMB group and afford acceptor **57** in 73% yield (Scheme 64).



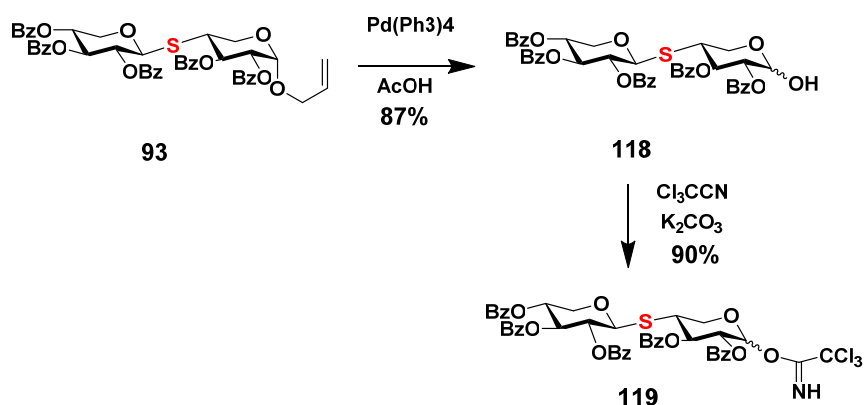
Scheme 64: Deprotection of PMB yielding compound **57**

Compound **57** is a common building block for the synthesis of all the desired final targets, while the donor **119** would have been employed only for the synthesis of target **1**. To obtain donor **119**, *S*-disaccharide **93** has to be deprotected on the anomeric position by hydrolysis followed by conversion of the free hydroxyl to the trichloroacetimidate.

As mentioned before the removal of the allyl group can be challenging when combined with a molecule containing sulfur^{116,167}. However, there are a few examples in literature, where Pd complexes have been successfully employed in removal of allyl group in sulfur-bearing molecules^{214,215}.

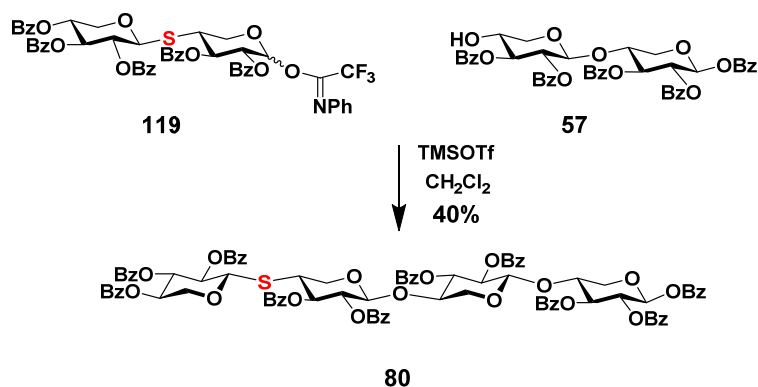
Our first attempt for removing the allyl group was performed with PdCl₂ and morpholine, yet without success. Therefore, we moved to the utilization of Pd(Ph₃)₄ in acetic acid²¹⁴. Satisfyingly, the procedure afforded the desired hemiacetal **118** in 87% yield. Compound **118** was consequently converted to the trichloroacetimidate donor **119** with Cl₃CCN and K₂CO₃ in 90% yield (Scheme 65).

The employment of a trichloroacetimidate rather than the more stable trifluoroacetimidate was based on previous results obtained during the assembly of *S*-disaccharide **91**.



Scheme 65: Deprotection of the anomeric position and conversion of the hemiacetal **118** in trichloroacetimidate donor **119**

The employment of a trichloroacetimidate rather than the more stable trifluoroacetimidate was based on previous results obtained during the assembly of *S*-disaccharide **93**. The final coupling was conducted in CH_2Cl_2 by using a catalytic amount of TMSOTf in accordance to standard procedure⁷³ (Scheme 66). The glycosylation reaction afforded tetrasaccharide **80** in 40% yield. However, TLC and LC-MS analysis of the reaction mixture showed large amount of unreacted acceptor **57**, suggesting the maybe the reaction did not go to completion. Therefore, further optimizations on the reaction are currently ongoing.



Scheme 66: Coupling glycosylation to obtain tetrasaccharide **80**

Tetrasaccharide **80** is the precursor of target **1**. The overall strategy has proven to be feasible, and therefore we decided to apply the same procedure for the synthesis of the branched target compounds, described earlier. Complete deprotection of compound **78** with Zémpfen conditions to afford final target **1** is currently ongoing.

5.8.1 Concluding remarks

From the results obtained from the *O*-glycosylation reaction providing compound **115** we can deduce that the amount of NIS and TMSOTf employed but also the temperature have a large influence on the final yield. In particular the coupling was found to be enhanced by excess amount of NIS and a temperature range of - 30 to - 20 °C. The reaction though, did not show to be negatively influenced by the presence of PMB when the combination NIS/TMSOTf was used, excluding the probable formation of the iodinated analogue. Another successful result obtained was the coupling reaction providing tetrasaccharide **78**, which also showed to proceed smoothly in the first test reaction. In conclusion the overall procedure affording tetrasaccharide **78** has shown to be reliable and reproducible and therefore applicable also to the other target molecules.

5.9 Synthesis of the branched targets

As discussed before, the synthesis of pentasaccharides **2** and **53** require the orthogonal protection of the 2- and 3-positions on the second sugar from the non-reducing end (Figure 22).

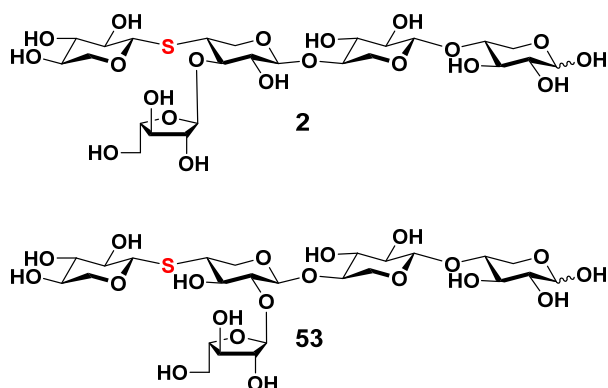
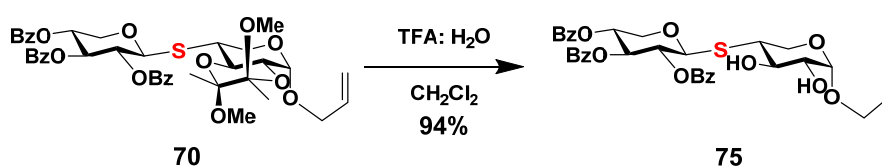


Figure 22: Target tetraxylans branched in the 3-position (**2**) and 2-position (**53**).

The pursued strategy to yield compound **2** and **53** described earlier, employs a silyl ether group on the 3-position and a chloroacetyl on the 2-position in order to discriminate and selectively deprotect the two positions when appropriate (Scheme 30).

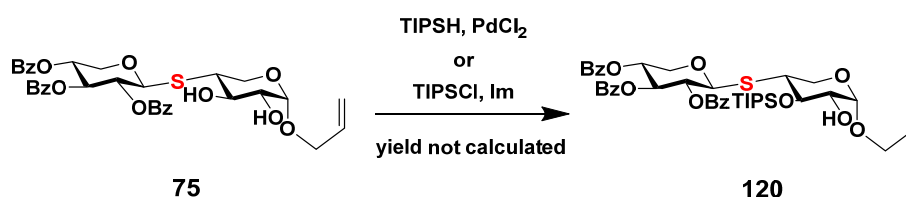
5.9.1 Functionalization of the branching positions

The next step of the synthetic strategy, as shown earlier in the retrosynthetic analysis, was the removal of the temporary diacetalic PG (BDA) by TFA^{172,173}, followed by the introduction of two orthogonal PGs on the two hydroxyl groups. The removal of BDA was afforded in 94% yield and is illustrated in Scheme 67.



Scheme 67: Removal of BDA protecting group

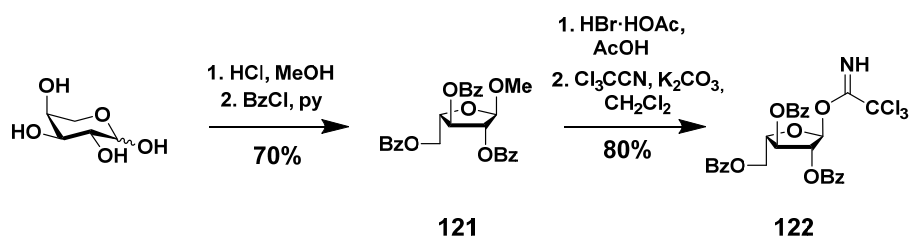
The 3-position on glucose-like pyranoses is usually more reactive than the 2-position and there are examples in literature of the selective protection of O-3 over O-2 with silyl ethers, also on xyloses²⁰³. For example, the selective protection of 3-position with silyl ethers by using a silyl hydride in combination with PdCl₂ has been reported²¹⁶. When the 4-position is protected there are other procedures that afforded the protection of the 3-position in high yield by employing the silyl chloride in presence of a base, for example imidazole²¹⁷. Regrettably, both reported procedures failed to afford the desired product **120** from compound **75** (Scheme 68).



Scheme 68: Attempts for synthesis of compound **120**

Since no major product was obtained from the reaction, it was difficult to predict the reactivity of the 2- and the 3-position and which one would have been more nucleophilic in a reaction. Therefore, we decided to directly perform the glycosylation reaction with the arabinofuranose branch **122** we intended to insert, to investigate the regioselectivity of the coupling reaction. We envisioned the experiment would provide us information on the different nucleophilicity of the two positions and hence of the necessity of having orthogonal protecting groups.

The branching arabinofuranoside donor **122** was synthesized in four steps from L-arabinose by standard procedure (Scheme 69)^{218,219}.



Scheme 69: Synthesis of compound **122**

Donor **122** was reacted with acceptor **75** following the general procedure with catalytic amount of Lewis acid in CH_2Cl_2 at $-35\text{ }^\circ\text{C}$, affording both trisaccharide **123** and tetrasaccharide **124**⁷³. To overcome the issue of the separation between **120** and **124**, trisaccharide **123** was benzoylated on the 2-position prior to separation (Scheme 70). After chromatographic separation we could verify that the glycosylation reaction yielded the trisaccharide **126** in 43% yield and only traces of the isomer **127**.

To verify which regioisomer was obtained from the glycosylation reaction, 2D NMR HMBC spectra were analyzed. The obtained trisaccharide shows a long range coupling between H-3 of the xyloside in the reducing and the anomeric carbon of the arabinofuranoside. For additional confirmation, the NMR also showed coupling between H-2 and a carbon in the aromatic zone. The 2D NMR analysis confirmed that the obtained trisaccharide is hence **126** (Figure 23). The result suggests that probably the glycosylation proceed first by coupling of the donor **122** on the 3-position followed by a second attack of the 2-position on donor **122**, thus showing a preference for the 3- over the 2-position. Since the 3-position intuitively should be more hindered than the 2-, it is possible to hypothesize a change of conformation of acceptor **75** during the glycosylation to allow for the attack of the donor on the 3-position.

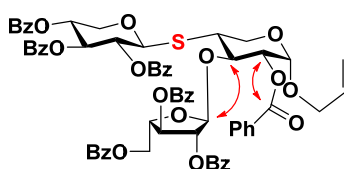
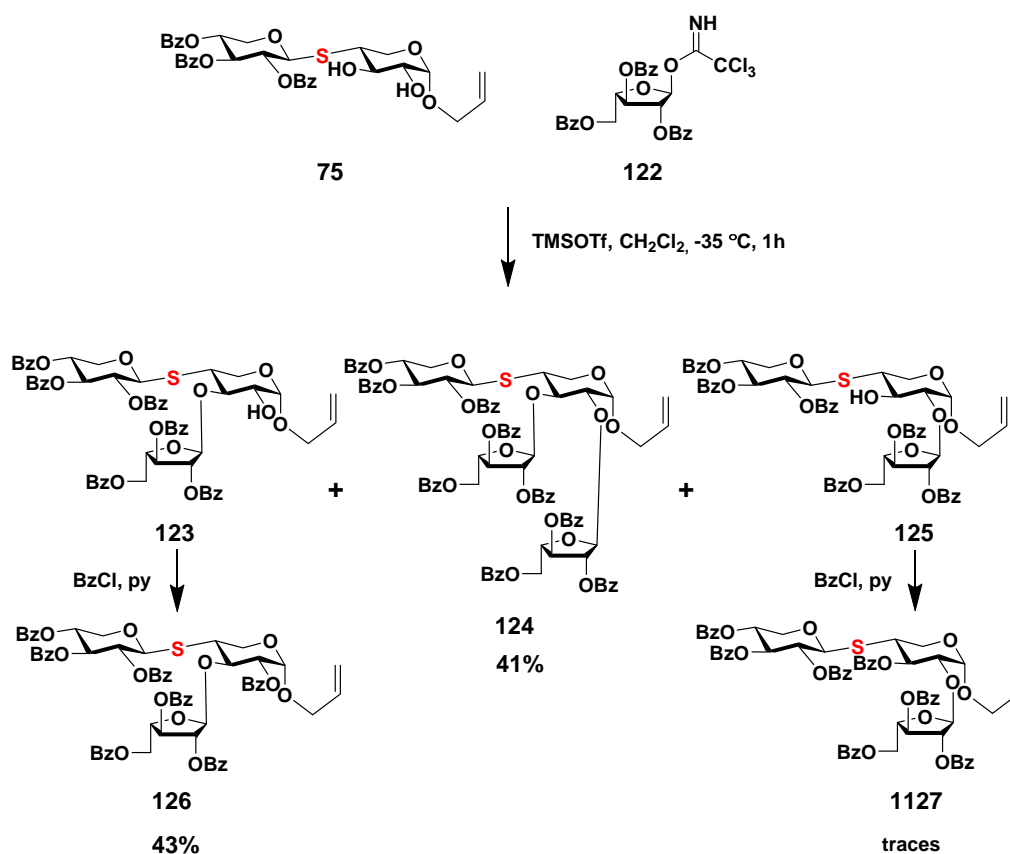


Figure 23: Long range coupling showed in the 2D HMBC spectrum of **126**

Scheme 70: Synthesis of tetrasaccharide **124** and trisaccharide **126**

We can also assume that the above-described coupling reaction can provide a fair suggestion on the possible need or not for orthogonal PGs in those positions. Since the coupling reaction seems to have a preference for the 3-position, it might be possible to avoid the orthogonal protections and proceed with the branching reaction. Moreover, compounds **126** and **124** are only few synthetic steps away from target **2** and **3** and therefore could be employed for their synthesis. However, their reactivity as donors is still unknown. Furthermore, based on these observations a different synthetic strategy would be needed to acquire pentasaccharide **53**.

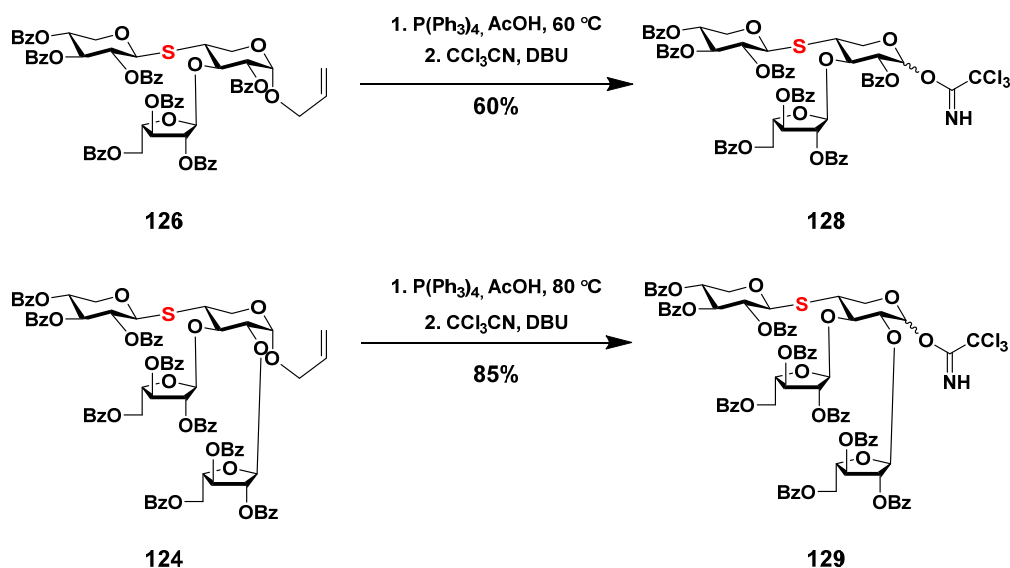
5.9.2 Concluding remarks

The first attempts of orthogonal protection have shown to be more challenging than expected. Due to time limitations other synthetic options for the orthogonal protection of the two branching positions were not further investigated. The glycosylation reaction between the *S*-disaccharide acceptor **73** and arabinofuranosyl donor **119** was performed to investigate a possible regioselectivity. Interestingly, the glycosylation reaction showed a significant preference for the 3-position suggesting that the orthogonal protections can potentially be omitted.

5.9.3 Future perspective

The ongoing work in collaboration with Dr. Irene Boos is focused on the final steps in the assembly of target **2** and **3**. We wanted to investigate whether the direct coupling between the trichloroacetimidate donor analogues of trisaccharide **126** and tetrasaccharide **124** and the *O*-disaccharide acceptor would succeed. In order to do that, trisaccharide **126** and tetrasaccharide **124** need to be subjected to hydrolysis of the allylic group on the anomeric position and converted to the trichloroacetimidate analogues **128** and **129** (Scheme 71).

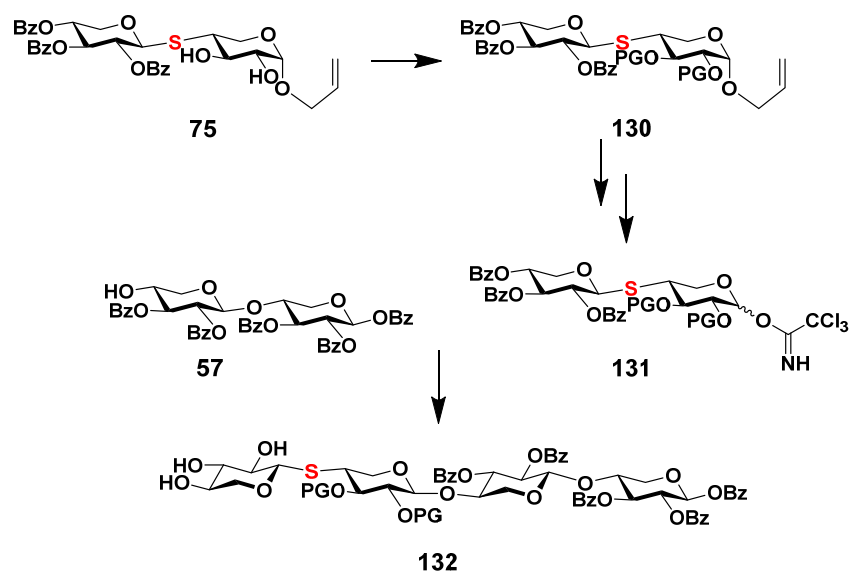
First test reactions to remove the allyl group from trisaccharide **126** and tetrasaccharide **124** with Pd tetrakis in acidic acid have required higher temperatures but moderate to high yields were obtained in both cases. The conversion of the corresponding hemiacetal into the trichloroacetimidate donors **128** and **129** have also required small adjustments, like the employment of a stronger base (e.g. DBU) than K_2CO_3 which was used for the synthesis of *S*-disaccharide **81**.



Scheme 71: Conversion of compounds **126** and **124** into their trichloroacetimidate analogues **128** and **129**

We expect the reactivity of the donors to be lower than the corresponding analogue **81** due to the higher complexity and bulkiness of **128** and **129**. We envision of obtaining a preference for 1,2-*trans*-anomeric product based on the bulkiness of the branches. We believe that the arabinofuranoses on the 2- and 3-position could in theory hinder the formation of the corresponding 1,2-*cis*-anomer enhancing thus the formation of the desired 1,2-*trans*-anomer.

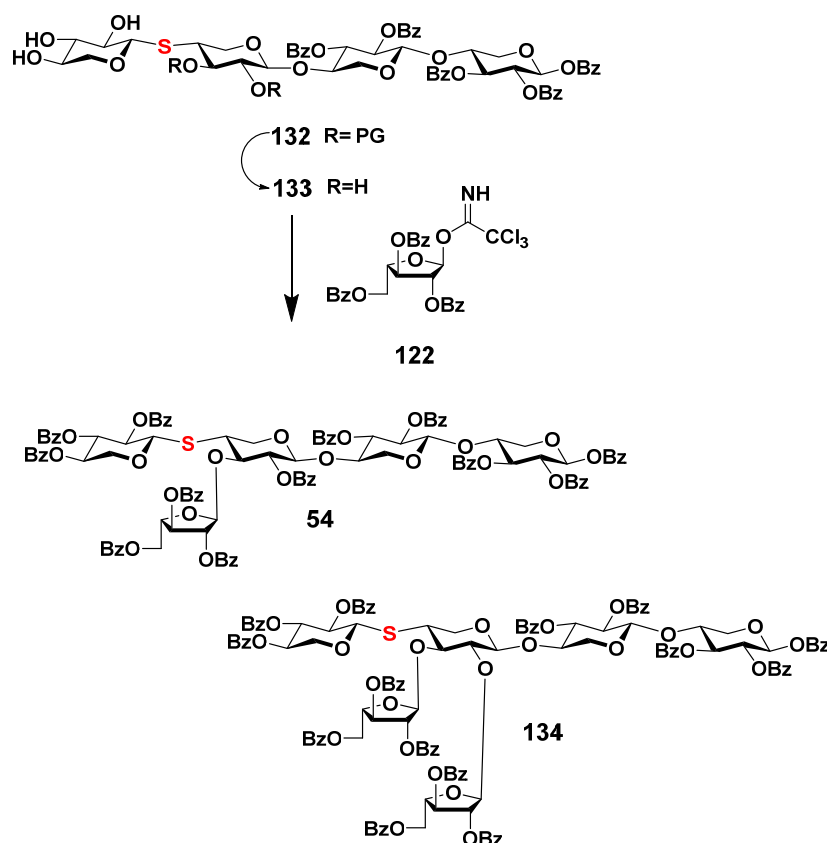
However, in case the afore-mentioned strategy would fail, a different approach has been also discussed. We envisioned that *S*-disaccharide **75** could be temporary protected (*S*-disaccharide **130**) with an ester PG orthogonal to the benzoyl group but still capable of participation in the glycosylation reaction (e.g. chloroacetyl group, levulinoyl group). Compound **130** would consequently be deprotected on the anomeric position and converted into the trichloroacetimidate **131** (Scheme 72). The donor **131** could then react with acceptor **57** to yield tetrasaccharide **132**.



Scheme 72: Possible synthesis of tetrasaccharide **132**

The product would be selectively deprotected on the 2- and 3-positions on the second sugar from the non-reducing end to obtain the tetrasaccharide **133**. Acceptor **133** would be subjected to the glycosylation reaction with the branch donor **122** (Scheme 73).

In this case, we envisioned that the separation of the final products would potentially be challenging.



Scheme 73: Alternative synthesis of compounds **54** and **134**

The final reaction will be the conversion of the products **54** and **134** into the fully unprotected oligosaccharides **2** and **3** under Zémlen conditions.

5.9.4 Biological future perspectives

The physicochemical and functional properties of the xylan chains in their native and modified forms depend intrinsically on their conformational behaviour²²⁰. Therefore, a detailed knowledge of such fundamental properties is important to understand conformational changes in relation to the enzyme binding site²²¹. NMR conformational analysis are among others important tools for the investigation of these properties^{221,222}. Therefore, NMR conformational analyses are going to be performed on the final targets and on the natural *O*-linked analogue. The results are

going to be also compared with X-ray crystallographic analysis. At first, the aforementioned homo-xylan chains are going to be considered in the study, followed by the effects of the branches and their influence on the conformational flexibility of the molecule. Degradation analysis on target **1** is also going to be performed, following reported procedures³⁰. The products of the hydrolysis of the natural *O*-linked substrates are going to be analyzed and compared with the *S*-linked analogues in order to evaluate the differences. Possible collaborations are currently under evaluation.

6 Identification and characterization of glycosyltransferases involved in rhamnogalacturonan I biosynthesis in plants.

The present chapter is the result of a three month external stay as Research Scholar at the Joint BioEnergy Institute (JBEI) part of Lawrence Berkeley Laboratories in Emeryville, California, under the supervision of Professor Henrik Vibe Scheller.

6.1 Pectin

As mentioned in the introduction chapter, pectin is one of the major components of the primary cell wall and includes a family of polysaccharides that contains galacturonic acid (α -D-GalA) linked at both the 1-and the 4-positions. Pectin contributes to a range of cell functions, including support, defense, cell signaling and adhesion²²³.

Three major types of pectic polysaccharides are usually recognized: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG, which is a homopolymer of D-GalA linked in an α -1,4-linked configuration, constitutes approximately 65% of pectin. RG-II constitutes around the 10% of pectin and is the most complex pectic polysaccharide, while RG-I constitutes 20–35% of pectin. Differently from HG and RG-II, RG-I has a disaccharide repeated backbone of 4- α -D-GalA-(1,2)- α -L-Rhamnoside in which the GalA residues are highly acetylated at O-2 or O-3 (Figure 24)²²⁴.

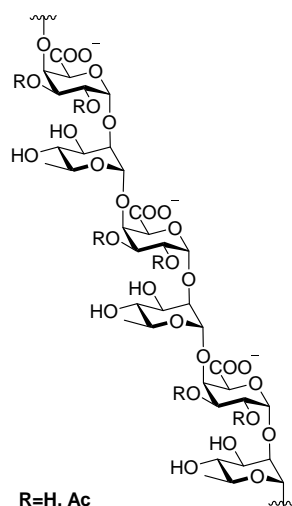


Figure 24: Structure of the RG-I backbone

Some plant cell walls also contain additional substituted galacturonans, known as apiogalacturonan (AGA) and xylogalacturonan (XGA)²²⁵. Glycosidic linkage data indicate that these different pectin polysaccharides are covalently linked to each other in the wall, although the full structure for a complete pectin macromolecule has not been yet determined^{226,227}.

6.2 Rhamnogalacturonan I biosynthesis

Cell walls polysaccharides and other glycans are synthesized by addition of sugar residues onto acceptor molecules. The activated donor substrates are nucleotide sugars and the acceptors are generally glycans¹⁸. Different nucleotide sugars are required in pectin biosynthesis in form of activated-sugar donor substrates. The nucleotide sugars are generated from a few nucleotide-sugar precursors (i.e. Uracil diphosphate Glucose (UDP-Glc)), by means of interconversion enzymes or through the so-called “salvage pathway”, which involves the conversion of free sugars to nucleotide sugars in two sequential reactions catalyzed by sugar kinases and UDP-sugar pyrophosphorylase²²³. Although the identification and characterization of pectin biosynthetic transferases and the encoding genes are major challenges,

pectin biosynthetic enzymes have shown to be localized in the Golgi apparatus²²⁴. To generate the backbone, RG-I biosynthesis requires both galacturonic acid transferase (GalAT) and rhamnosyltransferase (RhaT) activities, while multiple galactosyltransferases (GalTs) and arabinosyltransferases (AraTs) are needed to initiate, elongate, and branch the side chains. Most of these glycosyltransferases involved in the RG-I biosynthesis, have been identified by a bioinformatics approach using available phylogenetic, transcriptomic and proteomic data^{228–234}. For example, multiple *in vitro* studies of the synthesis of galactan side chains in several plant species have demonstrated Golgi-localized β -1,4-GalT activities with different pH optima and acceptor substrate preferences^{230–232}. Among them, the most well-characterized β -1,4-GalT activity specifically elongates the galactan side chains^{234–238}. Several AraTs involved in the synthesis of arabinan and arabinogalactan side chains have also been characterized from mung bean^{239,240}. RG-I backbone synthesis *in vitro* studies, however, has not provided conclusive data on GalAT and RhaT, in part because of the lack of available donor substrate UDP-Rha²²⁴. Hence, efforts to obtain conclusive biochemical evidence for enzyme activities and acceptor substrate preferences still represent a major challenge in glycobiology.

6.3 Project description

The aim of this project is to identify the glycosyltransferases involved in the biosynthesis of RG-I and characterize their activity *in vitro*. With enzymatic cocktails obtained from the isolation of microsomes from the Golgi apparatus of mung beans we intend to investigate the galacturonic acid transferases (GalAT) activities. The enzymes catalyze the reaction between UDP-GalA **136** and a fragment of RG-I backbone represented by the hexasaccharide **135** composed by three repeated sequences of Rha-GluA, which was provided by a former member of Prof. Mads H. Clausen's group (Figure 25)²⁴¹.

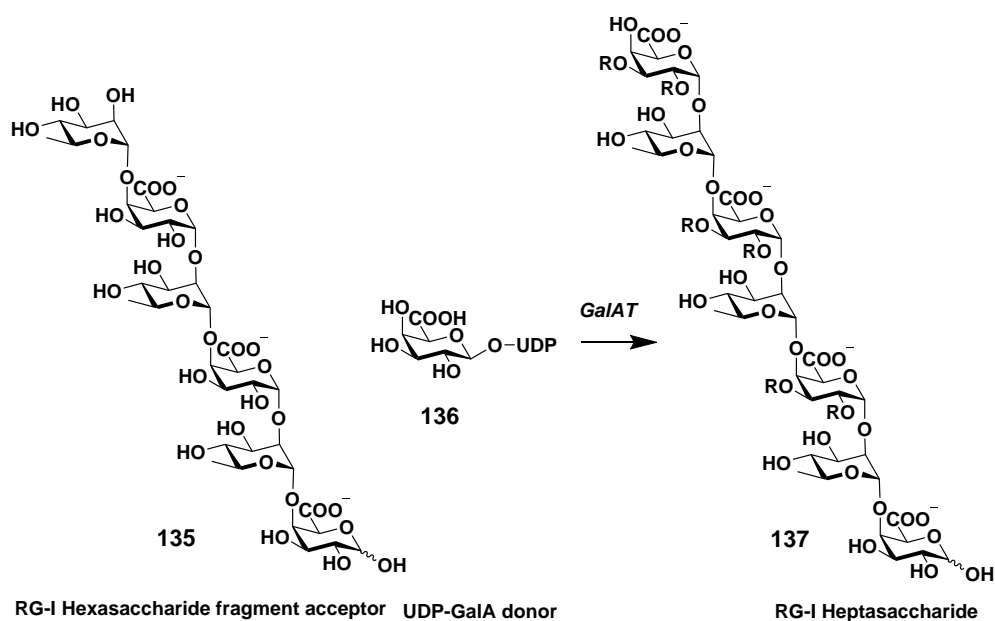


Figure 25: Enzymatic reaction catalyzed by GalAT

To determine the activity we wanted to employ enzymatic assays that allow the analysis of many reactions in parallel in a 96-well format. HPLC will be employed to determine the amount of product **137** obtained in the enzymatic reaction. Mass spectrometry will also be used as a method to verify the mass of the product **137**. With these experiments we wished not only to reproduce *in vitro* the natural elongation of RG-I backbone happening *in vivo*, but also to characterize the reaction by establishing conditions and substrate preferences.

6.3.1 Previous results

The assay was conducted following optimized conditions reported in the literature for other types of glycosyltransferases in *Arabidopsis Thaliana*^{242–244}. The procedure employs several components, which are all necessary for the feasibility of the reaction. The principal components are the acceptor molecule represented by the RG-I hexasaccharide acceptor **132**, the UDP-GalA donor **133** and an enzymatic mixture contained in the isolated microsome extracts, which contain the

glycosyltransferases. Other important components of the enzymatic assay are the pH buffer, a surfactant molecule to enhance the solubility of the microsomes in the buffer, and a source of metallic cations. Manganese has shown to be beneficial as co-factor for the catalysis of the many enzymatic processes^{242,244}. Therefore, $MnCl_2$ is used as manganese source in the reaction mixture. After method development (data not shown), it was decided that the detection of the enzymatic reaction products would be conducted by HPLC with an anion exchange column²⁴⁵. The analytical method was capable of identifying new peaks derived by the enzymatic reaction.

Following a standard enzymatic procedure, the reaction mixture was divided into 4 wells and terminated after 0, 2, 4 and 6 hours^{242,244}. The HPLC chromatogram showed indeed two possible product peak around 14 and 18 min as represented in Figure 26.

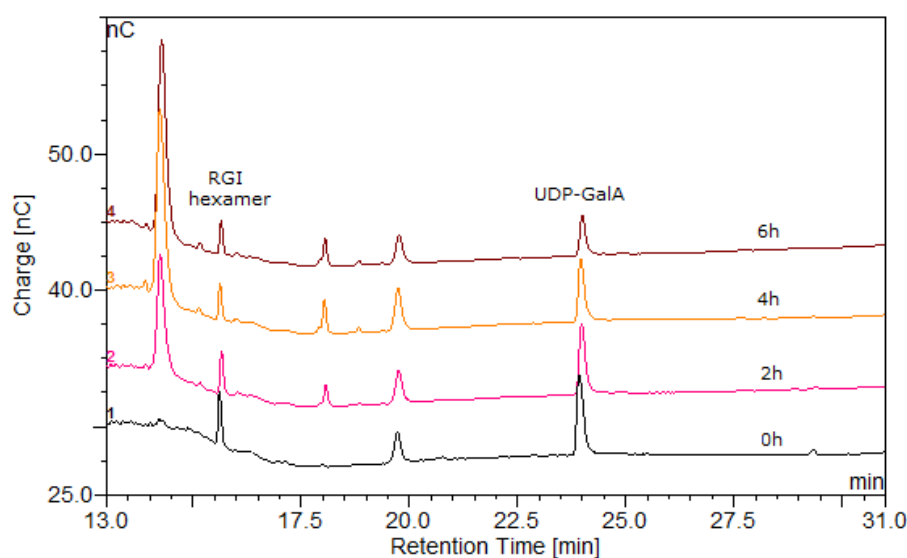


Figure 26: HPLC chromatogram of the enzymatic assay at 0h, 2h, 4h and 6h.

The peak at 14 min was investigated as a possible degradation product of UDP-GalA. For this reason comparative assays were run without hexasaccharide acceptor or UDP-GalA donor were performed (Figure 27).

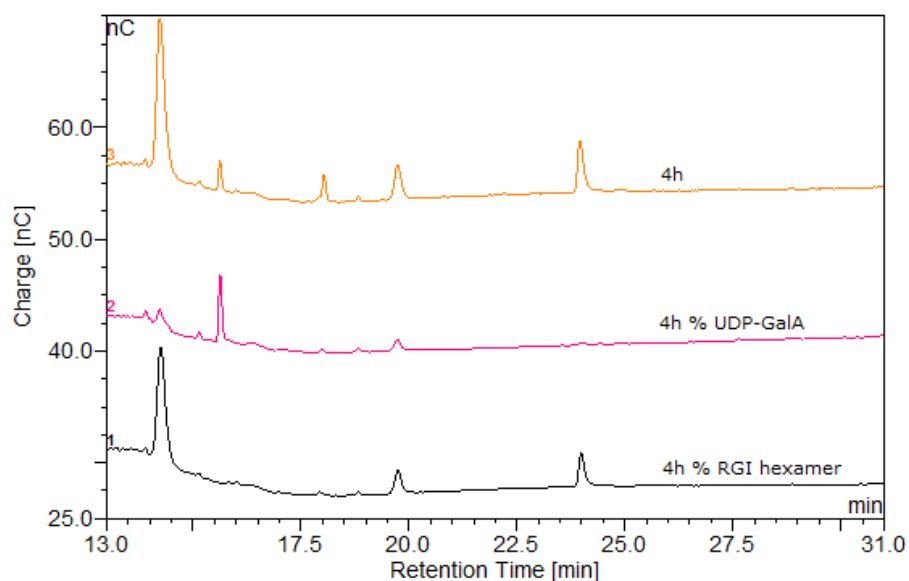


Figure 27: Comparative assays without RG-I hexamer acceptor (in black) and without UDP-GalA donor (in pink)

The peak at 14 min showed indeed a significant reduction when the assay was run without UDP-GalA donor indicating a possible connection to it. From Figure 27 we can also evince that the peak at 18 min is dependent on the presence of both acceptor and donor and therefore could be a possible product peak candidate. The peak was therefore collected and analyzed by mass spectrometry. The mass $[M + Na]^+$ was confirmed for the peak at 18 min (Figure 28).

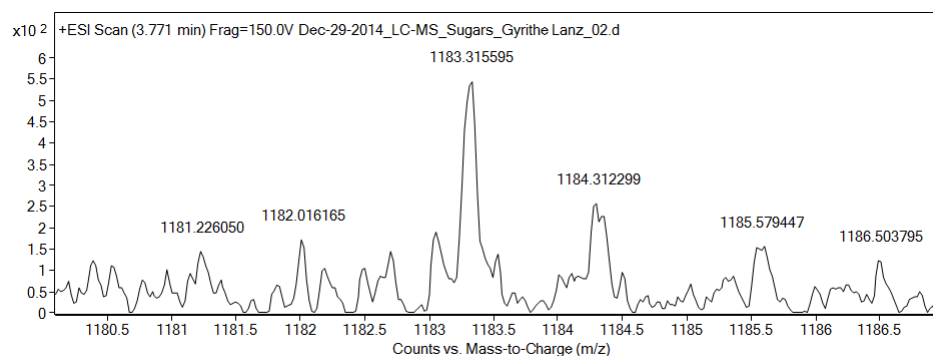


Figure 28: Mass spectrometer result on peak at 18 min: 1183.3156 (calc. 1183.3019)

However, due to high signal/noise ratio a more concentrated sample was envisioned to be necessary to conclusively confirm the mass of the heptasaccharide product **134**.

6.4 Optimization process

At my arrival to JBEI, one of the objects of the project was optimization of the assay. The aim was to identify the optimal conditions for the GalAT to catalyze the transfer and establish a general procedure for the enzymatic assay. The major parameters to optimize were the reaction time, the cation type and its relative concentration in the assay, the temperature, the buffer type and the pH. The parameters were investigated one by one maintaining the others constant.

6.4.1 Reaction time

Previous results have not shown a significant improvement in product yield between assays terminated after 4h and 6h. However, the reaction never showed evidence of reaching the completion (Figure 26). Therefore, we decided to elongate the reaction time to 24h before termination. The enzymatic reaction showed degradation products formed and no significant improvement in the amount of product. Therefore, we decided to fix the reaction time at 4h.

6.4.2 Cation type and relative concentration

The assay was initially performed employing MnCl_2 as cation source. Several concentrations of the cation salt were employed to analyze the influence on the transglycosylation reaction outcome. MgCl_2 at different concentrations was also investigated since there are examples in literature of transglycosylases that have shown a significant preference for Mg over Mn^{228,231,240,244}. The cation solutions were freshly prepared and added in different concentrations to the reaction mixtures. Preliminary results showed that the product peak at 18 minutes was highly influenced by different quantities of the two cations, suggesting a significant

preference for Mg^{2+} over Mn^{2+} . The different values were extrapolated by the HPLC chromatograms and plotted in Chart 1.

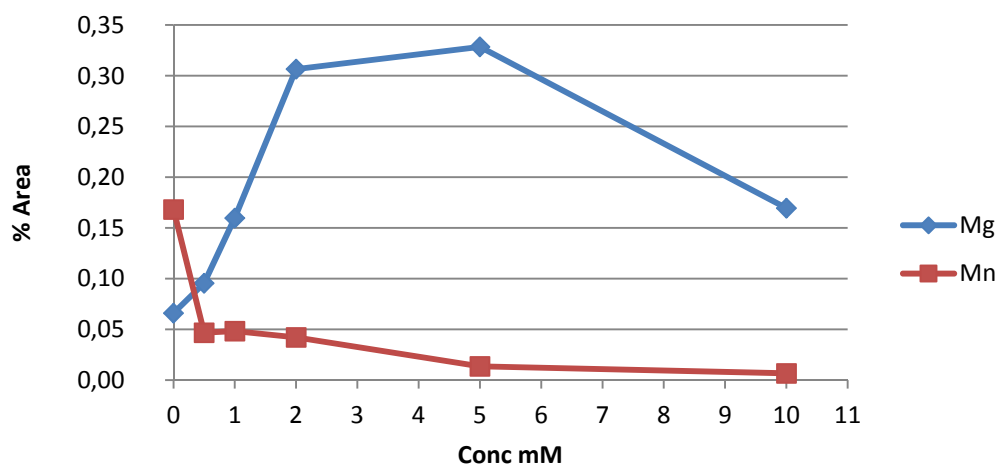


Chart 1: Influence of cation type and concentration on the product peak area. The graph has been plotted by reporting the integrated area of the product peak (% Area) on the y-coordinates and the final concentration of the cation salt in the enzymatic reaction mixture on the x-coordinates (Conc. in mM). HPLC chromatograms and procedures are collected in Appendix.

The plot showed significantly different trends associated with the two cations. According to these preliminary results, manganese seems in fact to inhibit the catalytic process especially at higher concentrations (5 mM). On the other hand magnesium shows to be a beneficial cofactor of the catalytic process suggesting an improvement in product formation at concentrations between 3 mM and 5 mM.

6.4.3 Buffer solution and pH

2-(*N*-Morpholino)ethanesulfonic acid (MES) is a water soluble buffer that owns poor affinity to metal cations, like manganese or magnesium²⁴⁶. It has formerly been used as biochemical buffering agent in the enzymatic assay. The buffer only covers a limited range of pH (from 5.0 to 6.5). Other types of buffering agents are often employed in analogous enzymatic assays. One of them is the

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), a zwitterionic buffering agent, which is active between a pH range of 6.8 and 8.5²⁴⁶.

A buffering agent able to cover a higher pH range is the [[1,3-dihydroxy-2-(hydroxymethyl)propanyl]amino]propane-1-sulfonic acid (TAPS). It is employed for the preparation of buffer solutions with a range between 7.7 and 9.1 and is used in biological assays that require alkaline environments²⁴⁶. Several assays were performed with the three different buffering agents at different pH values

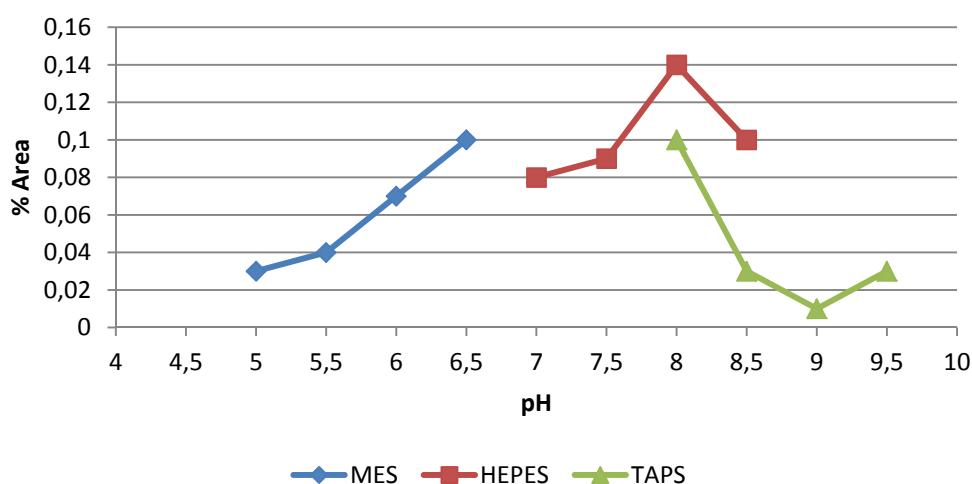


Chart 2: Influence of buffer type and pH on the product peak area. Data were extrapolated by plotting the integrated area of the product peak (% Area) on the y-coordinates and the pH of the enzymatic reaction mixture on the x-coordinates. HPLC chromatograms and procedures are collected in Appendix.

Four solutions at different pH values were prepared for each buffering agent and the enzymatic reaction performed with all of them individually. The preliminary results showed a significant improvement of the product amount when the pH of the reaction mixture was maintained around 8 (Chart 2). Two buffer solutions at pH 8 were prepared employing both HEPES and TAPS. The diagram showed a preference for HEPES-based buffer solutions and therefore the latter buffering agent at pH 8 was set as optimized parameter for the assay.

6.4.4 Reaction temperature

The temperature of the reaction was set at 25 °C in the previous assays. For the optimization the temperature range was established between 4 °C and 50 °C. Higher and lower temperatures were excluded from the study due to incompatibility (i. e. the enzymes are not active outside this temperature range). The temperatures were maintained constant by means of a temperature control system for the entire incubation time.

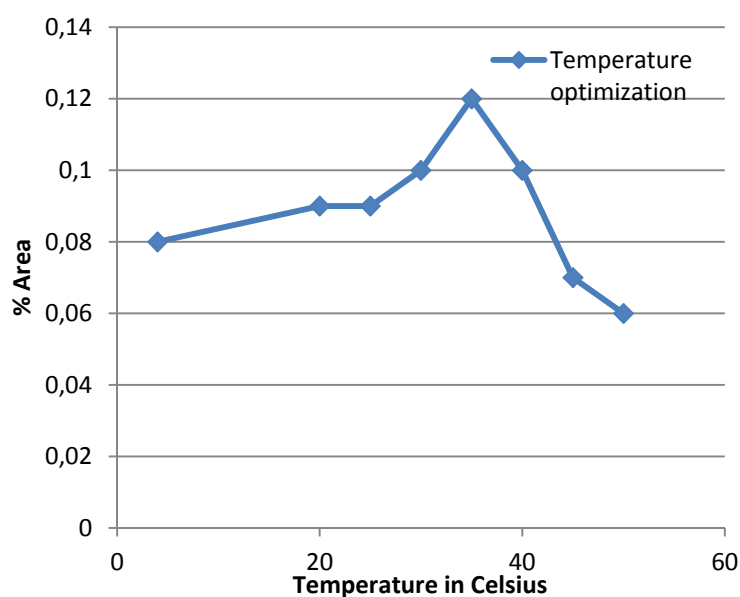


Chart 3: Influence of the reaction temperature on the product peak area. HPLC chromatograms were recorded and the data extrapolated by plotting the integrated area of the product peak (% Area) on the y-coordinates and the reaction temperature on the x-coordinates. HPLC chromatograms and procedures are collected in Appendix.

The results plotted in the diagram show the enzymatic reaction to peak around 35 °C (Chart 3).

The enzymatic reaction was consequently performed following the optimized conditions in regards to buffer, cation type, time and temperature and the product

peak collected and analyzed by mass spectrometer to confirm the mass of heptasaccharide **137**.

6.5 Future prospective

The preliminary results above described will be repeated in order to verify the reproducibility of the assay. The optimized reaction conditions have been also performed on different types of glycosyltransferases. Preliminary tests indicated a possible product peak when the RG-I hexamer acceptor was employed in presence of the branching donor UDP-Galactose (UDP-Gal). These results will also be repeated to characterize the product in more detail. Positive responses are going to possibly initiate screening of other nucleotidic donors. The study will be further pursued to identify substrate preferences and to produce important information on the RG-I backbone biosynthesis.

7 Conclusion

The aim of the present work was to develop a reliable method for the incorporation of thiolinkages in the synthesis of oligoxylans. Different strategies for assembly of *S*-linked disaccharides have been approached, both involving 1-thioglycoside donors and thioacceptors. In the latter strategy, the protecting groups present on the anomeric position of the donor proved to be critical for the success of the coupling. On the other hand when the procedure involved 1-thioglycosides, the protecting groups present on the acceptor was shown to highly influence the stability of the C4-triflate acceptor and therefore the yield of the coupling reaction. Both routes have been thoroughly investigated by byproduct determination and both approaches have shown to be employable for the synthesis of thiolinkages in oligoxylans assembly. The synthesis of target **1** has been performed by a 2+2 block-strategy, which involves the final coupling of an *S*-disaccharide and an *O*-disaccharide. The synthesis of the *O*-disaccharide was afforded with the employment of a common building block able to react as thiophenyl donor **106** itself but also to be converted to the desired acceptor **115** in few steps. The coupling reaction has been approached with different promoters and optimized to employ the combination NIS/TMSOTf. In the final coupling between the two disaccharides a trichloroacetimidate donor in the presence of TMSOTf has been employed. For the synthesis of the branched target **2** and **3**, orthogonal protecting groups have shown not to be required. The result was obtained by performing the branching glycosylation on the *S*-disaccharide **75**, which showed a significant preference for the 3-position over the 2-position. Final steps for the glycosylation between the branched units and the *O*-disaccharide are currently ongoing.

The enzymatic assay, developed prior my arrival, has been repeated in order to optimize the reaction conditions. Parameters like temperature, reaction time but also cationic cofactors and pH have been investigated. Reaction time was confirmed at 4h while the reaction temperature was increased to 35 °C. The pH at

which the reaction was run was also increased to 8, by employment of a HEPES-based medium. The cation was switched from Mn^{2+} to Mg^{2+} at the concentration of 3-5 mM. Moreover, other preliminary applications have been approached, like in the enzymatic reaction of GalT, which branches the RG-I backbone with galactosyl residues. The promising results are now currently under further investigation.

8 Experimental data

8.1 General Considerations

Starting materials, reagents and solvents were purchased from commercial suppliers and have been used without further purification. All solvents are HPLC-grade. The anhydrous solvents were obtained from an Innovative Technology PS-MD-7 Pure-solv solvent purification system. All reactions under nitrogen or argon atmosphere were carried out in flame dried glassware and in anhydrous solvents. Thin-layer chromatography (TLC) was performed on Merck Aluminium Sheets pre-coated with silica, C-60 F₂₅₄ plates. Compounds were visualized by charring after dipping in CAM stain (Ce(SO₄)₂ (1.6 g) and (NH₄)₆Mo₇O₂₄ (4 g) in 10% sulfuric acid (200 mL)). Eluent systems are specified for each R_f-value, and ratios are given as volume ratios. Evaporation of solvents was performed with a VWR International Laborota 400 under reduced pressure (*in vacuo*) at temperatures ranging between 35 – 55 °C. Trace solvent was removed under reduced pressure by means of a high-vacuum pump.

Flash chromatography was performed using Matrex 60 Å silica gel (35-70 μm) as the stationary phase by the general procedure developed by Still *et al.*²⁴⁷ The eluent system is specified in the synthesis protocols. Eluent ratios are given as volume ratios. Automated flash chromatography was performed using a Teledyne Isco Combiflash Rf200 and Redisep Rf Gold Silica columns. All columns were treated with a gradient of heptane-EtOAc from 0% EtOAc to 100% EtOAc. The intervals indicated for the eluent system under each entry implies where the compound eluted.

IR analysis was performed on a Bruker Alpha-P FT-IR instrument where solid compound was applied directly onto the instrument. Optical rotation was measured on a Perkin Elmer Model 241 Polarimeter. Melting points were measured on a Stuart melting point SMP30 and reported in °C uncorrected. UPLC/MS analysis was performed on a Waters AQUITY UPLC system equipped with a PDA and a

SQD MS detector. Column: AQUITY UPLC BEH C18 1.7 μm , 2.1 x 50mm. Column temp: 65 $^{\circ}\text{C}$. Flowrate: 0.6 ml/min. Solvent A: 0.1% formic acid in water, Solvent B: 0.1% formic acid in CH_3CN . Gradient: 5% B to 100% B over 2.4 min, hold 0.1 min, total run time – 2.6 min. High-resolution LC-DAD-MS was performed in an Agilent 1100 system equipped with a PDA and coupled to a LCT orthogonal time-of-flight mass spectrometer (Waters-Micromass, Manchester, UK) with Z-spray electrospray ionization (ESI) source and a LockSpray probe and controlled MassLynx 4.0 software. LC-MS calibration from m/z 100 – 900 was done with a PEG mixture. Standard separation involved a LUNA 2 column with a CH_3CN (50 ppm TFA) in water gradient starting from 15% to 100% over 25 minutes with a flow rate of 0.3 mL/min. The new compounds were characterized by ^1H NMR, ^{13}C NMR, IR, HRMS (ESI), melting point, and optical rotation.

8.2 NMR assignment of new compounds

1D and 2D NMR spectra were recorded on a Bruker Ascend 400 MHz at 298 K. Spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C , respectively. The correlation spectroscopy (COSY) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 3 k \times 3 k, collecting 4 FIDs and 1 k \times 128 data points. The heteronuclear single quantum coherence (HSQC) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 4.8 k \times 16.6 k, collecting 4 FIDs and 1 k \times 256 data points. Heteronuclear multiple-bond correlation (HMBC) spectra were recorded with a relaxation delay of 1.3 sec before each scan, a spectral width of 3.5 k \times 22.3 k, collecting 32 FIDs and 2 k \times 256 datapoints NMR chemical shifts are reported in ppm relative to deuterated solvent peaks as internal standards (δ ^1H , D_2O 4.79 ppm, δ ^1H , CD_3OD 3.31 ppm, δ ^1H , CDCl_3 7.26 ppm, δ ^{13}C , CD_3OD 49.86 ppm, δ ^{13}C , CDCl_3 77.16 ppm). Coupling constants (J) are given in hertz (Hz). Multiplicities of ^1H NMR signals are reported as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet. When trifluoroacetimidate compounds were characterized the quartet of the CF_3 group of low intensity in the ^{13}C that should

have been around 160 pm has not been identified. The oligosaccharides protons have been assigned starting from the monosaccharide on the reducing end, unless otherwise reported (Figure 29).

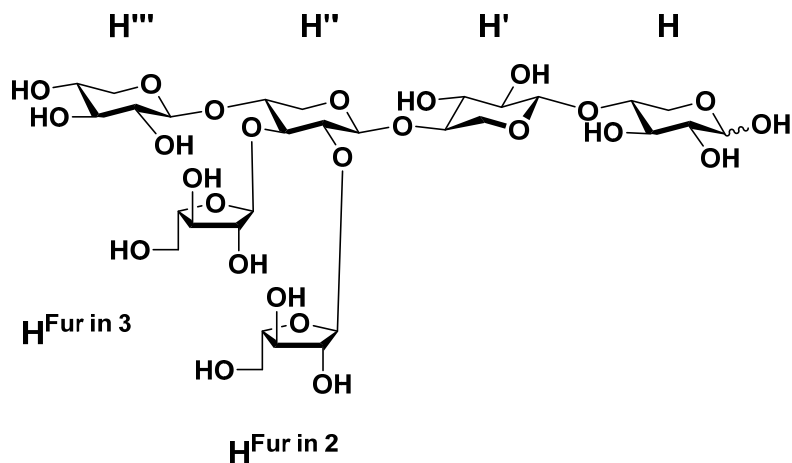
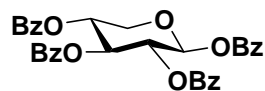


Figure 29: ^1H NMR assignment starting from the reducing end

^{13}C peaks are generally reported with one decimal value, unless discrimination between two distinct peaks was needed. In those cases two decimal values are reported.

8.3 Procedures

Tetrabenzoyl- β -D-xylopyranoside (61)

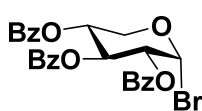


To a solution of D-xylose (5 g, 33.3 mmol) in dry pyridine (30 mL) at 0 °C benzoyl chloride (17 mL, 146 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 15 h and then diluted with CH_2Cl_2 and 1% HCl. The organic layer was separated and washed with saturated aqueous Na_2CO_3 (30 mL). The organic layer was washed with brine (30 mL), and the combined aqueous fractions were extracted with EtOAc (3×30 mL). The combined organic extract was dried with MgSO_4 , and concentrated *in vacuo* to give compound **61** as a white solid (18 g,

33.3 mmol, 100%). $R_f = 0.49$ (EtOAc:heptane, 2:3, v/v) The compound analyses were in accordance with data from the literature¹⁶⁸.

mp: 118 – 120 °C. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.79 – 8.08 (m, 8H, Ar-*H*); 7.15 - 7.57 (m, 12H, Ar-*H*), 6.70 (d, $J = 7.8$ Hz, 1H, H-1), 6.21 (t, $J = 10.3$ Hz, 1H, H-3), 5.57 (dd, $J = 7.8, 10.3$ Hz, 1H, H-2), 4.23 (dd, $J = 5.4, 10.7$ Hz, 1H, H-5a), 5.48 (m, 1H, H-4), 3.97 (t, $J = 10.7$ Hz, 1H, H-5b). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 165.3, 165.4, 165.8, 165.4, 165.3, 164.5, 133.8, 133.5, 133.4, 133.4, 133.3, 129.9, 129.86, 129.83, 129.7, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 90.2, 70.2, 69.9, 69.4, 61.2.

Bromo 2,3,4-Tri-O-benzoyl- α -D-xylopyranoside (62)



D-xylopyranose tetrabenzoate **61** (18 g, 33.3 mmol) was dissolved in ethylene dichloride (28 mL). The stirred solution was cooled in an ice-bath, treated with 30% hydrogen bromide in glacial acetic acid (50 mL) and allowed to stand tightly stoppered at room temperature for two hours. Glacial acetic acid (50 mL) was stirred into the crystalline magma and, after thirty minutes in the ice-box, the solid material was filtered off and washed successively with cold glacial acetic acid (20 mL) and isopentane (50 mL) to give compound **62** as a yellow solid (17.5 g, 33.3 mmol, 100%). $R_f = 0.85$ (acetone:toluene, 2:8, v/v). The compound analyses are in accordance to data from the literature²⁴⁸.

mp: 135 – 136 °C. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.96 – 7.88 (m, 4H, Ar-*H*), 7.89 - 7.83 (m, 2H, Ar-*H*), 7.50 – 7.44 (m, 2H, Ar-*H*), 7.43 – 7.30 (m, 5H, Ar-*H*), 7.30 – 7.23 (m, 2H, Ar-*H*), 6.75 (d, $J = 4.0$ Hz, 1H, H-1), 6.16 (t, $J = 9.8$ Hz, 1H, H-3), 5.47 – 5.37 (m, 1H, H-4), 5.21 (dd, $J = 9.8, 4.0$ Hz, 1H, H-2), 4.29 (dd, $J = 11.3, 5.9$ Hz, 1H, H-5a), 4.06 (t, $J = 11.1$ Hz, 1H, H-5b). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 165.6, 165.5, 165.4, 133.8, 133.6, 133.4, 130.1, 129.9, 129.8, 128.7, 128.6, 128.5, 128.4, 87.9, 71.4, 70.0, 68.8, 62.9.

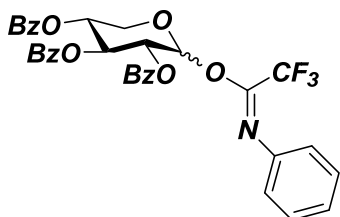
2,3,4-Tri-O-benzoyl-D-xylopyranoside (63)



Compound **62** (2.33 g, 4.43 mmol) was dissolved in acetone (22 mL), H₂O (0.9 mL) and CH₂Cl₂ (2 mL). Ag₂CO₃ (2.0 g, 7.3 mmol) was added in portions and the reaction was stirred for 1 h at room temperature, then the mixture was filtered through a bed of celite and anhydrous MgSO₄. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (hexane:EtOAc, 7:3, v/v) to give **63** (α : β = 5:1 determined by ¹H NMR) as a white foam (1.74 g, 3.76 mmol, 85%). *R_f* = 0.43 and 0.45 (hexanes/EtOAc, 7:3, v/v). The compound analyses are in accordance to data from the literature¹⁶⁹.

mp: 131 – 132 °C. **63 α** : ¹H NMR (400 MHz, CDCl₃) δ 7.98 – 7.85 (m, 6H, Ar-*H*), 7.47 – 7.37 (m, 3H, Ar-*H*), 7.34 – 7.24 (m, 6H, Ar-*H*), 6.12 (t, *J* = 9.3 Hz, 1H, H-3), 5.61 (d, *J* = 3.5 Hz, H-1), 5.33 (dd, *J* = 9.3, 15.9 Hz, 1H, H-4), 5.21 (dd, *J* = 3.5, 9.3 Hz, 1H, H-2), 4.10 – 4.02 (m, 2H, H-5a, H-5b). **63 β** : ¹H NMR (400 MHz, CDCl₃) δ 7.98 – 7.85 (m, 6H, Ar-*H*), 7.47 – 7.37 (m, 3H, Ar-*H*), 7.34 – 7.24 (m, 6H, Ar-*H*), 5.84 (t, *J* = 9.6 Hz, 1H, H-3), 5.33 (dd, *J* = 7.3, 17.6 Hz, 1H, H-4), 5.21 (dd, *J* = 7.3, 9.6 Hz, 1H, H-2), 4.92 (d, *J* = 7.3 Hz, H-1), 4.39 (dd, *J* = 5.3, 11.8 Hz, 1H, H-5a), 3.58 (dd, *J* = 17.6, 11.8 Hz, 1H, H-5b). **63 α** : ¹³C NMR (100 MHz, CDCl₃) δ 165.8, 165.7, 165.6, 133.5, 133.4, 133.2, 129.9, 129.8, 129.7, 129.3, 129.0, 128.9, 128.5, 128.4, 128.3, 90.7, 71.9, 69.9, 69.5, 59.1. **63 β** : ¹³C NMR (100 MHz, CDCl₃) δ 165.8, 165.7, 165.6, 133.5, 133.4, 133.2, 130.1, 129.9, 129.8, 129.2, 129.1, 128.9, 128.5, 128.4, 128.3, 97.6, 74.7, 69.1, 68.4, 60.8.

2,3,4-Tri-O-benzoyl-D-xylopyranosyl-1-(N-phenyl)-2,2,2-trifluoroacetimidate (58)

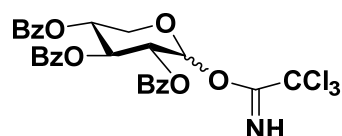


N-phenyl-2,2,2-trifluoro-acetimidoyl chloride (1.0 mL, 6.20 mmol) and K₂CO₃ (1.00 g, 7.20 mmol) was added to a solution of compound **63** (1.40 g, 3.02 mmol) in acetone (150 mL). After stirring at room temperature for 3h TLC showed complete conversion (EtOAc: heptane, 3:7, v/v). The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The crude purified

by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give **58** as white foam (1.97 g, 2.98 mmol, 99%, $\alpha:\beta = 1:3.3$, determined by $^1\text{H NMR}$). $R_f = 0.41$ (EtOAc:heptane, 3:7, v/v). The compound analyses are in accordance to data from the literature⁹¹.

mp = 70 – 72 °C. **58 α** : $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 8.15 – 8.00 (m, 6H, Ar-*H*), 7.64 - 7.26 (m, 11 H, Ar-*H*, NPh), 7.12 - 7.09 (m, 1H, NPh), 6.87 – 6.84 (m, 2H, NPh), 6.41 (bs, 1 H, H-1), 5.74 (t, $J = 4.0$ Hz, 1H, H-3), 5.50 (t, $J = 3.3$ Hz, 1H, H-2), 5.30 (d, $J = 3.3$ Hz, 1H, H-4), 4.58 (dd, $J = 3.3, 13.2$ Hz, 1H, H-5a), 4.07 (d, $J = 13.2$ Hz, 1H, H-5b). **58 β** : $^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ : 8.19 – 7.90 (m, 6H, Ar-*H*), 7.60 – 7.00 (m, 12H, Ar-*H*), 6.81 (bs, 1H, H-1), 6.52 – 6.48 (m, 2H, NPh), 6.25 (t, $J = 9.9$ Hz, 1H, H-3), 5.65 – 5.50 (m, 2H, H-2, H-4), 3.35 (dd, $J = 5.5, 11.0$ Hz, 1H, H-5a), 4.07 (t, $J = 11.0$ Hz, 1H, H-5b). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 165.6, 165.5, 165.3, 143.2, 142.9, 133.7, 133.6, 133.4, 133.3, 130.1, 130.0, 129.97, 129.93, 129.8, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 124.5, 124.4, 119.3, 119.1, 92.5, 92.4, 70.4, 69.6, 69.3, 67.3, 67.1, 61.3, 60.8.

2,3,4-Tri-*O*-benzoyl-*D*-xylopyranosyl-2,2,2-trichloroacetimidate (**95**)

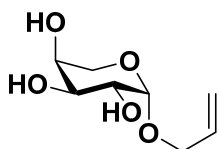


To a solution of compound **63** (723 mg, 1.51 mmol) in dry CH_2Cl_2 (70 mL) CCl_3CN (610 μL , 1.9 mmol) and anhydrous K_2CO_3 (725 mg, 5.20 mmol) were added. The mixture was stirred overnight at room temperature, filtered, and the filtrate concentrated *in vacuo*. The residue was purified by flash column chromatography (petroleum ether:EtOAc, 3:1, v/v) to give **95** as a foamy solid (734 mg, 1.21 mmol, 80%, $\alpha:\beta = 1:5$, determined by $^1\text{H NMR}$). $R_f = 0.48$ (heptane:EtOAc, 7:3, v/v). The compound analyses are in accordance to data from the literature¹⁹⁴.

$[\alpha]_D^{20} = -18.6^\circ$ (c 1.1, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 8.80 (s, 0.85H, NH (95 β)), 8.62 (s, 0.15H, NH (95 α)), 8.16–7.32 (m, 15H, Ar-*H*), 6.73 (d, $J = 3.6$ Hz, 0.15H, H-1 (95 α)), 6.44 (d, $J = 2.6$ Hz, 0.85H, H-1 (95 β)), 6.25 (t, $J = 10.0$ Hz, 0.15H, H-3 (95 α)), 5.72 (t, $J = 4.2$ Hz, 0.85H, H-3 (95 β)), 5.57 – 5.55 (m, 0.15H, H-2 (95 α)), 5.53 – 5.51 (m, 0.85H, H-2 (95 β)), 5.51 (m, 0.15H, H-4 (95 α)), 5.30 (m, 0.85H, H-4 (95 β)), 4.61 (dd, $J = 3.5$ Hz, 0.85H, H-5a (95 β)), 4.30 (dd, $J =$

5.8 Hz, 11.2 Hz, 0.15H, H-5a (95 α)), 4.08 (dd, $J = 3.5, 13.0$ Hz, 0.85H, H-5b(95 β)), 4.08 (m, 0.15H, H-5b(95 α)). ^{13}C DEPT NMR (100 MHz, CDCl_3) δ : 133.6, 133.5, 133.4, 133.3, 130.2, 130.1, 130.0, 129.9, 129.7, 128.6, 128.5, 128.44, 128.4, 94.6, 93.5, 77.2, 70.6, 69.7, 69.4, 67.5, 67.2, 67.1, 61.2, 60.9.

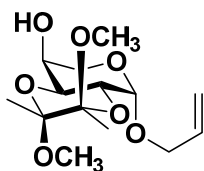
Allyl α -L-arabinopyranoside (65)



Allylic alcohol (140 mL) was stirred overnight with granulated calcium sulphate (5 g) at room temperature (20 °C). Finely powdered L-arabinose (10 g) was dissolved into the mixture under argon atmosphere and sulfuric acid (1 mL) was added dropwise. The reaction mixture was heated up to 80 °C and vigorously stirred for 24h. TLC (CH_2Cl_2 :MeOH, 10:1, v/v) showed complete conversion. The mixture was cooled to room temperature and filtered. The insoluble material was washed with allyl alcohol (60 mL), and the combined eluate and washings were concentrated *in vacuo*. The residue was purified by flash column chromatography (CH_2Cl_2 :MeOH, 10:1, v/v) to afford **65** as a white crystalline powder (13.59 g, 61%). $R_f = 0.51$ (CH_2Cl_2 :MeOH, 10:1, v/v). The compound analyses were in accordance with data from the literature¹⁷⁰.

mp: 118 - 120 °C. ^1H NMR (400 MHz, CD_3OD) δ : 5.98 (dddd, $J = 17.4, 10.5, 6.1, 5.0$ Hz, 1H, Allyl $\underline{\text{C}}\text{H}=\text{CH}_2$), 5.37 (ddt, $J = 17.4, 1.6, 1.4$ Hz, 1H, Allyl $\text{CH}=\underline{\text{C}}\text{H}_2$), 5.27 (ddt, $J = 10.5, 1.6, 1.1$ Hz, 1H, Allyl $\text{CH}=\underline{\text{C}}\text{H}_2$), 4.99 (d, 1H, $J = 3.2$ Hz, H-1), 4.22 (ddt, $J = 12.8, 5.0, 1.4$ Hz, 1H, Allyl O- $\underline{\text{C}}\text{H}_2$), 4.07 (ddt, $J = 12.8, 6.1, 1.1$ Hz, 1H, Allyl O- $\underline{\text{C}}\text{H}_2$), 4.01-3.98 (m, 1H, H-4), 3.92 (dd, $J = 12.6, 1.2$ Hz, 1H, H-5a), 3.90-3.82 (m, 2H, H-2, H-3), 3.65 (dd, $J = 12.6, 2.1$ Hz, 1H, H-5b). ^{13}C NMR (100 MHz, CD_3OD) δ : 133.5, 118.2, 97.9, 68.9, 68.8, 68.7, 68.1, 62.7.

Allyl O-(2,3-dimethoxybutane-2,3-diyl)- α -L-arabinopyranoside (66)

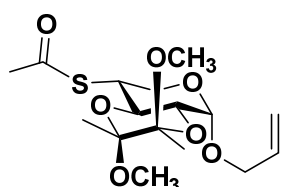


To a solution of allyl α -L-arabinopyranoside (**65**) (5.80 g, 30.5 mmol) in MeOH (100 mL) were added 2,3-butanedione (2.90 mL, 33.0 mmol), trimethylorthoformate (9.9 mL,

90.0 mmol) and camphorsulfonic acid (1.05 g, 4.5 mmol). The reaction mixture was stirred under reflux for 8 h. The reaction was quenched with triethylamine (5 mL) and concentrated under reduced pressure. The obtained residue was diluted with EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (2 × 20 mL) and brine (30 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (toluene:acetone, 8:2, v/v) to afford compound **66** (5.36 g, 17.6 mmol, 59%) as a yellow wax. $R_f = 0.44$ (toluene:acetone, 8:2, v/v).

$[\alpha]_D^{20} = -15.2^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 3600-3300, 2935.9, 1732.2, 1452.7, 1377.6, 1134.0, 1117.5. ¹H NMR (400 MHz, CDCl₃) δ 5.95 (dddd, $J = 17.1, 10.4, 6.4, 5.2$ Hz, 1H, Allyl CH=CH₂), 5.32 (dd, $J = 17.1, 1.4$ Hz, 1H, Allyl CH=CH₂), 5.19 (dd, $J = 10.4, 1.4$ Hz, 1H, Allyl CH=CH₂), 4.92 (d, $J = 3.4$ Hz, 1H, H-1), 4.24-4.07 (m, 4H, Allyl O-CH₂, H-2, H-3), 3.96 - 3.91 (m, 1H, H-4), 3.85 (dd, $J = 12.6, 1.4$ Hz, 1H, H-5a), 3.73 (dd, $J = 12.6, 1.7$ Hz, 1H, H-5b), 3.26 (s, 3H, OCH₃), 3.23 (s, 3H, OCH₃), 1.32 (s, 3H, CH₃), 1.30 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ : 134.2, 117.7, 100.2, 100.1, 96.3, 68.2, 68.1, 65.8, 65.1, 62.8, 47.8, 47.9, 17.8, 17.7. HRMS (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₁₄H₂₄O₇: 327.1414; Found 327.1414.

Allyl O-(2,3-dimethoxybutane-2,3-diyl)-4-S-acetyl- α -D-xylopyranoside (**67**)

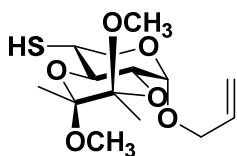


Compound **66** (1.18 g, 3.87 mmol) was dissolved in dry CH₂Cl₂ (12 mL) under argon atmosphere and cooled down to 0 °C. Pyridine (1.25 mL, 15.48 mmol) and triflic anhydride (1.3 mL, 7.74 mmol) were added dropwise. The reaction was stirred at room temperature (20 °C) for 20 min. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed once with saturated aqueous NaHCO₃ (20 mL), and the aqueous phase was extracted three times with CH₂Cl₂ (3 × 10 mL). The combined organic phases were collected and dried with Na₂SO₄, filtered and concentrated *in vacuo* to give a brown oil, which was used directly in the next step. The crude was dissolved in dry DMF (50 mL) and KSAc (1.77 g, 15.48 mmol) was added to the solution. The reaction

mixture was heated up to 40 °C for 2h where TLC (EtOAc:heptane, 3:7, v/v) showed complete conversion. Reaction mixture was diluted in water and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried with Na₂SO₄, filtered and concentrated *in vacuo* to give **67** as a brown oil, which was purified by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give the pure compound as a brown wax (786 mg, 56% over 2 step). *R_f* = 0.53 (acetone:toluene, 2:8, v/v).

$[\alpha]_D^{20} = -6.6^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 2948.3, 2919.7, 1697.1, 1454.7, 1378.9, 1132.7, 1030.3. ¹H NMR (400 MHz, CDCl₃) δ 5.86 (dddd, *J* = 17.1, 10.4, 6.4, 5.1 Hz, 1H, Allyl CH=CH₂), 5.26 (dq, *J* = 17.1, 1.4 Hz, 1H, Allyl CH=CH₂), 5.13 (ddd, *J* = 10.4, 1.6, 1.4 Hz, 1H, Allyl CH=CH₂), 4.82 (d, *J* = 3.5 Hz, 1H, H-1), 4.11 (ddt, *J* = 13.2, 5.1, 1.4 Hz, 1H, Allyl O-CH₂), 4.02 (ddt, *J* = 13.2, 6.4, 1.6 Hz, 1H, Allyl O-CH₂), 3.89 (dd, *J* = 10.8, 9.8 Hz, 1H, H-3), 3.74 (dd, *J* = 9.8, 3.5 Hz, 1H, H-2), 3.71 – 3.58 (m, 2H, H-5a, H-4), 3.58 – 3.45 (m, 1H, H-5b), 3.18 (s, 3H, OCH₃), 3.17 (s, 3H, OCH₃), 2.26 (s, 3H, AcS CH₃), 1.24 (s, 3H, CH₃), 1.21 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 194.2, 133.9, 117.8, 100.1, 99.9, 96.0, 70.3, 68.1, 64.5, 61.7, 47.9, 47.8, 42.5, 30.8, 17.7, 17.6. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₁₆H₂₆O₇S: 385.1291; Found 385.1301.

Allyl O-(2,3-dimethoxybutane-2,3-diyl)-4-thiol-α-D-xylopyranoside (59)

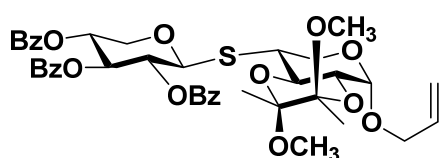


Compound **67** (178 mg, 0.49 mmol) was dissolved in MeOH (10 mL) and solid NaOMe (77 mg, 1.43 mmol) was added, where the orange suspension turned yellow, immediately. The mixture was stirred for 1h at room temperature (20 °C) and acidic resin (Amberlite H+) was added. The mixture was stirred until TLC (EtOAc:heptane, 3:7, v/v) showed complete conversion (1h). The reaction mixture was filtered and concentrated *in vacuo*. The crude was purified by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give a light brown oil (70 mg, 0.46 mmol, 91%). *R_f* = 0.70 (EtOAc:heptane, 3:7, v/v).

$[\alpha]_D^{20} = -69.4^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 2990.7, 2931.3, 2832.7, 1724.4, 1452.8, 1377.5, 1270.3, 1134.2, 1114.6, 1027.8. ¹H NMR (400 MHz, CDCl₃) δ:

5.93 (dddd, $J = 17.1, 10.4, 6.4, 5.1$ Hz, 1H, Allyl $\underline{CH=CH_2}$), 5.32 (ddd, $J = 17.1, 3.0, 1.6$ Hz, 1H, Allyl $CH=\underline{CH_2}$), 5.20 (ddd, $J = 10.4, 3.0, 1.2$ Hz, 1H, Allyl $CH=\underline{CH_2}$), 4.87 (d, $J = 3.5$ Hz, 1H, H-1), 4.19 (ddt, $J = 13.2, 5.1, 1.6$ Hz, 1H, Allyl O- $\underline{CH_2}$), 4.09 (ddt, $J = 13.2, 6.4, 1.2$ Hz, 1H, Allyl O- $\underline{CH_2}$), 3.83 (t, $J = 10.2$ Hz, 1H, H-3), 3.74 – 3.54 (m, 3H, H-2, H-5a, H-5b), 3.31 (s, 3H, OCH₃), 3.23 (s, 3H, OCH₃), 3.08 – 2.95 (m, 1H, H-4), 1.54 (d, $J = 6.6$ Hz, 1H, S-H), 1.31 (s, 3H, CH₃), 1.31 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ : 133.9, 117.8, 100.1, 99.9, 96.3, 70.1, 69.9, 68.1, 64.2, 47.96, 47.93, 38.2, 17.8, 17.5. HRMS (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₁₄H₂₄O₆S: 343.1186; Found 343.1195.

2,3,4-Tri-O-benzoyl-S- β -D-xylopyranosyl-(1 \rightarrow 4)-allyl-O-(2,3-dimethoxybutane-2,3-diyl)-4-S- α -D-xylopyranoside (70)



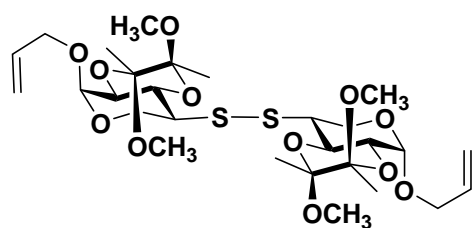
Compound **95** (338 mg, 0.56 mmol) and **59** (110 mg, 0.34 mmol) were dissolved in dry CH₂Cl₂ (3 mL). The solution was cooled down to - 35 °C in a cooling bath (dry ice in

acetone). TMSOTf (15 μ L, 0.2 mmol) was added dropwise to the reaction mixture. Stirring was continued until TLC (EtOAc:heptane, 3:7, v/v) showed complete consumption of the donor (20 min). The reactant in excess was quenched with Et₃N (100 μ L) and the reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give compound **70** as a white solid (167 mg, 0.22 mmol, 64%). $R_f = 0.27$ (EtOAc:heptane, 3:7, v/v).

mp: 82 - 84°C. $[\alpha]_D^{20} = -179.3^\circ$ (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2928.9, 2334.9, 1726.6, 1601.5, 1452.0, 1259.9, 1093.2, 710.2. **¹H NMR** (400 MHz, CDCl₃) δ : 8.07 – 7.95 (m, 6H, Ar-*H*), 7.58 – 7.51 (m, 3H, Ar-*H*), 7.40 – 7.31 (m, 6H, Ar-*H*), 5.91 (dddd, $J = 17.0, 10.7, 6.3, 5.2$ Hz, 1H, Allyl $\underline{CH=CH_2}$), 5.68 (t, $J = 5.8$ Hz, 1H, H-3'), 5.36 – 5.16 (m, 5H, H-1', Allyl $CH=\underline{CH_2}$, H-2', H-4'), 4.86 (d, $J = 3.5$ Hz, 1H, H-1), 4.72 (dd, $J = 3.5, 12.5$ Hz, 1H, H-5a'), 4.16 (ddt, $J = 13.3, 5.2, 1.4$ Hz, 1H, Allyl O- $\underline{CH_2}$), 4.07 (ddt, $J = 13.3, 6.3, 1.2$ Hz, 1H, Allyl O- $\underline{CH_2}$), 3.95 (t, $J = 10.6$ Hz, 1H, H-3), 3.80 - 3.68 (m, 4H, H-2, H-5b', H-5), 3.26 (s, 3H, OCH₃), 3.25 (s, 3H, OCH₃), 3.20 - 3.11 (m, 1H, H-4), 1.31 (s, 3H, CH₃), 1.27 (s,

3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 165.5, 165.1, 165.0, 134.0, 133.5, 133.4, 130.0, 129.95, 129.94, 129.3, 129.2, 128.9, 128.4, 128.3, 117.9, 100.2, 100.0, 96.0, 81.7, 70.3, 70.1, 69.5, 68.3, 68.1, 66.6, 62.4, 47.9, 43.6, 17.8, 17.6. **HRMS**: (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₄₀H₄₄O₁₃S: 787.2395; Found: 787.2395

Bis[Allyl O-(2,3-dimethoxybutane-2,3-diyl)-4-thiol-α-D-xylopyranosyl]-disuphide

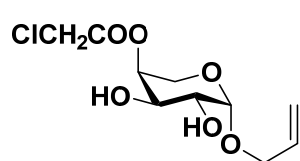


(69)

Compound **69** was one of the major byproduct of the procedure used to obtain compound **70**. It can also be obtained through oxidation of compound **59** by exposure to air at room temperature.

[α]_D²⁰ = -177.8° (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2925.72, 1730.83, 1455.59, 1379.48, 1135.22, 1028.71. ¹H NMR (400 MHz, CDCl₃) δ: 5.88 (dddd, *J* = 17.1, 10.4, 6.4, 5.1 Hz, 1H, Allyl CH=CH₂), 5.27 (dd, *J* = 17.1, 1.5 Hz, 1H, Allyl CH=CH₂), 5.15 (dd, *J* = 10.4, 1.2 Hz, 1H, Allyl CH=CH₂), 4.80 (d, *J* = 3.5 Hz, 1H, H-1), 4.13 (ddt, *J* = 13.3, 5.1, 1.5 Hz, 1H, Allyl O-CH₂), 4.04 (ddt, *J* = 13.3, 6.4, 1.2 Hz, 1H, Allyl O-CH₂), 3.91 - 3.84 (m, 2H, H-3, H-5a), 3.71 - 3.62 (m, 2H, H-2, H-5b), 3.20 (s, 3H, OCH₃), 3.19 (s, 3H, OCH₃), 3.04 - 2.95 (m, 1H, H-4), 1.26 (s, 3H, CH₃), 1.24 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 133.8, 117.5, 99.9, 99.7, 95.7, 70.1, 67.8, 66.1, 62.1, 49.8, 47.7, 47.6, 17.5, 17.4. **HRMS** (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₂₈H₄₆O₁₂S₂: 661.2323; Found 661.2324.

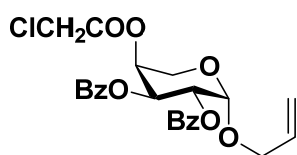
Allyl 4-O-chloroacetyl-α-L-arabinopyranoside(82)



L-arabinose (691 mg, 3.60 mmol) was dissolved in CH₃CN (20 mL) at 35 °C. *p*-TsOH (19 mg, 0.12 mmol) and 2-chloro-orthoformiate (1.00 g, 6.5 mmol) were added slowly to the reaction mixture, which was stirred at room temperature (20 °C) for 10 min. TLC (MeOH:CH₂Cl₂, 1:10, v/v) showed complete

consumption of starting material (1h). The reaction mixture was concentrated *in vacuo* and re-solubilized in the same amount of CH₃CN. Then TFA (676 μL) was added and the reaction mixture was stirred for 1h at room temperature. The reaction mixture was then concentrated *in vacuo*, and the resulting crude was purified by flash column chromatography (MeOH:CH₂Cl₂, 1:10, v/v) to give compound **82** as a pale yellow wax (950 mg, 2.4 mmol, 66%). *R_f* = 0.46 (acetone:toluene, 4:6, v/v). $[\alpha]_D^{20} = +91.3^\circ$ (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 3600-3100, 2927.5, 2344.0, 1675.3, 1453.0, 1200.7, 1130.6. **¹H NMR** (400 MHz, CDCl₃) δ: 5.91 (ddd, *J* = 17.2, 10.4, 5.3 Hz, 1H, Allyl CH=CH₂), 5.30 (ddd, *J* = 17.2, 3.0, 1.5 Hz, 1H, Allyl CH=CH₂), 5.21 (dd, *J* = 10.4, 1.3 Hz, 1H, Allyl CH=CH₂), 4.94 (d, *J* = 3.5 Hz, 1H, H-1), 4.21 (ddt, *J* = 12.8, 5.3, 1.3 Hz, 1H, Allyl O-CH₂), 4.10 - 3.69 (m, 8H, Allyl O-CH₂, H-2, H-3, H-4, H5, ChloroAc CH₂), 2.47 (bs, 2H, OH). **¹³C NMR** (100 MHz, CDCl₃) δ: 167.7, 133.4, 118.1, 97.7, 73.5, 69.3, 68.8, 68.4, 60.5, 41.0. **HRMS** (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₄H₂₃ClO₈: 289.0449; Found 289.0450.

Allyl 2,3-O-benzoyl-4-O-chloroacetyl-α-L-arabinopyranoside(83)

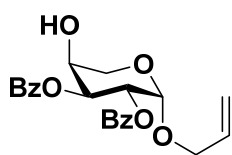


Compound **82** (64 mg, 0.24 mmol) was dissolved in dry CH₂Cl₂ (5 mL) under argon atmosphere. Triethylamine (64 μL) and DMAP (28 mg) were added to the solution, which was cooled down to 0 °C. Then benzoyl chloride (58 μL, 0.51 mmol) was added dropwise and the reaction mixture was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (18h) The reaction was diluted with EtOAc (15 mL) and washed with HCl (0.1 M, 10 mL), saturated aqueous NaHCO₃ (10 mL), and brine (10 mL). The organic phase was then dried with Na₂SO₄, filtrated and concentrated *in vacuo*. The waxy crude was purified by flash column chromatography (EtOAc:Heptane, 1:1, v/v) to give **83** as a pale yellow wax (80 mg, 0.21mmol, 91%). *R_f* = 0.64 (EtOAc:Heptane, 1:1, v/v).

$[\alpha]_D^{20} = +157.2^\circ$ (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2927.7, 1765.4, 1722.8, 1601.6, 1451.6, 1273.2, 1097.6, 1071.3, 709.7. **¹H NMR** (400 MHz, CDCl₃) δ: 7.95 - 7.89 (m, 2H, Ar-*H*), 7.88 - 7.82 (m, 2H, Ar-*H*), 7.48 - 7.40 (m, 2H, Ar-*H*), 7.35 - 7.26 (m, 4H, Ar-*H*), 5.83 - 5.72 (m, 2H, H-3, Allyl CH=CH₂), 5.57 - 5.50 (m, 2H, H-1,

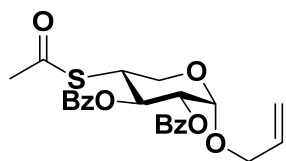
H-4), 5.27 – 5.19 (m, 2H, H-2, Allyl CH=CH₂), 5.09 (ddd, $J = 10.4, 2.7, 1.3$ Hz, 1H, Allyl CH=CH₂), 4.18 (ddt, $J = 13.2, 5.1, 1.3$ Hz, 1H, Allyl O-CH₂), 4.13 - 4.04 (m, 3H, ChloroAc CH₂, H-5a), 3.98 (ddt, $J = 13.2, 6.0, 1.4$ Hz, 1H, Allyl O-CH₂), 3.78 (dd, $J = 13.2, 1.9$ Hz, 1H, H-5b). ¹³C NMR (100 MHz, CDCl₃) δ : 166.8, 165.9, 165.6, 133.4, 133.3, 129.8, 129.7, 129.2, 129.1, 128.4, 117.7, 95.9, 71.6, 68.9, 68.8, 67.7, 60.3, 40.7. HRMS (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₂₄H₂₃ClO₈: 497.0974; Found 497.0978.

Allyl 2,3-O-benzoyl- α -L-arabinopyranoside(84)



Compound **83** (5.47 g, 11.4 mmol) was dissolved in dry THF (300 mL). Thiourea (2.60 g, 34.2 mmol), Bu₄NI (827 mg, 2.28 mmol) and NaHCO₃ (2.96 g, 35.3 mmol) were added, and the reaction mixture was heated to 55 °C for 12h, where TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion. The mixture was filtered, concentrated and purified by flash column chromatography (heptane:EtOAc, 7:3, v/v) to give **84** as a pale yellow wax (4.240 g, 10.6 mmol, 93%). $R_f = 0.55$ (heptane:EtOAc, 1:1, v/v)

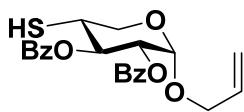
$[\alpha]_D^{20} = -151.1^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 3600-3400, 2928.7, 1720.5, 1601.5, 1451.7, 1279.0, 1110.0, 1069.2, 709.9. ¹H NMR (400 MHz, CDCl₃) δ : 8.03 – 7.96 (m, 4H, Ar-H), 7.54 – 7.47 (m, 2H, Ar-H), 7.41 – 7.34 (m, 4H, Ar-H), 5.85 (ddd, $J = 22.4, 10.7, 5.5$ Hz, 1H, Allyl CH=CH₂), 5.71 (m, 2H, H-3, H-2), 5.38 - 5.23 (m, 2H, H-1, Allyl CH=CH₂), 5.15 (dd, $J = 10.4, 1.2$ Hz, 1H, Allyl CH=CH₂), 4.38 – 4.31 (m, 1H, H-4), 4.25 (dd, $J = 13.2, 5.0$ Hz, 1H, Allyl O-CH₂), 4.15 – 3.98 (m, 2H, Allyl O-CH₂, H-5a), 3.82 (dd, $J = 12.5, 1.9$ Hz, 1H, H-5b), 2.25 (bs, 1H, OH). ¹³C NMR (100 MHz, CDCl₃) δ : 167.5, 166.0, 133.46, 133.44, 133.3, 129.89, 129.85, 129.24, 129.20, 128.5, 128.4, 117.6, 95.2, 74.8, 71.3, 69.6, 68.4, 61.8. HRMS (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₂₂H₂₂ClO₇ : 421.1258; Found 421.1264.

Allyl 4-S-acetyl-2,3-O-benzoyl- α -D-xylopyranoside(85)

Compound **84** (410 mg, 1.03 mmol) was dissolved in dry CH_2Cl_2 (3.5 mL) under argon atmosphere and the resulting solution was cooled down to 0 °C. Pyridine (331 μL , 4.12 mmol) and triflic anhydride (347 μL , 2.06 mmol) were added dropwise and the mixture was stirred at room temperature (20 °C) for 20 min. The reaction mixture diluted with EtOAc (15 mL), washed once with saturated aqueous NaHCO_3 (10 mL), and the aqueous phase was extracted three times with CH_2Cl_2 (3×10 mL). The combined organic phases were collected and dried with Na_2SO_4 , filtered and concentrated *in vacuo* to give a brown oil. The resulting crude was dissolved in dry DMF (14 mL) and KSAc (470 mg, 4.12 mmol) was added to the solution. The reaction mixture was heated to 40 °C until TLC (EtOAc:heptane, 3:7, v/v) showed complete reaction (2h). Reaction mixture was diluted in water (20 mL) and extracted 3 times with EtOAc (3×20 mL). The combined organic phases were dried with Na_2SO_4 , filtered and concentrated *in vacuo* to give a brown oil. The product was purified by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give **85** as a brown wax (344 mg, 0.75 mmol, 71% over 2 step). $R_f = 0.54$ (EtOAc:heptane, 3:7, v/v).

$[\alpha]_D^{20} = +149.8^\circ$ (c 1.0, CHCl_3). IR (neat, cm^{-1}): 2926.4, 2339.6, 1725.6, 1601.6, 1451.9, 1276.4, 1264.7, 1047.4, 710.1. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.98 - 7.93 (m, 4H, Ar-H), 7.51 - 7.45 (m, 2H, Ar-H), 7.38 - 7.32 (m, 4H, Ar-H), 5.87 (dd, $J = 11.3, 9.3$ Hz, 1H, H-3), 5.86 - 5.73 (m, 1H, Allyl $\underline{\text{CH}}=\text{CH}_2$), 5.32 - 5.24 (m, 3H, H-1, H-2, Allyl $\text{CH}=\underline{\text{CH}}_2$), 5.13 (ddd, $J = 10.5, 2.7, 1.3$ Hz, 1H, Allyl $\text{CH}=\underline{\text{CH}}_2$), 4.23 (ddt, $J = 13.2, 5.0, 1.3$ Hz, 1H, Allyl O- $\underline{\text{CH}}_2$), 4.15 - 3.95 (m, 2H, H-4, Allyl O- $\underline{\text{CH}}_2$), 3.92 - 3.74 (m, 2H, H-5), 2.24 (s, 3H, CH_3). $^{13}\text{C DEPT NMR}$ (100 MHz, CDCl_3) δ : 133.4, 133.3, 133.2, 129.9, 129.8, 128.40, 128.38, 117.6, 95.7, 73.2, 68.7, 68.6, 60.9, 43.3, 30.7. HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{24}\text{H}_{24}\text{O}_7\text{S}$: 479.1135; Found 479.1116.

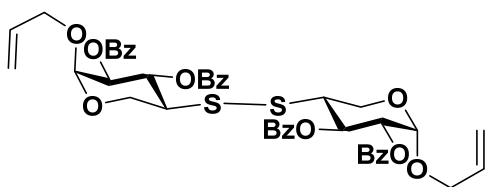
Allyl 2,3-O-benzoyl-4-thiol- α -D-xylopyranoside(79)



Compound **85** (200 mg, 0.44 mmol) was dissolved in a mixture of MeOH (2 mL) and CH₂Cl₂ (2 mL) and NaSCH₃ (31 mg, 0.44 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 30 min until LC-MS showed completed consumption of the starting material. The reaction was quenched with HCl (0.1 M) and extracted two times with CH₂Cl₂ (2 × 10 mL). The combined organic phases were dried with MgSO₄, filtered and concentrated *in vacuo*. R_f = 0.54 (EtOAc:heptane, 3:7, v/v).

$[\alpha]_D^{20}$ = -125.2.1° (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 3500 - 3500, 2979.1, 2938.5, 2885.3, 1720.2, 1601.7, 1451.8, 1261.7, 1107.5, 1025.4, 709.4. **¹H NMR** (400 MHz, CDCl₃) δ : 7.95 – 7.82 (m, 4H, Ar-*H*), 7.44 – 7.33 (m, 2H, Ar-*H*), 7.30 - 7.19 (m, 4H, Ar-*H*), 5.80 – 5.68 (m, 1H, Allyl CH=CH₂), 5.60 (t, J = 9.5 Hz, 1H, H-3), 5.20 (dq, J = 17.2, 1.5 Hz, 1H, Allyl CH=CH₂), 5.17 – 5.08 (m, 2H, H-1, H-2), 5.04 (dd, J = 10.4, 1.5 Hz, 1H, Allyl CH=CH₂), 4.15 (ddt, J = 13.2, 5.0, 1.5 Hz, 1H, Allyl O-CH₂), 3.97 – 3.88 (m, 2H, H-4, Allyl O-CH₂), 3.80 – 3.64 (m, 2H, H-5), 3.04 (s, 1H, SH). **¹³C NMR** (100 MHz, CDCl₃) δ : 165.9, 165.5, 133.1, 132.97, 132.90, 129.5, 129.4, 129.2, 129.1, 128.07, 128.04, 117.2, 95.4, 72.8, 68.28, 68.25, 60.5, 42.9. **HRMS** (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₂₂H₂₂O₆S: 437.1029; Found 437.1029.

Bis(Allyl 2,3-O-benzoyl-4-thiol- α -D-xylopyranosyl)-disulphide (94)

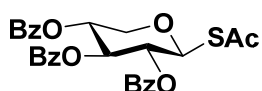


Compound **94** was one of the major byproduct of the procedure used to obtain compound **93** starting from compound **79** and **58**. It can also be obtained through oxidation of compound **79** by exposure to air at room temperature. R_f = 0.54 (EtOAc:heptane, 3:7, v/v).

$[\alpha]_D^{20}$ = +135.6° (c 1.0, CDCl₃). **IR** (neat, cm⁻¹): 2934.3, 1723.8, 1601.7, 1451.9, 1276.2, 1111.1, 1027.8, 709.7. **¹H NMR** (400 MHz, CDCl₃) δ : 7.96 – 7.85 (m, 8H, Ar-*H*), 7.46 – 7.39 (m, 4H, Ar-*H*), 7.3 – 7.26 (m, 8H, Ar-*H*), 5.81 - 5.64 (m, 4H,

Allyl $\underline{CH}=\underline{CH}_2$, H-3), 5.25 – 5.17 (m, 4H, Allyl $\underline{CH}=\underline{CH}_2$, H-1), 5.11 – 5.04 (m, 4H, Allyl $\underline{CH}=\underline{CH}_2$, H-2), 4.17 (ddt, $J = 13.2, 5.1, 1.5$, 2H, Allyl O- \underline{CH}_2), 3.95 (ddt, $J = 13.2, 6.0, 1.3$, 2H, Allyl O- \underline{CH}_2), 3.84 – 3.70 (m, 4H, H-5a, H-5b), 3.13 (m, 2H, H-4). ^{13}C NMR (100 MHz, CDCl_3) δ : 167.7, 166.0, 133.5, 133.4, 133.3, 129.9, 129.8, 129.2, 129.1, 128.4, 117.6, 95.2, 75.0, 71.2, 69.7, 68.5, 61.8. HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{44}\text{H}_{44}\text{O}_{12}\text{S}_2$: 849.2017; Found 849.2017.

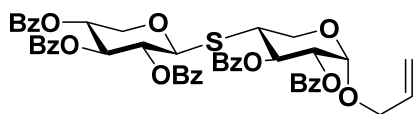
Acetyl 2,3,4-Tri-O-benzoyl-1-thio- β -D-xylopyranose (99)



Compound **62** (300 mg, 0.57 mmol) was dissolved in dry DMF (3 mL) and KSAC (308 mg, 2.28 mmol) was added at room temperature. The reaction mixture was stirred until TLC (EtOAc:heptane, 3:7, v/v) showed complete consumption of the starting material (2h). Reaction mixture was diluted in water (10 mL) and extracted 3 times with EtOAc (3×10 mL). The combined organic phases were dried with Na_2SO_4 , filtered and concentrated *in vacuo* to give a brown oil, which was purified by flash column chromatography (3:7= Ethyl Acetate:Heptane v/v) to give a mixture of the two anomers, which was further crystallized in Ethanol. The solid was filtrated and dried under vacuum to give **99** as a white solid. (169 mg, 0.32 mmol, 57 %). $R_f = 0.68$ (EtOAc:heptane, 2:3, v/v).

mp: 143 - 152 °C. $[\alpha]_D^{20} = +28.6^\circ$ (c 1.0, CHCl_3). **IR** (neat, cm^{-1}): 2925.5, 1727.8, 1601.4, 1452.0, 1278.4, 1258.9, 1092.3, 1069.5, 710.0. **^1H NMR** (400 MHz, CDCl_3) δ : 8.02 – 7.95 (m, 6H, Ar-*H*), 7.56 – 7.50 (m, 3H, Ar-*H*), 7.43 – 7.32 (m, 6H, Ar-*H*), 5.83 – 5.76 (m, 2H, H-1, H-3), 5.48 (t, $J = 6.8$ Hz, 1H, H-3), 5.31 (dt, $J = 6.8, 4.2$ Hz, 1H, H-4), 4.45 (dd, $J = 12.3, 4.2$ Hz, 1H, H-5a), 3.88 (dd, $J = 12.3, 6.8$ Hz, 1H, H-5b), 2.37 (s, 3H, CH_3). **^{13}C NMR** (100 MHz, CDCl_3) δ : 165.5, 165.2, 165.1, 133.5, 133.4, 130.0, 129.9, 129.9, 129.1, 128.86, 128.85, 128.5, 128.46, 128.43, 80.3, 70.5, 69.6, 68.5, 64.9, 30.9. **HRMS**: (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{28}\text{H}_{24}\text{O}_8\text{S}$: 543.1084; Found: 543.1089.

2,3,4-Tri-O-benzoyl-S-β-D-xylopyranosyl-(1→4)-allyl-2,3-O-benzoyl-4-thio-α-D-xylopyranoside (93)

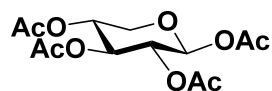


Compound **84** (139 mg, 0.35 mmol) was dissolved in dry CH₂Cl₂ (3.5 mL) under argon atmosphere and cooled down to 0 °C. Pyridine (90 μL, 1.40 mmol) and triflic anhydride (152 μL, 0.70 mmol) were added dropwise. The reaction was stirred at room temperature (20 °C) for 20 min and the reaction mixture was washed once with saturated aqueous NaHCO₃ (10 mL), and the aqueous phase was extracted three times with CH₂Cl₂ (3 × 10 mL). The combined organic phases were collected and dried with Na₂SO₄, filtered and concentrated *in vacuo* to give intermediate **98** as pale yellow oil. Intermediate **98** was added dropwise to an ice-cold mixture of compound **99** (300 mg, 0.58 mmol), 1,4-dithioerythritol (DTE) (77 mg, 0.35 mmol) and cysteamine (58 mg, 0.52 mmol) in DMF (4 mL) under argon atmosphere. The reaction mixture was stirred at room temperature for 14h. TLC (EtOAc:heptane, 3:7, v/v) showed complete consumption of the acceptor (intermediate 13). Crushed ice was added to the mixture and the resulting suspension was filtered. The precipitate was dissolved in CH₂Cl₂ and purified by flash column chromatography (EtOAc:heptane, 3:7, v/v) to give compound **93** as a white solid (236 mg, 0.27 mmol, 78%). *R_f* = 0.43 (EtOAc:heptane, 3:7, v/v).

mp: 85 - 89 °C. $[\alpha]_D^{20} = +23.4^\circ$ (c 1.0, CHCl₃). **IR** (neat, cm⁻¹) 2924.6, 2854.4, 2191.9, 1724.9, 1601.7, 1451.9, 1274.9, 1262.0, 1108.2, 1069.5, 708.4. **¹H NMR** (400 MHz, CDCl₃) δ: 7.95 - 7.78 (m, 10H, Ar-*H*), 7.54 - 7.32 (m, 7H, Ar-*H*), 7.32- 7.28 (m, 4H, Ar-*H*), 7.24 - 7.13 (m, 4H, Ar-*H*), 5.88 - 5.68 (m, 2H, H-3, Allyl CH=CH₂), 5.60 (t, *J* = 5.6 Hz, 1H, H-3'), 5.31 - 5.13 (m, 5H, H-1, H-1', H-2, H-2', Allyl CH=CH₂), 5.06 (dd, *J* = 10.4, 1.3 Hz, 1H, Allyl CH=CH₂), 4.99 (dd, *J* = 9.1, 5.6 Hz, 1H, H-4'), 4.32 (dd, *J* = 12.5, 3.5 Hz, 1H, H-5a'), 4.15 (ddt, *J* = 13.2, 5.0, 1.3 Hz, 1H, Allyl O-CH₂), 4.01 - 3.83 (m, 3H, Allyl O-CH₂, H-5), 3.55 (dd, *J* = 12.5, 5.6 Hz, 1H, H-5b'), 3.32 (td, *J* = 11.6, 5.5 Hz, 1H, H-4). **¹³C NMR** (100 MHz, CDCl₃) δ: 165.9, 165.8, 165.4, 165.1, 164.8, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 130.1, 130.0, 129.9, 129.8, 129.7, 129.4, 129.2, 129.1, 128.9, 128.7,

128.5, 128.44, 128.40, 128.3, 117.6, 95.7, 81.5, 73.3, 69.7, 68.9, 68.8, 68.6, 68.1, 62.05, 61.9, 45.3, 31.9. **HRMS:** (ESI-TOF) m/z : $[M + Na]^+$ Calcd for $C_{48}H_{42}O_{13}S$: 881.2238; Found: 881.2239.

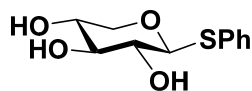
1,2,3,4-Tetra-*O*-acetyl- β -D-xylopyranose (**102**)



D-xylose (15 g, 0.1 mol) was dissolved in pyridine (100 mL). Acetic anhydride (50 g, 0.49 mol) was added drop wise and the reaction mixture was stirred at room temperature for 24 h. Pyridine and excess acetic anhydride were evaporated *in vacuo*, and the residue was poured into water. The mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4 . The solution was concentrated *in vacuo*, and the resulting crude was purified by flash column chromatography (MeOH: CH_2Cl_2 , 3:97, v/v) to afford compound **102** as a yellow wax (30.2 g, 0.47 mol, 95%). $R_f = 0.5$ (EtOAc:heptane, 1:1, v/v). The compound analyses are in accordance to data from the literature²⁴⁹.

1H NMR (400 MHz, $CDCl_3$), δ : 6.19 (d, $J = 9.6$ Hz, 1H, H-1), 5.41 (dd, $J = 9.6, 9.9$ Hz, 1H, H-2), 5.01 – 4.92 (m, 2H, H-3, H-4), 3.87 (dd, $J = 10.1, 5.4$ Hz, 1H, H-5a), 3.65 (t, $J = 10.1$ Hz, 1H, H-5b), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.98 (s, 3H, CH_3). **^{13}C NMR** (100 MHz, $CDCl_3$) δ : 170.0, 169.7, 169.2, 168.9, 89.2, 69.5, 69.3, 68.6, 60.6, 20.8, 20.7, 20.6, 20.4.

Phenyl 1-thio- β -D-xylopyranoside (**103**)

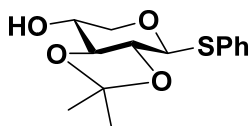


To a solution of compound **102** (2.0 g, 6.3 mmol) in dry CH_2Cl_2 (8 mL) were added thiophenol (0.77 ml, 7.6 mmol) and $BF_3 \cdot OEt_2$ (2.32 mL, 18.9 mmol) at 0 °C under argon atmosphere. The solution was stirred for 2h at room temperature and then diluted with CH_2Cl_2 (8 mL). The resulting solution was washed successively with saturated aqueous $NaHCO_3$ (2×25 mL) and water (2×15 mL), dried over Na_2SO_4 , and concentrated *in vacuo*. The residue was dissolved in MeOH (6 mL) and a 0.1 M solution of NaOMe in MeOH was added. After 15 min the mixture was

neutralized with Amberlite IR-120 (H⁺) resin, filtered, and concentrated. After recrystallization of the residue from acetone-hexane, compound **103** was obtained as a yellow solid (0.96 g, 4.0 mmol, 64%). The compound analyses are in accordance to data from the literature¹⁹⁸.

mp: 143 – 145 °C, ¹H NMR (400 MHz, CDCl₃) δ: 7.53 – 7.50 (m, 2H, S-Ph), 7.30 - 7.26 (m, 3H, S-Ph), 5.47 (d, *J* = 9.4 Hz, 1H, H-1), 4.09 – 4.07 (m, 1H, H-5a), 3.71 – 3.68 (m, 1H, H-4), 3.58 (d, *J* = 12.0 Hz, 1H, H-5b), 3.85 – 3.81 (m, 3H, H-2, H-3, OH), 3.10 (bs, 2H, OH). ¹³C NMR (100 MHz, CDCl₃) δ: 135.0, 133.4, 130.3, 128.9, 90.3, 79.2, 73.9, 71.1, 70.5.

Phenyl 3,4-O-isopropyliden-1-thio-β-D-xylopyranoside (104).



A solution of compound **103** (7 g, 27.3 mmol) in dry DMF (50 mL) was added camphorsulfonic acid (953 mg, 4.1 mmol). The resulting mixture was stirred at 60 °C for 2h. 2-Methoxy propene (7.85 mL, 82.02 mmol) was added to the mixture in portions and the reaction was stirred for another 2h. The reaction mixture was cooled to room temperature and quenched with Et₃N. The resulting mixture was concentrated and purified by flash column chromatography (EtOAc:heptane:CH₂Cl₂, 4:1:1, v/v) to afford compound **104** as a pale yellow solid (6.3 g, 21.3 mmol, 78%). *R_f* = 0.10 (EtOAc:heptane:CH₂Cl₂, 4:1:1, v/v). The compound analyses are in accordance to data from the literature²⁰¹.

¹H NMR (400 MHz, CDCl₃) δ: 7.59 – 7.56 (m, 2H, S-Ph), 7.33 – 7.31 (m, 3H, S-Ph), 4.81 (d, *J* = 9.5 Hz, 1H, H-1), 4.13 – 4.11 (m, 1H, H-5a), 3.99 – 3.97 (m, 1H, H-4), 3.55 (t, *J* = 9.2 Hz, 1H, H-3), 3.28 – 3.24 (m, 2H, H-2, H-5b), 2.24 (s, 1H, OH), 1.50 (s, 3H, CH₃), 1.46 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 132.9, 131.8, 128.9, 128.1, 111.9, 85.5, 82.8, 75.1, 69.9, 69.1, 26.6, 26.5.

Phenyl 4-O-p-methoxybenzyl-1-thio-β-D-xylopyranoside (105)



Compound **104** (6.3 g, 21.3 mmol) was dissolved in DMF (40 mL), followed by addition of NaH (1 g, 25.6 mmol) and PMBCl (3.76 mL, 27.7 mmol). After stirring at room temperature for 12h, the

reaction was quenched with 10% HCl solution and diluted with CH₂Cl₂ (100 mL). The organic phase was extracted with saturated aqueous NaHCO₃ (50 mL) and dried with Na₂SO₄. The resulting crude was purified by flash column chromatography (hexane:EtOAc, 4:1, v/v) obtaining a white solid (7.54 g, 18.1 mmol, 85%). *R_f*: 0.5 (EtOAc:heptane, 1:1, v/v).

The white solid was dissolved in CH₂Cl₂/MeOH (1:1, 60 mL), followed by addition of camphorsulfonic acid (4.23 g, 18.1 mmol). The resulting mixture was stirred under room temperature for 15h. After the reaction was complete, the excess of reactant was quenched with Et₃N and concentrated. The residue was purified by flash column chromatography (hexanes:EtOAc, 1:1, v/v) to afford compound **105** as a yellow solid (6.68g, 17.7 mmol, 98%). *R_f* = 0.2 (EtOAc:heptane, 1:1, v/v). The compound analyses are in accordance to data from the literature²⁵⁰.

¹H NMR (400 MHz, CDCl₃) δ: 7.47 – 7.41 (m, 2H, S-Ph), 7.28 – 7.21 (m, 3H, S-Ph), 7.20 – 7.16 (m, 2H, H, Ar-H^{PMB}), 6.90 – 6.67 (m, 2H, Ar-H^{PMB}), 4.58 – 4.48 (m, 2H, CH₂^{PMB}), 4.48 (d, *J* = 8.5 Hz, 1H, H-1), 3.99 (dd, *J* = 11.5, 4.8 Hz, 1H, H-5a), 3.73 (s, 3H, OCH₃), 3.58 (t, *J* = 8.5 Hz, 1H, H-3), 3.43 – 3.36 (m, 1H, H-4), 3.32 (t, *J* = 8.5 Hz, 1H, H-2), 3.19 (dd, *J* = 11.5, 9.7 Hz, 1H, H-5b), 2.39 (s, 2H, OH). ¹³C NMR (100 MHz, CDCl₃) δ: 159.5, 132.6, 132.0, 129.9, 129.5, 129.0, 128.1, 114.0, 88.7, 76.4, 76.3, 72.6, 71.9, 67.0, 55.3.

Phenyl 2,3-O-benzoyl-4-O-p-metoxibenzoyl-1-thio-β-D-xylopyranoside (106)

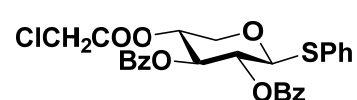


Compound **105** (1.00 g, 2.75 mmol) was dissolved in freshly distilled pyridine (20 mL) under argon atmosphere. The solution was cooled down to 0 °C and benzoyl chloride (705 μL, 6.07 mmol) was added dropwise. The reaction mixture was stirred at room temperature until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (16h). The reaction mixture was diluted with EtOAc (50 mL) and washed with 0.2 M H₂SO₄ (40 mL), saturated aqueous NaHCO₃ (40 mL) and brine (40 mL). The combined organic phase was dried with Na₂SO₄, filtrated and concentrated *in vacuo* to give a yellow wax. The crude was purified by flash

column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **106** as a pale yellow wax (1.57 g, 2.75 mmol, 100%). $R_f = 0.65$ (EtOAc:heptane, 1:1, v/v).

$[\alpha]_D^{20} = +58.3^\circ$ (c 1.0, CHCl_3). **IR** (neat, cm^{-1}): 2933.5, 1726.5, 1611.5, 1513.2, 1451.6, 1250.6, 1069.5, 709.1. **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ : 7.92 – 7.87 (m, 3H, Ar-*H*), 7.45 – 7.20 (m, 12H, Ar-*H*), 7.09 – 7.02 (m, 2H, Ar- H^{PMB}), 6.68 – 6.60 (m, 2H, Ar- H^{PMB}), 5.52 (t, $J = 7.9$ Hz, 1H, H-3), 5.26 (t, $J = 7.9$ Hz, 1H, H-2), 4.96 (d, $J = 7.9$ Hz, 1H, H-1), 4.49 – 4.40 (m, 2H, CH_2^{PMB}), 4.20 (dd, $J = 11.9, 4.6$ Hz, 1H, H-5a), 3.72 – 3.66 (m, 4H, H-4, CH_3^{PMB}), 3.49 (dd, $J = 11.9, 8.5$ Hz, 1H, H-5b). **$^{13}\text{C NMR}$** (100 MHz, CDCl_3) δ : 165.6, 165.3, 159.4, 133.7, 133.3, 133.1, 132.4, 130.2, 130.0, 129.9, 129.6, 129.5, 129.4, 129.3, 129.0, 128.5, 128.4, 128.3, 127.9, 113.8, 86.8, 73.8, 73.6, 72.4, 66.3, 55.2. **HRMS** (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{33}\text{H}_{30}\text{O}_7\text{S}$: 593.1604; Found 593.1611.

Phenyl 2,3-O-benzoyl-4-O-chloroacetyl-1-thio- β -D-xylopyranoside (113)

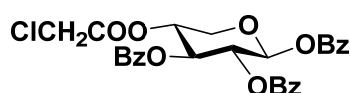


Compound **103** (445 mg, 1.84 mmol) was dissolved in benzene (15 mL) under argon atmosphere and Bu_2SnO (505 mg, 2.02 mmol) was added to the mixture. The suspension was refluxed at 95°C with a Dean-Stark apparatus for 3 h. The mixture was cooled to room temperature and chloroacetyl chloride (146 μL , 1.84 mmol) was added. After stirring for 2h at room temperature pyridine (1 mL) and benzoyl chloride (470 μL , 4.05 mmol) were added. The resulting mixture was stirred at room temperature for 14h. TLC (EtOAc:heptane, 3:7, v/v) showed complete conversion. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 0.1 M HCl (15 mL), saturated aqueous NaHCO_3 (15 mL) and brine (15 mL). The organic phase was dried with Na_2SO_4 , filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 3:7 v/v) to give compound **113** as a yellow amorphous solid (493 mg, 0.94 mmol, 51% over 3 steps). $R_f = 0.60$ (EtOAc:heptane, 1:1, v/v)

$[\alpha]_D^{20} = +38.1^\circ$ (c 1.0, CHCl_3). **IR** (neat, cm^{-1}): 3061.1, 2955.7, 1728.4, 1601.1, 1584.2, 1451.7, 1262.2, 1068.3, 709.4. **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ : 8.04 - 7.99 (m, 4H, Ar-*H*), 7.58 – 7.48 (m, 4H, Ar-*H*, *S*-Ph), 7.45 – 7.39 (m, 4H, Ar-*H*),

7.33 - 7.29 (m, 3H, S-Ph), 5.60 (t, $J = 7.0$ Hz, 1H, H-2), 5.40 (t, $J = 7.0$ Hz, 1H, H-3), 5.20 - 5.16 (m, 2H, H-1, H-4), 4.54 (dd, $J = 12.3, 4.3$ Hz, 1H, H-5a), 4.03 - 3.99 (m, 2H, ChloroAc CH_2), 3.73 (dd, $J = 12.3, 7.00$ Hz, 1H, H-5b). ^{13}C DEPT NMR (100 MHz, $CDCl_3$) δ : 133.6, 133.5, 132.7, 130.0, 129.9, 129.1, 128.5, 128.4, 128.2, 86.4, 70.7, 69.8, 69.7, 63.4, 40.4. HRMS (ESI-TOF) m/z : $[M + Na]^+$ Calcd for $C_{27}H_{23}ClO_7S$: 549.0745; Found 549.0757.

Benzoyl 2,3-di-O-benzoyl-4-O-chloroacetyl- β -D-xylopyranoside (114)

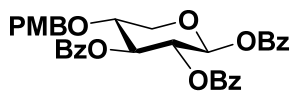


Compound **113** (424 mg, 0.80 mmol) was dissolved in a mixture acetone (70 mL) and H_2O (7 mL) and NBS (215 mg, 1.20 mmol) was added at room temperature. The reaction was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion. Extra portions of NBS were added if needed. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (30 mL) and the solution was extracted with EtOAc (2×20 mL). The combined organic phases were dried with Na_2SO_4 , filtered and concentrated *in vacuo*. The resulting crude was co-evaporated with toluene and subjected to high vacuum for 2h. Then it was dissolved in dry CH_2Cl_2 (20 mL) at room temperature and Et_3N (2 mL) was added. After stirring for 10 min, benzoyl chloride (92 μ L, 0.8 mmol) was added dropwise. The reaction mixture was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (14h). The suspension was washed with water (10 mL) and brine (10 mL), dried with Na_2SO_4 , filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **114** as a white amorphous solid (258 mg, 0.48 mmol, 60% over 2 step). $R_f = 0.45$ (EtOAc/Heptane, 1:1, v/v)

$[\alpha]_D^{20} = +37.4^\circ$ (c 1.0, $CHCl_3$). IR (neat, cm^{-1}): 2953.30, 1730.29, 1601.33, 1451.74, 1260.23, 1069.17, 709.36. 1H NMR (400 MHz, $CDCl_3$) δ : 8.06 - 7.97 (m, 6H, Ar-H), 7.61 - 7.51 (m, 3H, Ar-H), 7.44 - 7.34 (m, 6H, Ar-H), 6.28 (d, $J = 5.0$ Hz, 1H, H-1), 5.68 (t, $J = 6.4$ Hz, 1H, H-3), 5.60 (dd, $J = 6.4, 5.1$ Hz, 1H, H-2), 5.29 (td, $J = 6.2, 4.0$ Hz, 1H, H-4), 4.43 (dd, $J = 12.6, 3.9$ Hz, 1H, H-5a), 4.06 (d, $J = 6.9$ Hz, 2H, ChloroAc CH_2), 3.91 (dd, $J = 12.6, 6.2$ Hz, 1H, H-5b). ^{13}C DEPT

NMR (100 MHz, CDCl₃) δ : 133.8, 133.7, 133.6, 130.0, 129.97, 129.92, 128.6, 128.5, 128.5, 92.1, 69.3, 69.3, 68.4, 61.6, 40.4. . **HRMS** (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₂₈H₂₃ClO₉: 561.0923; Found 561.0937.

Benzoyl 2,3-di-O-benzoyl-4-O-p-metoxibenzoyl- β -D-xylopyranoside (116)

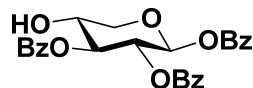


Compound **106** (1.5 g, 2.85 mmol) was dissolved in a mixture acetone (50 mL) and H₂O (5 mL) and NBS (1.01 g, 5.70 mmol) was added at room temperature. The reaction was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion. Extra portions of NBS were added if needed. The reaction was stopped by addition of saturated aqueous NaHCO₃ and the solution was extracted with EtOAc (2 \times 30 mL). The combined organic phases were dried with Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude was co-evaporated with toluene and subjected to high vacuum for 2h. Then it was dissolved in dry CH₂Cl₂ (40 mL) at room temperature and Et₃N (4 mL) was added. After stirring for 10 min benzoyl chloride (182 μ L, 1.56 mmol) was added dropwise. The reaction mixture was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (14h). The suspension was washed with water (30 mL) and brine (30 mL), and then dried over Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **116** as a white amorphous solid (1.16 g, 1.99 mmol, 70% over 2 step). R_f = 0.50 (EtOAc:heptane, 1:1, v/v).

$[\alpha]_D^{20}$ = +21.9° (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2924.1, 2853.6, 1729.9, 1628.5, 1602.0, 1498.6, 1451.9, 1260.3, 1053.2, 710.3. **¹H NMR** (400 MHz, CDCl₃) δ : 7.96 – 7.84 (m, 6H, Ar-*H*), 7.52 – 7.37 (m, 3H, Ar-*H*), 7.37 – 7.22 (m, 6H, Ar-*H*), 7.14 – 7.07 (m, 2H, Ar-*H*^{PMB}), 6.72 – 6.63 (m, 2H, Ar-*H*^{PMB}), 6.07 (d, J = 6.2 Hz, 1H, H-1), 5.62 (t, J = 7.6 Hz, 1H, H-3), 5.48 (dd, J = 7.6, 6.2 Hz, 1H, H-2), 4.55 - 4.45 (m, 2H, CH₂^{PMB}), 4.14 (dd, J = 11.9, 4.2 Hz, 1H, H-4), 3.84 – 3.75 (m, 1H, H-5a), 3.73 – 3.65 (m, 4H, H-5b, CH₃^{PMB}). **¹³C NMR** (100 MHz, CDCl₃) δ : 165.4, 165.2, 164.7, 159.4, 133.6, 133.3, 130.0, 129.9, 129.8, 129.6, 129.4, 129.3,

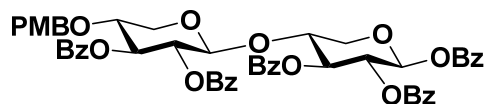
128.9, 128.8, 128.5, 128.4, 128.3, 113.8, 92.8, 73.0, 72.3, 71.7, 69.7, 63.6, 55.2.
HRMS (ESI-TOF) m/z : $[M + K]^+$ Calcd for $C_{34}H_{30}O_9$:622.9521; Found 622.9206.

Benzoyl 2,3-di-O-benzoyl- β -D-xylopyranoside (115)



Compound **116** (440 mg, 0.76 mmol) was dissolved in a mixture of CH_2Cl_2 (70 mL) and water (7 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (313 mg, 1.2 mmol) was added. The reaction mixture was stirred at room temperature until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (15h). The reaction mixture was filtrated through celite and the filtrated was washed with saturated aqueous $NaHCO_3$ (40 mL). The organic phase was dried with $MgSO_4$, filtrated and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **115** as a white amorphous solid. (259 mg, 0.56 mmol, 74%). $R_f = 0.43$ (EtOAc:heptane, 1:1, v/v). $[\alpha]_D^{20} = +25.0^\circ$ (c 1.0, $CDCl_3$). **IR** (neat, cm^{-1}): 3600-3300, 2928.2, 1729.3, 1601.4, 1451.9, 1261.4, 1069.4, 1042.8, 709.1. **1H NMR** (400 MHz, $CDCl_3$) δ : 8.00 – 7.84 (m, 6H, Ar-H), 7.54 – 7.39 (m, 3H, Ar-H), 7.37 – 7.24 (m, 6H, Ar-H), 6.14 (d, $J = 5.9$ Hz, 1H, H-1), 5.57 (dd, $J = 7.3, 5.9$ Hz, 1H, H-2), 5.36 (t, $J = 7.3$ Hz, 1H, H-3), 4.27 (dd, $J = 12.1, 4.3$ Hz, 1H, H-5a), 4.04 (td, $J = 7.3, 4.3$ Hz, 1H, H-4), 3.73 (dd, $J = 12.1, 7.3$ Hz, 1H, H-5b), 2.65 (bs, 1H, OH). **^{13}C NMR** (100 MHz, $CDCl_3$) δ : 166.7, 165.1, 164.7, 133.8, 133.7, 133.6, 130.1, 130.0, 129.8, 128.9, 128.8, 128.8, 128.6, 128.54, 128.52, 92.6, 74.5, 69.2, 68.1, 65.3. **HRMS** (ESI-TOF) m/z : $[M + Na]^+$ Calcd for $C_{26}H_{22}O_8$:485.1207; Found 485.1218.

2,3-O-benzoyl-4-O-p-metoxibenzoyl-O- β -D-xylopyranosyl-(1 \rightarrow 4)-benzoyl-2,3-di-O-benzoyl- β -D-xylopyranoside (117)

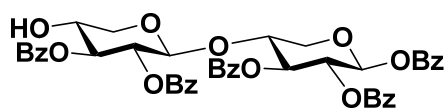


Compound **115** (98 mg, 0.21 mmol), compound **106** (181 mg, 0.32 mmol) and NIS (83 mg, 9.37 mmol) were dissolved in dry CH_2Cl_2 (5 mL) and the mixture was stirred at room temperature for 5 min.

Then the reaction mixture was cooled to -35 °C and TMSOTf (16 µL, 0.9 mmol) was added. The resulting reaction mixture was stirred at -20 °C until TLC (heptane:toluene:EtOAc, 4:2:3, v/v) showed full conversion of the acceptor **117** (20 min). The reaction mixture was washed with 1 M NaS₂O₃ (5 mL), 0.1 M HCl (5 mL) and the organic phase was dried with MgSO₄, filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **32** as a white amorphous solid. (175 mg, 0.19 mmol, 92%). *R_f*: 0.41 (EtOAc:heptane, 1:1 v/v).

$[\alpha]_D^{20} = +22.5^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 2924.2, 2853.5, 1727.1, 1260.6, 1214.2, 1093.1, 1069.1, 748.8. ¹H NMR (400 MHz, CDCl₃) δ: 7.95 – 7.80 (m, 10H, Ar-*H*), 7.49 – 7.40 (m, 5H, Ar-*H*), 7.36 – 7.26 (m, 10H, Ar-*H*), 6.98 – 6.93 (m, 2H, Ar-*H*^{PMB}), 6.64 – 6.58 (m, 2H, Ar-*H*^{PMB}), 6.01 (d, *J* = 6.4 Hz, 1H, H-1), 5.66 (t, *J* = 7.5 Hz, 1H, H-3), 5.46 (dd, *J* = 7.5, 6.4 Hz, 1H, H-2), 5.41 (t, *J* = 8.3 Hz, 1H, H-3'), 5.12 (dd, *J* = 8.3, 6.4 Hz, 1H, H-2'), 4.72 (d, *J* = 6.4 Hz, 1H, H-1'), 4.35 – 4.25 (m, 2H, CH₂^{PMB}), 4.08-3.99 (m, 2H, H-4, H-5a), 3.62 – 3.51 (m, 3H, CH₃^{PMB}), 3.63 - 3.51 (m, 2H, H-5b, H-5a'), 3.15 (dd, *J* = 11.9, 8.4 Hz, 1H, H-5b'). ¹³C NMR (100 MHz, CDCl₃) δ: 165.5, 165.3, 165.2, 165.1, 164.6, 159.3, 133.6, 133.31, 133.30, 133.22, 133.20, 130.0, 129.9, 129.8, 129.78, 129.71, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.7, 128.5, 128.4, 128.3, 113.8, 101.2, 92.7, 77.3, 77.0, 76.7, 75.3, 73.6, 72.4, 72.2, 71.4, 71.2, 69.5, 63.2, 62.9, 55.2. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₅₃H₄₆O₁₅: 945.2729; Found 945.2746.

2,3-O-benzoyl-O-β-D-xylopyranosyl-(1→4)-benzoyl-2,3-di-O-benzoyl-β-D-xylopyranoside (57)



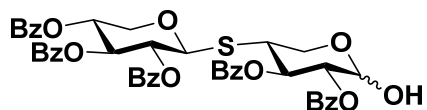
Compound **117** (156 mg, 0.17 mmol) was dissolved in a mixture of CH₂Cl₂ (20 mL) and water (2 mL) and DDQ (69 mg, 0.30 mmol)

was added. The reaction mixture was stirred at room temperature until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (15h). The reaction mixture was filtrated through celite and the filtrated was washed with saturated aqueous NaHCO₃. The organic phase was dried with MgSO₄, filtrated and concentrated *in*

vacuo. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **57** as a white amorphous solid. (99 mg, 0.12 mmol, 73%). $R_f = 0.35$ (EtOAc:heptane, 1:1, v/v).

$[\alpha]_D^{20} = +19.1^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 3600-3400, 2924.3, 2853.8, 1728.0, 1263.1, 1094.7, 1069.7, 1026.5, 708.9. ¹H NMR (400 MHz, CDCl₃) δ : 7.98 – 7.84 (m, 10H, Ar-H), 7.53 – 7.43 (m, 5H, Ar-H), 7.37 – 7.28 (m, 10H, Ar-H), 6.02 (d, $J = 6.4$ Hz, 1H, H-1), 5.68 (t, $J = 7.6$ Hz, 1H, H-3), 5.49 (dd, $J = 7.6, 6.4$ Hz, 1H, H-2), 5.23 (dd, $J = 7.8, 6.1$ Hz, 1H, H-2'), 5.12 (t, $J = 7.8$ Hz, 1H, H-3'), 4.77 (d, $J = 6.1$ Hz, 1H, H-1'), 4.11 – 4.02 (m, 2H, H-4, H-5a), 3.75 – 3.60 (m, 3H, H-5b, H-4', H-5a'), 3.18 (td, $J = 9.2, 2.3$ Hz, 1H, H-5b'), 2.87 (bs, 1H, OH). ¹³C NMR (100 MHz, CDCl₃) δ : 167.2, 165.3, 165.2, 164.9, 164.7, 133.7, 133.5, 133.4, 133.3, 130.1, 130.0, 129.9, 129.7, 129.6, 129.5, 129.0, 128.9, 128.76, 128.74, 128.6, 128.56, 128.52, 128.42, 128.40, 101.0, 92.8, 75.5, 75.4, 71.5, 70.5, 69.6, 68.4, 64.5, 63.2. HRMS: (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₄₅H₃₈O₁₄: 825.2154; Found: 825.2169.

2,3,4-O-benzoyl-S- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-O-benzoyl-D-xylopyranoside
(**118**)



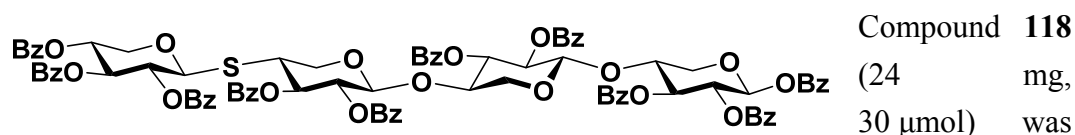
Compound **93** (30 mg, 0.035 mmol) was dissolved in acetic acid (250 μ L) and Pd(Ph₃)₄

(21 mg, 0.019 mmol) was added at room temperature. The reaction mixture was stirred at 40 °C until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (28h). An extra portion of catalyst was added if necessary. The reaction was diluted with EtOAc (5 mL) and washed with brine (5 mL). The inorganic phase was separated and extracted with EtOAc (2 \times 3 mL). The combined organic phases were dried over MgSO₄, filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **118** as a yellow amorphous solid. (25 mg, 0.030 mmol, 87%, α : β = 5 : 2, determined by ¹H NMR). $R_f = 0.26$ (EtOAc:heptane, 1:1, v/v).

IR (neat, cm⁻¹): 3500 – 3500, 2924.1, 2854.4, 1728.3, 1452.0, 1315.7, 1278.9, 1092.8, 1069.6, 709.5. **118 α** : ¹H NMR (400 MHz, CDCl₃) δ : 7.93 – 7.79 (m, 10H,

Ar-H), 7.50 – 7.14 (m, 15H, Ar-H), 5.85 (dd, $J = 9.7, 10.9$ Hz, 1H, H-3), 5.61 - 5.56 (m, 2H, H-3', H-1), 5.28 – 5.14 (m, 3H, H-2', H-2, H-1'), 5.02 – 4.97 (m, 1H, H-4'), 4.33 (dd, $J = 3.5, 12.4$ Hz, 1H, H-5a'), 4.18 (t, $J = 11.9$ Hz, 1H, H-5a), 3.91 (dd, $J = 5.3, 11.9$ Hz, H-5b), 3.56 (dd, $J = 5.00, 12.4$ Hz, 1H, H5b'), 3.33 (dt, $J = 5.3, 11.9$ Hz, 1H, H-4), 1.60 (bs, 1H, OH). **118 β** : $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.93 - 7.79 (m, 10H, Ar-H), 7.50 – 7.14 (m, 15H, Ar-H), 5.61 – 5.56 (m, 1H, H-3), 5.52 (dd, $J = 9.4, 10.9$ Hz, 1H, H-3'), 5.28 – 5.14 (m, 3H, H-2', H-2, H-1), 5.02 - 4.97 (m, 1H, H-4'), 4.76 (d, $J = 7.7$ Hz, 1H, H-1'), 4.35 – 4.24 (m, 2H, H-5a, H-5a'), 3.36 – 3.54 (m, 2H, H-5b, H-5b'), 3.38 (dt, $J = 4.9, 11.2$ Hz, 1H, H-4), 1.60 (bs, 1H, OH). **118 α** : $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 165.9, 165.8, 165.5, 165.1, 164.9, 133.6, 133.4, 133.38, 133.33, 133.2, 130.1, 129.93, 129.87, 129.7, 129.3, 129.2, 129.1, 128.9, 128.8, 128.5, 128.44, 128.43, 128.32, 128.31, 91.00, 81.6, 73.5, 71.2, 69.6, 68.9, 68.5, 68.1, 62.00, 44.2. **118 β** : $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 166.9, 165.7, 165.4, 165.1, 164.8, 133.5, 133.41, 133.38, 133.3, 133.1, 130.0, 129.9, 129.8, 129.7, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.44, 128.43, 128.32, 128.31, 96.5, 81.5, 75.6, 70.7, 69.4, 67.9, 68.8, 66.6, 61.9, 44.9. **HRMS** (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{45}\text{H}_{38}\text{O}_{13}\text{S}$: 841.1925; Found 841.1941.

2,3,4-O-benzoyl-S- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-O-benzoyl-O- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-O-benzoyl-O- β -D-xylopyranosyl-(1 \rightarrow 4)-1,2,3-tri-O-benzoyl- β -D-xylopyranoside (80)



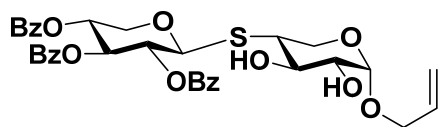
dissolved in dry CH_2Cl_2 (300 μL) and K_2CO_3 (15 mg, 0.1 mmol) and Cl_3CCN (12 μL , 0.05 mmol) were added to the mixture at room temperature (20 $^\circ\text{C}$). The reaction mixture was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete conversion (5h). The reaction mixture was diluted with CH_2Cl_2 and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography

(EtOAc:heptane, 3:7, v/v) to give intermediate **119** (26 mg, 27 μmol , 90%). $R_f = 0.58$ (EtOAc:heptane, 1:1, v/v).

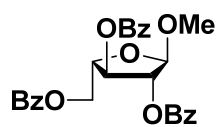
Intermediate **119** and compound **57** (18 mg, 22 μmol) were co-evaporated two times with toluene and subjected to high vacuum for 2h. The solid mixture was dissolved in dry CH_2Cl_2 (1 mL) and reaction mixture was cooled to $-35\text{ }^\circ\text{C}$ and TMSOTf (1 μL , 4 μmol) was added. Reaction mixture was stirred at $-30\text{ }^\circ\text{C}$ until TLC showed complete consumption of compound **119** (1h). The reactant in excess was quenched with Et_3N and the resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give compound **80** as an amorphous white solid. (15 mg, 8.8 μmol , 40%). $R_f = 0.30$ (EtOAc:heptane, 1:1, v/v).

$[\alpha]_D^{20} = +3.2^\circ$ (c 1.0, CHCl_3). IR (neat, cm^{-1}): 2923.1, 2853.8, 1725.8, 1601.5, 1451.9, 1259.3, 1068.4, 1026.0, 757.3, 707.7. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.94 - 7.82 (m, 15H, Ar-H), 7.81 - 7.73 (m, 5H, Ar-H), 7.51 - 7.36 (m, 10H, Ar-H), 7.36 - 7.25 (m, 17H, Ar-H), 7.23-7.21 (m, 1H, Ar-H), 7.18 - 7.13 (m, 2H, Ar-H), 5.99 (d, $J = 6.3$ Hz, 1H, H-1), 5.61 (t, $J = 7.3$ Hz, 1H, H-3), 5.53 (t, $J = 5.9$ Hz, 1H, H-3'), 5.48 - 5.39 (m, 2H, H-2, H-3''), 5.28 (dd, $J = 10.0, 8.6$ Hz, 1H, H-3''), 5.16 - 5.05 (m, 3H, H-2', H-2'', H-2'''), 4.96 (d, $J = 5.3$ Hz, 1H, H-1'''), 4.95 - 4.88 (m, 1H, H-4'), 4.67 (d, $J = 6.5$ Hz, 1H, H-1''), 4.42 (d, $J = 7.1$ Hz, 1H, H-1'), 4.19 (dd, $J = 12.5, 3.5$ Hz, 1H, H-5a'), 4.03 - 3.91 (m, 2H, H-5a, H-4), 3.67 (td, $J = 8.6, 5.2$ Hz, 1H, H-4'''), 3.62 - 3.51 (m, 2H, H-5b, H-5a''), 3.45 (dd, $J = 12.5, 5.2$ Hz, 1H, H-5b'), 3.41 (dd, $J = 12.4, 5.2$ Hz, 1H, H-5a'''), 3.19 - 3.12 (m, 1H, H-5b''), 3.09 - 2.97 (m, 2H, H-5b''', H-4''). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ : 165.7, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 164.8, 164.6, 133.7, 133.5, 133.4, 133.3, 133.29, 133.25, 133.1, 133.0, 130.0, 130.00, 129.94, 129.93, 129.8, 129.78, 129.75, 129.72, 129.68, 129.64, 129.4, 129.2, 129.19, 129.16, 129.0, 128.96, 128.90, 128.8, 128.7, 128.6, 128.5, 128.4, 128.38, 128.34, 128.32, 128.28, 101.6, 101.1, 92.8, 81.6, 76.04, 75.25, 72.65, 72.05, 71.42, 71.23, 69.54, 69.42, 69.09, 68.10, 65.37, 65.35, 63.13, 62.59, 44.1. HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{90}\text{H}_{74}\text{O}_{26}\text{S}$: 1625.4081; Found 1625.4110.

2,3,4-Tri-O-benzoyl-S-β-D-xylopyranosyl-(1→4)-allyl-4-thio-α-D-xylopyranoside (75)

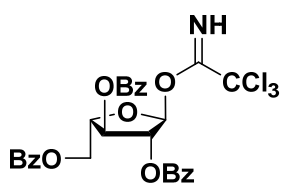


Compound **70** (117 mg, 0.15 mmol) was dissolved in mixture of CH₂Cl₂ (10 mL) and water (200 μL) and TFA (1.5 mL) was added at room temperature. The reaction mixture was stirred until TLC (EtOAc:heptane, 1:1, v/v) showed complete consumption of the starting material (1h). The reaction was quenched with saturated aqueous NaHCO₃ (10 mL) and the organic layer was separated. The inorganic phase was extracted twice with CH₂Cl₂ (2 × 10 mL) and the combined organic phases were washed with brine (10 mL), dried with Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (EtOAc:heptane, 1:1, v/v) to give **75** as a yellow amorphous solid (93 mg, 0.14 mmol, 94%). *R_f* = 0.25 (MeOH:CH₂Cl₂, 1:100, v/v). $[\alpha]_D^{20} = -28.3^\circ$ (c 1.0, CHCl₃). IR (neat, cm⁻¹): 3500 – 3500, 2924.1, 2854.4, 1728.3, 1452.0, 1315.7, 1278.9, 1092.8, 1069.6, 709.5. ¹H NMR (400 MHz, CDCl₃) δ 8.02 – 7.84 (m, 6H, Ar-*H*), 7.51 – 7.39 (m, 3H, Ar-*H*), 7.37 – 7.29 (m, 6H, Ar-*H*), 5.83 (ddd, *J* = 22.4, 10.4, 5.8 Hz, 1H, Allyl CH=CH₂), 5.74 (t, *J* = 7.5 Hz, 1H, H-3'), 5.40 (t, *J* = 7.5 Hz, 1H, H-2'), 5.32 – 5.17 (m, 2H, H-4', Allyl CH=CH₂), 5.14 (d, *J* = 10.4 Hz, 1H, Allyl CH=CH₂), 4.95 (d, *J* = 7.5 Hz, 1H, H-1'), 4.87 (d, *J* = 3.6 Hz, 1H, H-1), 4.53 (dd, *J* = 12.0, 4.4 Hz, 1H, H-5a'), 4.13 (dd, *J* = 12.8, 5.8 Hz, 1H, Allyl O-CH₂), 3.94 (dd, *J* = 12.8, 5.8 Hz, 1H, Allyl O-CH₂), 3.74 – 3.50 (m, 5H, H-5b', H-2, H-3, H-5a, H-5b), 2.97 (td, *J* = 11.6, 5.4 Hz, 1H, H-4), 2.14 (bs, 2H, OH). ¹³C NMR (100 MHz, CDCl₃) δ: 165.5, 165.4, 165.3, 133.6, 133.5, 133.4, 129.9, 129.9, 128.9, 128.8, 128.7, 128.4, 118.0, 97.6, 83.1, 77.3, 77.0, 76.7, 73.6, 72.1, 71.1, 70.6, 68.8, 68.6, 64.8, 61.8, 47.9. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₃₄H₃₄O₁₁S :673.1714; Found 673.1728.

Methyl-2,3,5-Tri-O-benzoyl- α -L-arabinofuranoside (121)

To a solution of L-arabinose (10 g, 67 mmol) in dry MeOH (200 mL) was added a solution of HCl in MeOH (freshly prepared by adding slowly acetyl chloride (5 mL) in anhydrous methanol (60 mL) at 0 °C). After stirring for 19h at room temperature, the reaction mixture was quenched by addition of pyridine (130 mL) at 0 °C and concentrated *in vacuo*. Three co-evaporations with CH₂Cl₂ (3 × 50 mL) gave a yellow oil, which was dissolved in pyridine (80 mL). Benzoyl chloride (62 mL, 536 mmol) was added dropwise at 0 °C to the reaction mixture. After stirring 40h at room temperature, the solution was cooled 0 °C. Water (10 mL) was added at 0 °C to the reaction mixture, followed by CH₂Cl₂ (100 mL). The resulting organic layer was washed with 1M HCl (5 × 30 mL) and saturated aqueous NaHCO₃ (5 × 30 mL), dried with MgSO₄ and concentrated *in vacuo*. The crude was purified by flash column chromatography (hexane:EtOAc, 3:2, v/v) to give yellow oil, which was crystallised from EtOH to give compound **121** as a white solid (22.28 g, 70%) *R_f* = 0.32 (hexane:EtOAc, 5:5, v/v). The compound analyses are in accordance to data from the literature²¹⁸.

mp: 101 – 102 °C. ¹H NMR (400 MHz, CDCl₃) δ : 8.17 – 7.28 (m, 15 H, Ar-*H*), 5.58 (dd, *J* = 5.6, 1.6 Hz, 1H, H-3), 5.51 (d, *J* = 5.6 Hz, 1H, H-2), 5.18 (s, 1H, H-1), 4.84 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5a), 4.69 (dd, *J* = 12.0, 4.8 Hz, 1H, H-5b), 4.57 (ddd, *J* = 4.8, 3.6, 1.6 Hz, 1H, H-4), 3.49 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ : 166.2, 165.8, 165.5, 134.5, 133.5, 133.0, 130.5, 129.9, 129.8, 129.7, 129.1, 129.0, 128.8, 128.5, 128.4, 128.3, 106.8, 82.2, 80.8, 77.9, 63.7, 55.0.

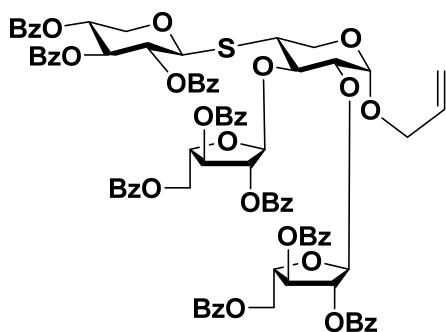
2,3,5-Tri-O-benzoyl- α -L-arabinofuranosyl-2,2,2-trichloroacetimidate (122)

To a solution of compound **121** (440 mg, 0.92 mmol) in acetic acid (3 mL), was added 33% HBr·HOAc (2.5 mL). The mixture was stirred for 4h at room temperature. TLC showed complete consumption of the starting material, and the mixture was diluted with EtOAc (15 mL), washed three times with brine (3 × 10 mL), then with water (10 mL), dried over Na₂SO₄,

and concentrated *in vacuo*. The crude product was subjected to automatic flash chromatography (12 g column, heptane:EtOAc, 10:1→5:1, v/v) to afford the corresponding hemiacetal as a pale yellow foam (340 mg, 0.74 mmol, 80%).^[8] The resulting hemiacetal was dissolved in dry CH₂Cl₂ (8 mL) and CCl₃CN (305 μL, 1.3 mmol) and anhydrous K₂CO₃ (368 mg) were added. The mixture was stirred overnight at room temperature and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane:EtOAc, 3:1, v/v) to give **122** as a foamy solid (373 mg, 0.62 mmol, 80%). *R_f* = 0.47 (heptane:EtOAc, 7:3, v/v). The compound analyses are in accordance to data from the literature²¹⁹.

$[\alpha]_D^{25} = -24.0^\circ$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ: 8.66 (s, 1H, NH), 8.10 - 8.01 (m, 2H, Ar-H), 8.01 - 7.90 (m, 4H, Ar-H), 7.59 - 7.48 (m, 2H, Ar-H), 7.46 - 7.29 (m, 6H, Ar-H), 7.25 - 7.14 (m, 3H, Ar-H), 6.59 (s, 1H, H-1), 5.74 (s, 1H, H-2), 5.61 (d, *J* = 3.5 Hz, 1H, H-3), 4.81 - 4.61 (m, 3H, H-4, H-5a, H-5b). ¹³C DEPT NMR (100 MHz, CDCl₃) δ: 133.8, 133.7, 133.0, 130.1, 130.0, 129.8, 128.6, 128.5, 128.3, 103.1, 84.3, 80.7, 63.5.

2,3,4-Tri-O-benzoyl-S-β-D-xylopyranosyl-(1→4)-[2,3,5-tri-O-benzoyl-O-α-L-arabinofuranosyl-α-(1→3)]-[2,3,5-tri-O-benzoyl-O-α-L-arabinofuranosyl-(1→2)]allyl-4-thio-α-D-xylopyranoside (124)



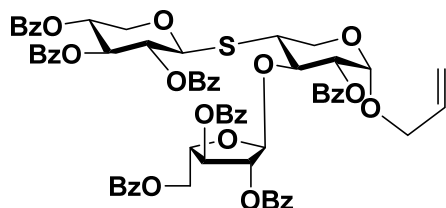
Compound **75** (84 mg, 0.13 mmol) and compound **122** (102 mg, 0.17 mmol) were co-evaporated with toluene twice and subjected to high vacuum for 15h. The mixture was dissolved in anhydrous CH₂Cl₂ (3 mL) and cooled to -35 °C. TMSOTf (27 μL, 0.02 mmol) was added and the reaction mixture was stirred at -35 °C until TLC

(EtOAc:heptane, 1:1, v/v) showed complete conversion (10 min). The reaction mixture was quenched with Et₃N (300 μL), evaporated and purified by flash

column chromatography (MeOH:CH₂Cl₂, 1:99, v/v) to give compound **124** as a white solid (82 mg, 0.053 mmol, 41%). $R_f = 0.7$ (MeOH:CH₂Cl₂, 1:99, v/v).

mp: 87 - 97 °C. $[\alpha]_D^{20} = +1.9^\circ$ (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2925.1, 2854.7, 1722.8, 1601.7, 1451.8, 1264.0, 1110.0, 1069.5, 1026.7, 709.7. **¹H NMR** (400 MHz, CDCl₃) δ : 8.11 – 8.04 (m, 2H, Ar-H), 7.95 - 7.82 (m, 12H, Ar-H), 7.79 - 7.75 (m, 2H, Ar-H), 7.73 – 7.65 (m, 2H, Ar-H), 7.43 – 7.21 (m, 21H, Ar-H), 7.16 – 7.03 (m, 6H, Ar-H), 5.79 (s, 1H, H-1^{FUR in 3}), 5.62 (t, $J = 7.5$ Hz, 1H, H-3'), 5.56 – 5.48 (m, 4H, Allyl CH=CH₂, H-3^{FUR in 2}, H-2^{FUR in 2}, H-1^{FUR in 2}), 5.45 – 5.40 (m, 2H, H-4^{FUR in 3}, H-2^{FUR in 3}), 5.20 (t, $J = 7.5$ Hz, 1H, H-2'), 5.13 (dd, $J = 8.2, 4.2$ Hz, 1H, H-4^{FUR in 2}), 5.09 – 4.99 (m, 3H, Allyl CH=CH₂, H-1, H-4'), 4.94 (d, $J = 7.5$ Hz, 1H, H-1'), 4.82 (dd, $J = 10.5, 1.0$ Hz, 1H, Allyl CH=CH₂), 4.75 – 4.66 (m, 2H, H-5a^{FUR in 2}, H-5a^{FUR in 3}), 4.61 (dd, $J = 12.0, 4.5$ Hz, 1H, H-5b^{FUR in 2}), 4.54 (dd, $J = 12.0, 5.1$ Hz, 1H, H-5b^{FUR in 3}), 4.50 – 4.43 (m, 1H, H-3^{FUR in 3}), 4.15 (dd, $J = 12.0, 4.5$ Hz, 1H, H-5a'), 4.07 (dd, $J = 6.0, 6.8$ Hz, 1H, H-3), 3.99 (dd, $J = 12.8, 5.1$ Hz, 1H, Allyl O-CH₂), 3.82 – 3.69 (m, 2H, Allyl O-CH₂, H-2), 3.69 – 3.60 (m, 2H, H-5a, H-5b), 3.38 (dd, $J = 12.0, 7.7$ Hz, 1H, H-5b'), 3.23 - 3.10 (m, 1H, H-4). **¹³C NMR** (100 MHz, CDCl₃) δ : 166.3, 166.1, 165.8, 165.7, 165.5, 165.3, 165.24, 165.23, 165.0, 133.5, 133.4, 133.3, 133.2, 133.0, 132.9, 132.8, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.2, 129.1, 129.0, 128.9, 128.8, 128.46, 128.45, 128.43, 128.37, 128.34, 128.2, 128.1, 117.3, 107.6, 106.5, 98.1, 82.2, 82.1, 81.8, 78.0, 77.5, 72.6, 71.3, 71.0, 68.9, 68.4, 63.9, 63.8, 60.4, 53.4, 45.4. **HRMS** (ESI-TOF) m/z : $[M + Na]^+$ Calcd for C₈₆H₇₆O₂₅S : 1561.4132; Found 1561.4155.

2,3,4-Tri-O-benzoyl-S-β-D-xylopyranosyl-(1→4)-[2,3,5-tri-O-benzoyl-O-α-L-arabinofuranosyl-(1→3)]-allyl-4-thio-2-O-benzoyl-α-D-xylopyranoside (126)



Compound **123** was obtained as described in the previous procedure as a co-product (see compound **124**). The resulting mono-substituted trisaccharide **123** (57 mg, 0.056 mmol) was solubilized in pyridine (1 mL) and the reaction mixture was cooled to 0 °C. Benzoyl chloride (9 μL,

0.08 mmol) was added and the reaction mixture was stirred at room temperature until TLC (MeOH:CH₂Cl₂, 1:99, v/v) showed complete conversion (16h). The reaction mixture was diluted in CH₂Cl₂ (10 mL), washed with 1M HCl (5 mL), saturated aqueous NaHCO₃ (5 mL), and brine (5 mL). The organic phase was dried over MgSO₄, filtered, and concentrated *in vacuo*. The resulting crude was purified by flash column chromatography (MeOH:CH₂Cl₂, 1:99, v/v) to give compound **126** as a white solid (66 mg, 55 μmol, 43% over 2 step). *R_f* = 0.62 (MeOH:CH₂Cl₂, 1:99, v/v).

mp: 85 - 95 °C. [α]_D²⁰ = +12.0° (c 1.0, CHCl₃). **IR** (neat, cm⁻¹): 2925.8, 1725.1, 1601.6, 1452.0, 1265.3, 1108.1, 1069.4, 709.3. **¹H NMR** (400 MHz, CDCl₃) δ: 7.98 – 7.85 (m, 12H, Ar-*H*), 7.50 – 7.05 (m, 23H, Ar-*H*), 5.74 – 5.57 (m, 2H, Allyl CH=CH₂, H-3'), 5.51 (s, 1H, H-1^{FUR in 3}), 5.48 (d, *J* = 5.4 Hz, 1H, H-2^{FUR in 3}), 5.37 (d, *J* = 1.5 Hz, 1H, H-2'), 5.26 (t, *J* = 6.8 Hz, 1H, H-4'), 5.18 – 5.12 (m, 2H, Allyl CH=CH₂, H-2), 5.12 – 5.01 (m, 3H, H-1, H-1', H-3^{FUR in 3}), 5.00 – 4.89 (m, 2H, Allyl CH=CH₂, H-4^{FUR in 3}), 4.71 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5a^{FUR in 3}), 4.53 (dd, *J* = 12.0, 4.3 Hz, 1H, H-5b^{FUR in 3}), 4.34 (dd, *J* = 12.1, 4.3 Hz, 1H, H-5a'), 4.25 (dd, *J* = 12.0, 12.8 Hz, 1H, H-3), 4.03 (dd, *J* = 13.3, 5.1 Hz, 1H, Allyl O-CH₂), 3.86 - 3.70 (m, 3H, Allyl O-CH₂, H-5a, H-5b), 3.51 (dd, *J* = 12.1, 7.1 Hz, 1H, H-5b'), 3.34 – 3.19 (m, 1H, H-4). **¹³C NMR** (100 MHz, CDCl₃) δ: 166.1, 165.7, 165.5, 165.2, 165.1, 164.77, 164.73, 133.4, 133.3, 133.0, 132.9, 130.0, 129.9, 129.85, 129.81, 129.6, 129.5, 129.4, 129.2, 129.1, 129.0, 128.8, 128.7, 128.48, 128.46, 128.43, 128.3, 128.28, 128.26, 128.0, 117.4, 106.7, 95.7, 82.3, 82.3, 81.3, 77.6, 75.6, 72.4, 70.82, 70.81, 68.7, 68.2, 63.9, 63.5, 62.5, 62.2, 45.5. **HRMS** (ESI-TOF) *m/z*: [M + K]⁺ Calcd for C₆₆H₅₆O₁₉S: 1221.2768; Found 1221.3206.

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Appendix

10 JBEI Experimental data

Materials and Methods

10.1.1 Microsomes preparation

All microsome preparation steps took place at 4 °C. Mung beans stems were ground in buffer containing 50 mM HEPES-KOH, pH 7.0, 400 mM Suc, 1 mM phenyl-methanesulfonyl fluoride, 1% (w/v) polyvinylpolypyrrolidone, and protease inhibitors (Roche Complete protease inhibitor tablets). The homogenate was filtered through two layers of Miracloth (EMD Millipore) and centrifuged at 3,000g for 10min, then the supernatant was centrifuged at 50,000g for 1 h. The pellet was re-suspended in 50 mM HEPES-KOH, pH 7.0, and 400 mM Suc. Microsomes were used immediately or frozen in liquid nitrogen and stored at 280°C. No significant loss of activity was detected after freezing.

10.1.2 HPAEC system

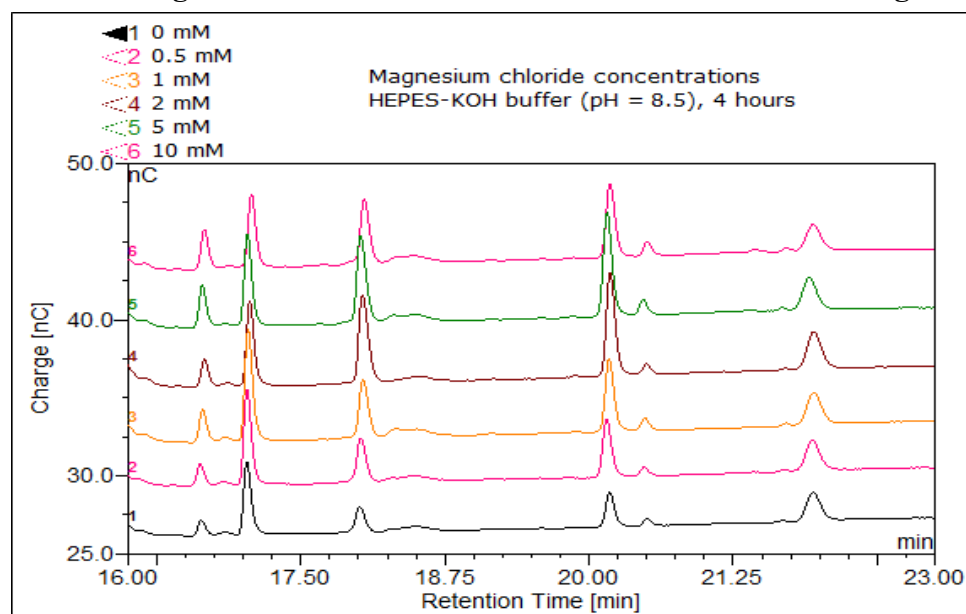
Polysaccharide composition was determined by HPAEC of reaction material. Chromatography was on a PA20 column (Dionex, CA, USA) at a flow rate of 0.5 ml/min. Before injection of each sample (up to 0.2 lg monosaccharide), the column was washed with 200 mM NaOH for 10 min, then equilibrated with 10 mM NaOH for 10 min. The elution program consisted of a linear gradient from 10mMNaOH to 5mMNaOH from 0 to 1.5 min, followed by isocratic elution with 5 mM NaOH from 1.5 to 20 min, followed by a linear gradient up to 800 mM NaOH from 20 to 43 min.

Procedure

Cation type/concentration determination: *Magnesium cation concentration assay*

Six of the following reactions were contemporaneously made with different concentration of MgCl_2 (0 mM, 0.5 mM, 1mM, 2 mM, 5 mM, 10 mM). 50 μg of microsomal proteins was incubated with 50 mM HEPES-KOH, pH 8.5, 0.20 μM RG-I hexasaccharide 134, Triton X-100 1%, and in 50 μL of milliQ water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h at 25 $^\circ\text{C}$ by addition of 10 μL water and heating at 95 $^\circ\text{C}$ for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 μm , cat. no. 82031-360) along with 80 μL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 $^\circ\text{C}$ until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column)

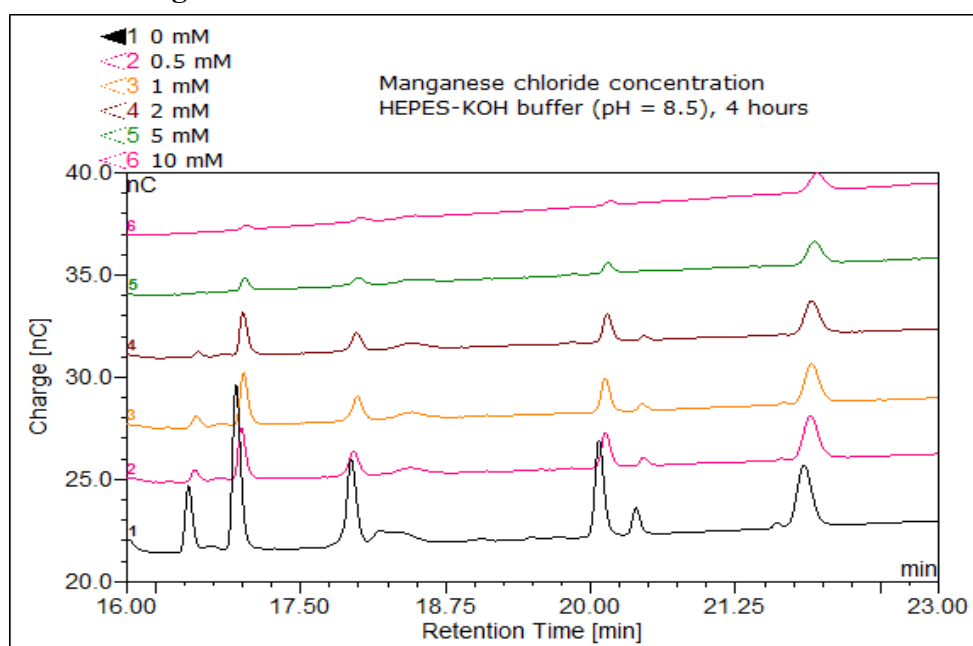
Chromatogram of the reaction in different concentrations of MgCl_2 :



Cation type/concentration determination: *Manganese cation concentration assay*

Six of the following reactions were contemporaneously made with different concentration of MnCl_2 (0 mM, 0.5 mM, 1mM, 2 mM, 5 mM, 10 mM). 50 μg of microsomal proteins was incubated with 50 mM HEPES-KOH, pH 8.5, 0.20 μM RG-I hexasaccharide 134, Triton X-100 1%, and in 50 μL of milliQ water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h at 25 $^\circ\text{C}$ by addition of 10 μL water and heating at 95 $^\circ\text{C}$ for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 μm , cat. no. 82031-360) along with 80 μL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 $^\circ\text{C}$ until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column)

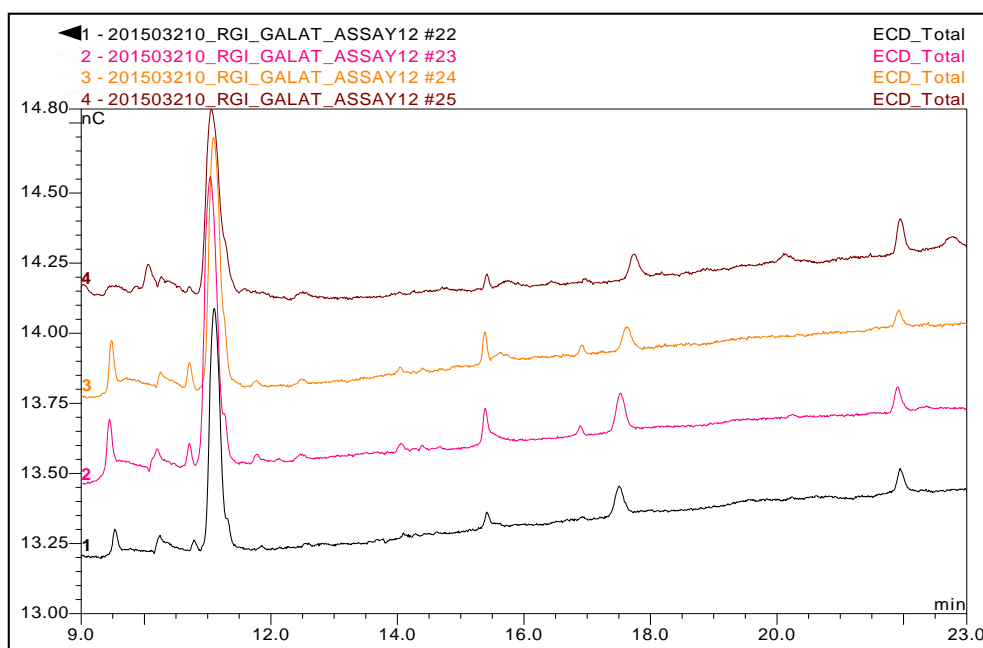
Chromatogram of the reaction in different concentrations of MnCl_2 :



pH optimum determination: *MES-KOH buffering agent assay*

Four of the following reactions were contemporaneously made with different MES-based pH solution (pH: 5.0, 5.5, 6.0, 6.5). 50 µg of microsomal proteins was incubated with 50 mM, RG-I hexasaccharide 134, MgCl₂ 5mM, Triton X-100 1%, and in 50 µL of milliq water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h at 25 °C by addition of 10 µL water and heating at 95 °C for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 µM, cat. no. 82031-360) along with 80 µL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 °C until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column).

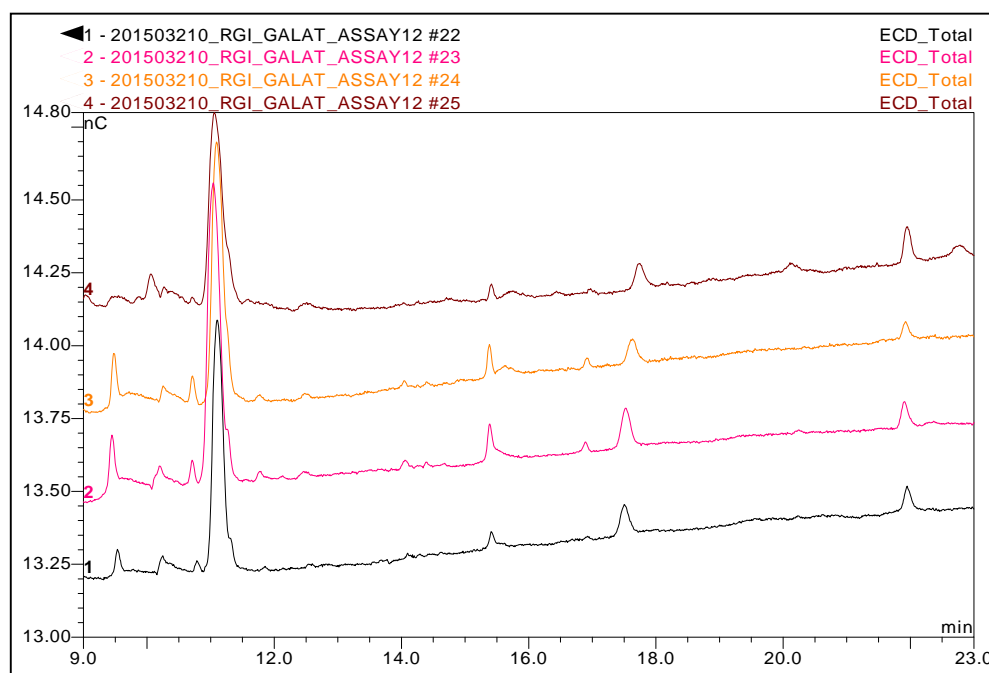
MES assay: n.1=pH 5.0, n.2=pH 5.5, n.3=pH 6.0, n.4=pH 6.5



pH optimum determination: *HEPES-KOH buffering agent assay*

Four of the following reactions were contemporaneously made with different HEPES-based pH solution (pH: 7.0, 7.5, 8.0, 8.5). 50 µg of microsomal proteins was incubated with 50 mM, RG-I hexasaccharide 134, MgCl₂ 5mM, Triton X-100 1%, and in 50 µL of milliq water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h at 25 °C by addition of 10 µL water and heating at 95 °C for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 µM, cat. no. 82031-360) along with 80 µL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 °C until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column).

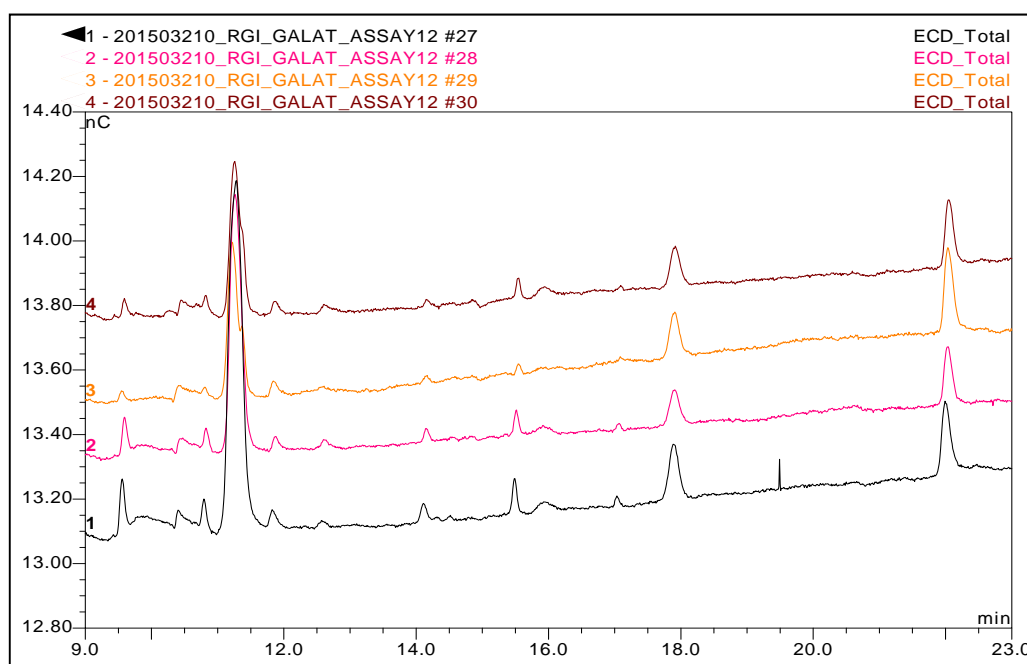
HEPES assay: n.1=pH 7.0, n.2=pH 7.5, n.3=pH 8.0, n.4=pH 8.5



pH optimum determination: *TAPS-KOH* buffering agent assay

Four of the following reactions were contemporaneously made with different TAPS-based pH solution (pH: 8.0, 8.5, 9.0, 9.5). 50 µg of microsomal proteins was incubated with 50 mM, RG-I hexasaccharide 134, MgCl₂ 5mM, Triton X-100 1%, and in 50 µL of milliq water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h at 25 °C by addition of 10 µL water and heating at 95 °C for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 µM, cat. no. 82031-360) along with 80 µL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 °C until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column).

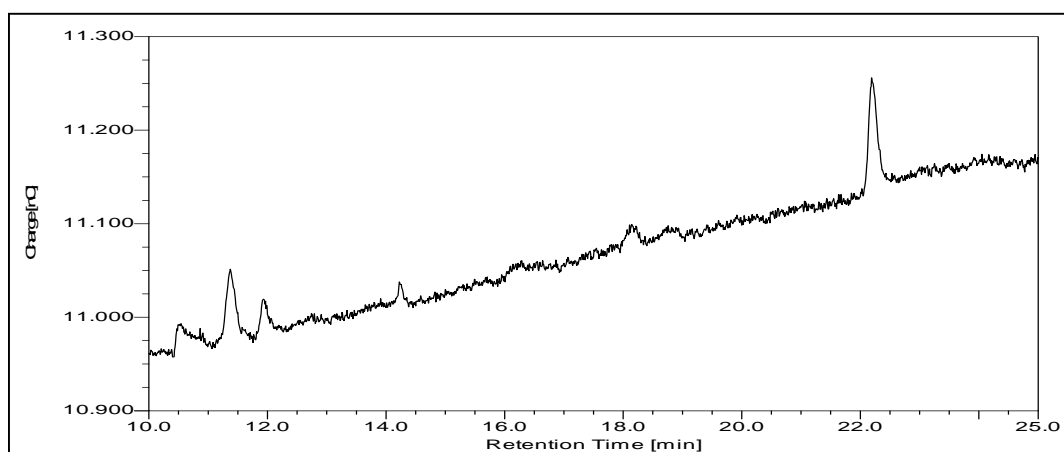
TAPS assay: Mg Assay012 n.1=pH 8.0, n.2=pH 8.5, n.3=pH 9.0, n.4=pH 9.5



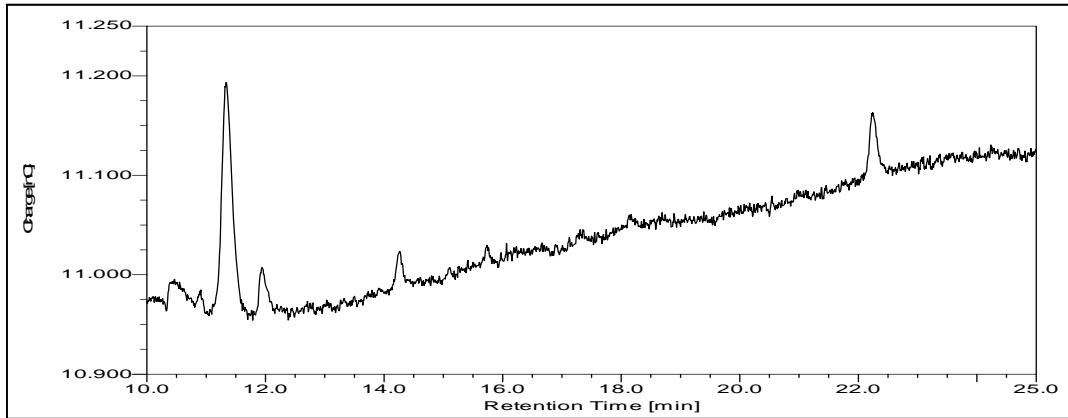
T optimum determination:

Six of the following reactions were contemporaneously made and incubated at different temperatures for 4h (4, 20, 25, 30, 35, 40, 45, 50 °C). 50 µg of microsomal proteins was incubated with 50 mM HEPES-KOH, pH 8.5, 0.20 µM RG-I hexasaccharide 134, MgCl₂ 5mM, Triton X-100 1%, and in 50 µL of milliQ water. 1 mM UDP-GlcA was added to the mixture after 10 min. The incubation was terminated after 4h by addition of 10 µL water and heating at 95 °C for 5 min. The terminated samples were transferred to a VWR centrifugal filter (nylon, 0.45 µM, cat. no. 82031-360) along with 80 µL water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5 °C until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis, new column and guard column)

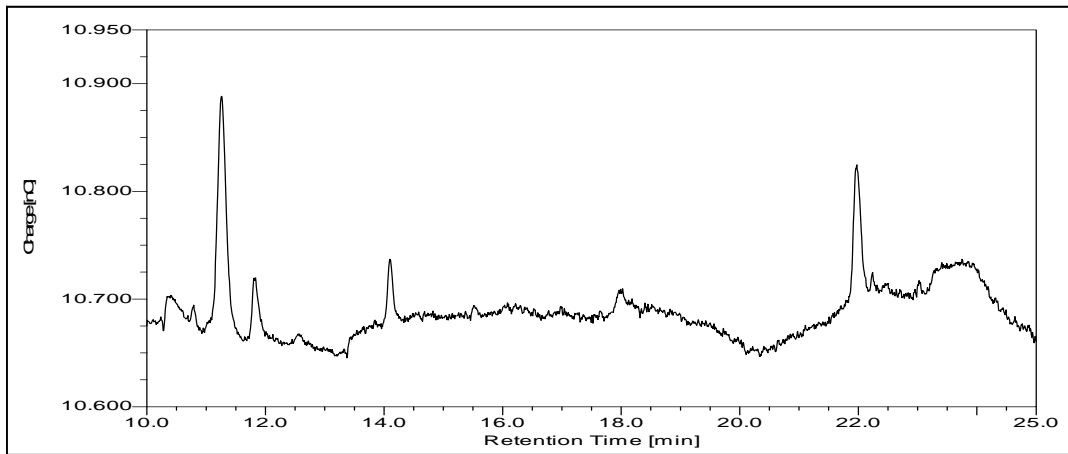
4 °C assay chromatogram:



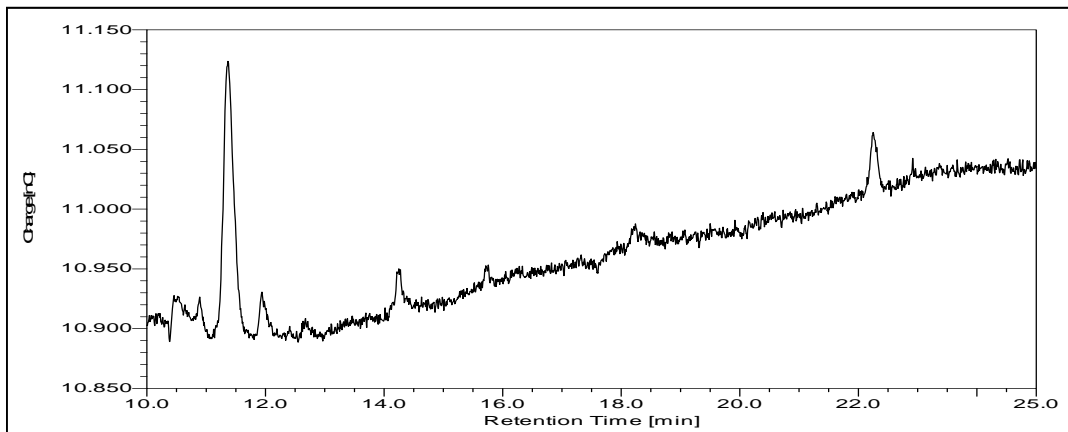
20 °C assay chromatogram:



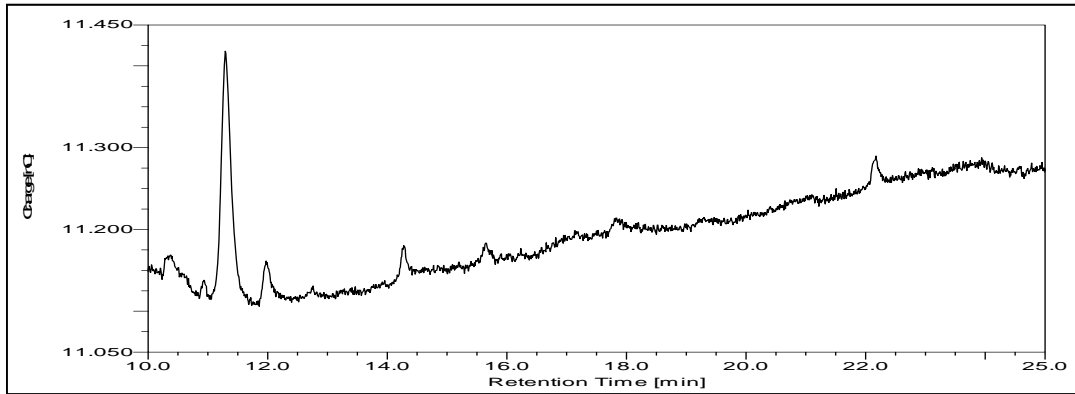
25 °C assay chromatogram:



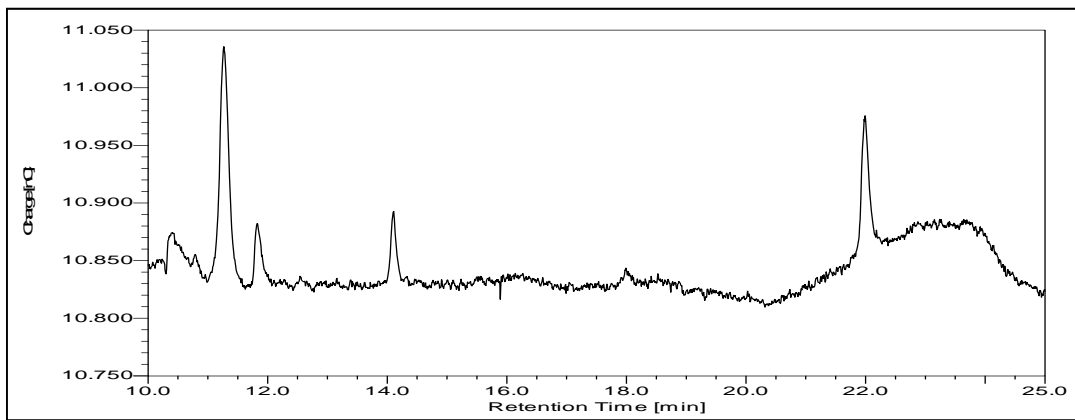
30 °C assay chromatogram:



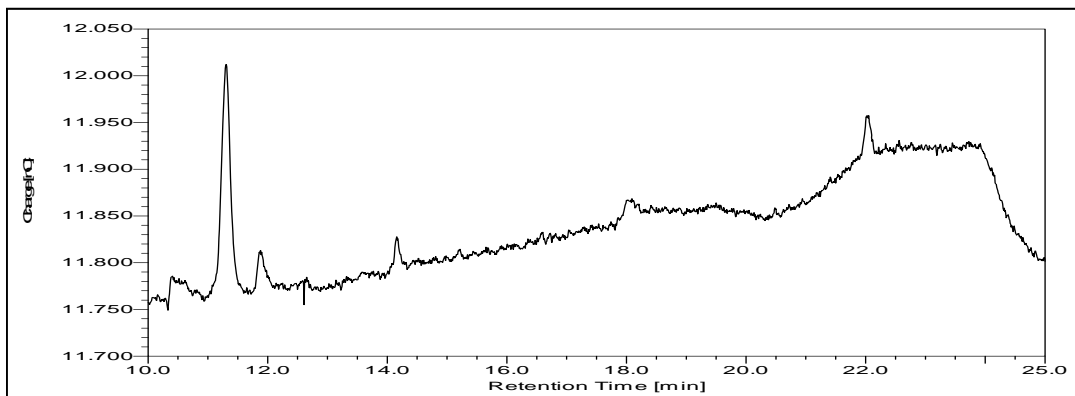
35 °C assay chromatogram:



40 °C assay chromatogram:



45 °C assay chromatogram:



50 °C assay chromatogram:

