UNIVERSITY OF ALBERTA

Thio-oligosaccharides: Synthesis, Conformational Analysis, and Immunochemistry

by

Henry N. Yu 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Fall 2002

University of Alberta

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Degree: Doctor of Philosophy

Year this Degree Granted: 2002

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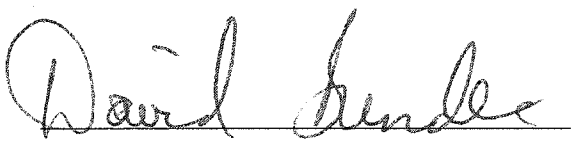

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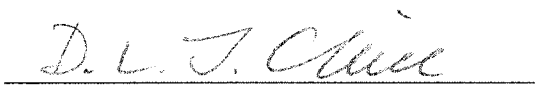

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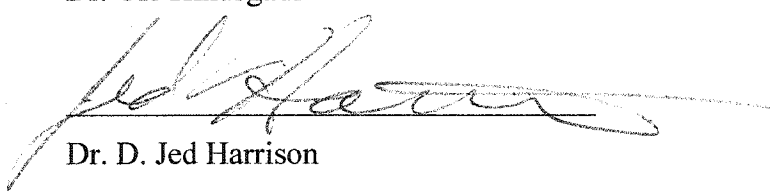

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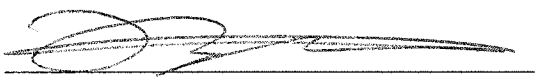

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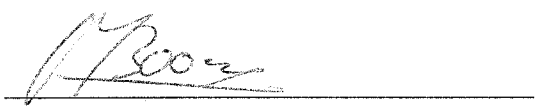


Dr. Geert-Jan Boons (External Examiner)

Dated: September 17th, 2002

Dedicated to my beautiful wife Lan

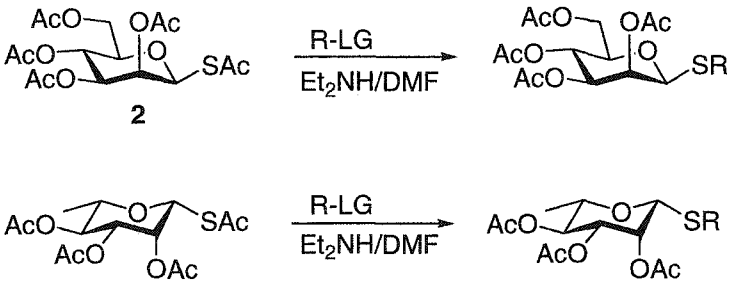
and our lovely daughter Annie

Abstract

Aspects of the synthetic chemistry of thio-glycosidic linkage formation have been addressed and the target molecules were used to explore the role of thio-glycosidic linkages in molecular recognition involving the immune system.

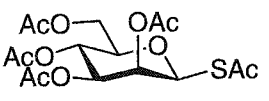
A new methodology for the formation of the synthetically challenging 1,2-cis-β-thio-glycosidic linkages has been developed. It was employed for the synthesis of a series of biologically interesting 1-thio-β-mannopyranosides and 1-thio-β-rhamnopyranosides.

This method employs the simple, easy-to-make 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-mannopyrannose **2** and 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-L-rhamnopyranose **27** as starting materials to conduct an *in situ* selective de-*S*-acetylation, and subsequent S_N2 reaction with an acceptor bearing a leaving group. The high nucleophilicity and slow anomerization of the intermediate thiol allowed the synthesis of 1,2-cis-β-thioglycosides in a simple and practical manner.



X-ray crystallographic and NMR data for the first free example of a deprotected β-thio-linked disaccharide (**49**) and three other protected thioglycosides (**2**, **27**, and **42a**) with a similar linkage are reported. Comparison of the analogous linkage in α-Man-

(1→3)-β-Man-(1→4)-GlcNAc trisaccharide and a available thioglycoside crystal structure confirmed that substitution of oxygen by a sulfur atom in the glycosidic linkage changes the coordinates of all the atoms in the vicinity, and the effect is larger for β-glycosides. A longer C1'···C4 distance strongly suggests that β-thioglycosides are more flexible than the α-form. This feature was explained by a reduced *endo*- and *exo*-anomeric effect. Although the *exo*-anomeric effect is weakened, it still governs the specific orientation preference of the aglycon. This finding contradicts to the earlier steric effect argument.



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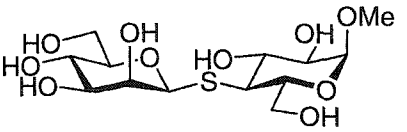

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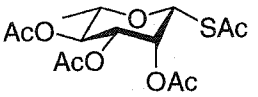

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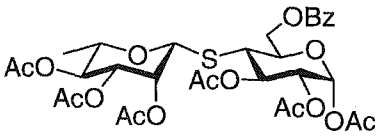
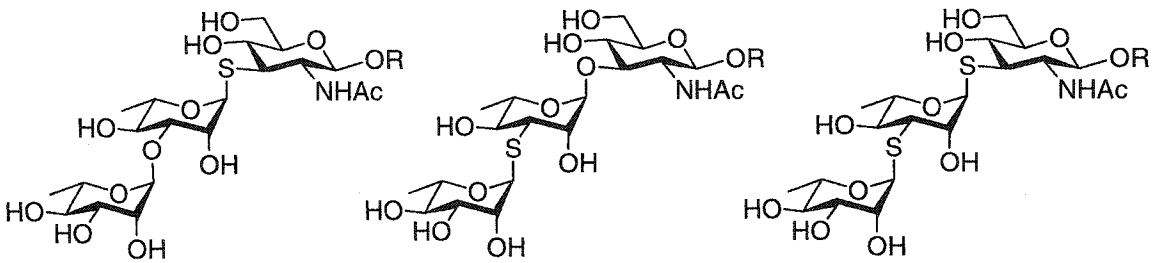


42a

Three analogs of the trisaccharide epitope (Rha-Rha-GlcNAc) from the lipopolysaccharide (LPS) antigen of *Shigella flexneri* variant Y, in which we sequentially replaced by sulfur at each glycosidic linkage, were synthesized to address two fundamental issues in the field of synthetic carbohydrate vaccines: It was shown that the inhibitory power of *S*-linked oligosaccharides is generally significantly reduced compared to corresponding *O*-linked analogs, secondly, antibody produced against glycoconjugates of glycosidase resistant thio-analogs induce a strong immune response and the antibodies cross-react with the natural *O*-linked oligosaccharides.



R = Tether for covalent attachment to protein

Acknowledgements

I am most grateful to my supervisor, Professor David R. Bundle for his expert guidance, generosity, support, and patience. To put it simply, if I had to choose a supervisor for Ph. D studies again, I would certainly join his group one more time.

I would like to thank our "walking synthesis encyclopedia" Dr. Chang-Chun Ling. Without his help, none of this work would have been possible. Dr. Ping Zhang, who has been sharing her superior organic synthesis knowledge and techniques, is greatly appreciated. I am very grateful to Joanna Sadowska, for not only her expertise in ELISA, but also the affection that keeps us in a big warm "Bundle family". I would also like to thank all of the other members of the Bundle group over the years: Lesley Liu (especially for her friendship and proof reading ability), Dr. Soren Anderson, Dr. Bernd Becker, Dr. Stephane Chambert, Dr. Rodney Gagné, Dr. Pavel Kitov, Dr. Elena Kitova, Scott McGavin, Dr. Mark Nitz, Corwin Nycholat, Dr. Eugenia Paszkiewicz, Jamie Rich, Dr. Amanda Seago, Dr. Shirley Wacowich-Sgarbi, Dean Williams, Hongmei Shang.

I sincerely thank Dr. Robert McDonald and Dr. Michael J. Ferguson for solving those beautiful crystal structures and sharing their expertise on X-ray crystallography. I gratefully acknowledge Dr. Albin Otter for his expert NMR guidance and Dr. Angelina Morales for her professional cooperation and enthusiasm. Lynne Lechelt's excellent organization skills will never be forgotten.

Outside the group I would like to thank: Dr. Yuming Zhao, whose different chemistry perspective never fail to amaze me; Dr. Xiangping Qian, who helped and encouraged me especially in the early stages of my Ph. D studies; Dr. Steve Trepanier, who has shown me many fun things to do besides chemistry.

Finally, I'd like to give a big thanks to my wife, Lan, for her continuous love, support and understanding during my studies. Our little Annie, who came into our life about two and half years ago, has brought endless joy and opened a brand new page of our life.

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List of Abbreviations

Ac	acetyl
Bn	benzyl
t-Boc	*t*-butoxycarbonyl
t-Bu	*t*-butyl
BSA	bovine serum albumin
Bz	benzoyl
2D	two dimensional
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMTST	dimethyl(methylthio)sulfonium triflate
ELISA	enzyme-linked immunosorbent assay
ES HRMS	electrospray high resolution mass spectrometry
Et	ethyl
EXSIDE	excitation-sculptured indirect-detection
Fab	antigen-binding fragment
GCOSY	gradient coupling correlated spectroscopy
Glc	glucose
GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-glucose
HMQC	heteronuclear multiple quantum coherence
HPLC	high pressure liquid chromatography
HRP	horseradish peroxidase
IC_{50}	inhibitor concentration required to give 50% inhibition
IUPAC	International Union of Pure and Applied Chemistry
Mab	monoclonal antibody
MALDI-TOF	matrix assistant laser desorption ionization time of flight
Man	mannose
Me	methyl

M.S.	molecular sieves
NIS	*N*-iodosuccinamide
NOE	nuclear overhauser effect
NMR	nuclear magnetic resonance
n-Pr	*n*-propyl
PBS	phosphate buffer saline
PBST	phosphate buffer saline containing Tween 20
Pent	Pentenyl
Ph	phenyl
ppm	parts per million
Pyr	pyridine
ROE	rotating-frame
R_f	retention factor
Rha	L-rhamnose, 6-deoxy-L-mannose
S_N2	bimolecular nucleophilic substitution
TBDPS	*t*-butyldiphenylsilyl
TEA	triethylamine
Tf	trifluoromethanesulphonate
THF	tetrahydrofuran
TLC	thin layer chromatography
T-ROESY	transverse rotating-frame nuclear Overhauser effect
UV	ultraviolet

Chapter 1

Introduction

Thio-oligosaccharides and their Synthesis

1.1 Introductory remarks

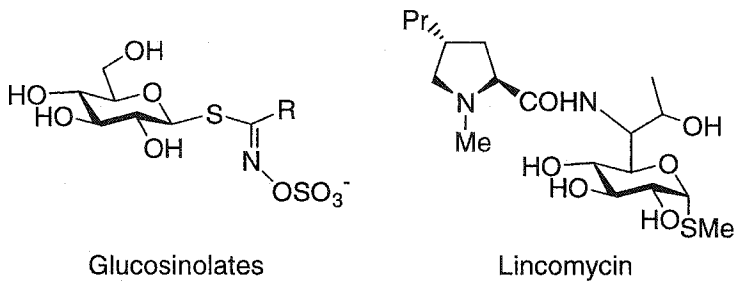
Carbohydrates are ubiquitous and important biomolecules. Besides their role in energy storage, they form much of the structural framework of cells and tissues. As part of glycoproteins, glycolipids, and other conjugates, they are key elements in a variety of processes such as signaling, cell-cell communication, and molecular and cellular targeting.[1,2] Detailed knowledge of carbohydrate-protein interactions is crucial in the understanding of these processes, which consequently pave the way for the development of carbohydrate-based therapeutics as well as prophylactic drugs and vaccines.

One of the versatile approaches used to probe carbohydrate-protein interactions is to apply modified carbohydrates as substrates or ligands. This was first demonstrated by Lemieux's "chemical mapping"strategies[3] and then by several laboratories.[4] Recently, the use of non-hydrolysable oligosaccharide mimics has gained much attention. This can be achieved, in most respects, by substituting the natural glycosidic linkage (O) with a methylene group (CH_2)[5], nitrogen (NR)[6] or sulfur atom (S).[7] The last class of compounds are named thio-oligosaccharides, and their synthesis is the focus of this chapter.

1.2 Natural thio-oligosaccharides

Oligosaccharides that contain a thio-glycosidic bond are not very common in nature. The only examples found are the glucosinolates (β-thioglucoside-*N*-

hydroxysulfates)[8] and the simple alkyl thio-glycosides of Lincomycin and structurally related antibiotics found in *Streptomyces* species[9,10,11] (Figure 1.1).



Glucosinolates Lincomycin

Figure 1.1. The structure of glucosinolates and Lincomycin

1.3 Synthesis of thio-oligosaccharides

1.3a Introduction

Three general strategies can be envisioned in the synthesis of thio-oligosaccharides (Figure 1.2). The first two strategies take advantage of the nucleophilicity of the sulfur atom in the reaction of a thiolate with an electrophilic donor. The donor is armed with a leaving group, such as bromide, chloride, iodide, or sulfonate.[12,13] A 1,6-anhydro saccharide has also been used as glycosyl donor.[14] Michael addition of thiolates to α,β-unsaturated system,[15] as well as epoxide[16] and aziridine[17] ring opening have been applied as alternatives. The third strategy for the synthesis of thio-linked oligosaccharides is the Lewis acid catalyzed condensation of a glycosyl acceptor containing an SH group with a suitable glycosyl donor. The trichloroacetimidate method is usually the method of choice. In principle, all three strategies can produce any of the four possible classes of products: 1,2-*trans*-α, 1,2-*trans*-β, 1,2-*cis*-α, 1,2-*cis*-β, which are conveniently divided based on the stereochemistry at C-1 and its relationship to the functional group at C-2 of the sugar ring (Figure 1.3). Analogous to the synthesis of the

corresponding *O*-linked oligosaccharides, stereoselective formation of *trans* linkages is most easily achieved, and 1,2-trans-β thioglycosides are the most abundant in the literature.[18] Literature syntheses of the 1,2-cis-α linkage are less numerous. The 1,2-cis-β linkage is the most difficult to prepare. In fact, there is only one literature procedure available for the preparation of 1-thio-β-*D*-mannosides.[19] No procedure has been reported for the synthesis of 1-thio-β-*L*-rhamnosides, which also have a 1,2-cis-β linkage.

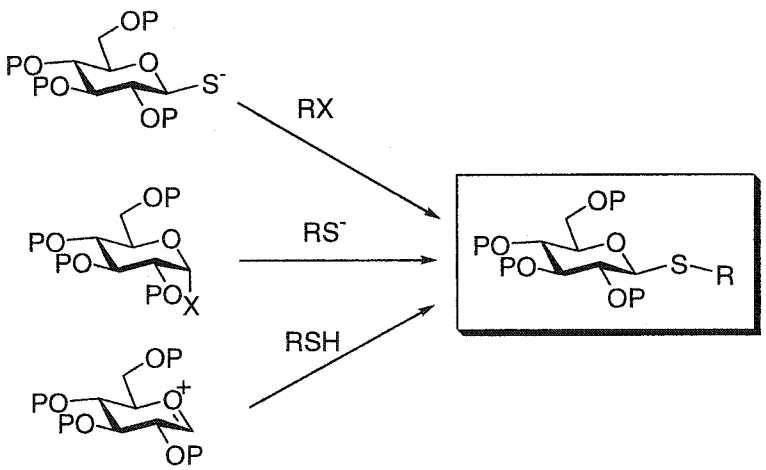


Figure 1.2. General strategies for the synthesis of thio-oligosaccharides

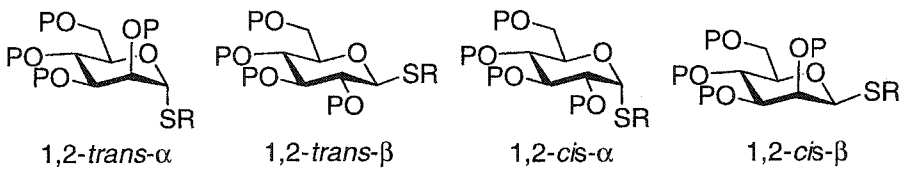


Figure 1.3. Classification of thio-glycosidic linkages

General problems associated with the synthesis of thio-glycosidic bonds, as compared to the formation of O-glycosides, are (a) the easy formation of disulfides from thiols, (b) the exclusion of glycosylation methods using thiophilic promoters, (c) the

incompatibility with methods frequently used to manipulate sugar protecting groups. For example, catalytic hydrogenolysis of benzyl ethers protecting groups can be problematic, although Birch reduction has been used as an alternative.[20]

1.3b Formation of 1-thioglycoses

1-thioglycoses (anomeric thiols) are the most widely used synthons for the synthesis of thio-oligosaccharides. Their synthesis usually employs S_N2 displacement of glycosyl halides with thiolates (Figure 1.4).[21,22] Mild acid catalyzed glycosidations of peracetylated sugars[23], Fisher type glycosylation[16], and radical addition to glycals with thioacetic acid[24] have also been applied (Figure 1.5). More recently, Lawesson's reagent was employed to prepare 2-acetamido-2-deoxy-2,3,4-tri-*O*-acetyl-1-thio-α-*D*-galactopyranose in excellent yield.[25] Glycosidation of the glycosyl fluoride[26] donor or trichloroacetimidate[27] provides alternative routes to its corresponding 1-thioglycoses (Figure 1.6).

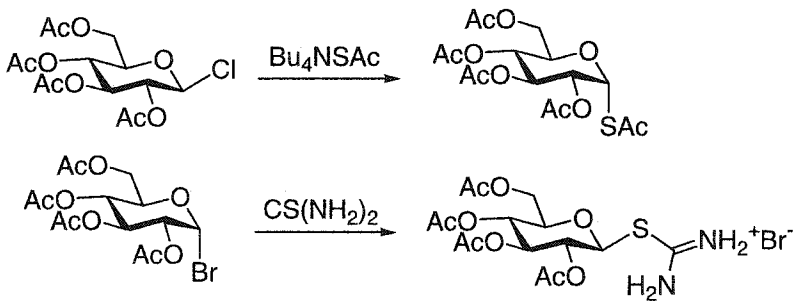


Figure 1.4. Synthesis of 1-thioglycoses by S_N2 reaction

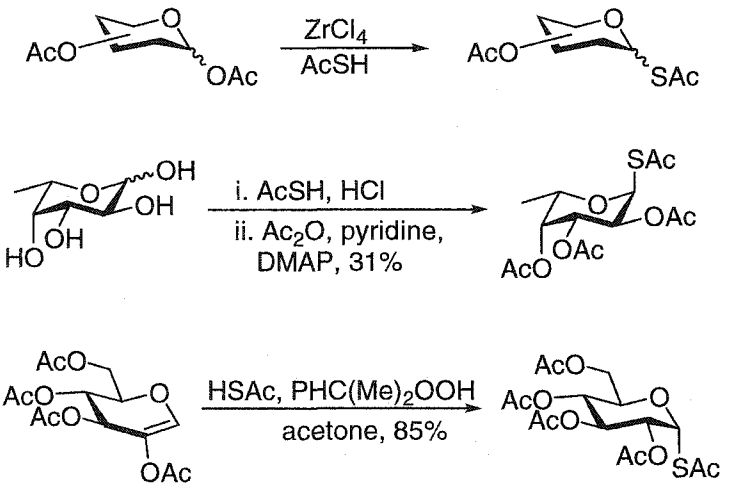


Figure 1.5. Acid catalyzed and radical reactions for the synthesis of 1-thioglycoses

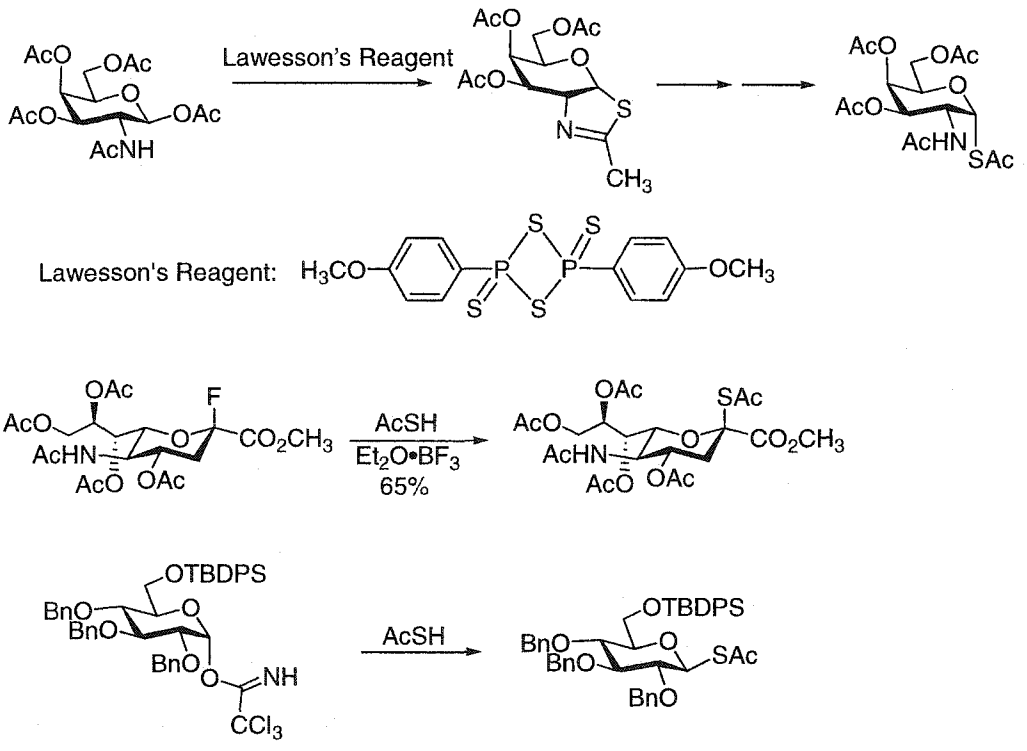


Figure 1.6. Recent methods for the synthesis of 1-thioglycoses

Synthetic 1-thioglycoses are prepared almost exclusively in the form of thioacetates or thiourea salts to avoid disulfide formation; and have been used as intermediates in the synthesis of thio-oligosaccharides. Selective de-*S*-acetylation of the peracetylated 1-thioglycoses can be done either in a two-step procedure or *in situ* (Figure 1.7). There are several methods available. The classical one is demercuration of phenylmercury thioglycoses.[28] Sodium methoxide at temperatures below -40 °C sometimes works.[29,30] A more preferential chemoselective deprotection has been achieved by the action of diethylamine in DMF[31], cysteamine in acetonitrile[32], or hydrazine acetate in DMF.[33] Recently, a highly hindered iminophosporane base BEMP (Schwesinger base) was used *in situ* for direct alkylation coupling.[34] This non-nucleophilic base avoids elimination reactions of the donor and thus increases the reaction yield.

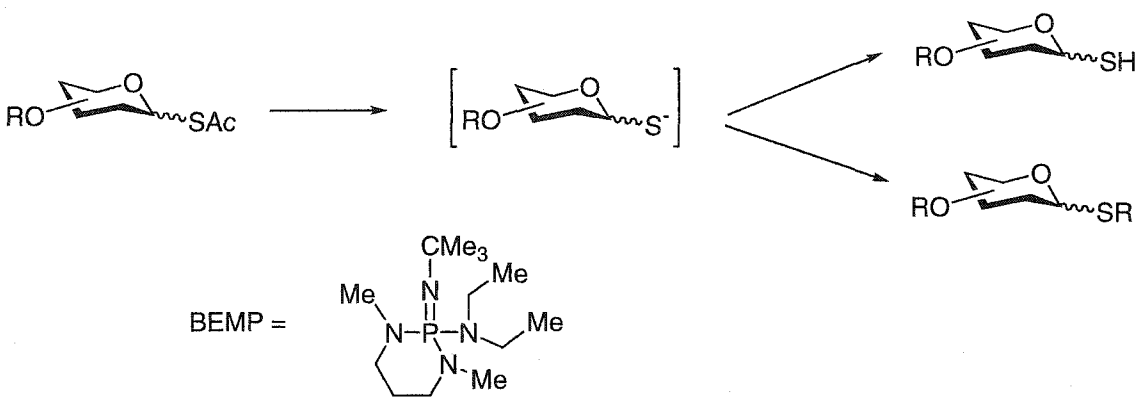


Figure 1.7. Activation of 1-thioglycoses and the structure of BEMP

1.3c Synthesis of the 1,2-*trans*-α and β linkages

1,2-*trans*-α and β linkages can be formed by either S_N2 reaction or Lewis acid catalyzed glycosylation. The thio functionality is introduced first to yield an anomeric thiol or thiolate in the desired configuration, which is then reacted with a sugar electrophile to form the inter-*S*-glycosidic bond through a S_N2 displacement reaction. A reciprocal approach has been undertaken by installing a non-anomeric thiol in the acceptor to act as the nucleophile in the opening of the manno-1,2-epoxide.[35] Trichloroacetimidates function well as donors with saccharide thiol acceptors to yield either 1,2-*trans*-α or β linkages depending on the configuration of the acceptors and the conditions used.[13,36,37] General approaches are shown in Figure 1.8 and 1.9 for establishing 1,2-*trans*-α and 1,2-*trans*-β thio-linkages respectively. Recently, the cyclic sulfate group has been targeted as a means of establishing thio linkages in good yield (Figure 1.9).[13] Application of Michael addition of sugar thiols to levoglucosenone resulted in the synthesis of the thio-oligosaccharide containing anhydrides[15,38] A more traditional approach has been chosen by Pinto and Andrews[39] toward establishing 1,2-*trans*-β thio-linkages (Figure 1.9). Interestingly, a stable orthoester was formed at low temperature (-40 °C), and hydrolysis of the orthoester at 0 °C gave β-linked disaccharides as the major product; in contrast, the coupling of the trichloroacetimidates donor with the acceptor thiol at room temperature gave no anomeric selectivity. More recently, the same group has developed an elegant 1→2 intramolecular thioglycosyl migration method to synthesize thio-linked disaccharides (α/β ratio, 1/3) (Figure 1.10).[40] An oxocarbenium ion is postulated to react with non-hindered alcohols (i.e. MeOH) to produce α/β

glycoside mixtures in proportions partially governed by the relative steric hindrance to attack from either the α- or β- face.

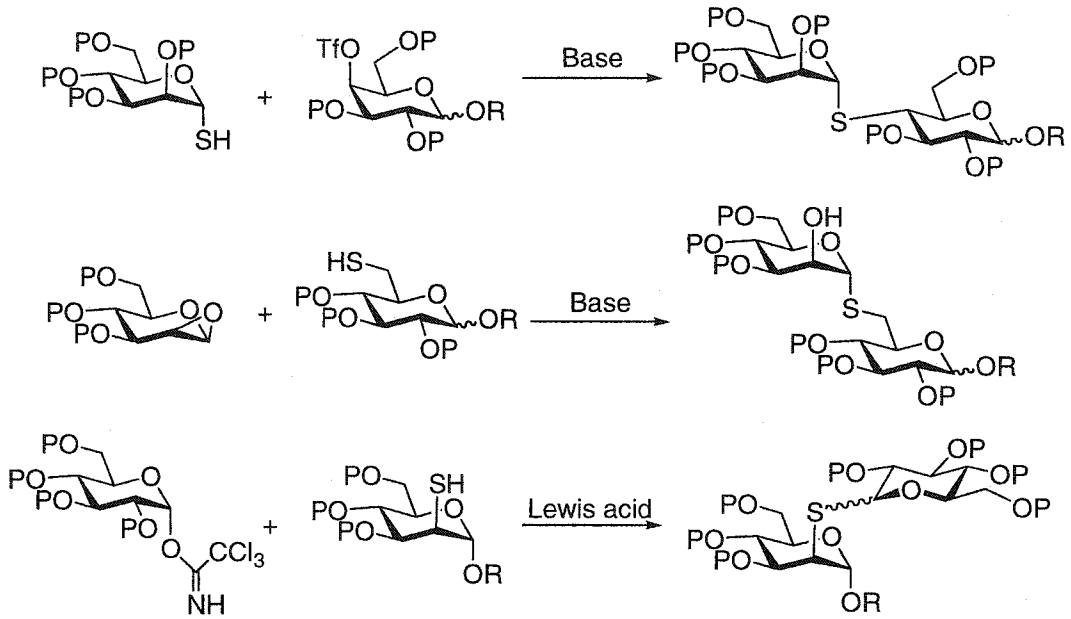


Figure 1.8. General approaches to form 1,2-*trans*-α linkage

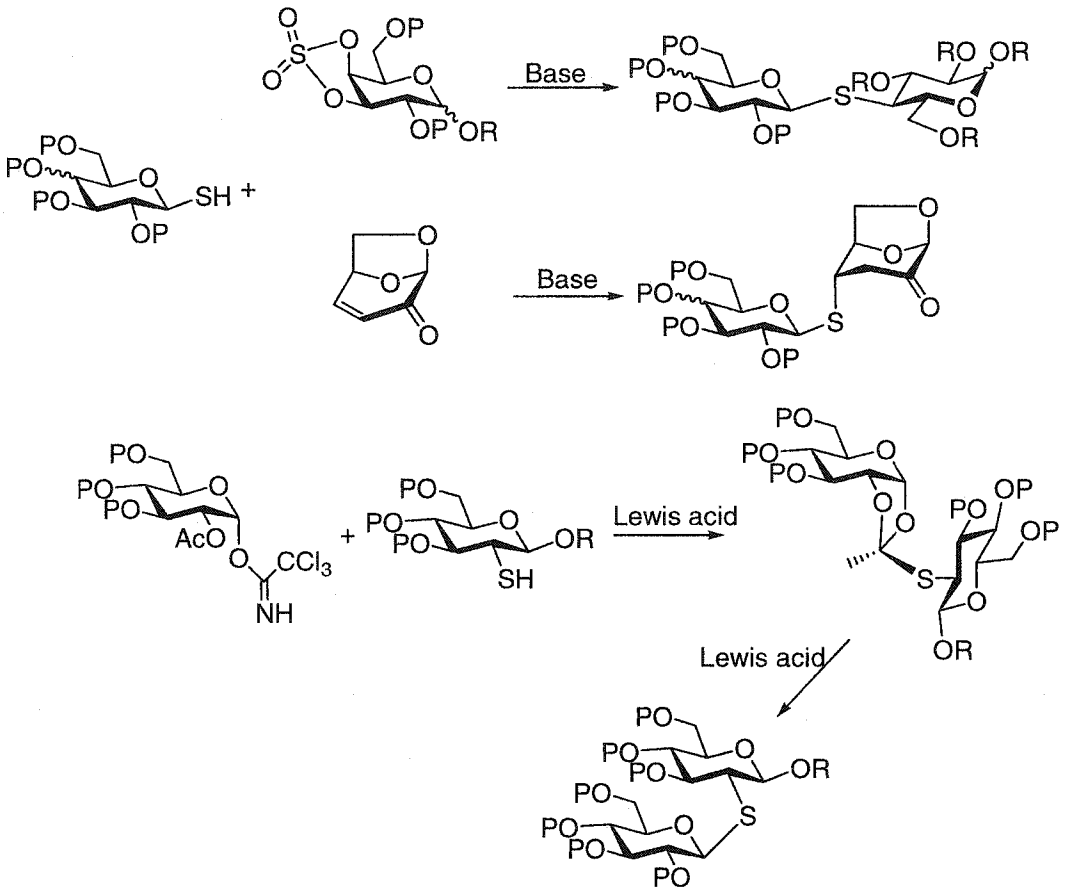


Figure 1.9. General approaches to form 1,2-*trans*-β thio-linkages

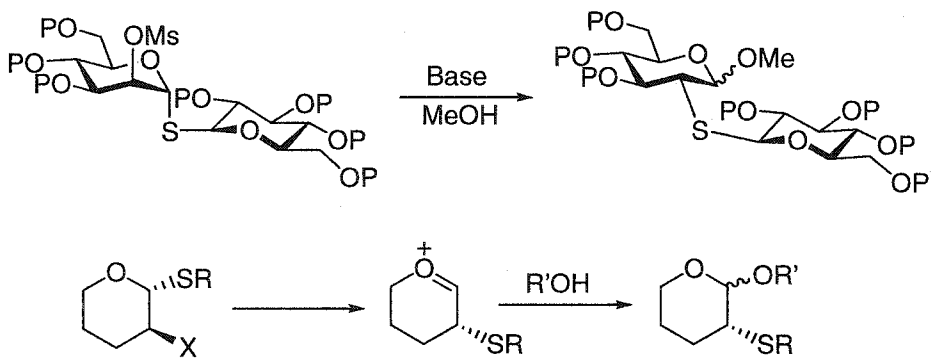


Figure 1.10. 1→2 Intramolecular thioglycosyl migration method and the postulated mechanism

1.3d Formation of the 1,2-*cis*-α linkages

Formation of 1,2-*cis*-α linkages has been accomplished most frequently by the direct alkylation approach: either by using the anomeric thiol to carry out a S_N2 displacement reaction[41,42], or employing a donor glycosyl halide as electrophile to condense with a thiolate.[43] The yields range from 40 to 85%, and the low yields are usually the result of competing elimination reactions. This can be avoided by using the iminophosporane base (BEMP) discussed above. Another strategy has been to couple the furanose form of the electrophile, which is less likely to eliminate, and subsequently to convert it to the desired pyranoside (Figure 1.11).[44]

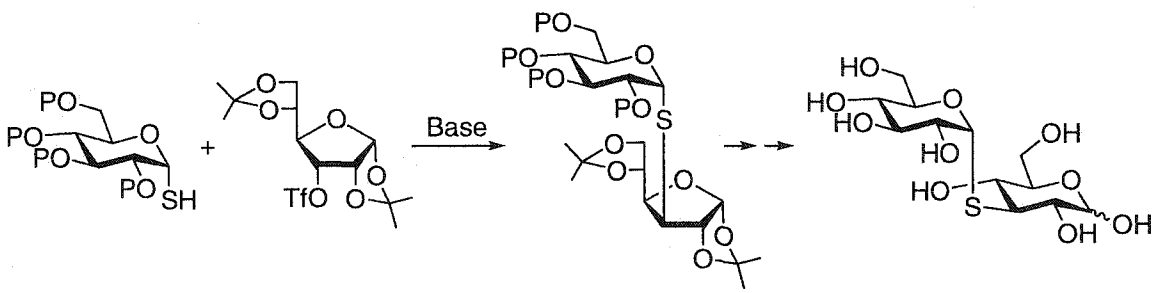


Figure 1.11. Using furanose electrophile in S_N2 coupling to avoid competing elimination

1.3e Establishment of 1,2-*cis*-β linkages

The final class of compounds, 1,2-*cis*-β thio-linked glycosides, is the least explored. This class of compounds includes *S*-linked analogs of the β-*D*-mannopyranosides and β-*L*-rhamnopyranosides. Although an enormous effort has been applied to the synthesis of *O*-linked β-*D*-mannopyranosides and β-*L*-rhamnopyranosides, their synthesis remains one of the most challenging to carbohydrate chemists.[45] While

there are no literature procedures for the synthesis of 1-thio-β-*L*-rhanmopyranosides, Crich reported the synthesis of 1-thio-β-mannopyranosides: a glycosylation approach involving reaction of the anomeric sulfoxide, 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thia-α-*D*-mannopyranoside *S*-oxide, with acceptors containing a thiol group under activation by triflic anhydride.[19] This method allowed the stereoselective preparation of several 1-thio-β-manno-disaccharides in 61-69% yield. The proposed reaction mechanism is shown in Figure 1.12. Triflic anhydride serves to activate the donor as a sulfonium salt. This collapses immediately to the sulfenyl triflate and the oxocarbenium ion. The latter species was trapped by the triflate anion to give the α-mannosyl triflate. On subsequent addition of the glycosyl acceptor, the thiol participates in an S_N2-like reaction with the formation of the β-thiomannosides.

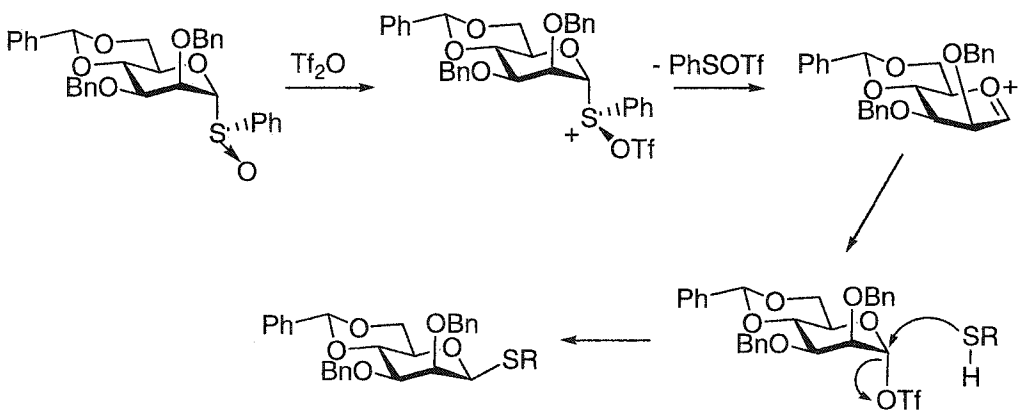


Figure 1.12. Synthesis of 1-thio-β-*D*-mannopyranosides

1.4 Solid-phase and chemoenzymatic synthesis of thio-oligosaccharides

Solid-phase synthesis of thio-oligosaccharides in excellent yields has been described by Hummel and Hindsgaul.[46] Unprotected monosaccharides were attached to a

resin via their primary hydroxyl group. Activation through the formation of the sodium thiolate and subsequent reaction with a sugar triflate gave thiodisaccharides, which can be cleaved from the resin by the treatment with TFA. If the electrophile contains an anomeric ethyl disulfide group, the reaction sequence can be repeated to give thio-oligosaccharides (Figure 1.13). A chemoenzymatic synthesis of regioselectively substituted cyclodextrins has also been reported.[47,48] In this fashion, the self condensation of 4-thio-α-maltosyl fluoride in the presence of pure GGTases has afforded the hemithiocellodextrins (n = 4-6) (Figure 1.14).

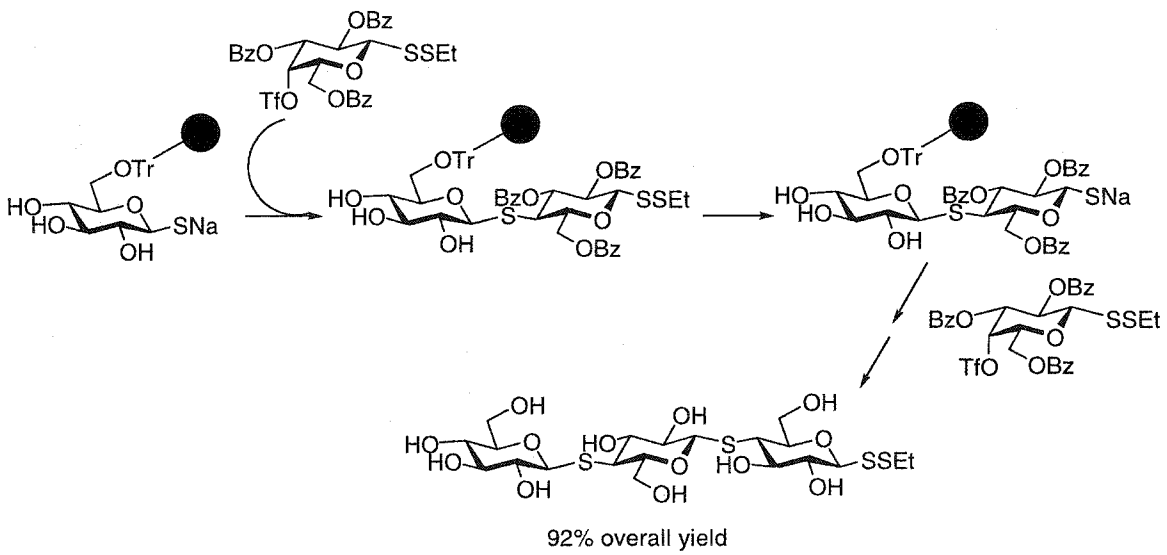


Figure 1.13. Solid-phase synthesis of a thio-linked trisaccharide.

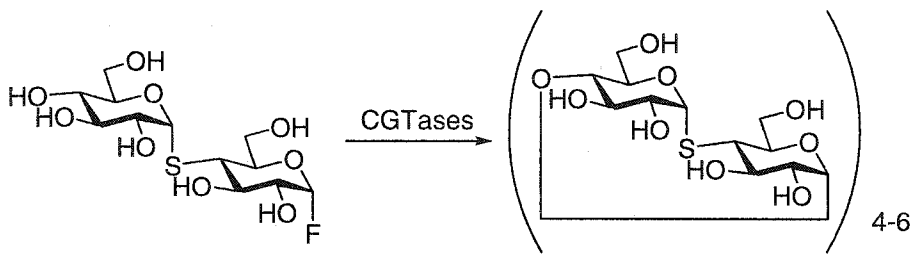


Figure 1.14. Chemoenzymatic synthesis of hemithiocellodextrins (n = 4-6)

1.5 Scope of projects

Several aspects of the synthetic chemistry of thio-glycosidic linkage formation have been addressed, and the target molecules were used to explore the role of thio-glycosidic linkages in molecular recognition involving the immune system.

A new methodology for the formation of the synthetically challenging 1,2-cis-β-thio-glycosidic linkages has been developed, and employed for the synthesis of a series of biologically interesting 1-thio-β-mannopyranosides and 1-thio-β-rhamnopyranosides.

Natural *O*-linked oligosaccharides populate a range of three-dimensional conformations, which are critical in protein-carbohydrate interactions. For the most part these conformations are determined by the accessible range of the two glycosidic torsional angles, ϕ and ψ. When considering the ability of thio-linked oligosaccharides to mimic their *O*-linked counterparts, it is important to compare the populated conformational families sampled by each linkage type. This can be achieved by a combination of X-ray crystallography, NMR analysis, and molecular modelling. Unfortunately, due to the difficulties in obtaining crystalline samples of many oligosaccharides, including thio-linked oligosaccharides, the X-ray structure of only one unprotected thio-linked disaccharide, methyl α-*D*-thio-maltoside crystalline has been reported.[49] In the work reported in this thesis we describe X-ray crystallographic and NMR data of a deprotected 1,2-*cis*-β-thio-linked disaccharide and three other protected thioglycosides with a similar linkage. These results will be discussed in Chapters 4 and 5.

Three analogs of a natural trisaccharide (Rha-Rha-GlcNAc), related to the lipopolysaccharide (LPS) antigen of *Shigella flexneri* variant Y, in which we sequentially

replaced the oxygen atom by sulfur at each glycosidic linkage, were synthesized to address two fundamental issues in the field of synthetic vaccines: Firstly, using a crystallographically defined antibody, the inhibitory power of *S*-linked oligosaccharides is compared to *O*-linked analogs; and secondly, we address the question of whether antibody produced against glycosidase resistant thio-analogs will bind to the natural *O*-linked oligosaccharides.

Chapter 2

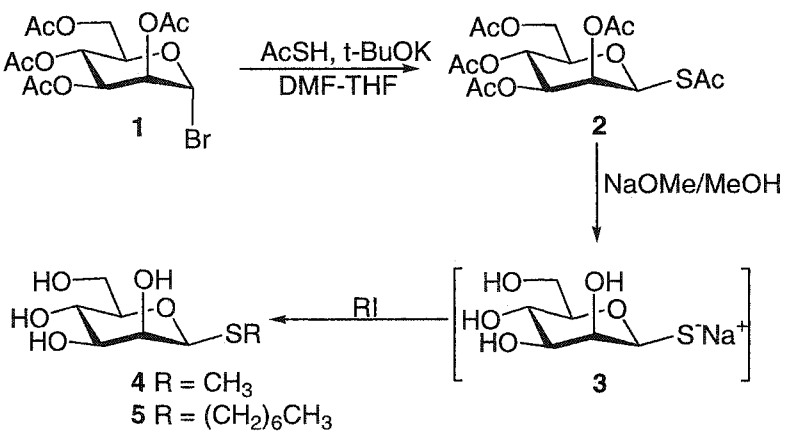
Studies on the efficient and stereoselective synthesis of 1-thio-β-D-mannopyranosides

2.1 Introduction

The examples of stereoselective synthesis of 1-thio-β-mannopyranosides in the literature are limited, and the few examples reported have been limited to simple aglycons such as thiophenyl or its analogs.[50,51] In 1984, Defaye *et al.*[52] prepared 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose using an S_N2 approach by reacting 2,3,4,6-tetra-*O*-acetyl-α-*D*-manopyranosyl bromide with tetrabutylammonium thioacetate in toluene. More recently, Crich's group[19] reported a glycosylation approach by reacting a rather elaborate anomeric sulfoxide, namely 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thia-α-*D*-mannopyranoside *S*-oxide, with acceptors containing a thiol group, under the activation of triflic anhydride (Figure1.12, Chapter 1). Typically, this method allowed the stereoselective preparations of two 1-thio-β-mannopyranosides bearing simple alkyl aglycons in 74–77% yield, and several more elaborate 1-thio-β-manno-disaccharides in 61-69% yield. These developments in the synthesis of thioglycosides prompted us to explore the potential of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose as a convenient starting material in the stereoselective synthesis of 1-thio-β-mannopyranosides.

2.2 Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-mannopyranose

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose (**2**) was prepared by a simple nucleophilic displacement of the anomeric α-bromide of 2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyanosyl bromide (**1**) with thioacetate. In contrast to Defaye's procedure, we found the reaction worked equally well using the cheaper potassium thioacetate. The reaction was carried out in DMF and the potassium thioacetate was prepared *in-situ* by mixing thioacetic acid and potassium *tert*-butoxide, leading to exclusive formation of the 1-thio-β-mannopyranoside **2** (Scheme 2.1). The reaction can be easily scaled and compound **2** was obtained in 79% isolated yield on a 30 g scale, following chromatography, or in 63% yield by crystallization.



Scheme 2.1. Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose

The β-anomeric configuration of **2** was unambiguously established by different NMR experiments. As a general rule, the homonuclear three bond coupling constants ($J_{1,2}$) in 1-thio-β-mannopyranosides are smaller than 1.3 Hz and the heteronuclear one

bond coupling constant ($J_{C1,H1}$) at the anomeric center ranges from 148 to 160 Hz; in addition, TROESY experiments should show dipolar coupling between H-1, H-3 and H-5 within the same pyranose ring, while the corresponding 1-thio-α-mannopyranosides show no such interactions. In fact, for compound **2**, a 1D-TROESY experiment showed strong NOEs between H-1, H-3 and H-5 and a 2D-HMQC experiment confirmed the one bond heteronuclear coupling constant, $^1J_{CH}$ for C-1 to be 155.6 Hz.[53]

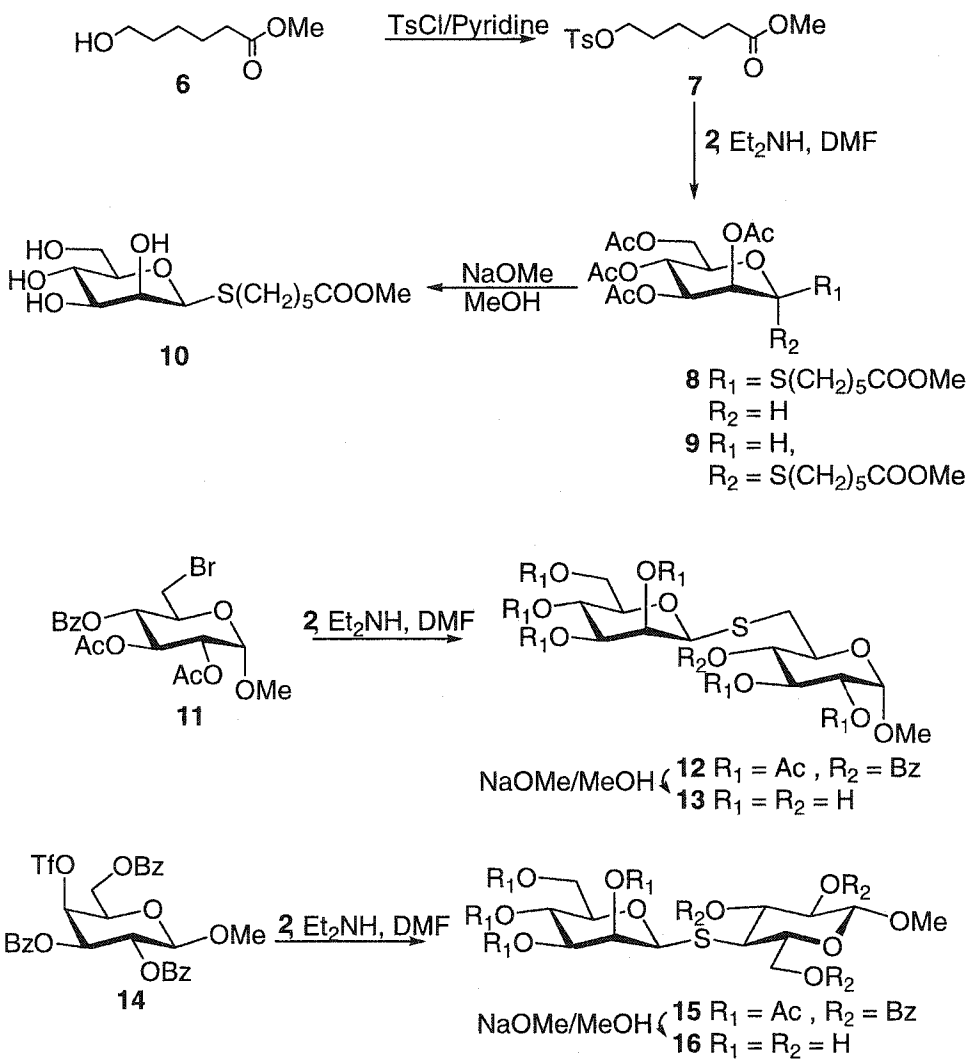
It is well known that some of the fully deprotected glycosyl 1-thiolates are stable, and anomerizations are so slow that in the case of 1-thio-β-*D*-glucopyranose and 1-thio-β-*D*-galactopyranose, the sodium salts can even be purchased from commercial sources. The salts can often be used to conduct S_N2 glycosylations and the corresponding thioglycosides can be obtained in pure anomeric form. In this context, we were interested in determining the stability of the fully deprotected β-manno-1-thiolate **3**. This was carried out by periodically recording the NMR spectrum of a sample of compound **2** in CD_3ONa/CD_3OD. The NMR experiment clearly showed that compound **3** was relatively stable, and that anomerization to the α-thiolate proceeded at a very slow pace, such that after three days in this basic solution, almost 90% of the compound remained as the β-anomer. Therefore, by exploiting the high nucleophilicity of thiolate **3** addition of simple alkyl halides to the solution should trap the corresponding 1-thio-β-mannosides to give the corresponding alkyl glycoside in high yield. In fact, the methyl (**4**) and heptyl (**5**) glycosides were both efficiently synthesized in excellent yield (→ **4**, 91% and → **5**, 97%) by adding iodomethane or iodoheptane directly to a solution of **2** in CH_3ONa/CH_3OH. No 1-thio-α-mannopyranosides were detected.

2.3 Synthesis of β-thiomannosides with thioacetate 2 as starting material

Glycosides bearing the ω-(methoxycarbonyl)octyl group (often called the "Lemieux tether") are widely used in the preparation of various carbohydrate conjugates. We were interested in synthesizing a 1-thio-β-mannopyranoside bearing this tether group, or more accurately its six-carbon counterpart. Thus 5-(methoxycarbonyl)pentanol (**6**) was treated with *p*-toluenesulfonyl chloride in pyridine to afford the corresponding sulfonate ester (**7**) (75%) (Scheme 2.2). In their thiosialoside synthesis, von Itzstein and co-workers[9] reported a convenient procedure for the *in-situ* selective deprotection of the *S*-acetate in the presence of *O*-acetates *via* reaction with diethylamine in DMF; the intermediate thiol can then be used directly for attacking a sugar triflate to afford the corresponding thioglycoside. We applied this procedure to prepare compound **8**. At room temperature the reaction proceeded rapidly, however a 1:1 α/β mixture of glycoside was obtained. When the reaction was carried out at lower temperatures, a longer reaction time was needed to reach completion, however, the ratio of the β-mannopyranoside improved. The optimum result was obtained when the reaction was carried out at –55 °C, and under these conditions the reaction was complete after 48 h and compound **8** was obtained in 64% yield along with its α-isomer **9** in 21% yield. The relatively low β/α selectivity may be due to the leaving group properties of tosylate in compound **7**. The β-mannoside **9** was smoothly deprotected by Zemplen methanolysis (→ **10**, 91% yield).

Encouraged by the initial success in synthesizing 1-thio-β-mannopyranosides bearing simple aglycons, we proceeded to explore the generality for the preparation of more complicated oligosaccharides. The preparation of a 1,6 linked disaccharide was the first choice. The easily accessible 6-bromo-glucopyranoside **11** was chosen. When the

reaction between **2** and **11** was carried out at –15 °C under conditions reported by von Itzstein,[31] the expected disaccharide **12** was obtained in 74% yield with excellent β-selectivity (α:β, 1:15 judged from NMR). However, at higher temperature, poor β-selectivity was observed. Deprotection of **12** was conducted under standard Zemplen transesterification conditions, and the fully deprotected disaccharide **13** was obtained pure and in excellent yield (92%) after reverse phase chromatography.



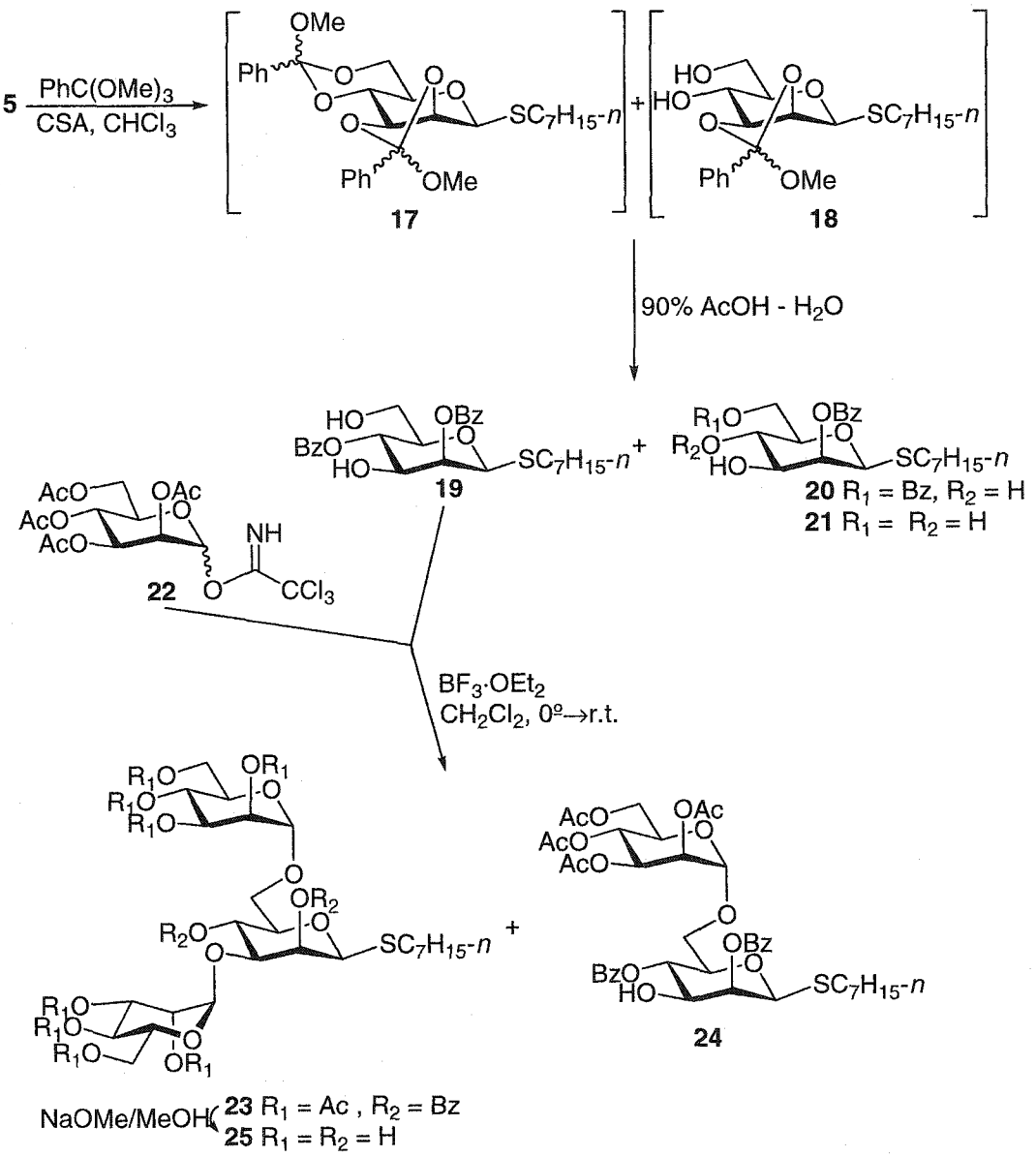
Scheme 2.2. Synthesis of β-thiomannosides

This success encouraged us to attempt the synthesis of the 1,4 linked disaccharide **15**. Here we employed a galactosyl triflate **14**[54] as starting material; the reaction with **2** gave **15** in 80 % yield. The reaction was carried out at –5 °C and the β/α ratio was 19:1 as judged by NMR. A similar deprotection step as described above was conducted to afford disaccharide **16** in 93% yield after reverse phase chromatography on C18 silica gel.

2.4 Efficient synthesis of a thio analog of the core mannotrioside structure

With compound **5** in hand, we prepared the biologically relevant structure **25** (Scheme 2.3) – an analog of the core mannotrioside structure located in the glycan chains of all *N*-linked glycoproteins. In this structure, a β-mannopyranoside is branched at both the 3 and 6 positions by α-mannopyranoside residues. The synthesis of the *O*-linked analogs were reported by Kaur *et al.*[55] and recently by Lichtenthaler *et al.*.[56] In the former case, the *O*-linked β-mannopyranoside was synthesized from 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-*D*-mannopyranosyl bromide in 27% yield, whilst in the latter case, the authors had to create the *O*-linked β-mannopyranoside in a long oxidation-reduction sequence starting from a glucose derivative. In our hands, since **5** can be easily synthesized in large quantity and in pure form, we used it as starting material. Thus, under camphor sulfonic acid catalysis, two moles of trimethyl orthobenzoate reacted with **5** to afford mainly intermediate **17**[57], plus the mono-orthoester **18**. Without purification, the mixture of intermediates **17** and **18** was subsequently treated with 90% acetic acid – water in order to open the 2,3 and 4,6 orthoesters. The 2,3-orthoester opened in a highly regioselective fashion to give the 2-benzoate almost exclusively, whilst the 4,6-orthoester opened in a less regioselective manner to give both the 4-benzoate and 6-benzoate. Thus hydrolysis

of **17** afforded the desired 2,4-dibenzoylated **19** (32%) and the undesired 2,6-dibenzoylated **20** (40%) while the hydrolysis of **18** lead only to monobenzoylated mannoside **21** (13%). Although not of high yield, this approach to **19** was efficient since it only involved a single purification step, and a readily available starting material **5**. Compounds **20** and **21** can be recycled to regenerate the starting material **5**. The two α-mannopyranosyl units at the 3 and 6 positions were subsequently installed after a glycosylation step with imidate **22**[58] under catalysis by boron trifluoride etherate. The desired trimannoside **23** was obtained in 51% yield together with the 1,6 linked disaccharide **24** (48%). Trisaccharide **23** was fully deprotected by methanolysis to give the free trimannoside **25** in 91% yield.



Scheme 2.3. Synthesis the thio analog of the core mannotrioside structure

2.5 Conclusion

We have developed an efficient route for the preparation of 1-thio-β-mannopyranosides using the easily accessible 2,3,4,6-tetra-O-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose **2** as starting material. The synthesis can generally be carried out in high yield and with a high degree of stereoselectivity. Considering the significant

difficulties in preparing the *O*-linked β-mannopyranosides, this route offers an alternative to the design and synthesis of carbohydrate analogs containing a β-mannoside linkage. As reported in the following chapter, the method has been extended to the synthesis of 1-thio-β-rhamnopyranosides.

Chapter 3

Studies on stereoselective establishment of the 1-thio-β-L-rhamnopyranosyl linkage

3.1 Introduction

Rhamnose is a ubiquitous constituent of bacterial polysaccharide antigens, and although the occurrence of the α-pyranose form is more frequent, *O*-linked β-L-rhamnopyranosides exist in several bacterial capsular polysaccharides. For example, capsular polysaccharides of *Streptococcus pneumoniae* [59,60,61], the causative agent of pneumonococcal meningitis, and otitis media[62], as well as several other bacterial strains[63,64] contain the 1,2-*cis*-β rhamnopyranosyl linkage. Notwithstanding numerous attempts to synthesize oligosaccharides containing this linkage, its stereospecific synthesis remains one of the most challenging targets in carbohydrate chemistry.

While numerous methodologies for the synthesis of β-mannopyranosides have been reported in the literature[45,65], practical synthetic routes to the related β-*L*-rhamnopyranosides have been confined to Koenigs-Knorr methods[66], intermolecular S_N2 reaction via 1,2-*O*-*cis*-stannylene acetals[67] and intramolecular glycosylation[68]. These methods either suffer from low yield, involve toxic metal, or are complicated by lengthy manipulation before coupling glycosyl units. The two popular routes to β-*D*-mannopyranosides use either a 2-ulosyl bromide as the glycosyl donor with subsequent stereoselective reduction of the ketone, or S_N2-like displacement of α-glycosyl triflates recently developed by Crich. Rigidity of the pyranose ring is a central requirement of the

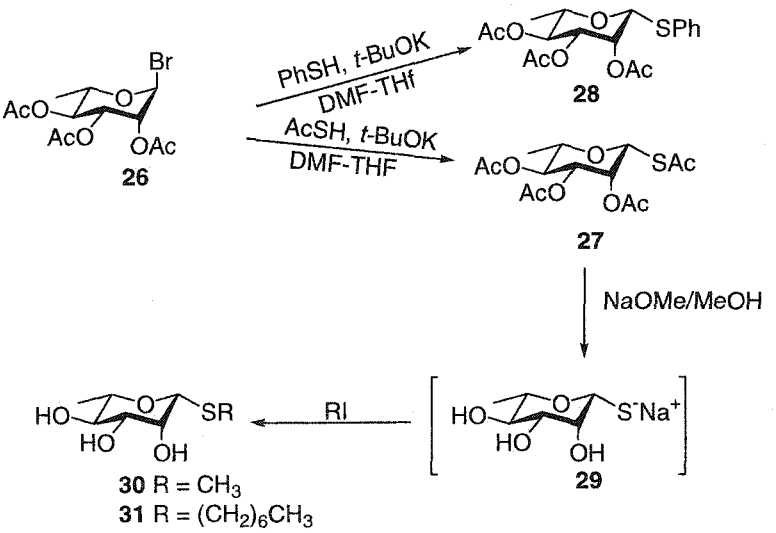
latter methodology and is accomplished by locking the pyranose ring via a 4,6-*O*-benzylidene acetal synthon.[69] Neither this approach nor the ulosyl bromide[70] provide β-*L*-rhamnopyranosides in a concise and straightforward manner.

Due to their unique properties thioglycosides are often an attractive target compared to their natural *O*-linked counterparts. These properties include a) ease of synthesis via a simple S_N2 reaction due to the superior nucleophilicity of sulphur[71,72], (b) resistance to hydrolysis by acid and by hydrolases[73,74], (c) greater flexibility of the thio-glycoside linkages. Together these properties may provide longer-lived bioactive saccharide antigens due to enhanced metabolic stability that can sample a larger range of conformations in solution[71,75].

In Chapter 2, a general, efficient method to synthesize 1-thio-β-mannopyranosyl units via S_N2 glycosidation is described.[72] This method employs 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyrannose as starting material. The anomeric β-SAc was selectively deprotected *in situ* and the intermediate β-thiol reacted with acceptors bearing different leaving groups. Due to the high nucleophilicity and slow anomerization rate, the corresponding 1-thio-β-mannopyranosides were prepared in high yields. Here, we report our progress in extending this methodology to the chemistry of 1-thio-β-*L*-rhamnopyranosides. To the best of our knowledge, this is the first example of the stereoselective synthesis of oligosaccharides containing a 1-thio-β-*L*-rhamnopyranosyl linkage.[76]

3.2 Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-L-rhamnopyranose

The synthesis of the required β-thiol building block, namely, 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*L*-rhamnopyranose **27**, is shown in Scheme 3.1. Nucleophilic displacement of the anomeric α-bromide of 2,3,4,6-tetra-*O*-acetyl-α-L-rhamnopyranosyl bromide **26** with buffered potassium thioacetate – thioacetic acid (1 eqv. potassium *tert*-butoxide mixed with 1.2 eqv. thioacetic acid in DMF) rendered compound **27** free of the α anomer in 66% yield after crystallisation. Here the use of buffered thioacetate solution is crucial in providing a less basic nucleophile. In fact, when potassium thioacetate was employed, α/β mixtures along with unidentified side products were obtained (data not shown). Thioacetate **27** was then used as the common starting material for the synthesis of 1-thio-β-*L*-rhamnopyranosides. The 2,3,4-tri-*O*-acetyl-1-*S*-phenyl-1-thio-β-*L*-rhamnopyranoside **28** could be prepared by reaction of potassium thiophenolate with rhamnopyranosyl bromide **26** in a similar fashion (94%).

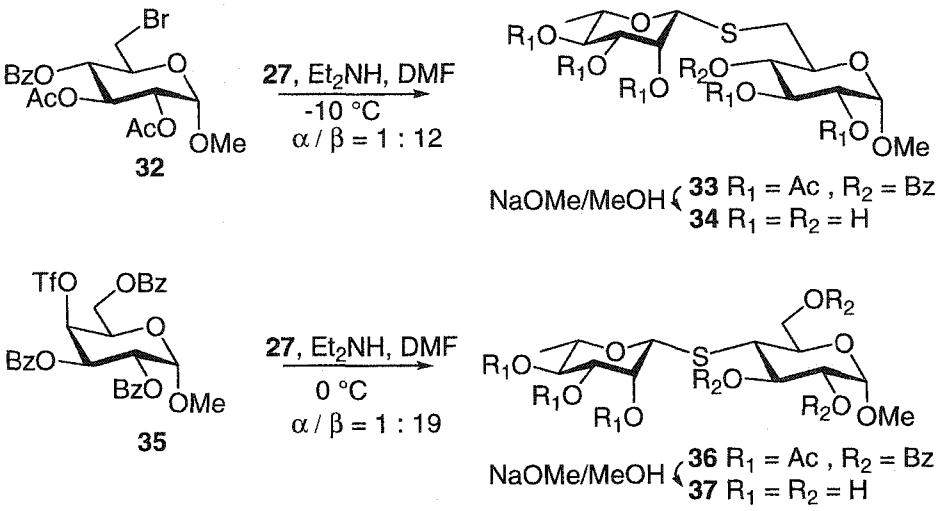


Scheme 3.1. Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*L*-rhamnopyranose

The high nucleophilicity of this thiol was demonstrated in the preparation of two simple 1-thio-β-*L*-rhamnopyranosides **30** and **31.** In fact, when compound **27** was treated with a solution of NaOMe in methanol, the intermediate thiolate **29** was formed. Results from NMR experiments recording the proton spectra of compound **27** in a mixture of $NaOCD_3/CD_3OD$, indicated that the intermediate **29** was formed almost instantly and remained as the β-anomer for an extended period of time. The anomerization rate of **29** was so low that even after 3 days, the NMR spectra showed more than 90% of the material remained as β-anomer. When iodomethane was added to the solution, the methyl β-thio-*L*-rhamnopyranoside **30** was formed within 5 min, NMR showed that a quantitative transformation had occurred. When the less reactive iodoheptane was added, the reaction was complete in 30 min, and NMR revealed compound **31** was formed in a clean and quantitative fashion. Both compound **30** and **31** were isolated by reverse phase chromatography (→ **30**, 98% and → **31,** 99%).

3.3 Synthesis of β-thio-linked disaccharides via thioacetate 27

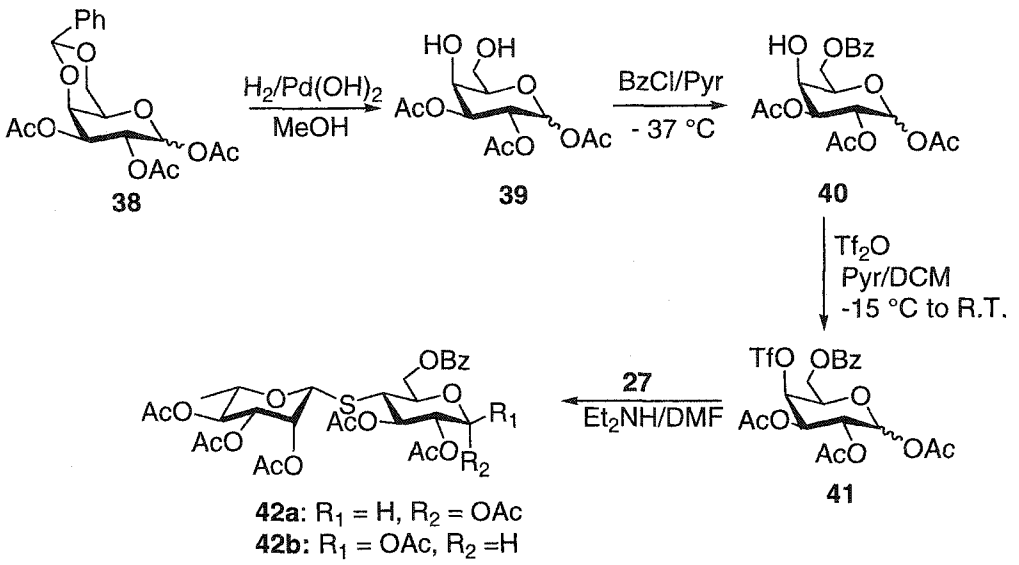
The general utility of **27** was next established in the synthesis of two *S*-linked disaccharides. Reaction of **27** with methyl 6-bromo-6-deoxy-α-*D*-glucopyranoside **32** gave β-anomer disaccharide **33b** (79%) and α-anomer **33a** (7%). Deprotection by transesterification gave anomerically pure **34** after reverse phase chromatography. The galactopyranoside triflate **35** reacted with **27** under similar conditions to give the 1,4 *S*-linked disaccharide **36** in 78% yield and improved stereoselectivity (β/α is 19:1). The mixture was not resolved and was transesterified to give the anomerically pure, deprotected disaccharide **37** (94%) after reverse phase chromatography.



Scheme 3.2. Synthesis of 1-thio-β-rhamnopyranosyl disaccharides

3.4 Synthesis of the thio-linked trisaccharide

In order to evaluate the robustness of the 1,4-*S*-linked disaccharide as a glycosyl donor, a thio-linked trisaccharide was synthesized. To this end, 1,2,3-tri-*O*-acetyl-4,6-*O*-benzylidene-α/β–D-galactopyranose **38** was prepared according to a literature procedure.[77] Recently, Váxquez *et. al.*[78] reported that deprotection of the benzylidene acetal (→ **39**) was carried out under acidic condition in 89% yield. In our hands, diol **39** was obtained in low yield with side products, presumably resulting from acetyl migration under acidic conditions. However, hydrogenation of **38** under neutral conditions with palladium hydroxide on charcoal, gave the triacetate **39** as an α/β mixture in excellent yield (95%) (Scheme 3.3). Selective benzoylation proceeded smoothly at –37° C with benzoyl chloride in pyridine, to give the anomeric mixture of 6-*O*-benzoates **40** in 80% yield. The galactose triflate **41** was prepared and glycosidated with **27** at 0° C. Disaccharide **42** was obtained in 71% yield and no α 1,4-linked isomer was detected.

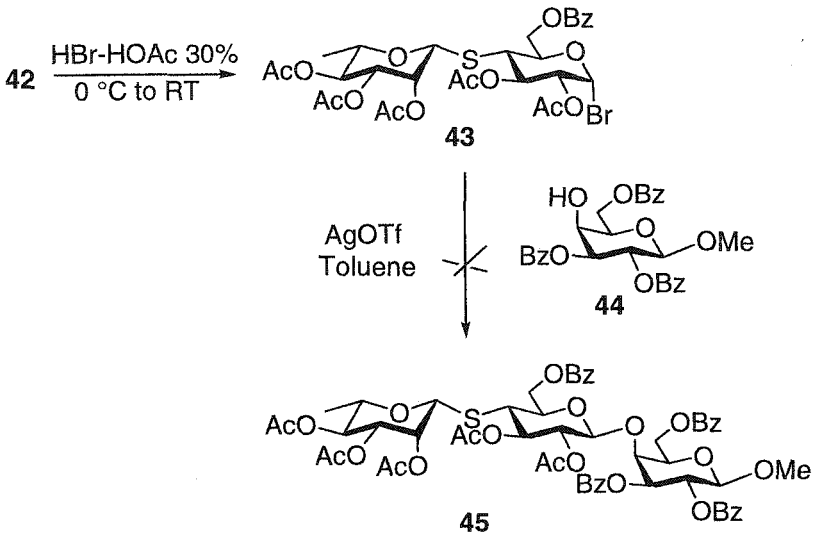


Scheme 3.3. Synthesis of β-thio-linked disaccharides **42a** and **42b**

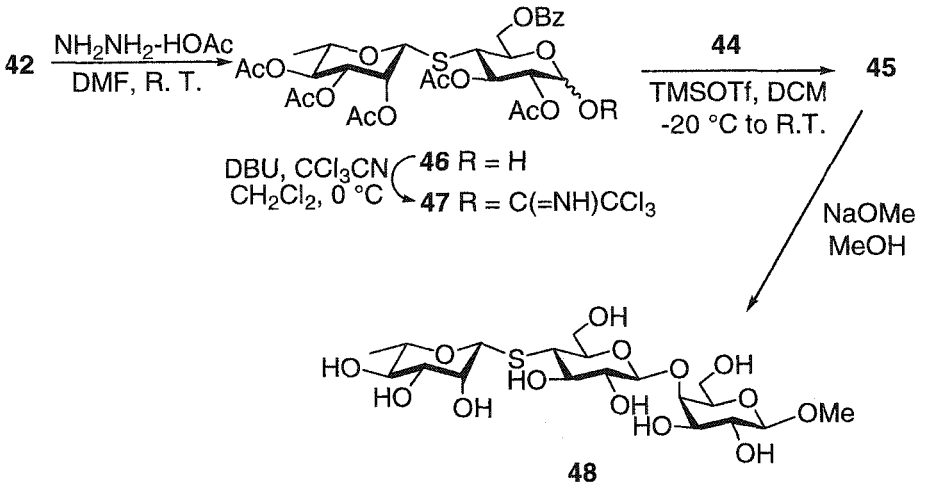
Treatment of **42** with HBr-HOAc gave the disaccharide glycosyl bromide **43** quantitatively (TLC) (Scheme 3.4). The glycosyl bromide was sufficiently pure as judged by NMR to perform the next glycosidation step without purification. Attempted glycosidation of **43** with acceptor **44** under promotion by silver triflate with 4 Å molecular sieves in anhydrous toluene failed to yield any trisaccharide. Changing the solvent to anhydrous dichloromethane with or without molecular sieves at various reaction temperatures did not show any improvement.

Glycosylation was then attempted with the trichloroacetimidate donor **47** (Scheme 3.5). Selective anomeric deacetylation of compound **42** at ambient temperature gave **46** in 86% yield. Higher temperatures gave an unidentified mixture. Trichloroacetimidate **47** was obtained swiftly under standard condition at 0° C. The dissaccharide donor **47** was then coupled to **44** by activation with trimethylsilyl trifluoromethanesulfonate in anhydrous dichloromethane. Under these conditions, the trisaccharide **45** was obtained in

good yield (77%). In our hands, adding molecular sieves lowered the reaction yield. The final compound **48** was obtained following transesterification in 87% yield.



Scheme 3.4 Attempted glycosylation with glycosyl bromide



Scheme 3.5 Glycosylation with trichloroacetimidate

The configuration of the newly synthesized glycosidic bonds in all compounds were confirmed by a range of NMR experiments. The 1-thio-β-rhamnopyranosides

showed similar characteristics compared to those of the 1-thio-β-mannopyranosyl linkages: (a) the homonuclear three bond coupling constants (J) are always smaller than 1.3 Hz, (b) the heteronuclear one bond coupling constant ($J_{C1,H1}$) at the anomeric centre ranged from 151 to 156 Hz, and (c) TROESY experiments show intrapyranose ring dipolar coupling between H-1, H-3 and H-5 while the corresponding α-anomer shows no such interactions.

3.5 Conclusion

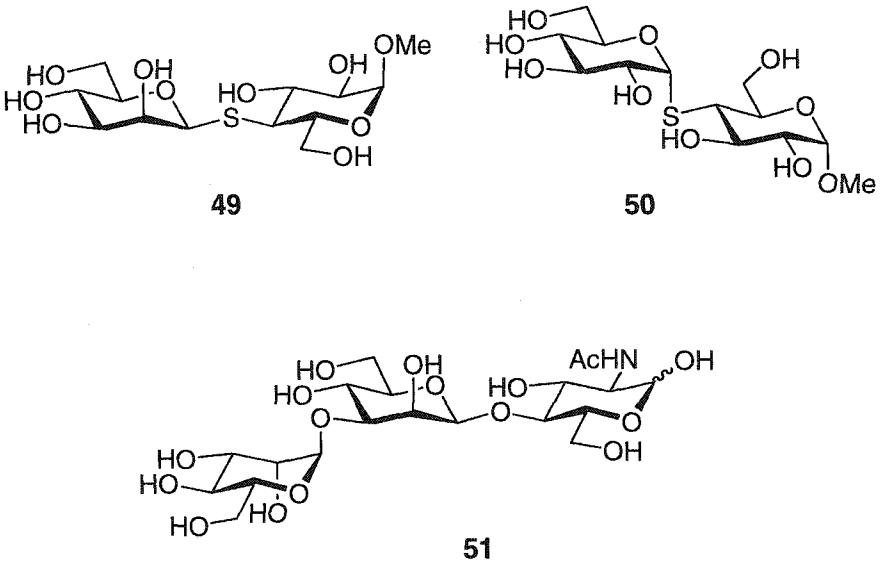
In summary, we have demonstrated that a facile S_N2 glycosidation not only works for 1-thio-β-*D*-mannopyranosides, but also for 1-thio-β-rhamnopyranosides. Using the easily accessible 1-thio-β-*L*-rhamnopyranose **27** as starting material, this methodology takes advantage of the superior nucleophilicity and slow anomerization of the thiolate, providing 1-thio-β-*L*-rhamnopyranosides in high yield. The thio-linkage is sufficiently robust to withstand further chemical manipulation, as shown by the synthesis of more complex structures such as **48**.

Chapter 4

X-ray crystallographic and NMR studies of methyl 4-*S*-(β-D-mannopyranosyl)-4-thio-α-D-glucopyranoside

4.1 Introduction

The success of any thiosugar acting as an enzyme inhibitor, carbohydrate mimic, or immunogen depends on one critical criterion: that is, how analogous these pseudosaccharides are to the natural *O*-linked counterparts.[79] Ever since the first and only crystal structure for an unprotected thio-linked disaccharide methyl α-*D*-thio-maltoside **50** (Figure 4.1) was reported in the early 1980s,[49] tremendous efforts employing NMR and theoretical methods have been expanded to map the conformational space available to thioglycosides.[43,80,81,82,83,84,85,86] X-Ray crystallographic data was used either as parameters for theoretical calculation or for comparative purposes in computer modeling and NMR studies. Unfortunately, no crystal data was available for the β-thioglycosides, other than for simple monosaccharides or protected disaccharides. Researchers interested in conformational analysis for β-thioglycosides relied on the available α-form crystal structure[84,85] and/or on *ab initio* simulation.[87] The accuracy and confidence in these approaches could be substantially improved if deprotected β-thio-linked disaccharide X-ray crystalline data became available.



49 50

51

Figure 4.1. Chemical structures of three X-ray crystal structures

In addition to the different conformational preferences of exocyclic substituents at the anomeric carbon of either an α- or β-pyranose, the *endo* and *exo*-anomeric effects govern bond length, valence geometry, reactivity and other properties.[88,89] The C1-*O* glycosidic bond length in β-glycosides is shorter than in α-glycosides. The underlying reason for this difference can be appreciated from the relative position of the orbitals containing the non-bonding electron pairs (Figure 4.2). In β anomers, both the unshared pairs of electrons of the ring oxygen are *syn*-clinal to the glycosidic bond and, therefore, unfavourably disposed for delocalization toward the anomeric carbon. Thus, there exists no important competition for delocalization of charge (back bonding) from the glycosidic oxygen that, in the sterically most favourable orientation, has an unshared pair of electrons *anti*-periplanar to the C1-ring oxygen bond. In contrast to the β anomers, the α-glycosides have the aglycon in axial orientation and therefore, as displayed in Figure 4.2, are importantly stabilized by an *exo*-anomeric effect. However, for these α-anomers, competition exists between the *endo*- and *exo*-anomeric effects for the electron deficiency

at the anomeric carbon. Consequently, The C1-O glycosidic bond length is shorter for β anomer. Recently, Pinto *et al.* shown that the combination of *endo*- and *exo*- anomeric interactions in the α anomer is more stabilizing than the exo-anomeric interaction alone in the β anomer.[90]

Figure 4.2. *Endo*-and *exo*-anomeric effect in α- and β-glycosides

The torsional angles defining the conformational preferences at the glycosidic bond that are influenced by the anomeric effects are termed Φ and Ψ. The conventions for defining these angles are shown in Figure 4.3. Here it must be noted that crystallographers follow the IUPAC convention, while NMR based conformational studies frequently use different reference atoms to define the torsional angle, Φ^H and Ψ^H. Both conventions are illustrated in Figure 4.3.

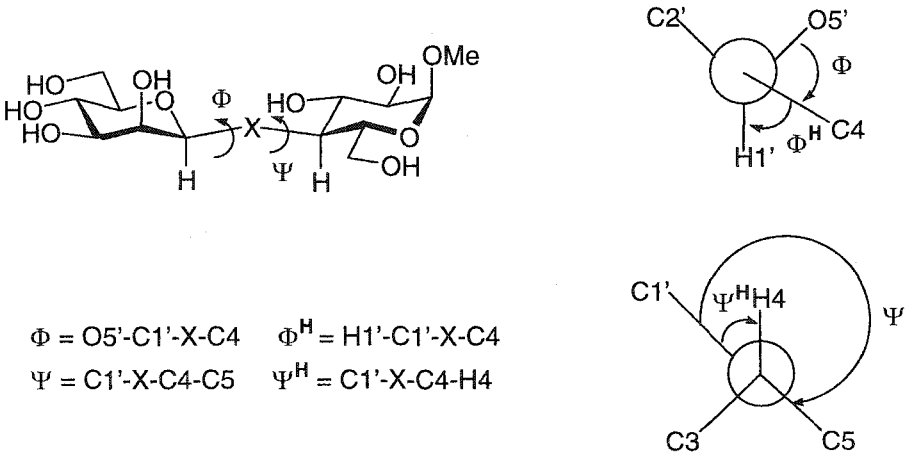


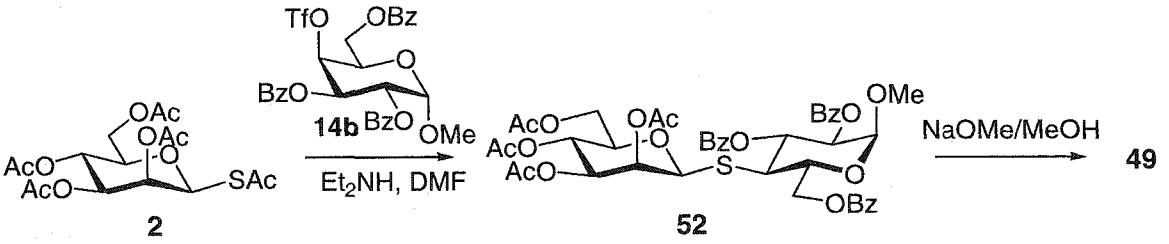
Figure 4.3. Definitions of glycosidic torsional angles

When the glycosidic oxygen atom is replaced by S, the magnitude of the anomeric effects must be re-evaluated.[87] The seemingly simple replacement might change not only the glycosidic bond, but also the pyranose ring, which in turn changes the internal coordinates, such as bond length, bond angle, ring torsional angle and pendent group orientation. While the conformation a molecule adopt in the solid state is not necessarily the same as those sampled in solution, crystal structure information with its greater detail and precision frequently complements structures determined for molecules in more mobile states. Here, we report the first crystallographic study of a free β-thio-linked disaccharide, methyl 4-*S*-(β-*D*-mannopyranosyl)-4-thio-α-*D*-glucopyranoside **49**. Detailed conformational analysis was performed by comparing the crystal structure of **49** to a) the crystalline *O*-linked α-*D*-Man*p*-(1→3)-β-*D*-Man*p*-(1→4)-α/β-*D*-GlcNAc*p* trisaccharide **51** (Figure 4.1)[91], b) all thio-pyranoside crystal structures (especially methyl α-thiomaltoside **50**[49]), and c) solution conformations of **49** obtained from NMR.

4.2 Synthesis of thio-linked disaccharide 49

The synthesis of methyl 4-*S*-(β-*D*-mannopyranosyl)-4-thio-α-*D*-glucopyranoside **49** started from 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-*D*-mannopyranose **2** using S_N2 glycosidation procedures that we developed previously for the synthesis of 1-thio-β-mannopyranosides.[72] Briefly, the intermediate thiol was generated *in-situ* by selectively deprotecting the *S*-acetate in the presence of *O*-acetates by reaction with diethylamine in DMF; it was then used directly for S_N2 reaction with galactosyl triflate **14b**[54] to afford **52**

(Scheme 4.1). Transesterification of **52** then gave deprotected disaccharide **49**.

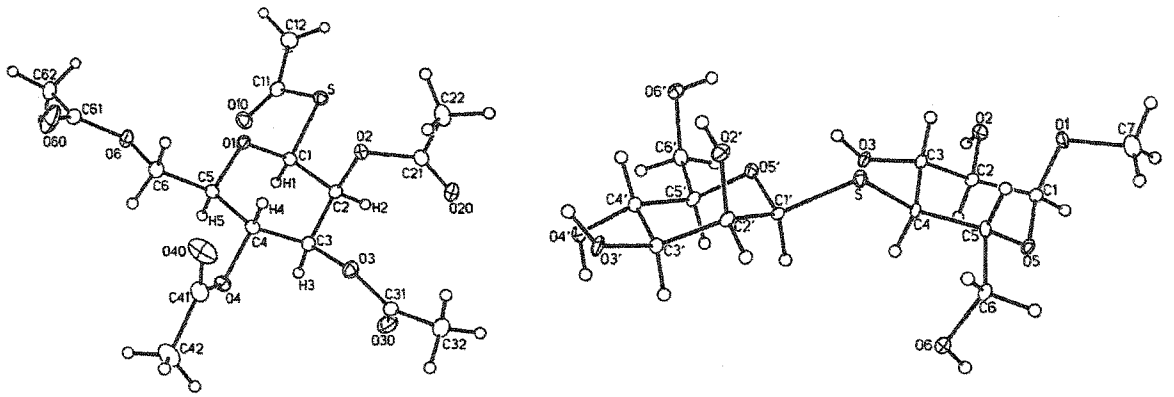


Scheme 4.1. Synthesis of compound **49**

4.3 Molecular structures

X-ray crystal structures[i] of both compound **49** and **2** are shown in Figure 4.4, followed by a stereo view of **49** (Figure 4.5). Different views of both molecules are shown in Figure 4.6. For disaccharide **49**, the ring C-C bond lengths (range 1.505-1.538 Å) are similar to the mean bond length in carbohydrate pyranose rings[92] (Table 4.1). As expected, the exocyclic C5'-C6' bond (1.505 Å) is shorter than endocyclic C-C bonds in the mannose residue, as C-C bond involving both primary and secondary carbon atoms are shorter than those that connect secondary atoms.[93] It is interesting to note that the C5-C6 (1.522 Å) bond of the glucosyl moiety is slightly longer than the C5'-C6' bond. Excluding the short C1-O1 bond (1.389 Å), for both pyranoses, the endocyclic C5-O5 bond distances are similar to the exocyclic ones, the average being 1.426 Å (mannose) and 1.437 Å (glucose). Due to the anomeric effect, the O5-C1 bond (1.420 Å) is significantly shorter than the C5-O5 bond (1.451 Å), while the O5'-C1' bond (1.434 Å) of the mannose ring (without *endo* anomeric effect) is similar to the C5'-O5' bond (1.433 Å).

[i] Crytal structures were solved by Dr. Robert McDonald and Dr. Michael J. Ferguson at the X-ray laboratory of this department



2 **49**

Figure 4.4. Molecules **2** and **49** with atomic notation

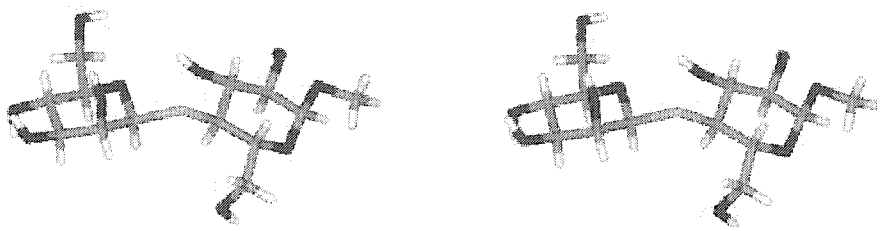


Figure 4.5. Stereo view of compound **49**

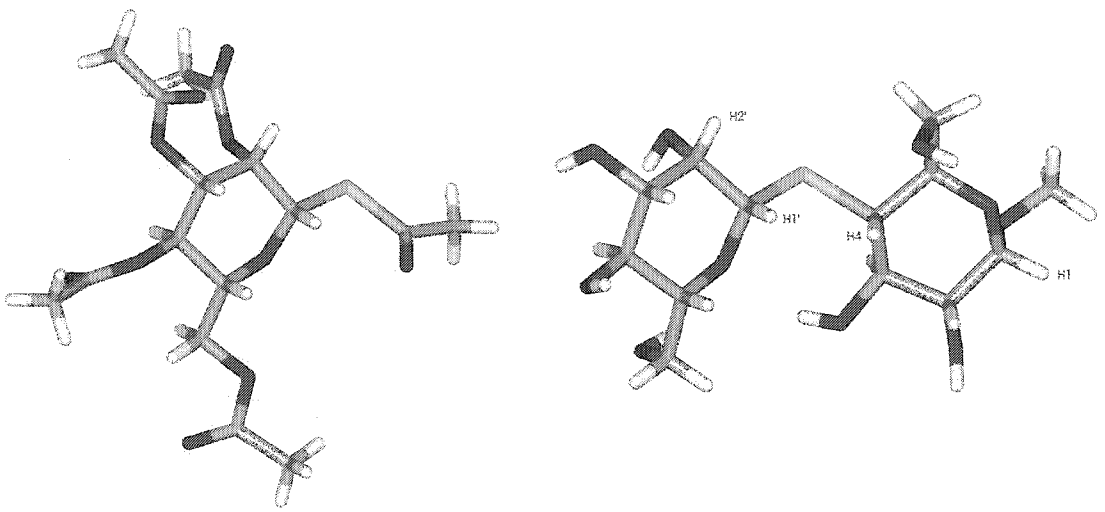


Figure 4.6 Different views of compound **2** and **49**

Table 4.1. Bond lengths (Å) in the mannose ring of **2**, **49**, and **51**

	2, Man	**49**, Glu	**49**, Man	**51**, Man
Endocyclic				
C1-C2	1.527(3)	1.517(7)	1.522(6)	1.550
C2-C3	1.525(3)	1.519(6)	1.536(6)	1.523
C3-C4	1.512(4)	1.517(6)	1.505(7)	1.511
C4-C5	1.529(3)	1.538(6)	1.522(6)	1.552
C5-O5	1.438(3)	1.451(5)	1.443(5)	1.432
O5-C1	1.424(3)	1.420(5)	1.434(5)	1.408
Exocyclic				
C5-C6	1.510(3)	1.522(6)	1.505(6)	1.510
C2-O2	1.440(3)	1.430(5)	1.415(5)	1.415
C3-O3	1.449(3)	1.422(5)	1.428(5)	1.429
C4-O4	1.456(3)		1.421(5)	1.406
C6-O6	1.433(3)	1.432(5)	1.433(5)	1.432
C1-O(Me)		1.389(5)		
Glycosidic linkage				
C1'-X	1.800(3)		1.798(5)	1.385
X-C4	1.791(3) (S-C11)	1.826(4)		1.438
C1'···C4			2.838	2.378

The per-acetylated mannopyranose derivative **2** shows striking similarity to the mannopyranosyl residue of **51** in the parameters mentioned above (Table 4.1, Figure 4.4 & 4.4). All three hexoses adopt a typical $^4C_1$ chair conformation with Creamer-Pople puckering parameter[94] of Q = 0.575 Å, Θ = 8.52° and Q_2 = 0.085 Å for compound **2**, Q = 0.573 Å, Θ = 6.96° and Q_2 = 0.069 Å for the mannose ring in **49**, and Q = 0.550 Å, Θ = 8.16° and Q_2 = 0.078 Å for glucose. All of the puckering amplitudes (Q) are slightly less than the Q value of an ideal cyclohexane chair that displays a value of 0.63 Å for C-C bond lengths of 1.54 Å and C-C-C angles of 109.4°.[93] This shows that all three pyranoses are slightly flattened from the ideal cyclohexane chair. The magnitude of the distortion is given by Θ's above. Except for the primary acetyl group, the carbonyl groups of compound **2** nearly eclipse the methine hydrogen geminal to the acetoxy group.[95] They are in good agreement with those generally observed in acetylated carbohydrates.[96]

The observed conformation about the exocyclic C5-C6 bond in **2** is the *gauche, trans* orientation, while both C5-C6 bonds in **49** are in the *gauche, gauche* orientation, where the C6-O6 bond is *gauche* to both the C5-O5 and C4-C5 bonds (Table 4.2). This difference is probably due to the different molecular hydrogen bond pattern (see below) and van der Waals forces of molecular packing, since there is not a large difference in energy between C5-C6 rotamers.

Table 4.2. Torsional angles (°) of the mannose ring in **2**, **49** and **51**

Torsion about glycosidic bond	**2**, Man	**49**, Glu	**49**, Man	**51**, Man
O5'-C1'-S-C4 (Φ)	-85.18 (O5-C1-S-C11)		-82.3(3)	-75.74
C1'-S-C4-C5 (Ψ)			-141.5(3)	-130.65
H1'-C1'-S-C4 (Φ^H)	34.5 (H1-C1-S-C11)		36.8	48.08
C1'-S-C4-H4 (Ψ^H)			-24.4	-0.56
Endocyclic Torsion angles				
C1-C2-C3-C4	-48.2(3)	-49.1(5)	-50.0(5)	-55.2
C2-C3-C4-C5	50.6(3)	164.6(3)	50.2(5)	57.9
C3-C4-C5-O5	-57.6(2)	-51.4(4)	-54.3(5)	-60.4
C4-C5-O5-C1	65.8(2)	61.7(4)	62.8(4)	+64.9
C5-O5-C1-C2	-65.1(2)	-64.0(5)	-65.4(4)	-62.6
O5-C-1-C2-C3	54.2(3)	56.2(5)	57.3(4)	+55.5
Exocyclic Torsion angles				
O5-C5-C6-O6 (χ)	60.8(3)	-77.4(4)	-53.4(5)	66.6
C4-C5-C6-O6 (χ')	179.4(2)	43.4(5)	68.1(5)	-172.1
S-C1-C2-O2	54.1(2)	55.7(5) [O1]	50.6(4)	
O2-C2-C3-O3	-48.8(3)	67.6(4)	-49.3(5)	
O3-C3-C4-O4	-70.5(2)	-75.1(4) [S]	-63.5(5)	
O4-C4-C5-C6	66.6(3)	66.7(4) [S]	63.0(5)	
O5-C1-O1-C(Me)		71.5(5)		
H1-C1-O1-C(Me)		-49.2		

4.4 Comparison with the natural *O*-linked glycoside crystal structure

When disaccharide **49** is compared to the crystalline α-Man-(1→3)-β-Man-(1→4)-GlcNAc trisaccharide **51**,[90] it is seen that the C1'-S bond length is 1.798 Å and the S-C4 distance 1.826 Å, each being about 0.4 Å longer than the corresponding C-O bond. Due to the smaller valence angle τ C1'-S-C4 (103.1°) vs C1'-O-C4 (114.6°) (Table 4.3), the non-bonded C1'···C4 distance in **49** is 2.838 Å. This is 0.46 Å longer than the analogous *O*-linkage in the β-Man-(1→4)-GlcNAc portion of **51**. This difference is somewhat greater (0.11 Å) than the C1'···C4 distance of methyl α-maltoside and its thio-

analog **50**. If this trend holds true, one might expect that the α-thio-linked glycosides are better carbohydrate mimics of their natural counterpart than are the β forms. The endocyclic O5'-C1' bond of **49** (1.434 Å) is significantly longer than the one in its *O*-linked counterpart (1.408 Å). The difference is due to the greater electronegativity of the oxygen over the sulfur atom.[97] The absence of the *endo*-anomeric effect in the β configuration results in a longer O5'-C1' bond and shorter exocyclic C1'-S bond than those in methyl α-thiomaltoside **50** (see later).[49]

The glycosidic linkage of compound **49** shows slightly varied torsional angles (Φ = -82.3° and Ψ = -141.5°) compared to the corresponding glycosidic linkage in **51**, albeit both show similar comformation. In both cases, the aglyconic residues locate in the region required by the *exo*-anomeric effect.

Table 4.3. Bond angles (°) of the mannose ring in **50**, **49** and **51**

	50, Man	**49**, Glu	**49**, Man	**51**, Man
Endocyclic				
C1-C2-C3	110.0(2)	111.8(4)	107.9(4)	108
C2-C3-C4	112.3(2)	112.0(4)	113.3(4)	111.3
C3-C4-C5	110.7(2)	112.1(3)	110.5(4)	106.9
C4-C5-O5	108.0(2)	109.4(3)	110.0(4)	108.4
Exocyclic				
O5-C1-O1	--	112.5(4)	--	--
C2-C1-O1	--	108.8(4)	--	--
C1-C2-O2	107.25(19)	109.8(4)	112.4(3)	110.9
C3-C2-O2	109.2(2)	110.1(4)	113.6(4)	110.5
C2-C3-O3	109.7(2)	105.6(4)	110.1(4)	108.4
C3-C4-O4	116.88(19)	--	112.0(3)	113.1
C5-C4-O4	109.2(2)	--	109.7(4)	110.6
C4-C5-C6	112.2(2)	113.6(3)	115.6(4)	115.0
O5-C5-C6	107.6(2)	106.5(4)	105.1(4)	108.1
C5-C6-O6	106.8(2)	112.3(4)	112.0(4)	108.3
C5-O5-C1	111.48(18)	113.4(3)	111.2(3)	112.6
Glycosidic linkage				
O5'-C1'-S	108.1(3)	--	108.1(3)	107.6
C2'-C1'-S	109.01(18)	--	109.9(3)	109.0
C1'-X-C4	100.71(14) (C1-S-C11)	--	103.1(2)	114.6
C5-C4-S	112.6(3) (C12-C11-S)	--	107.2(3)	107.1
C3-C4-S	122.9(2) (O10-C11-S)	--	111.8(3)	110.7

4.5 Molecular packing and hydrogen bonds

The packing and hydrogen bonding of the molecules in the unit cell are shown in Figures 4.7 & 4.8. The disaccharides align in a herringbone orientation. The three dimensional arrangement results from a hydrogen bond network, the geometries of which are given in Table 4.4. To correct for the shortened C-H and O-H bond distances, the bond lengths are normalized as follows: O-H of 0.84 Å, and C-H distances of 0.98, 0.99, and 1.00 Å for methyl, methylene and methine, respectively. The two weak

intramolecular hydrogen bonds (I & II) form a bifurcated bond.[98] The bifurcated acceptor O5' and HO-3 stabilize the conformation of the glycosidic linkages (HO3 and O5' H-bond is also found in the related trisaccharide crystal structure **51**[90] and other β-(1→4) disaccharide structures[99]). Three other bifurcated hydrogen bonds are in the network: a) II & III with a bifurcated donor O6', b) III & IV with a bifurcated acceptor O2, and c) V & VI with a bifurcated acceptor O6. A tandem hydrogen bond is formed by O3' and O6 (VI & VII).

Table 4.4. Hydrogen bond geometry for compound **49**

Entry	Hydrogen bonds	H···O (Å)	O···O (Å)	O-Ĥ···O (°)
I	O3-H···O5'	2.38	2.93	124
II	O6'-H··· O5'	2.43	2.71	101
III	[a]O6'-H···O2	2.06	2.89	167
IV	[a]O3-H···O2	2.10	2.85	149
V	[b]O4'-H···O6	2.01	2.82	162
VI	[d]O3'-H···O6	2.07	2.72	135
VII	[c]O6-H···O3'	1.94	2.72	155
VIII	[d]O2'-H···O5	2.17	2.90	145
IX	O2-H···O(MeOH)	1.94	2.72	154
X	[c]MeO-H···O6'	1.92	2.73	161
XI	[b]MeO-H···O3	1.93	2.73	161

[a]At $\bar{x}$, 1/2+y, $\bar{z}$. [b]At 1+$\bar{x}$, 1/2+y, 1+$\bar{z}$. [c]At x, −1+y, z. [d]At x, 1+y, z.

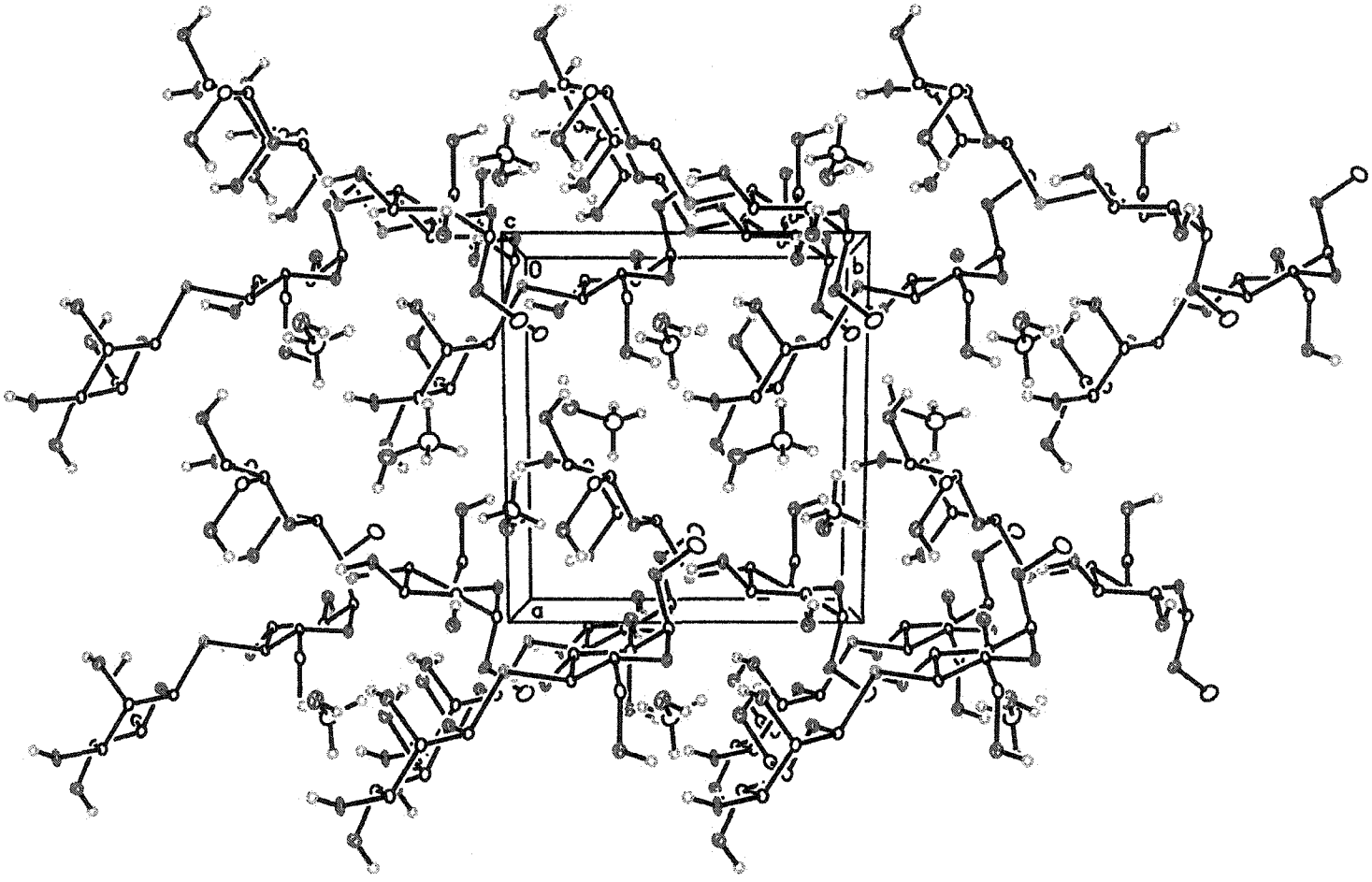


Figure 4.7. Molecular packing of **49**, a view down the C-axis showing the crystal packing of the disaccharide and methanol solvent molecules. Only the hydroxyl hydrogen atoms of the disaccharide and the methanol hydrogen atoms are shown for clarity

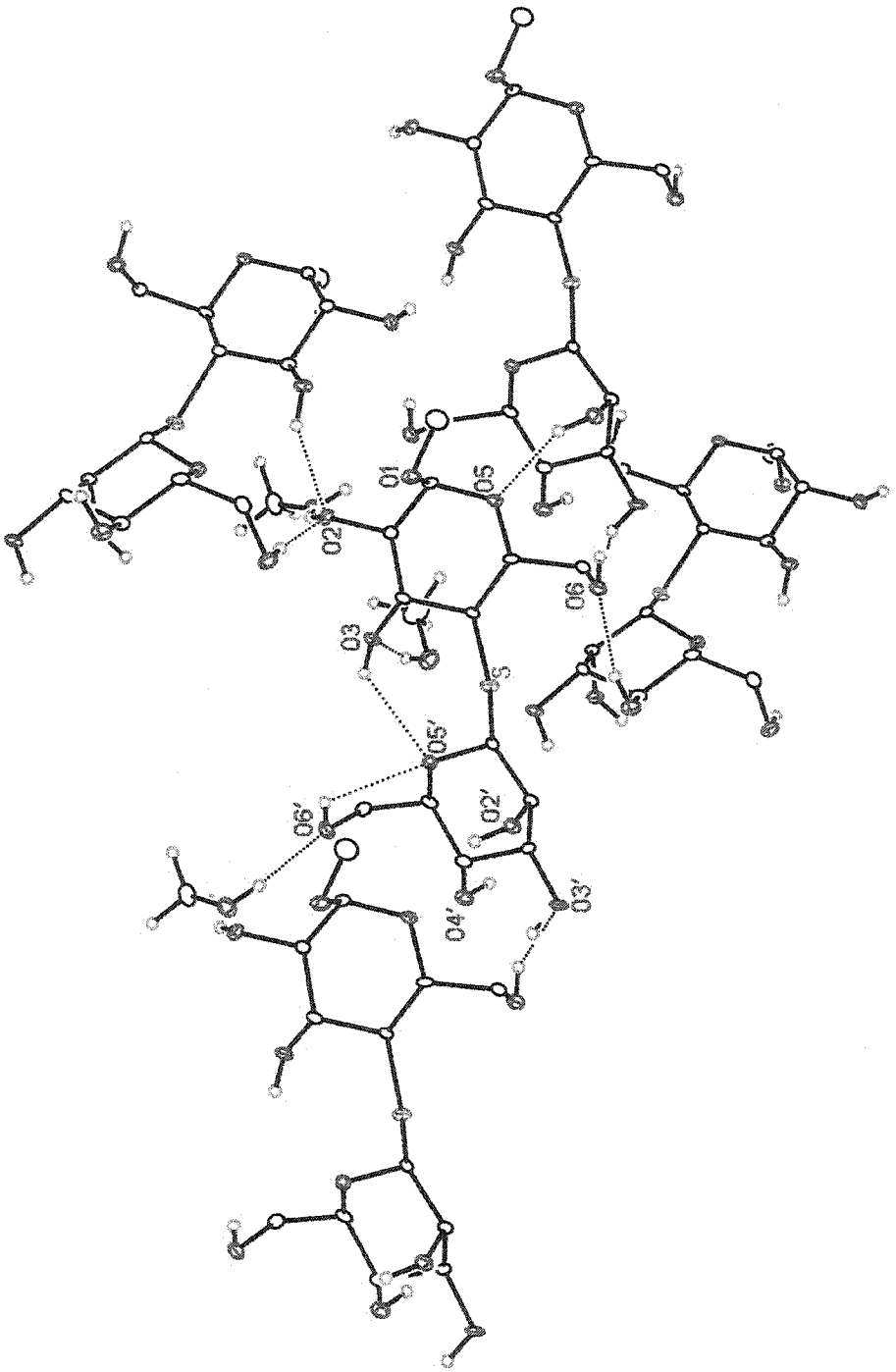


Figure 4.8. Hydrogen bonding of 49, only the hydroxyl hydrogen atoms of the disaccharide and the methanol hydrogen atoms are shown for clarity

4.6 Comparison with thio-linked pyranoside X-ray crystal structures

Besides the deprotected thio-linked disaccharide **50**, little more than a dozen mono- and protected disaccharide crystal structures are available in the literature. Table 4.5 surveys all reported thio-linked protected and deprotected pyranosides. Entries 1 to 5 are α-glycosides and entries 7 to 20 are β forms. The mean values of each parameter are derived as entries 6 and 21 for α- and β- thioglycosides, respectively. A few interesting trends can be established about the α- and β- glycosidic linkages:

a) the sterically unfavorable β-thioglycosidic torsion angles Φ (-67 to -85° for D-sugar and 83 to 96° for L-sugar) probably result from the diminished but still present *exo*-anomeric effect (see below),

b) one of the manifestations of the anomeric effect is shortening of the endocyclic O5-C1 bond and lengthening exocyclic C1-S bond. While the anomeric axial C1-S bonds are about 0.02 Å longer than the equatorial ones, the O5-C1 bonds show little difference between the α- and β-thio-analogs, these data supports that the anomeric effect in thioglycoside is weak compared to the *O*-linked counterpart.[100]

c) the S-C(sp^3) bond lengths remain within a narrow range (1.809-1.829) regardless of the configuration of the aglycons,

d) valence angles C5-O5-C1 in both series are close to the mean value of the corresponding natural analogs,[91]

e) more importantly, the valence angle τ C1-S-C(sp^3) displays 2.5° discrepancy between the two anomeric thioglycosides, while the same angle shows only a 0.7° difference between α- and β-*O*-linked sugars. The explanation relies on the

weaker *endo*-and *exo*-anomeric effect of thio-sugarswhich shorten the C1-S bond in the β-anomer to a larger extent than in the α-anomer (i.e. the difference between C1-S and C1-O bond length is 0.428 Å for α, and 0.404 Å for β). The shorter C1-S bond in β-anomer corresponds to more *s* character and an enlarged angle τ. Thus, the non-bonding distance C1'···C4 is in turn lengthened as discussed above. Based on this, one would expect that glycosidic linkages in thiomimetics for β-glycosides would be more flexible than those in thiomimetics for α-glycosides.

Table 4.5. Survey of thio-linked pyranoside X-ray crystal structures (in case of disaccharide, data only refer to the prime ring)

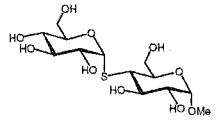
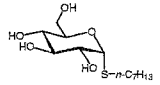
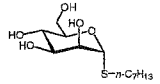
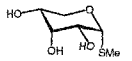
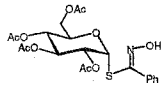
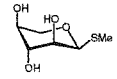
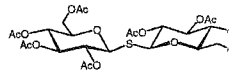
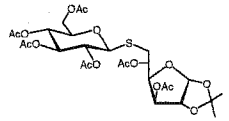
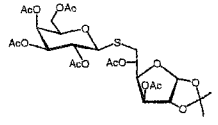
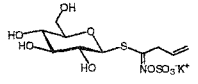
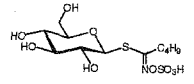
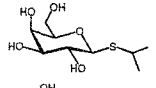
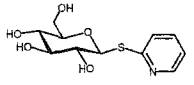
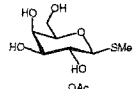
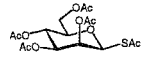
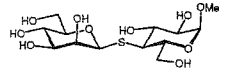
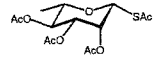
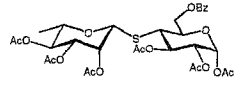
		Torsion		Bond length				Valence angle			Ref.
		Φ	Ψ	C5-O5	O5-C1	C1-S	S-C(sp^3)	C5-O5-C1	O5-C1-S	C1-S-C(sp^3)	
1		89.0	-116.8	1.439	1.427	1.826	1.828	114.4	113.9	100.3	49
2		53.1	-	1.441	1.433	1.831	1.819	113.6	112.0	96.7	98
3		67.7	-	1.448	1.433	1.824	1.819	114.8	113.7	98.0	99
4		75.2[a]		1.426	1.435	1.796	1.809	111	108	99	100
5		54.9	-	1.431	1.395	1.838	-	116.0	113.7	98.5[d]	101
6	**Mean Value**			**1.437**	**1.425**	**1.823**	**1.819**	**114.0**	**112.3**	**98.5**[e]	
7		-[a]	-	1.43	1.44	1.796	1.823	109.9	108.1	98.3	102
8[b]		-117.2 -67.1	-	1.43 1.43	1.42 1.43	1.780 1.784	-	114.2 111.9	109.0 107.9	-	103
9[c]		-73.0	-	1.44	1.42	1.80	1.82	111.3	108.8	99.9	104

Table 4.5. Survey of thio-linked pyranoside X-ray crystal structures (continued).

		Torsion		Bond length				Valence angle			Ref.
		Φ	Ψ	C5-O5	O5-C1	C1-S	S-C	C5-O5-C1	O5-C1-S	C1-S-C	
10[c]		-74.8		1.42	1.43	1.80	1.81	111.5	107.7	101.3	104
12		-89.5	-	1.441	1.411	1.809	-	112.0	109.2	104.0[d]	106
13		-73.9	-	1.439	1.415	1.809	-	110.9	109.0	103.5[d]	107
14		-72.1	-	1.446	1.422	1.801	1.827	110.7	108.4	101.1	108
15		-75	-	1.436	1.421	1.793	-	111.3	109.3	103.3[d]	109
16		-99	-	1.440	1.429	1.806	1.811	110.3	109.2	100.2	110
17		-85.18	-	1.438	1.424	1.800	-	111.48	108.1	100.71[d]	*f*
18		-82.3	-141.5	1.443	1.434	1.798	1.826	111.2	108.1	103.1	*f*
19		82.96	-	1.4376	1.4249	1.8046	-	110.62	108.54	99.26[d]	111
20		96.2	-106.5	1.437	1.417	1.826	1.827	112.5	108.5	103.0	111
21	**Mean Value**			**1.438**	**1.425**	**1.803**	**1.821**	**111.5**	**108.6**	**101.0**[e]	

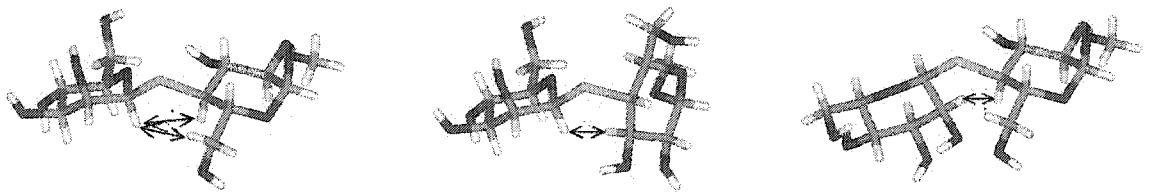
[a] Crystal conformation is $^{1}C_{4}$. [b] Except for C1-S and C1-S-C, all data were obtained from deposited information in the Cambridge Crystallographic Data Centre (CCDC). [c] Data was obtained from CCDC. [d] C1-S-C(sp^2) angle. [e] C1-S-C(sp^3) mean value. [f] This work

4.7 NMR Studies

Previous studies showed that while the crystalline conformation of α-thiomaltoside **50** is one of the low energy conformers derived from quantum-mechanical energy minimization, it is too high in energy to be present in water solvent as an equilibrium mixture.[80] We employed NMR spectroscopy to provide dynamic conformational information for compound **49**. The ^{1}H–NMR spectrum of compound **49** was assigned by GCOSY,[115] T-ROESY[115] and HMQC.[115] Good signal dispersion permitted the spin system of each hexose residue to be assigned. The intra-ring vicinal proton-proton coupling constants and the intra-residue ROE cross-peaks between H1/H3, H1/H5 pairs in the mannose ring proved that both six-membered rings adopt the $^{4}C_{1}$(D) conformation. The T-ROESY cross-peak volumes were quantified and the data were used to derive conformationally averaged inter-proton distance as shown in Table 4.6. For the β1,4 linked sugar such as **49**, the key interresidue NOEs unequivocally characterized the allowed regions of *syn-Φ/syn-Ψ, syn-Φ/anti-Ψ*, and *anit-Φ/syn-Ψ* in the potential energy map. The data in Table 4.6 indicate that two minimum energy configurations are heavily populated in solution (Figure 4.9): 1) *syn-Φ/syn-Ψ*, detected by the presence of NOEs between H1' and H4, and H1' and H-6b protons; and 2) *syn-Φ/anti-Ψ*, indicated by NOE between H1'/H3. The H2'-H4 NOE, which would reveal the presence of *anti-Φ/syn-Ψ* conformer, was not detected. Although this does not rule out the existence of the *anti-Φ/syn-Ψ* conformer, its population in solution must be minimal. Except for the H4-H6b distance, intra-residue inter-proton distances from T-ROESY agree well with those from X-ray crystal data, which strongly suggest that both hexoses adopt similar ring

conformations in solution and in the crystal lattice. The unconstrained hydroxymethyl group in glucose might assume orientations other than *gauche, gauche* in solution.

(a)



syn-Φ/syn-Ψ *syn-Φ/anti-Ψ* *anti-Φ/syn-Ψ*

(b)

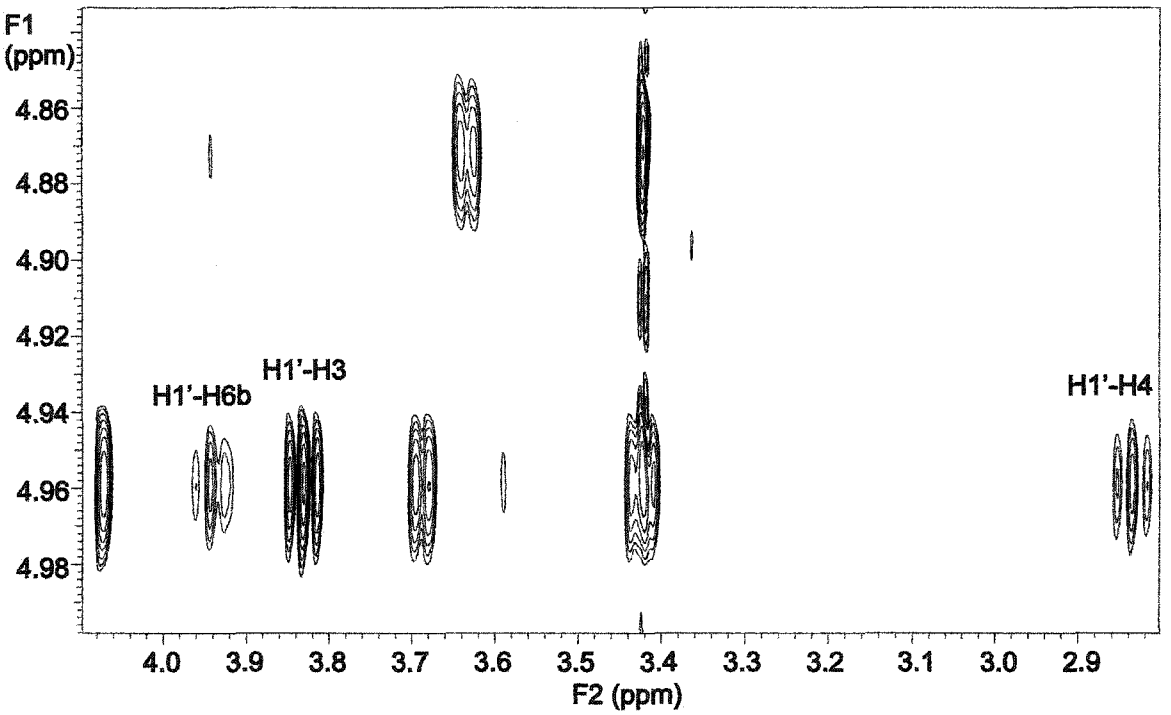


Figure 4.9. (a) Schematic view of the short inter-proton distances for the low energy minima, (b) Section of the T-ROESY experiment showing the inter-residue correlations

Table 4.6. Comparison of X-ray and ROE inter-proton distances (Å) for compound **49**

	X-ray	ROE	Difference
Inter-residue			
H1'-H4	2.35	2.67	0.32
H1'-H3	3.97	2.61	1.36
H1'-H6b	4.28	2.27	2.01
Intra-residue (Man)			
H1'-H2'	2.31	2.33	0.02
H1'-H3'	2.53	2.37	0.16
H1'-H5'	2.31	2.22	0.09
H2'-H3'	2.28	2.31	0.03
H6'a-H6'b	1.60	1.80	0.20
Intra-residue (Glu)			
H1-H2	2.33	2.33	0
H4-H6b	3.15	2.55	0.60
H2-H4	2.71	2.44	0.27

In order to compare the solid state conformation with the solution conformation, simple computer modelling was performed. Starting with the crystal conformer, the glycosidic linkage was systematically rotated in 10 degrees increments. An energy minimization was performed every 10 degrees. The inter-proton distances for H1'-H4, H1'-H3, and H1'-H6b, and torsional angles (Φ and Ψ) were recorded for each minimum energy conformers, and the Φ/Ψ map versus inter-proton distances (2 to 3 Å) maps were plotted. Clearly, conformation *syn-Φ/syn-Ψ* and *syn-Φ/anti-Ψ* occupy distinct regions (Figure 4.10). The crystal conformer falls within acceptable *syn-Φ/syn-Ψ* space if the H1'-H4 distance alone was considered, but it is not within the volume of conformational space consistent with both the H1'-H4 and H1'-H6b contour maps (Figure 4.11). This indicates that the crystal conformer is different from the *syn-Φ/syn-Ψ* conformation in solution, which suggests that in the crystal lattice, the molecular hydrogen bonds and van

der Waals forces of molecular packing direct the methylhydroxyl group around the C5-C6 bond away from the low energy rotamers.

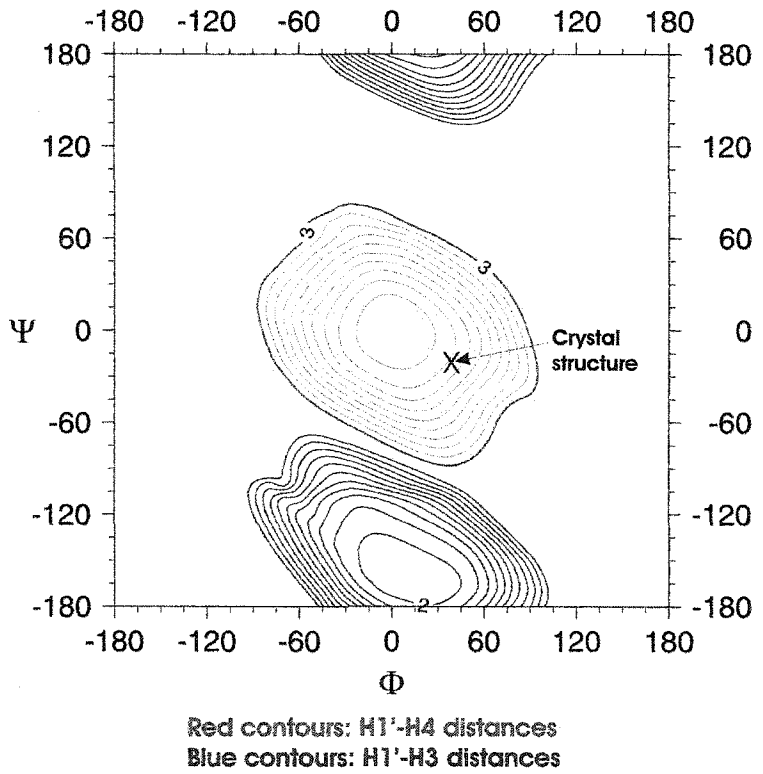


Figure 4.10. The Φ,Ψ conformational space constrained by inter-proton distance H1'-H4 (red) in the range 2 to 3 Å, and by the H1'-H3 (blue)

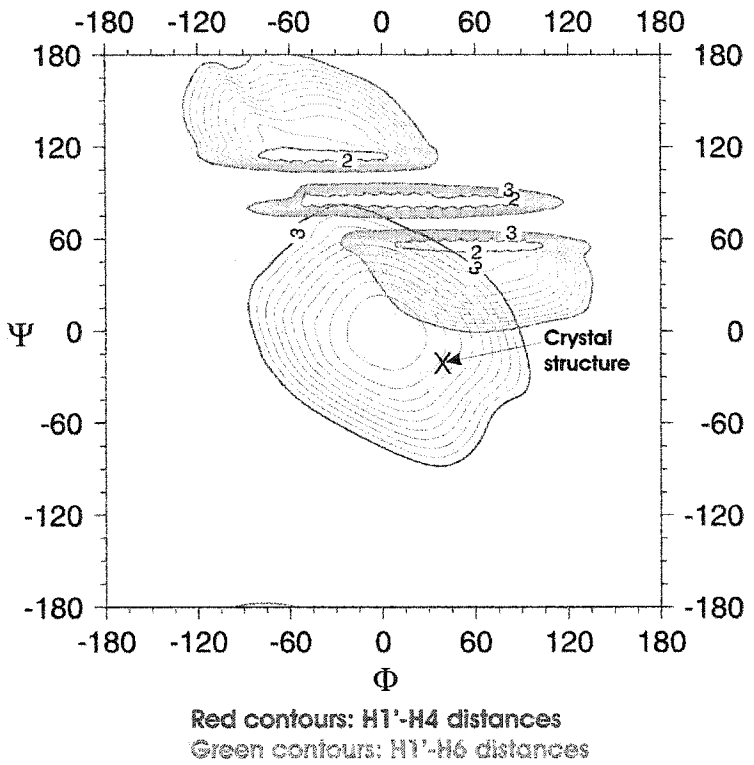


Figure 4.11. The Φ,Ψ conformational space constrained by inter-proton distance H1'-H4 (red) in the range 2 to 3 Å, and by the H1'-H6b (blue)

The cause of the *exo*-anomeric orientation around the Φ torsional angle has been under debate recently.[85] Jiménez-Barbero *et al.* argued that, in the *N, N'*-diacetyl-4-thiochitobiose system, the origin for this particular orientation preference stems from the 1,3-type steric effect in the non-*exo*-anomeric (*trans*) conformation instead of the *exo*-anomeric effect (Figure 4.12). In the mannose case, this non-*exo-anomeric* steric effect is removed. If the steric effect argument was important, we should see populated *trans* conformer in solution as well as the *-gauche* conformer. The fact that no *trans* type signal is detected in D_2O, indicates that the exo-anomeric effect plays a role here for the strong orientation preference. Because of the 1,3 type steric interaction in **49**, the *+gauche*

conformer can be expected to have a negligible existence, which is also confirmed by NMR studies as discussed above.

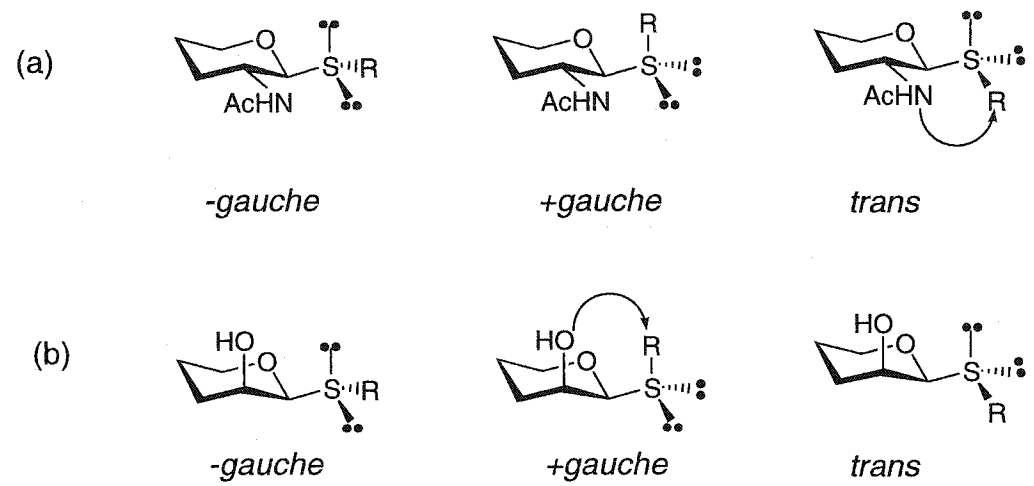


Figure 4.12. Schematic representation of the three basic orientations around the ϕ angle in (a) N-acetylglucopyranoside, and (b) mannopyranoside.

4.8 Conclusion

In summary, the first crystal structure of an unprotected β-*D*-thio-disaccharide has been solved by X-ray crystallography. Its dynamic conformation was probed by T-ROSEY NMR studies. The X-ray structure was compared to the analogous natural linkage in trisaccharide **51** and all other crystalline thio-sugars. Substituting oxygen by a sulfur atom in the glycosidic linkage results in slight changes in the coordinates of all the atoms in the vicinity of the anomeric center. The effect is larger for the β-glycosides. A longer non-bonding C1'···C4 distance strongly suggests that β-thioglycosides are more flexible than the α-form. This phenomenon can be explained by a reduced *endo*- and *exo*-anomeric effect. Although the *exo*-anomeric effect is weakened, it still governs the specific orientation preference of the aglycon. This finding contradicts an earlier steric

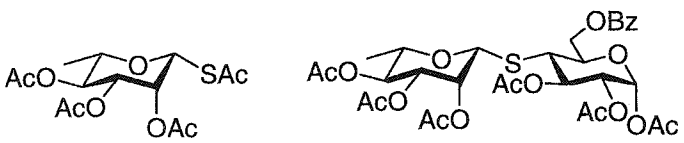
effect argument.[85] The information gathered here provides the dynamic atomic level structural information needed in the design of ligands for recognition by proteins and other receptor molecules.

Chapter 5

Crystal and molecular structures of peracetylated 1-thio-β-L-rhamnopyranosyl derivatives

5.1 Introduction

O-linked β-*L*-rhamnopyranosides exist in the capsular polysaccharides of *Streptococcus pneumoniae*[59,60,61], the causative agent of pneumonococcal meningitis, and otitis media[62], as well as several other bacterial strains.[63,64] In a recent effort to synthesize the 1,2-cis-β-thio-analogs[116] toward a potentially metabolically more stable vaccine, we obtained two crystalline compounds: a thiopyranose, 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-*L*-rhamnopyranose **27,** and the thio-linked disaccharide, 1,2,3-tri-*O*-acetyl-6-*O*-benzoyl-4-*S*-(2,3,4-tri-*O*-acetyl-β-*L*-rhamnopyranosyl)-4-thio-*D*-glucopyranose **42a** (Figure 5.1).[ii] X-Ray crystal structures are described here, along with the conformation analysis of **42a** by solution NMR. The present study adds to the information concerning the conformation of the β-thio-linked glycosides.[117]



27 **42a**

Figure 5.1. Chemical structures of compound **27** and **42a**

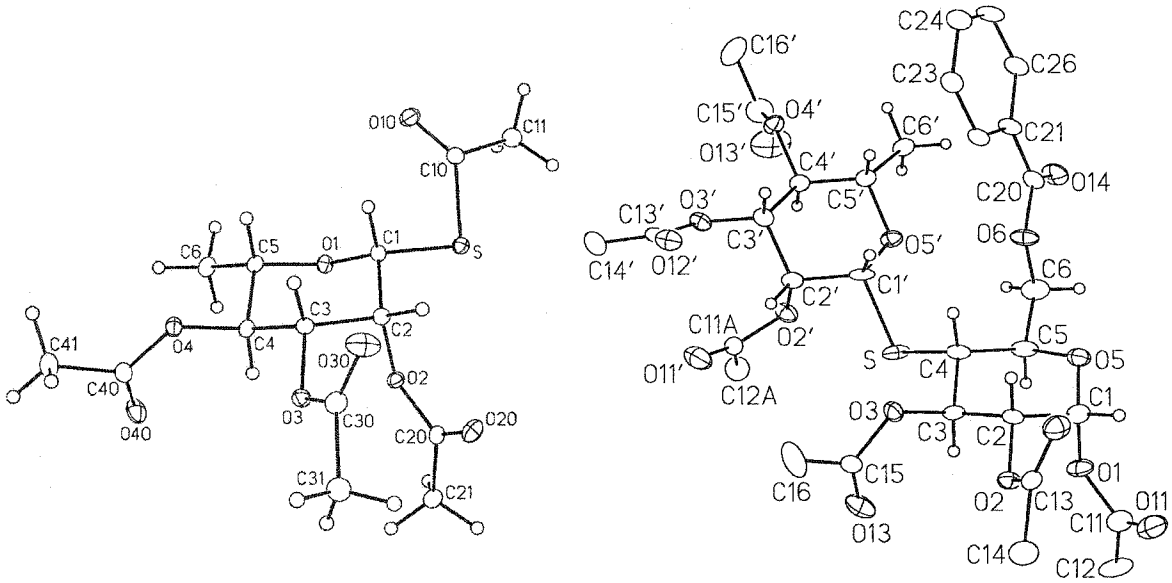
[ii] Crytal structures were solved by Dr. Robert McDonald at the X-ray laboratory of this department

5.2 Synthesis

The synthesis of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-*L*-rhamnopyranose **27** and 1,2,3-tri-*O*-acetyl-6-*O*-benzoyl-4-*S*-(2,3,4-tri-*O*-acetyl-β-*L*-rhamnopyranosyl)-4-thio-*D*-glucopyranose **42a** were reported in Chapter 3 (Scheme 3.1 & 3.3).

5.3 Molecular structures

Single crystals were obtained for compound **27** by crystallization from hexane-ethyl acetate, and for **42a** by crystallization from chloroform. The crystal data and details of the intensity data are showed in Appendix A. X-ray diffraction data were collected at 193 K using a $\omega/2\theta$ scan mode. Unit-cell dimensions were obtained by a least-squares fit. The structures were solved by direct methods with Bruker PLATFORM/SMART 1000 CCD and refined to a final R = 0.0289 and 0.0853 for compound **27** and **42a**, respectively. The positional and equivalent isotropic thermal parameters for the non-hydrogen atom are listed in Appendix B & C. The conformation of both molecules with the atomic notations is depicted in Figure 5.2.



27 **42a**

Figure 5.2. Molecular structures of **27** and **42a** with atomic notations

For both monosaccharide **27** and disaccharide **42a**, the ring C-C bond lengths (range 1.502-1.543 Å) are similar to the mean bond length value in carbohydrate rings[91] (Table 5.1 & 5.2). As expected, the exocyclic C5-C6 bond (1.471 Å) is shorter than endocyclic C-C bonds in the glucose residue.[92] For **42a**, the endo and exocyclic C-O bond distances are similar, the average being 1.423 and 1.436 Å, respectively. Compound **27** shows similar C-O bond lengths to those observed for the rhamnosyl residue of **42a**. All carbonyls of each acetyl group of both compounds nearly eclipse the methine hydrogen geminal to the acetoxy group.[94,95]

Table 5.1. Selected interatomic parameters for **27**

(a) interatomic distances (Å)

Atom1	Atom2	Distance		Atom1	Atom2	Distance
S	C1	1.8046(15)	O4	C4		1.4473(17)
S	C10	1.7986(16)	C1	C2		1.517(2)
O1	C1	1.4249(16)	C2	C3		1.526(2)
O1	C5	1.4376(17)	C3	C4		1.5215(19)
O2	C2	1.4498(17)	C4	C5		1.530(2)
O3	C3	1.4417(17)	C5	C6		1.522(2)

(b) interatomic angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C1	S	C10	9.26(7)	O3	C3	C4	107.02(11)
C1	O1	C5	110.62(11)	C2	C3	C4	110.72(12)
S	C1	O1	108.54(10)	O4	C4	C3	106.99(12)
S	C1	C2	109.58(9)	O4	C4	C5	109.13(11)
O1	C1	C2	110.90(11)	C3	C4	C5	109.80(12)
O2	C2	C1	107.66(11)	O1	C5	C4	108.94(11)
O2	C2	C3	108.38(11)	O1	C5	C6	106.23(12)
C1	C2	C3	108.93(11)	C4	C5	C6	113.31(13)
O3	C3	C2	109.58(11)				

Table 5.2. Selected interatomic parameters for **42a**

(a) interatomic distances (Å)

Atom1	Atom2	Distance	Atom1	Atom2	Distance
S	C4	1.827(7)	C1	C2	1.502(9)
S	C1'	1.826(6)	C2	C3	1.499(9)
O1	C1	1.443(8)	C3	C4	1.507(8)
O2	C2	1.445(7)	C4	C5	1.543(10)
O3	C3	1.422(8)	C5	C6	1.471(9)
O5	C1	1.394(8)	C1'	C2'	1.508(10)
O5	C5	1.444(9)	C2'	C3'	1.503(9)
O6	C6	1.438(8)	C3'	C4'	1.540(8)
O2'	C2'	1.438(8)	C4'	C5'	1.505(10)
O3'	C3'	1.439(8)	C5'	C6'	1.521(8)
O4'	C4'	1.426(7)			
O5'	C1'	1.417(7)			
O5'	C5'	1.437(7)			

(b) interatomic angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C4	S	C1'	103.0(3)	C4	C5	C6	113.9(6)
C1	O5	C5	116.4(5)	O6	C6	C5	108.8(5)
C1'	O5'	C5'	112.5(4)	S	C1'	O5'	108.5(4)
O1	C1	O5	107.6(5)	S	C1'	C2'	107.5(4)
O1	C1	C2	109.3(5)	O5'	C1'	C2'	112.0(5)
O5	C1	C2	109.7(5)	O2'	C2'	C1'	107.5(5)
O2	C2	C1	110.1(5)	O2'	C2'	C3'	109.6(5)
O2	C2	C3	107.8(5)	C1'	C2'	C3'	110.0(5)
C1	C2	C3	111.9(5)	O3'	C3'	C2'	111.5(5)
O3	C3	C2	108.5(5)	O3'	C3'	C4'	107.7(5)
O3	C3	C4	109.6(5)	C2'	C3'	C4'	108.9(5)
C2	C3	C4	109.0(5)	O4'	C4'	C3'	108.5(5)
S	C4	C3	109.3(4)	O4'	C4'	C5'	110.0(5)
S	C4	C5	110.2(4)	C3'	C4'	C5'	108.2(5)
C3	C4	C5	110.6(5)	O5'	C5'	C4'	108.8(5)
O5	C5	C4	110.6(5)	O5'	C5'	C6'	106.6(5)
O5	C5	C6	106.7(6)	C4'	C5'	C6'	112.5(5)

The S-C4 distance (1.827 Å) of compound **42a** is close to the mean value (1.821 Å).[113] The C1'-S bond length is 1.826 Å, and valence angle τ = C1'-S-C4 value is 103.0° (Table5.2). Both values are in the upper range for these parameters in β-thio-linked glycosides.[113] Both of the rhamnosyl units in compound **27** and **42a** occur in the $^1C_4$(L) chair conformation, and the glucose ring of **42a** is in the expected $^4C_1$ chair conformation. Despite the fact that it is fully protected, the glycosidic linkage of compound **42a** exists in the syn-Φ/syn-Ψ conformation with torsional angles of Φ = 96.2° and Ψ = -106.5° (Table 5.3).

Table 5.3. Linkage torsion angle (°) for **27** and **42a**

	27	**42a**
O5'-C1'-S-C4 (Φ)	83.96 (10) (O1-C1-S-C10)	96.2(5)
C1'-S-C4-C5 (Ψ)		-106.5(4)
H1'-C1'-S-C4 (Φ^H)	-36.2 (H1-C1-S-C10)	-23.5
C1'-S-C4-H4 (Ψ^H)		12.9

5.4 NMR studies of 42a

We employed NMR measurement to provide dynamic conformational information on **42a**. The ^{1}H NMR spectrum of compound **42a** was assigned by standard methods using GCOSY, T-ROESY and HMQC. Both six-member rings are in the conformation observed in the crystal structure, as shown by the intra-ring vicinal proton-proton coupling constants as well as the intra-residue ROE cross-peaks between the H1'/H3', H1'/H5' pairs of the rhamnose ring. As for the glycosidic linkage, the configuration in the solid state (*syn-Φ/syn-Ψ*) is heavily populated in solution, which was

indicated by the strong NOEs between H1' and H4 (Figure 4.9a and Figure 5.3). The other configuration *syn-Φ/anti-Ψ*, detected by NOE between H1'/H3, is usually populated for thio-linkage in free oligosaccharides.[7,43,80,81,82,83,84] as well as in the bound state with enzyme.[86] For the protected disaccharide **42a**, this conformation should be minimally populated due to steric hindrance around the glycosidic bond. In fact, the NOE between H1'/H3, characteristic for the *syn-Φ/anti-Ψ* conformer, was only weakly detected (~ 5%) (Figure 5.3). The H2'-H4 NOE, which would reveal the population of the *anti-Φ/syn-Ψ* conformer, was not detected.

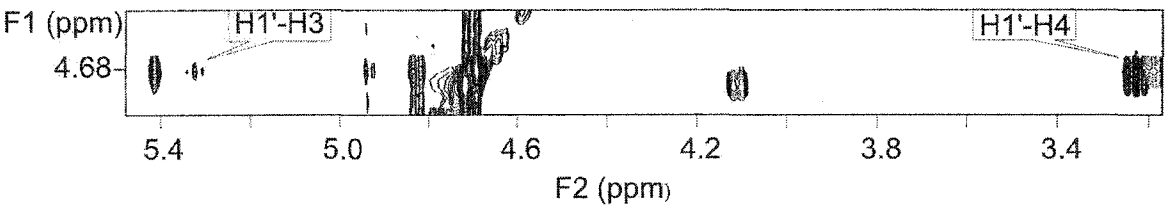


Figure 5.3. Section of the T-ROESY experiment of **42a** showing the inter-residue correlations

It is interesting to see that the chemical shift of H5' in **42a** at 3.19 ppm, "shifted" upfield from 3.67 ppm of the corresponding H5 in monosaccharide **27**. Clearly, the H5' proton, which is held below the aromatic ring of the O-6 benzoyl group (Figure 5.4), is shielded due to the aromatic ring current effect.

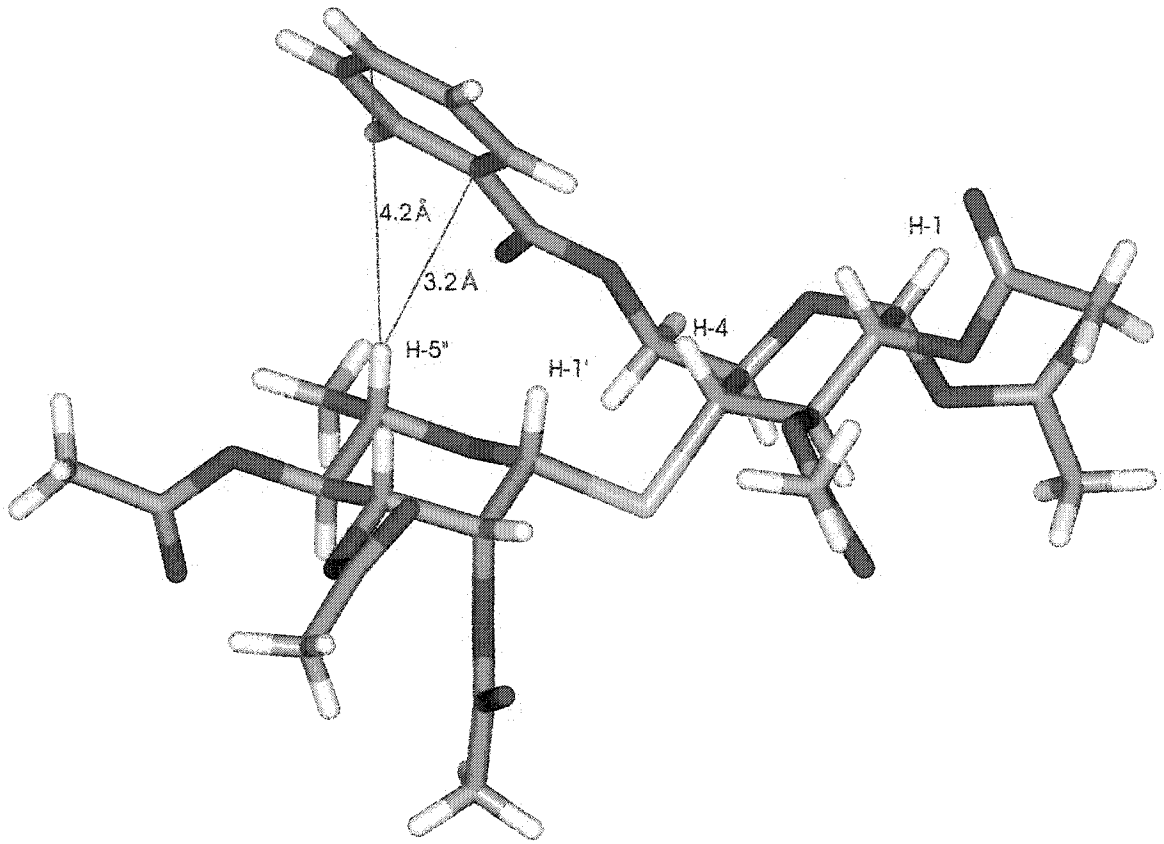


Figure 5.4. Crystal structure of **42a** showing relative position of H5' to benzene ring

Chapter 6

Thio-linked oligosaccharides: better immunogens?

Synthesis and immunization studies of *S*-linked trisaccharides related to *Shigella flexneri* variant Y LPS antigens

6.1 Introduction

Carbohydrate-based vaccines present a powerful weapon against infection[118] and even cancer.[119,120,121] This stems from the unique properties of carbohydrate molecules: the molecular structures are highly conserved, as they are secondary gene products. Unlike proteins, bacterial polysaccharides, with the exception of form variation, exhibit no mutation in their primary structure.[122] Aberrant cell surface glycosylation patterns have been targeted in the intensive development of anti-cancer vaccines.[123,124,125]

The immune response to polysaccharides is characterized by production of IgM antibody and the absence of immunological memory. This arises because polysaccharides are so called T-cell independent antigens – they activate B cells to produce antibody without the involvement of the T-cell, a cell type that is of crucial importance in the immune response to protein antigens.

Protein antigens are normally processed (hydrolysed to peptides) and then presented to T-cells when these peptides are bound by a histocompatibility complex (MHC). The complex is recognized by T-cells, which in turn signal B cells to start making antibody. Under the mediation of T-cells, the immune response also generates memory cells that can rapidly reactivate during future immune responses to the same

antigen. The T-cell also orchestrates an antibody class switch from low affinity IgM to higher affinity IgG antibody.

Carbohydrates since they are not processed by immunocompetent cells do not get presented by MHC molecules and do not stimulate T-cells. However, when covalently coupled to proteins, carbohydrates can become T-cell dependent antigens by virtue of the immunogenicity of the protein. In this way bacterial polysaccharides have been converted into T-cell dependent antigens and in this form are used as commercial vaccines.[122] A similar approach is being adopted to convert tumor associated ganglioside glycoshingolipids into conjugate vaccines for cancer therapy.

It was demonstrated that synthetic oligosaccharide protein conjugates elicit a stronger immune response in animals[126] and human[127] than isolated polysaccharide-protein conjugates. There remain, however, several obstacles to the further development of synthetic carbohydrate-based vaccines, such as the challenge of preparation of these complex molecules, low immune responses and *in vivo* enzyme hydrolysis of glycosidic bonds. Replacement of the glycosidic linkage(s) with unnatural but metabolically stable C-S bond(s) might shed some light on these problems.

Thio-oligosaccharides have been exploited extensively as enzyme inhibitors.[74] However, few studies have been conducted using thio-oligosaccharides as vaccines. Recently, Bousquet *et al.*[128] have shown that the *S*-linked Tn thioglycopeptide displayed immunostimulatory activity similar to that of the *O*-linked analog. Furthermore, the *S*-linked Tn thioglycopeptide reached its maximal effect at lower doses compared to the *O*-linked glycopeptide. One of the ongoing projects in this laboratory is the evaluation of a series of glycoconjugates, including a thio-oligosaccharide conjugate, for the

development of an anti *Candida albicans* vaccine.[129] A systematic immunochemical study of thio-oligosaccharide conjugates, especially in comparison with their *O*-linked glycoconjugate counterparts, has yet to be undertaken.

Here, we chose a well-studied monoclonal antibody SYA/J6 to address this issue. The SYA/J6 antibody recognizes a linear polysaccharide composed of a tetrasaccharide repeating unit [→2)α-*L*-Rha*p*(1→2)α-*L*-Rha*p*(1→3)α-*L*-Rha*p*(1→3)β-*D*-GlcNAc*p*(1-]. This polysaccharide is present on the cell wall of variant Y *Shigella flexneri*, a Gram negative bacterium. Crystal structures of SYA/J6 antibody Fab fragment were obtained with several synthetic oligosaccharides.[130,131] It was shown that the trisaccharide **53** (α-*L*-Rha*p*(1→3)α-*L*-Rha*p*(1→3)β-*D*-GlcNAc*p*) is the antigenic determinant, accounting for most of the binding with the antibody[132,133,134,135,136] (Figure 6.1).

Our objective is to address two issues: first, the inhibitory power of *S*-linked oligosaccharides as compared to *O*-linked analogs; and second, we want to address the question whether antibody produced against glycosidase-resistant thio-analogs will bind to the natural *O*-linked oligosaccharides antigen (Rha-Rha-GlcNAc). To this end, we have designed three analogs (**54-56**) of the natural trisaccharide with sequential replacement of oxygen by sulfur at glycosidic linkages. Here we report the chemical synthesis of these new analogs and the preliminary results of immunization studies with their protein conjugates.

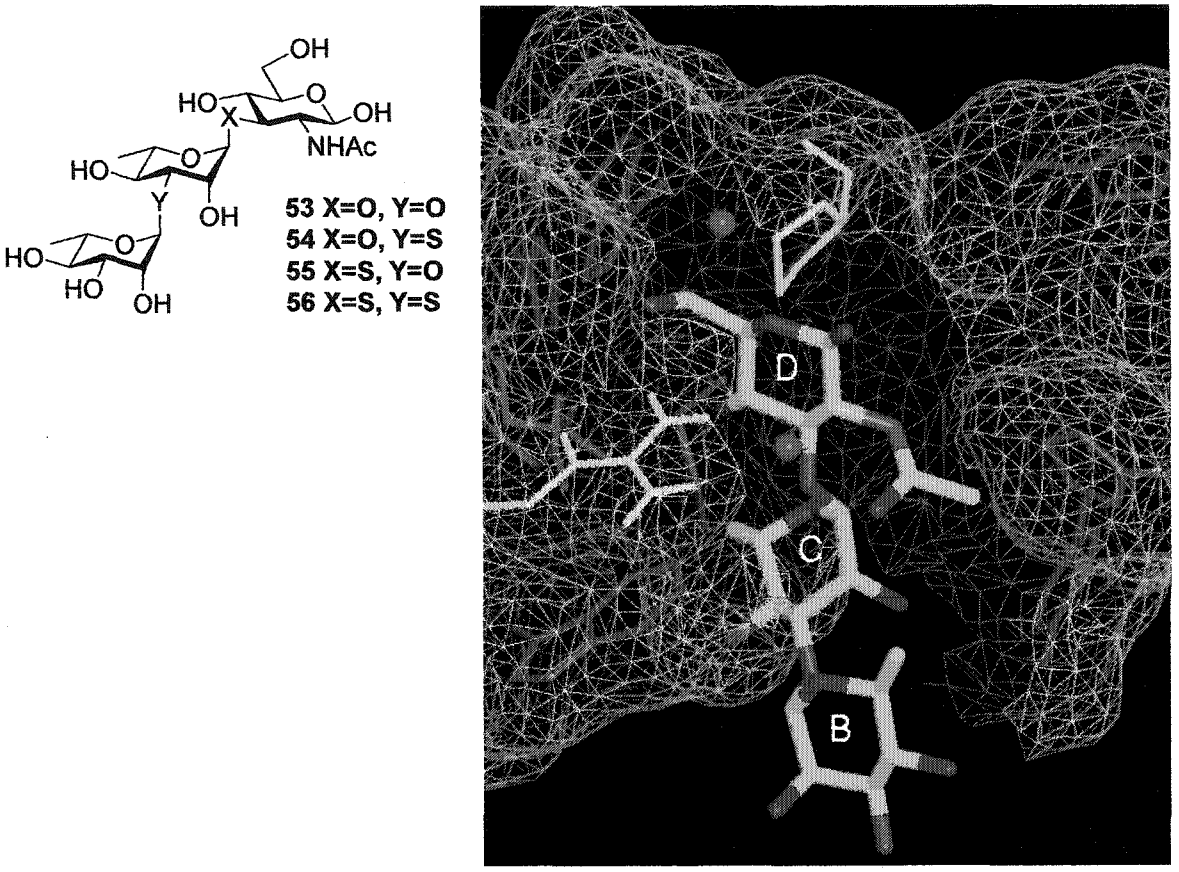


Figure 6.1 The trisaccharide (*L*-Rha*p*(1→3)α-*L*-Rha*p*(1→3)β-*D*-GlcNAc*p*) **53**, is shown complexed in the binding site of the SYA/J6 antibody. The D ring and C-methyl group of the C ring are buried in the binding pocket, while residue B is largely solvent exposed[130,131]

6.2 Retro-synthetic analysis

A convergent retro-synthetic scheme is shown in Figure 6.2. The three target thio-linked trisaccharides (**53**, **54**, and **55**) can potentially be constructed by glycosidation of the proper disaccharide trichloroacetimidate donors (**57** or **58**) with the corresponding glucosamine acceptors (**59** or **60**).

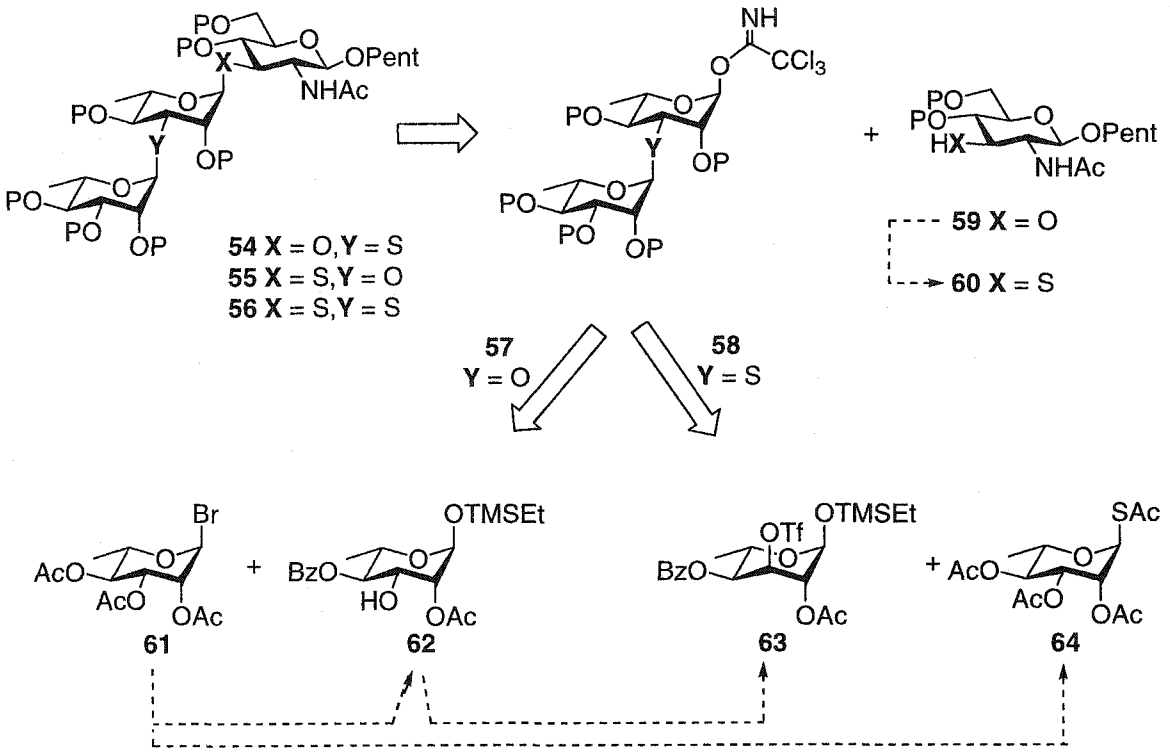


Figure 6.2. Retro-synthetic scheme

Two methods have been reported for the preparation of 3-thio-glucosamine derivatives. Hashimoto *et al.*[137] developed a method involving the opening of an aziridine ring by a thiolate anion to give a 2:1 mixture of the desired thio-linkage (D-*gluco*) and its D-*allo* isomer in 92% yield (Figure 6.3). More recently, Aguilera *et al.*[138] published a more efficient route that exploits the cyclic sulfamidate derived from D-allosamine as the substrate in a nucleophilic displacement by thiolate (Figure 6.4).[139] It was reported that using the conventional leaving groups, such as 3-triflate or 3-tosylate of D-allosamine derivatives, led to elimination products.

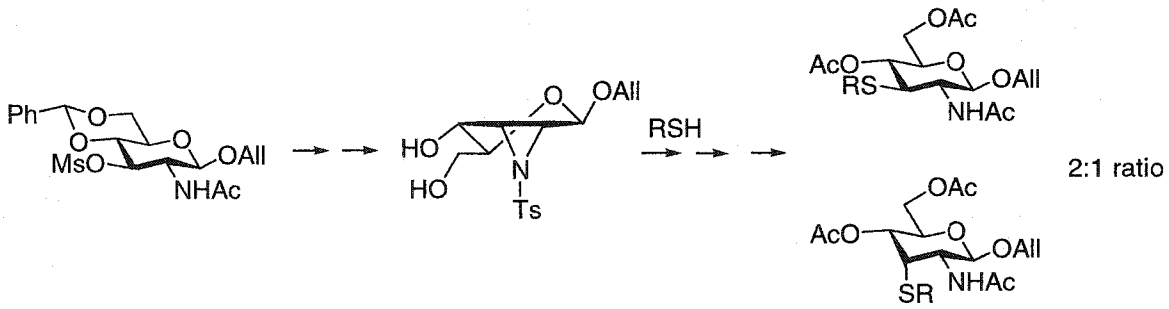


Figure 6.3. Aziridine ring opening to 3-thio allosamine derivatives

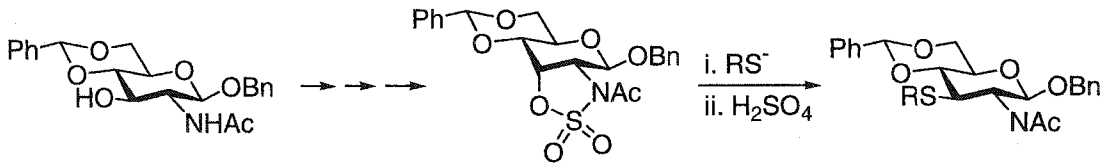


Figure 6.4. Synthesis of 3-thio-glucosamine derivatives via cyclic sulfamidate

The synthesis of the *O*-linked disaccharide donor **57** can be accomplished by coupling the easily accessible per-acetylated rhamnosyl bromide **61** with acceptor **62** (Figure 6.2). Migration of the acetyl group under acidic conditions from O-4 to O-3 in **62** can be circumvented by using a benzoyl group. Compound **62** can be obtained from bromide **61** through controlled glycosylation conditions. The 2-(trimethylsilyl)ethyl (TMSEt) ether is an ideal anomeric protecting group here, since it can withstand various reaction conditions and can be easily hydrolysed to afford the desired disaccharide hemiacetal.[140]

By applying the methodology we developed recently for the synthesis of 1,2-cis-β thio-linked glycosides,[72,141] nucleophilic displacement of triflate **63** at C-3 by a rhamnosyl thiolate anion derived from **64** should give the 1,2-*trans*-α thio-linkage in the *S*-linked disaccharide donor **58**. Alternatively, a thiol functionality can be preinstalled at

the C-3 position of rhamnose and the thiol can be glycosylated by a rhamnosyl donor (Figure 6.5).

Compound **63** can be prepared from rhamnopyranoside **62** followed by inversion of stereochemistry at C-3. The synthesis of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-*L*-rhamnopyranose **64** has not yet been reported, although our group recently reported an efficient synthesis of its β-anomer.[137] Hashimoto[16] has reported a two-step reaction to prepare 2,3,4-tri-O-acetyl-1-S-acetyl-1-thio-α-*L*-fucopyranose under Fisher glycosylation conditions followed by acetylation in only moderate 31% yield (Figure 6.6). We decided to use a different strategy to prepare **64** by reacting bromide **61** with thioacetic acid under glycosylation conditions, the participating acetyl group at C-2 should then control the α-stereochemistry and lead possibly to a better yield (Figure 6.6).

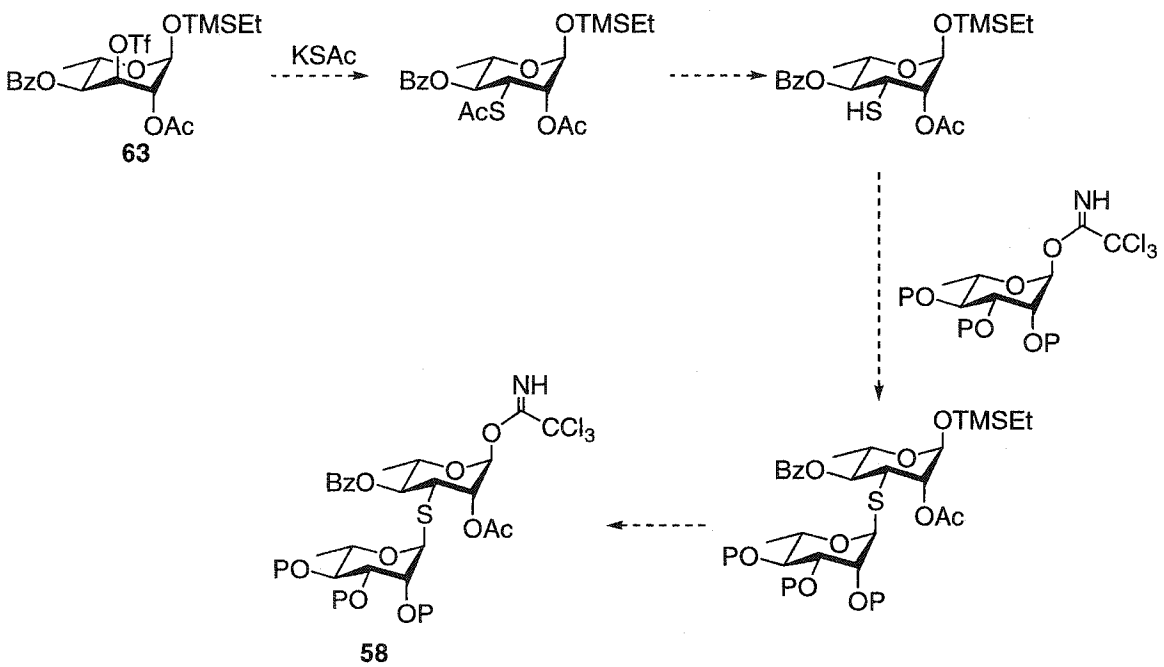


Figure 6.5. Alternative strategy to prepare the *S*-linked disaccharide donor **58**

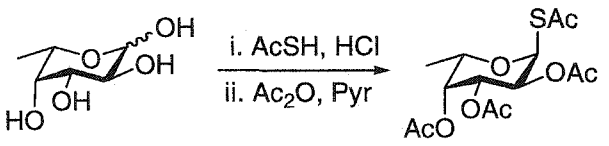


Figure 6.6. Preparation of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-*L*-fucopyranose

We elected to use the pentenyl group as the aglycon of the synthetic trisaccharides, since its double bond can be easily derivatized in order to couple to protein carriers.[142] The pentenyl aglycon was introduced by Fraser-Reid's lab[143] as a versatile and temporary protecting group for the anomeric position of sugars. It can be activated to conduct glycosylation under the promotion of *N*-iodosuccinimide. Fraser-Reid *et al.*[144] have also developed an armed-disarmed strategy to synthesize oligosaccharides using this aglycon.

6.3 Synthesis of *O*- and *S*-linked acceptors

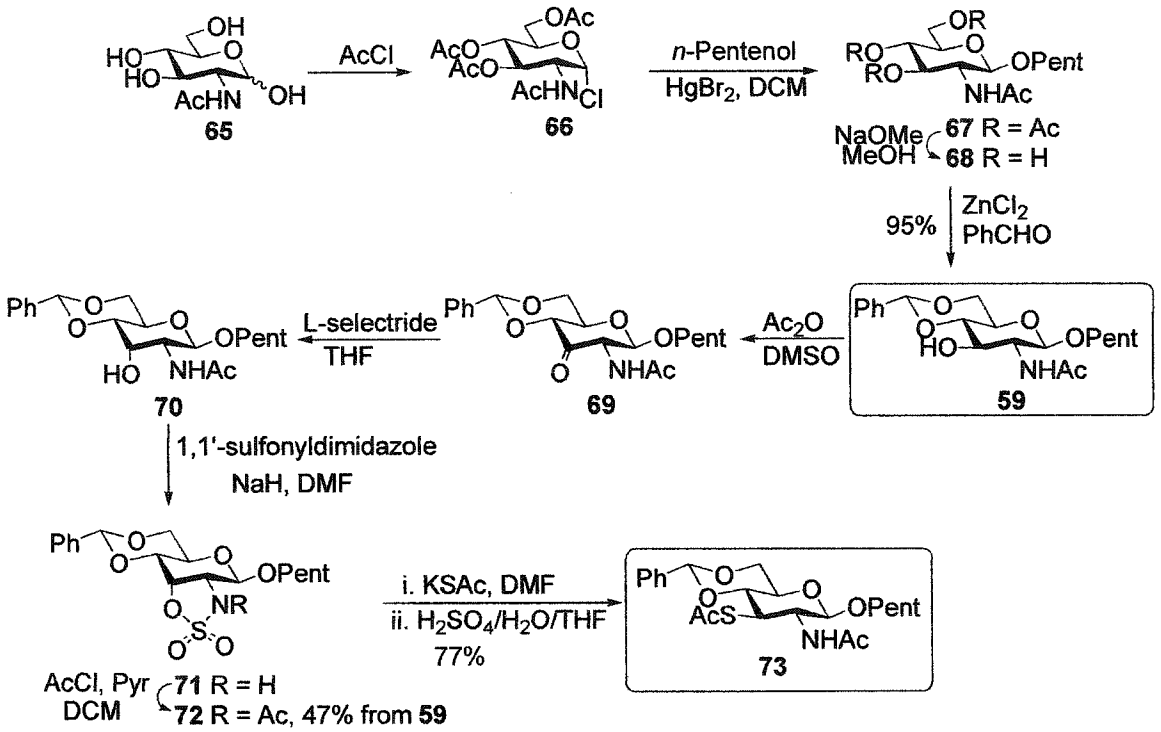
The synthesis of *O*- and *S*-linked acceptors is shown in Scheme 6.1. Pentenylated glucosamine derivative **68** was prepared in good yield in three steps from readily available *N*-acetyl-*D*-glucosamine **65** by following the literature procedure.[145] Different reaction conditions were attempted for the synthesis of benzylidene acetal **59** from **68**: treating **68** with benzaldehyde dimethyl acetal; using different acids (camphorsulfonic acid or *p*-toluenesulfonic acid) in different solvents (dry DMF or dry acetonitrile) at various temperatures (50 °C or refluxing temperature) led to **59** in only low to moderate yield; however, by employing dry $ZnCl_2$ powder in benzaldehyde, compound **68** was converted to **59** in excellent yield on a multi-gram scale. Next, the cyclic sulfamidate derivative **72** was prepared from **59** after a four-step transformation sequence that

involved only one purification step: the 3-OH was oxidized using DMSO-Ac_2O and the resulting ketone was reduced using L-selectride to afford exclusively the 3-epimer (**70**); the sulfamidate ring was introduced by treating **70** with 1,1'-sulfonyl diimidazole using NaH as base (→ **71**) and the sulfamidate was reacetylated with acetyl chloride to afford **72** in 47% overall yield.[132]

A single crystal of **72** was obtained and the structure was solved[iii] by X-ray crystallography (Figure 6.7).[146] As we can see from the Figure, in this tricyclic system, the pyranose ring and the benzylidene acetyl ring still adopt the chair conformation, with slight flattening at the pyranose ring. The five member ring of the cyclic sulfamidate adopts an envelope conformation with C-2, N, S and O-3 aligned in the same plane and C-3 out of the plane. The phenyl ring occupies the equatorial position at the acetal carbon center, and adopts a conformation where the plane of the phenyl ring is perpendicular to the dioxane ring. It is interesting to note that the carbonyl group is parallel to and the methyl group is antiparallel to the N-S bond.

Nucleophilic displacement by potassium thioacetate in DMF regioselectively opened the sulfamidate ring in **72,** and after the hydrolysis of the sulfate functionality, the desired glucosamine derivative **73** was obtained in 77% yield.

[iii] Crytal structures were solved by Dr. Robert McDonald at the X-ray laboratory of this department



Scheme 6.1. Synthesis of *O*- and *S*-linked acceptor **59** & **73**

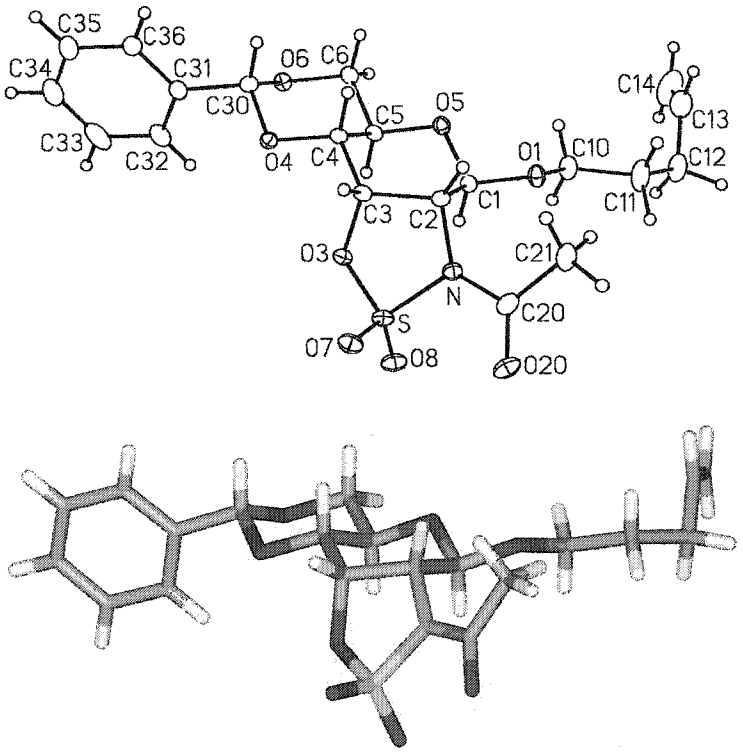
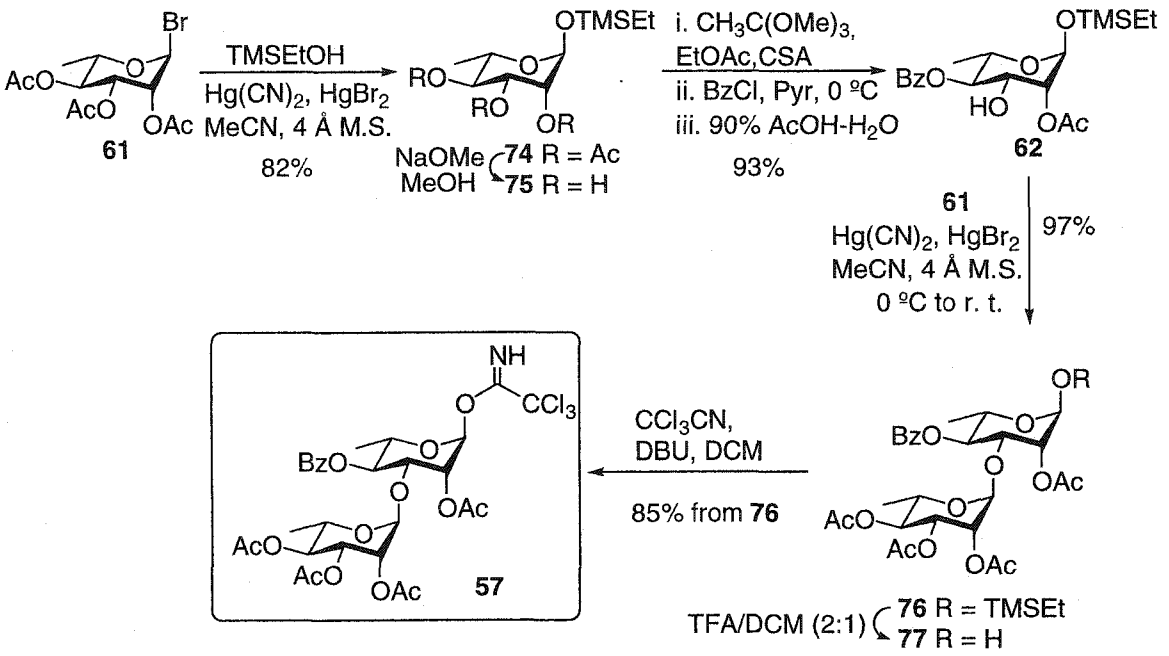


Figure 6.7. X-ray crystallography structure of sulfamidate **72**

6.4 Synthesis of *O*- and *S*-linked disaccharide donors

The synthesis of *O*-linked disaccharide donor **57** required the use of a rhamnosyl acceptor that has an anomeric OTMSEt protecting group (Scheme 6.2). The introduction of the OTMSEt group to rhamnose was previously investigated by Poszgay *et al.*[147] The authors reported some difficulty in using the 2,3,4-tri-*O*-acetyl-α-*L*-rhamnopyranosyl bromide **61** as a donor with silver triflate as promoter in dichloromethane as solvent; and the OTMSEt group could only be introduced in high yield by using the corresponding 2,3,4-tri-*O*-benzoyl-α-*L*-rhamnopyranosyl bromide as the donor. We have investigated the introduction of OTMSEt to rhamnose using the milder promoter mercury. If the glycosylation was carried out with tri-*O*-acetyl glycosyl halide **61** in anhydrous dichloromethane, none of the desired glycoside could be obtained; however, when we carried out the reaction in anhydrous acetonitrile at room temperature, the desired glycoside **74** was obtained in very good yield (82%). Following transesterification, the resulting triol **75** was treated with trimethyl orthoacetate under vacuum to form the 2,3-cyclic orthoester intermediate; the remaining 4-OH was benzoylated, and the 2,3-cyclic orthoester was regioselectively opened by aqueous acid to afford compound **62** in 93% yield. The 3-OH of acceptor **62** was glycosylated with donor **61** under Koenigs-Knorr glycosylation conditions to afford disaccharide **76** in excellent yield (97%). Trichloroacetimidate **57** was then prepared after selective deprotection of **76** with trifluoroacetic acid in dichloromethane (→ **77**) followed by treatment of the hemiacetal **77** with trichloroacetonitrile under standard conditions to give imidate **57** in 85% yield.



Scheme 6.2. Synthesis of *O*-linked disaccharide donor **57**

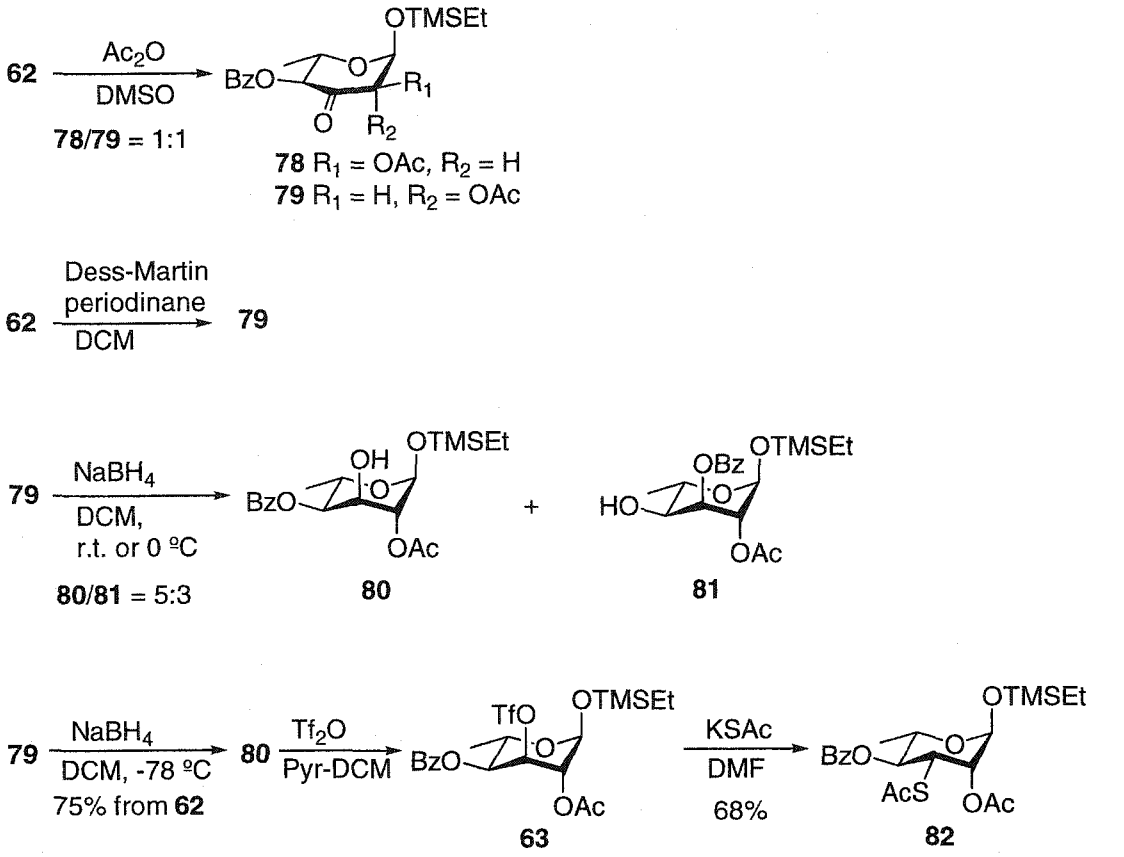
The key step in the synthesis of *S*-linked disaccharide donor **58** was the transformation of the equatorial 3-OH group of **62** to its axial epimer. This is carried out by an oxidation/reduction sequence using DMSO-Ac_2O as oxidation reagents (Scheme 6.3). Although the oxidation by DMSO-Ac_2O in almost quantitative yield according to TLC, it was discovered that the intermediate ketone **78** epimerized slowly to its 2-epimer which is inseparable from **78** (**78/79** ratio is 1:1). ^{1}H NMR showed $J_{1,2}$ coupling constants 1.8 Hz for **78**, and 4.3 Hz for **79**. Long-range coupling was observed in ketone **79** ($^4J_{2,4}$ = 1.1 Hz). The epimerisation likely occurred due to the slight acidity of the reaction mixture. We next tried Dess-Martin oxidation conditions, and to our satisfaction, this method afforded ketone **78** as the sole product according to TLC, no epimerisation at C-2 was observed. NMR revealed that the intermediate ketone **78** was sufficiently pure for the reduction step. Reduction with $NaBH_4$ at room temperature gave a mixture of the desired

4-benzoate **80** and undesired regioisomer **81** due to an intramolecular benzoate migration from O-4 to O-3 (Figure 6.7). In order to suppress this side reaction, we carried out the reduction at lower temperatures. The best reaction temperature was –78 °C, at which temperature the reaction was completed in 5 minutes and compound **80** was obtained in 75% yield. None of the migration product isomer **81** was detected.

With the altroside **80** in hand, the desired thioacetate **82** was prepared in 68% yield by first transforming the 3-OH to a 3-triflate, followed by displacement of the 3-*O*-triflate with KSAc (Scheme 6.3).

The synthesis of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-*L*-rhamnopyranose **64** was carried out by glycosylation of bromide **61** with thioacetic acid under promotion of AgOTf (Scheme 6.4). Compound **64** was obtained in excellent yield (90%). The preparation of 1,2-*trans*-α thio-linkage by this method is complementary to the method for 1,2-*cis*-β thio-linkage we developed earlier.[72]

The anomeric S-acetate of **64** was selectively deprotected and the resulting thiolate was used to carry out a direct *in situ* nucleophilic displacement on triflate **63** at 0° C to afford the *S*-linked disaccharide **83** in 46% yield. Although the yield was moderate, we were satisfied with the short route. The anomeric OTMSEt was successfully transformed to the disaccharide imidate donor **58** in 81% yield following the same reaction sequence as described above.



Scheme 6.3. Synthesis of thioacetate **82** *via* oxidation, reduction and S_N2 reaction

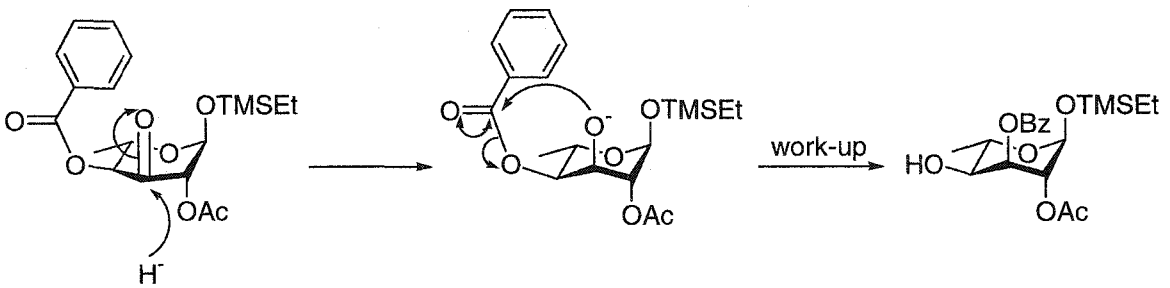
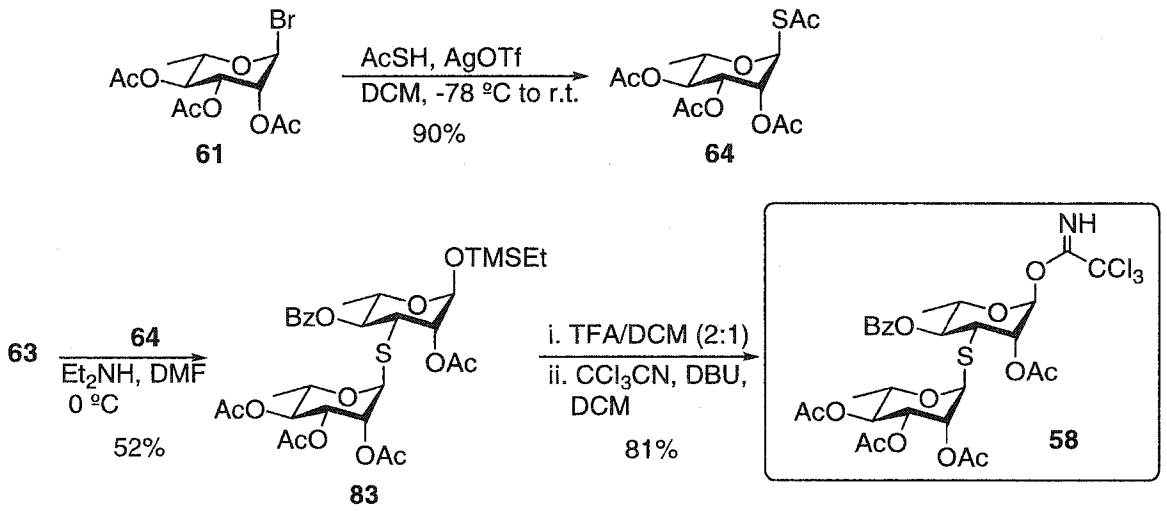


Figure 6.8. Postulated reaction mechanism for benzoyl migration during reduction of the ketone



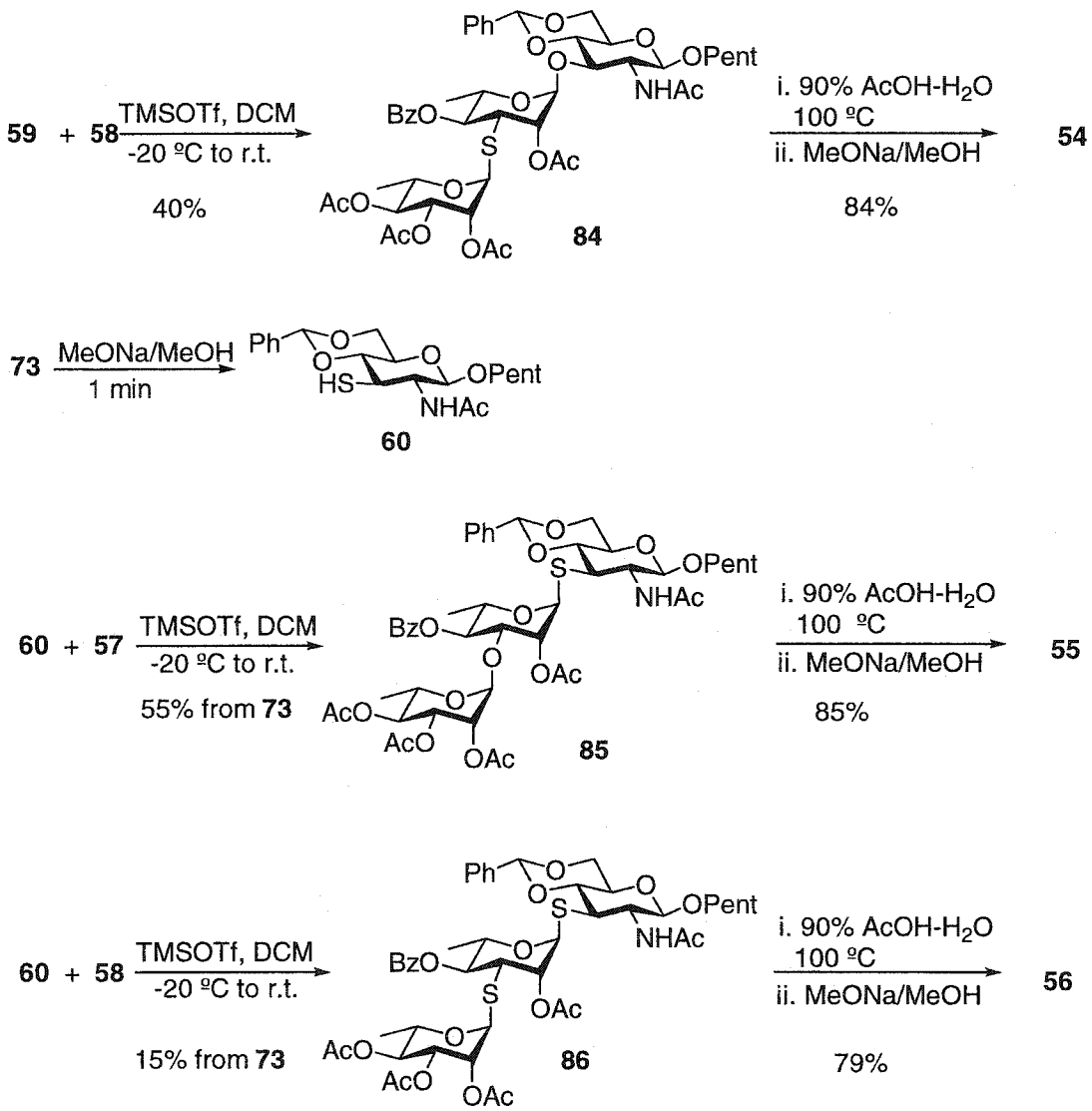
Scheme 6.4. Preparation of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-*L*-rhamnopyranose **64** and *S*-linked disaccharide donor **58**

6.5 Assembly of target thio-linked trisaccharides

With all *O*- and *S*-linked acceptors and donors in hand, the three target thio-linked trisaccharides were assembled as shown in Scheme 6.5. Coupling of **59** with **58** under activation by TMSOTf in dichlormethane afforded trisaccharide **84** in moderate yield (40%). Deprotection of **84** was accomplished by first hydrolysing the benzylidene acetal with 90% aqueous acetic acid at 100 °C, followed by removal of all ester groups by transesterification. Trisaccharide **54** was obtained in pure form (84% yield) after reverse–phase HPLC.

For the synthesis of the other two thio-linked trisaccharides, it was necessary to unmask the thiol of glucosamine derivative **73** before glycosylations. This was done by treating **73** with 10 equivalent of NaOMe in anhydrous degassed MeOH for 1 minute to give thiol acceptor **60**. Prolonged stirring at various temperatures resulted in the formation of disulfide. Compound **60** was reacted with donor **57** or **58** under similar

reaction conditions to those described for the synthesis of **84** to give the corresponding trisaccharides (→**85**, 55%; →**86**, 15%). In our hands, adding molecular sieves did not improve the yield for the three glycosylation reactions. The reason for the low yield of coupling S-linked acceptor **60** with S-linked donor **58** is unknown at this point. Free trisaccharides **55** and **56** were obtained in good yield after deprotection and purification by reverse-phase HPLC.



Scheme 6.5. Assembly of three target thio-linked trisaccharides **54**, **55** and **56**

6.6 Evaluation of the immunochemical activities of trisaccharide thioglycosides ligand and epitopes

There are two general ways to evaluate the immunochemical activities of compounds **54** - **56** and the corresponding glycoconjugates **91** and **92**. Inhibitory power of ligands **54** – **56** are measured using a monoclonal antibody SYA/J6, the binding site of which is optimally filled by the native trisaccharide Rha-Rha-GlcNAc **53**.[131] In this way we can assess how well *S*-linked oligosaccharide fit in the binding site generated by the native *O*-linked antigen.

However, this activity correlation may or may not reflect the ability of the same epitopes to raise specific antibody that is able to recognize the native antigen when the S-linked epitopes are coupled to a protein carrier and used to immunize mice or rabbits. In our work we coupled ligands **54** and **55** to BSA, immunized mice to generate mouse antibodies to these conjugates **91** and **92** and then measured the ability of the immune sera to bind to *S*-linked and *O*-linked epitopes **93** - **95** coated on ELISA plates.

6.7 Binding studies of trisaccharide 54 and 55

The thio-linked trisaccharides **54** and **55** were evaluated in a competitive ELISA (enzyme-linked immunosorbent assay), and their inhibitory power was compared *via* their IC_{50} values.[iv] A general outline of the ELISA is shown in Figure 6.6. The ELISA plate was coated with antibody SYA/J6, which is specific for the *O*-linked trisaccharide. Thio-linked trisaccharides **54** or **55** were tested for their ability to inhibit binding of biotinylated *O*-linked lipopolysaccharide (LPS) antigen to SYA/J6 antibody coated plates. The IC_{50} for compound **54** was 0.9 mM, **55** was more than 10 mM. In the same

[iv] All ELISA assays reported in this thesis were performed by Mrs. Joanna Sadowska

format, the natural *O*-linked trisaccharide (Rha-Rha-GlcNac-OMe) had an IC_{50} of 17 μM. The low affinity of **54** and **55** toward antibody SYA/J6 most likely arises from the more flexible thio-linkages. Based on this interpretation, the even more flexible trisaccharide **56** (with two thio-linkages) should have an even higher IC_{50}. A large quantity of compound is needed for ELSA to obtain a meaningful value. Therefore, it was not practical to assay compound **56** at this point due to the small quantity of compound available.

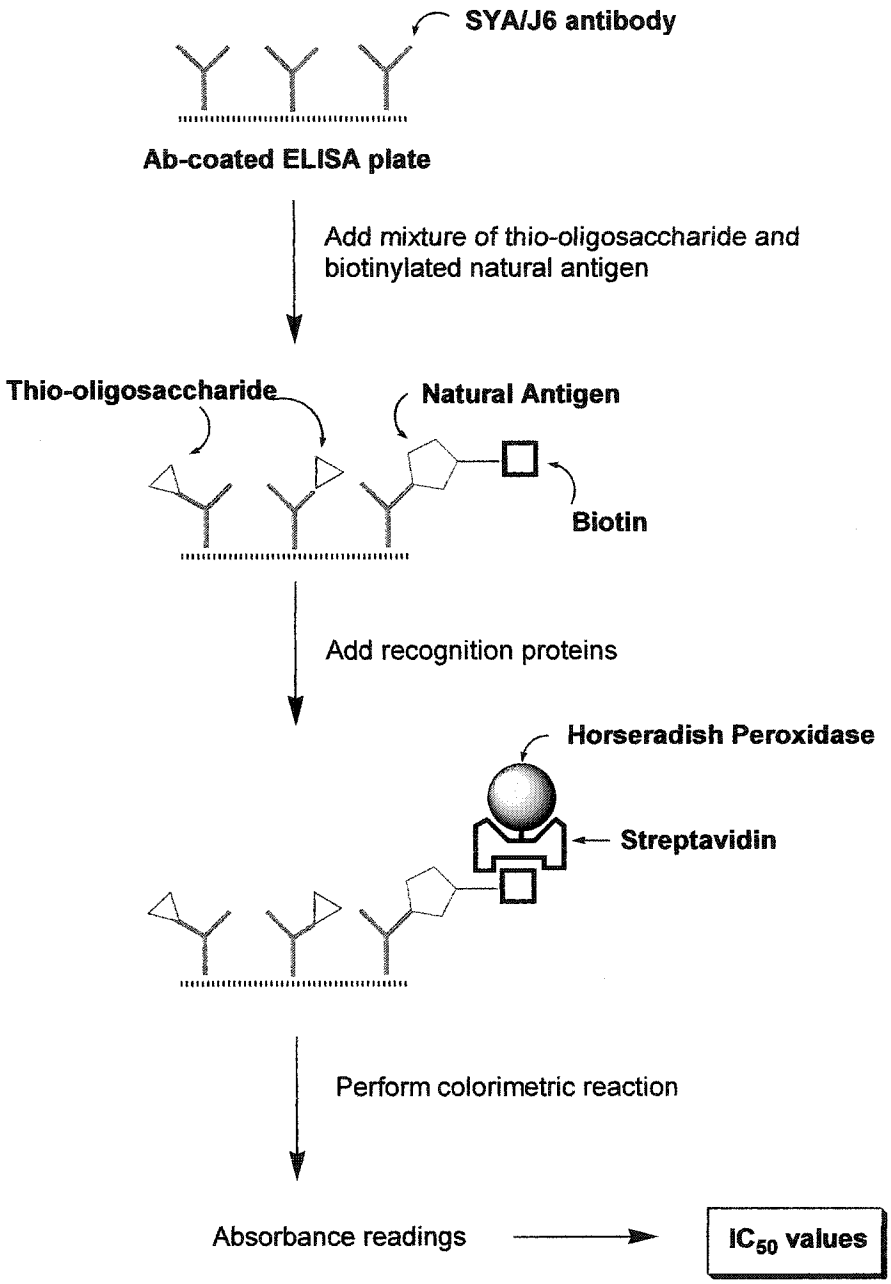
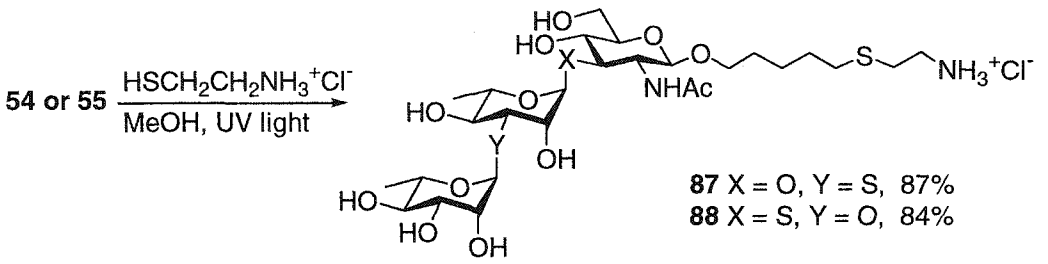


Figure 6.9. Competitive inhibition ELISA protocol for the evaluation of binding affinity of the synthetic ligands

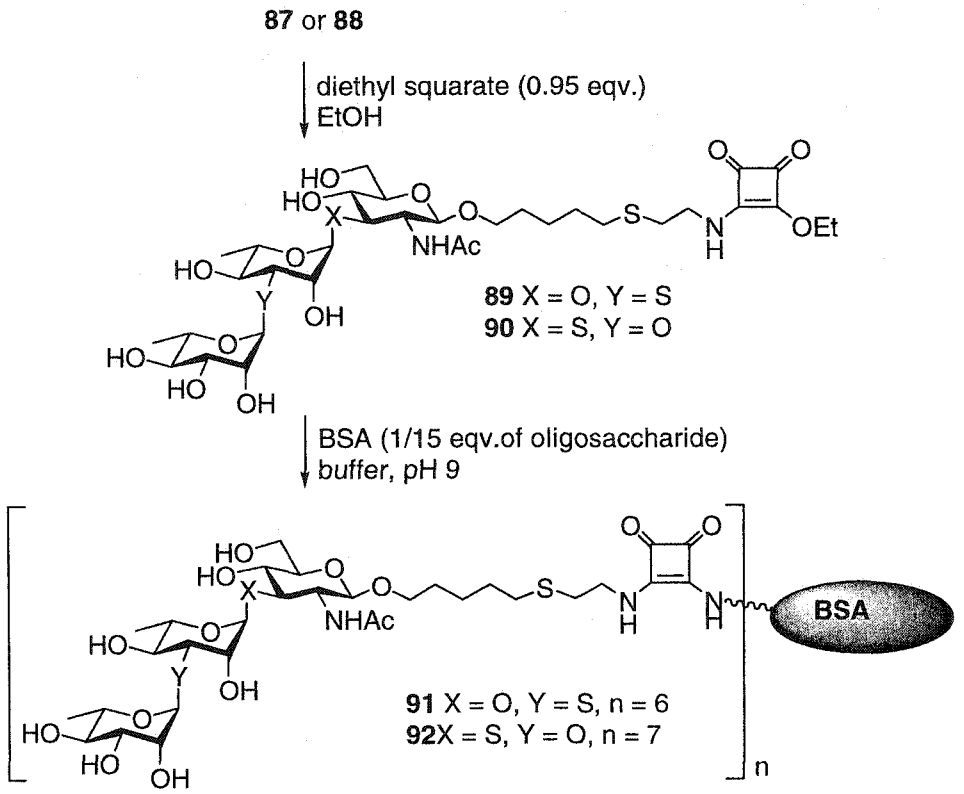
6.8 Synthesis of protein conjugates

The elongation and functionalization of the aglycon of pentenyl **54** and **55** was achieved through photoaddition of cysteamine hydrochloride to the terminal double bond to afford the desired trisaccharides **87** (87%) and **88** (84%) (Scheme 6.6).



Scheme 6.6. Fuctionalization of the thio-oligosaccharide linkers

Coupling of **87** and **88** to bovine serum albumin (BSA) employed diethyl squarate as developed by Hindsgaul *et al.*[148] (Scheme 6.7). Coupling efficiencies were 40-47%, which is at the lower end of published data. This corresponds to the incorporation of 6 or 7 ligands per BSA molecule using a 15-fold molar excess of activated thio-oliogosaccharide.

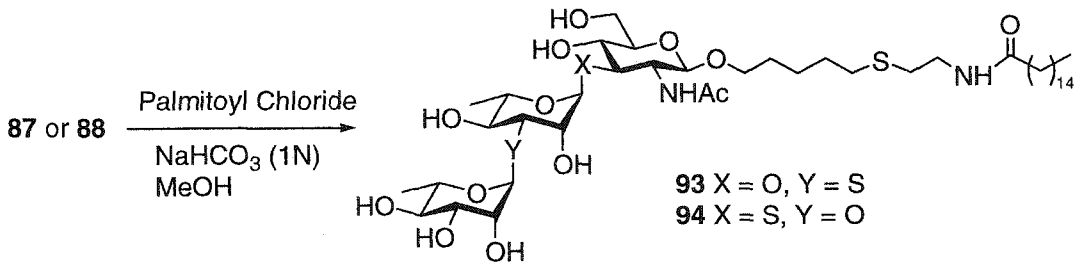


Scheme 6.7. Synthesis of protein conjugates

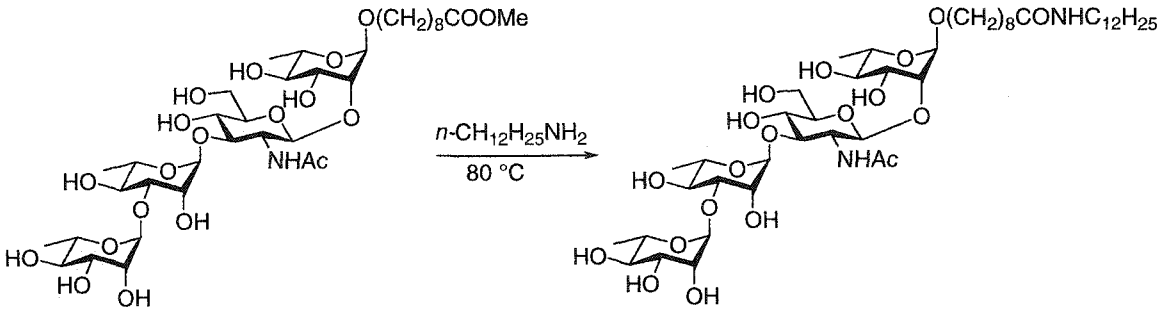
6.9 Synthesis of thio-oligosaccharide lipids for ELISA coating

Unpublished work from our group has suggested that the squarate moiety in the neoglycoconjugates might also induce antibodies.[126] In order to screen for antibodies in sera that specifically recognize the carbohydrate portion of the conjugates, two thio-oligosaccharide lipids **93** and **94** were synthesized for coating ELISA plates. The saturated hydrocarbon lipid we chose is 15 carbons long. After this work was completed, Wong *et al.* reported that the optimal length for saccharide lipids binding to microtiter plates through hydrophobic interaction is 13 to 15 carbon atoms.[149] A third glycoconjugate **95** was prepared from 8-methoxycarbonyloctanol glycoside of the native tetrasaccharide Rha-Rha-GlcNAc-Rha (Scheme 6.9).

A literature procedure[150] was modified for the synthesis of the glycolipids. Treatment of **87** with palmitoyl chloride and 1N $NaHCO_3$ in MeOH afforded **93** in moderate yield (47%). A similar procedure using **88** gave the desired compound **94** in 73% yield.



Scheme 6.8. Synthesis of thio-oligosaccharide lipids for ELISA coating



Scheme 6.9. Synthesis of glycoconjugate **95**, compounds were supplied by Dr. C-C. Ling and Dr. D. R. Bundle

6.10 ELISA titration of immune sera

Experimentally, an effective vaccine can be identified by monitoring the production of carbohydrate-specific antibody and the class switching from IgM to IgG antibody. Detection of carbohydrate specific IgM and IgG antibodies, with a characteristic rise in the latter as successive booster immunizations are administered, provides direct evidence of the class switch and indirect evidence of T-cell involvement. It also establishes the carbohydrate specific nature of the generated antibodies. Here we employed enzyme-linked immunosorbent assays (ELISA) to monitor the immune response to our synthetic conjugate vaccines.

As shown in Figure 6.11 and the following ELISA graphs, the curves indicate the extent to which mouse serum can be diluted and still exhibit binding to antigen. Here the cut-off is arbitrarily set at an optical density (OD) of 0.2 above background noise. It therefore provides a measure of the magnitude of the antibody response. A serum that can be diluted many fold is said to have a high titer. For example, if the dilution factor 10^{-5} corresponds to the cut-off OD of 0.2, the antibody titer is said to be 10^5 or a hundred thousand. Since antibody captured on the antigen-coated microtiter plate must survive numerous washing steps, ELISA titers provide a measure of both antibody concentration and antibody affinity/avidity.

6.11 Immunization of experimental animals

Balb/C mice were immunized 4 times with glycoconjugates **91** and **92** suspended in Freunds complete and incomplete adjuvant. Sera were collected after the 3rd injection and screened against the immunizing antigen by ELISA. Following a 4th injection the experiment was terminated, and final sera were collected and again tittered by ELISA.

Antibody titrations were made against three epitopes presented as lipid analogs **93** - **95** coated on ELISA plates. These glycolipid antigens corresponded to the respective homologous & heterologous *S*-linked trisaccharide antigens and native *O*-linked tetrasaccharide. Bound antibody was detected by goat anti-mouse IgG and IgM (Figure 6.10). The end point of the titration was taken as the antibody dilution required to give an OD of 0.2 above background. The end points were used to compare antibody levels.

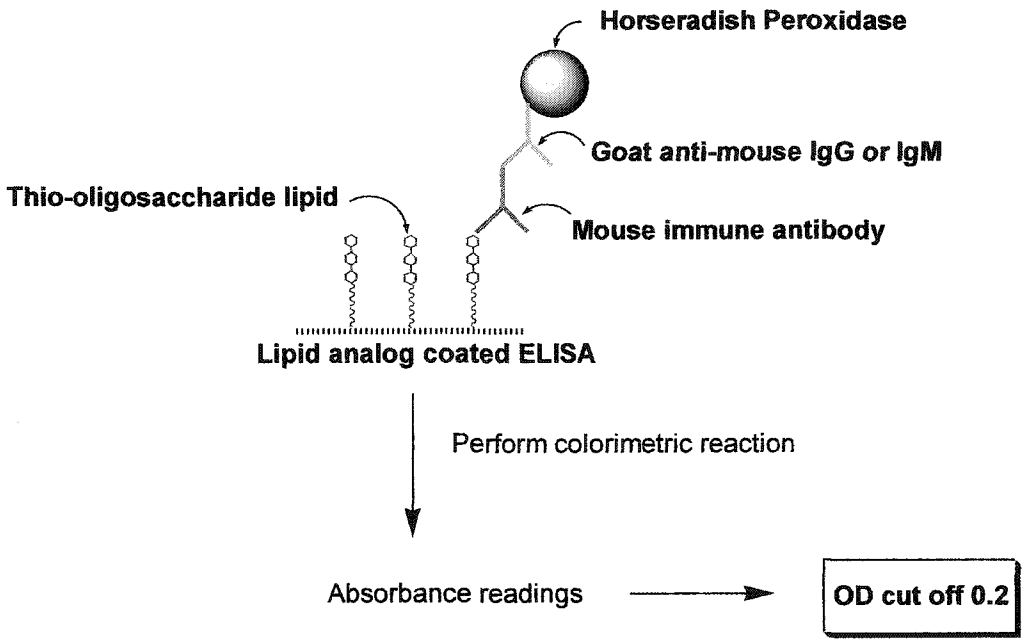


Figure 6.10. ELISA protocol for screening antibodies level in sera that specifically recognize the carbohydrate portion of the conjugates

6.12 Results of ELISA titration of immune mouse sera

Antibody levels for mice immunized with conjugates **91** and **92** as determined by titration are shown in Figure 6.11 and Table 6.1. Sera from mice immunized with conjugate **91** were evaluated against the homologous immunizing epitope **93** (Figure 6.11) and against the heterologous epitope **94**. The sera showed a specific response toward the carbohydrate epitope **93** with strong IgM and IgG titers (Table 6.1). Antibodies from these sera cross-reacted with the heterologous *S*-linked epitope **94**. A similar series of data were obtained with mouse sera from mice immunized with conjugate **92** (Table 6.1). The high titer against the homologous epitope **94** revealed a strong carbohydrate specific response, with moderate to high levels of IgM and IgG antibody. The antibody also cross-reacted with the heterologous *S*-linked epitope **93**. It is immediately evident that both *S*-linked antigens elicit a strong immune response and that these antibodies possess a relaxed binding fidelity to epitopes in which the location of the substitution of *S* for *O* varies.

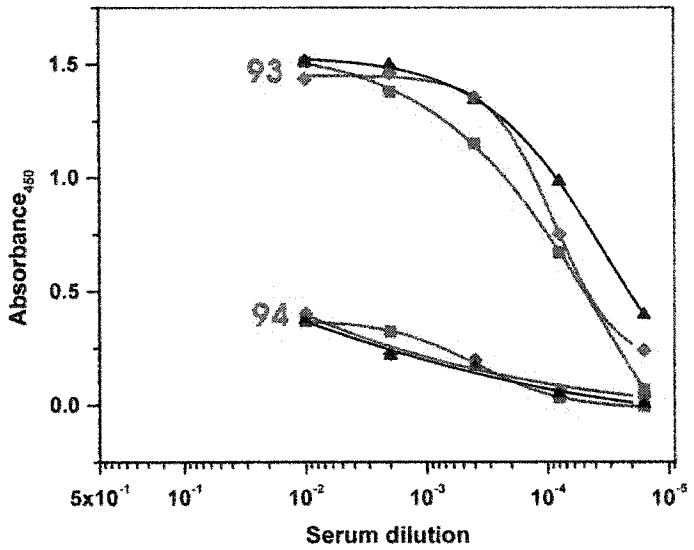


Figure 6.11. Titration of mouse sera generated against glycoconjugate **91** and screened against glycolipids **93** and **94**. Each line represents serum from one mouse

Table 6.1 Antibody titers of mice immunized by conjugates **91** and **92**

Immunogen[a]	Screening Antigen[b]	Titer[c,d]	
		IgM	IgG
91	**93**	2×10^3	1×10^5
91	**94**	2×10^3	3×10^2
92	**93**	2.5×10^3	5×10^4
92	**94**	2.5×10^3	5×10^2

[a] BSA-carbohydrate conjugates used to immunize mice
[b] glycolipid conjugates used to coat ELISA plates
[c] Titer is the reciprocal dilution giving an OD of 0.2 above background
[d] Titer were measured after 3 injections

The IgG antibodies raised against **92** show a higher titer with the heterologous *S*-linked epitope **93** than with the homologous epitope **94**. We attribute this to substantially different plate coating efficiency for conjugate **93** and **94**. Since only very small

quantities of these glycolipids were prepared, they were difficult to purify and the samples may not be as pure as the others. Alternatively the coating properties might be due to the different solvents used in the preparation of the stock solutions of **93** and **94** (i.e. stock solution of **93** was prepared with carbonate buffer and the stock solution of **94** with 1:1 MeOH-H_2O). Glycolipids have higher solubility in MeOH than in aqueous buffers. We also note that methanol has been used to detach glycolipids from microtiter plates.[149] Therefore, glycolipid **94** might not have coated the plates as well as lipid **93**, which may have resulted in a lower IgG titer against **94**. Similar IgM titers are observed for **92** when screening against **93** and **94**. This may be due to the multivalent nature of IgM molecules, which can bind to several epitopes on the plate at one time. IgM antibodies are not so dependent on epitope density as divalent IgG molecules. Glycolipid **93** appears to coat the plate in a normal manner. Thus, titers for sera from 3 mice immunized with immunogen **91** revealed a IgG response that has surpassed that for IgM when screened against glycolipid **93**.

The most crucial test is evaluation of the mouse sera raised to **91** and **92** for binding to the native sequence of glycolipid **95**, and to the native *O*-linked lipopolysaccharide (LPS) antigen. ELISA data are shown in Figures 6.12 to 6.15 and are summarized in Tables 6.1 and 6.2. Sera collected after the third and fourth immunizations show significant IgG antibody titers for both tetrasaccharide **95** and the LPS antigen (IgM levels were not measured for the cross-reactions). The shape of these titration curves and the absence of a plateau at low dilutions indicate that the binding is most likely the result of a strong cross-reaction rather than a close mimic of the nature epitope by the *S*-linked antigen.

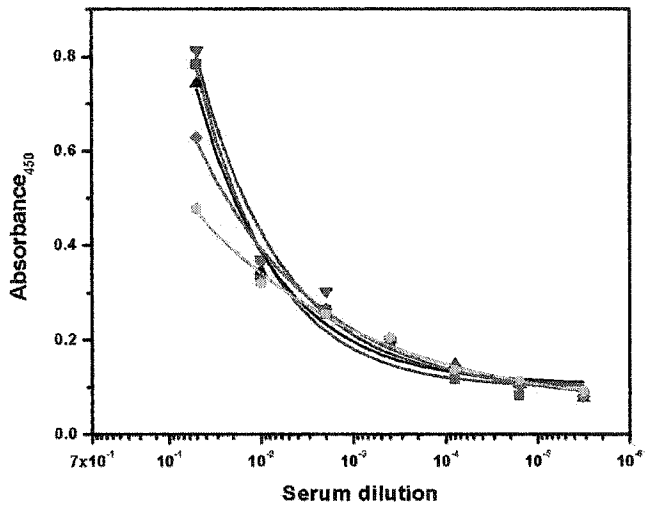


Figure 6.12. Titration of mouse sera (5 mice) generated against glycoconjugate **91** and screened against glycolipid **95**. Antigen captured antibody was detected by a goat anti-mouse IgG reagent

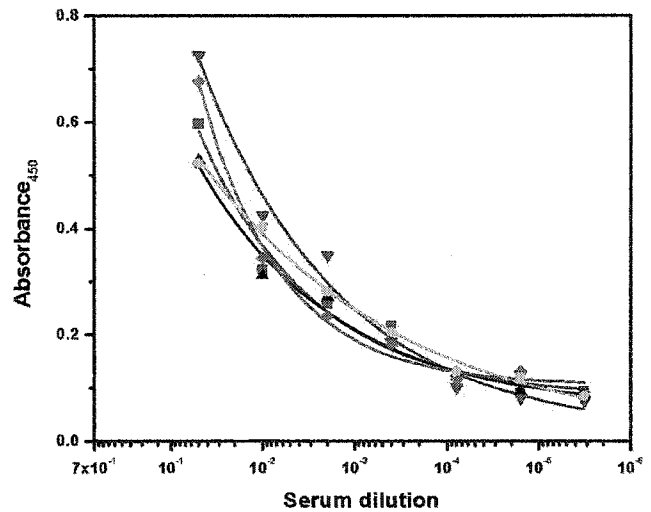


Figure 6.13. Titration of mouse sera (5 mice) generated against glycoconjugate **92** and screened against glycolipid **95**. Antigen captured antibody was detected by a goat anti-mouse IgG reagent

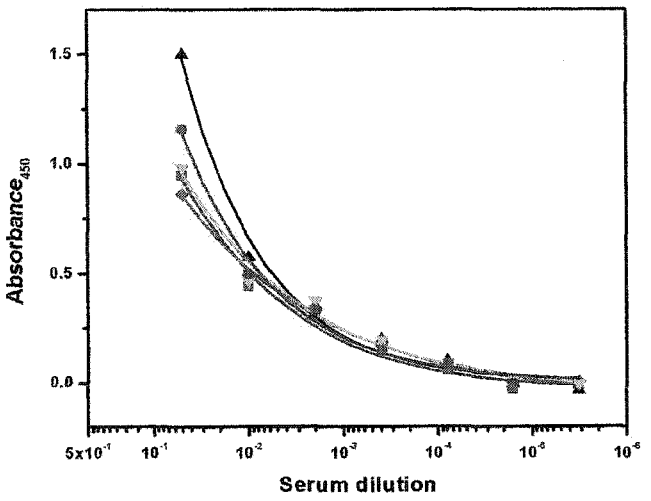


Figure 6.14. Titration of mouse sera (5 mice) generated against glycoconjugate **91** and screened against LPS antigen. Antigen captured antibody was detected by a goat anti-mouse IgG reagent

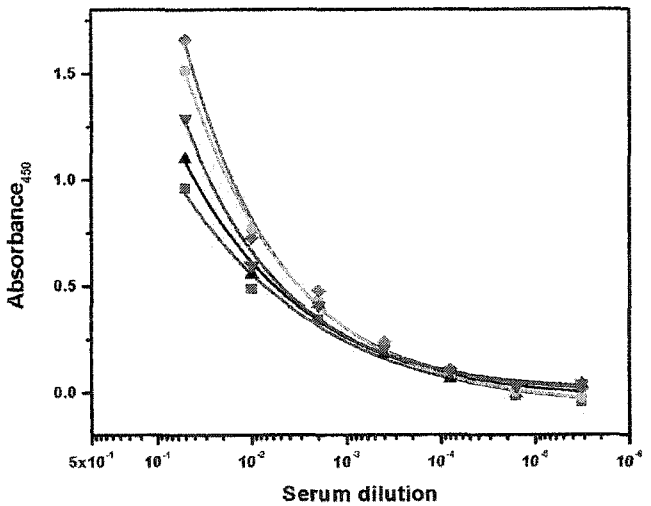


Figure 6.15. Titration of mouse sera (5 mice) generated against glycoconjugate **92** and screened against LPS antigen. Antigen captured antibody was detected by a goat anti-mouse IgG reagent

Table 6.2 Antibody titers of mice immunized by conjugates **91** and **92** and screened against native antigens.

Immunogen[a]	Screening Antigen[b]	IgG Titer[c,d]
91	**95**	1×10^3
91	**95**	1×10^3
92	LPS	1.5×10^3
92	LPS	5×10^3

[a] BSA-carbohydrate conjugates used to immunize mice
[b] glycolipid conjugates used to coat ELISA plates
[c] Titer is the reciprocal dilution giving an OD of 0.2 above background
[d] Sera collected 10 days after the 4th injection

One might not expect a cross-reaction because of the difference in the sequence of the immunogens and the native antigen. Hence, it is of considerable interest to note that the two antigens **91** and **92** are in fact internal epitopes, since the native *O*-antigen which is part of the LPS has the biological repeating sequence Rha-Rha-Rha-GlcNAc. Most often carbohydrate conjugates induce antibodies that recognize the terminal ends of the immunizing antigen. Antigens **91** and **92** are notable in that the antibodies to them recognize an internal sequence of the native antigen with very respectable antibody titers.

6.13 Conclusion

Three targeted thio-linked trisaccharides have been synthesized effectively in a highly convergent manner. A novel methodology for the synthesis of 1,2-*trans*-α thio-

linked oligosaccharides has been established that is complementary to the methodology developed earlier for the synthesis of the 1,2-*cis*-β thio-linkage[72]. From the easily accessable 1-thio-rhamnopyranose **64**, thio-linked disaccharide **83** was prepared smoothly by de-*S*-acetylation and *in situ* S_N2 reaction. Targeted thio-oligosaccharides **54** - **56** were synthesized under similar glycosylation conditions by using the corresponding glycosyl donors and acceptors. Neoglycoconjugates (**91** and **92**) and glycolipids (**93** and **94**) were also synthesized for the immunization studies.

The immunization studies revealed that *S*-linked antigens induce a strong carbohydrate epitope specific response with a good IgG antibody titer. The class switch from IgM to IgG indicates T-cell involvement in the response. The antibodies show cross-reactivity with the corresponding *O*-linked analogs. These data support the use of *S*-linked analogues to raise protective antibodies to selected microbial and most tumour carbohydrate associated antigens. This approach is the subject of intense investigation by our group.

Chapter 7

Experimental

General Methods

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 ± 2 °C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with sulfuric acid. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (E. Merck 40 – 60 μM, Darmstadt). ^{1}H NMR spectra were recorded on 300 MHz (Varian), 400 MHz (Varian), 500 MHz (Varian) or 600 MHz (Varian). The first order proton chemical shifts δ_H are referenced to either residual $CHCl_3$ (δ_H 7.24, $CDCl_3$) or internal acetone (δ_H 2.225, D_2O). HMQC NMR spectra were recorded on Varian Unity 300 MHz, 400 MHz, 500 MHz or 600 MHz NMR spectrometers. The ^{13}C chemical shifts, δ_C are referenced to internal $CDCl_3$ (δ_C 77.00, $CDCl_3$). Organic solutions were dried prior to concentration under vacuum at < 40 °C (bath). Reverse - phase chromatography was performed on a Waters 600 HPLC systems, using a Beckman semi-preparative C-18 column (10 × 250 mm, 5 μ), and the products were detected with a Waters 2487 UV detector or a Waters 2410 refractive index monitor. Microanalyses and electrospray mass spectra were performed by the analytical services of this department, and X-ray crystallography was carried out by the X-ray Crystallography Laboratory (XCL) of this department.

*2,3,4,6-Tetra-O-acetyl-1-S-acetyl-1-thio-β-*D*-mannopyranose* (**2**)

Method A: A solution of thioacetic acid (13.4 mL, 187.6 mmol) in dry DMF (130 mL) was cooled to 0 °C under argon, potassium *tert*-butoxide (12.63 g, 112.6 mol) was added in portions. After stirring for 15 minutes at room temperature, a dark red homogenous solution was obtained. A solution of 2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl bromide (**1**, 38.6 g, 93.8 mmol) in anhydrous THF (50 mL) was added dropwise over 20 minutes and the reaction was continued for 3 hours at room temperature. The mixture was diluted with EtOAc (2000 mL), the organic phase was successively washed with H_2O (3 × 600 mL), brine (1 × 500 mL), dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel (hexane - EtOAc 4:1 v/v) gave compound **2** as a white solid (30.1g, yield 79%).

Method B: The reaction was carried out as above starting from bromide **1** (35.0 g, 93.3 mmol), thioacetic acid (12.5 ml, 174.9 mmol) and potassium *tert*-butoxide (11.8 g, 105.1 mmol). After washing the organic phase with H_2O and brine, the organic solution was dried with Na_2SO_4, decolorized with charcoal and filtered through a thin layer of celite pad. Compound **2** (23.9 g, yield 63%) was obtained by crystallization from EtOAc and hexane; $[\alpha]_D^{22}$ –23.6° (*c* 3.6, $CHCl_3$); mp 128 °C (hexane – EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz): δ5.47 (m, 2H, H-1 + H-2), 5.24 (t, 1H, *J* 10.1 Hz, H-4), 5.13 (dd, 1H, *J* 3.5 Hz, H-3), 4.24 (dd, 1H, *J* 5.3 Hz, 12.5 Hz, H-6a), 4.10 (dd, 1H, *J* 2.2 Hz, H-6b), 3.80 (ddd, 1H, H-5), 2.34 (s, 3H, SAc), 2.16 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc); δ_C ($CDCl_3$, 600 MHz, from HMQC) 79.3 (C-1, $J_{C1,H1}$ 155.6), 76.4 (C-5), 71.5 (C-3), 70.4 (C-2), 65.1 (C-4), 62.4 (C-6), 30.9 (SAc), 20.8 (4 × OAc);

High Res. ES-MS $C_{16}H_{22}O_{10}SNa^+$: 429.0826; found: 429.083. Anal. Calcd. for $C_{16}H_{22}O_{10}S$: C 47.3, H 5.4, S 7.9; found: C 47.1, H 5.1, S 8.05.

*Methyl 1-thio-β-*D*-mannopyranoside* (**4**)

Compound **2** (100 mg, 246 μmol) was dissolved in a solution of dry NaOMe/MeOH (700 μL, 3.5 M) under argon. The mixture was stirred for 30 min. Iodomethane (28 μL, 615 μmol) was then added dropwise to the mixture and the reaction was continued under argon for 1 h at room temperature. The mixture was neutralized with Dowex 50W (H^+) resin and concentrated. Coumpound **4** was purified by reverse-phase chromatography (47 mg, 91%); $[\alpha]_D^{22}$ –129.1° (*c* 1.1, MeOH); ^{1}H NMR (D_2O, 600 MHz): δ4.78 (d, 1H, *J* 0.9 Hz, H-1), 4.04 (dd, 1H, *J* 3.5 Hz, H-2), 3.92 (dd, 1H, *J* 2.4 Hz, 12.3 Hz, H-6a), 3.73 (dd, 1H, *J* 6.2 Hz, H-6b), 3.66 (dd, 1H, *J* 3.5 Hz, H-3), 3.60 (t, 1H, *J* 9.7 Hz, H-4), 3.41 (ddd, 1H, H-5), 2.27 (s, 3H, OMe); δ_C (D_2O, 600 MHz, from HMQC) 86.8 (C-1, $J_{C1,H1}$ 156.4), 81.3 (C-5), 75.0 (C-3), 72.7 (C-2), 67.4 (C-4), 62.3 (C-6), 15.2 (OMe); High Res. ES-MS $C_7H_{14}O_5SNa^+$: 233.0454; found: 233.0456. Anal. Calcd. for $C_7H_{14}O_5S{\cdot}0.5\ H_2O$: C 38.35, H 6.4; found: C 38.3, H 6.5.

*Heptyl 1-thio-β-*D*-mannopyranoside* (**5**)

Under argon, a solution of compound **2** (100 mg, 246 μmol) in anhydrous NaOMe/MeOH (840 μL, 3.5 M) was stirred for 30 min, 1-iodoheptane (162 μL, 984 μmol) was added dropwise and the reaction was continued for 1 h at room temperature. After neutralization with Dowex 50W (H^+) resin, the residue was purified by reverse - phase chromatography using a MeOH - H_2O gradient to afford **5** (70 mg, 97%); $[\alpha]_D^{22}$ –

73.0° (*c* 2.7, MeOH); ^{1}H NMR (D_2O, 600 MHz): δ4.84 (s, 1H, H-1), 4.02 (d, 1H, *J* 3.5, H-2), 3.91 (dd, 1H, *J* 2.2, 12.3, H-6a), 3.73 (dd, 1H, *J* 6.2, H-6b), 3.66 (dd, 1H, 9.7, H-3), 3.60 (t, 1H, H-4), 3.40 (ddd, 1H, *J* 9.5, H-5), 2.75 (m, 2H, SCH_2), 1.64 (m, 2H, SCH_2CH_2), 1.38 (m, 2H, $S(CH_2)_2CH_2$), 1.35 – 1.24 (m, 6H, $(CH_2)_3CH_3$), 0.87 (t, 3H, *J* 6.8, CH_3); δ_C (D_2O, 600 MHz, from HMQC) 85.0 (C-1, $J_{C1,H1}$ 153.7), 81.0 (C-5), 74.8 (C-3), 73.2 (C-2), 67.5 (C-4), 62.0 (C-6), 31.7 (SCH_2). 30.0 (SCH_2CH_2), 28.5 – 13.3 ($(CH_2)_3CH_3$); High Res. ES-MS $C_{13}H_{26}O_5SNa^+$: 317.1393; found: 317.1384. Anal. Calcd. for $C_{13}H_{26}O_5$: C 53.0, H 8.9; found: C 52.6, H 9.1.

Methyl 6-p-toluenesulfonyloxyhexanoate (**7**)

A solution of 5-(methoxycarbonyl)pentanol (**6**, 0.5 g, 3.4 mmol) in anhydrous pyridine (10 mL) was ice-cooled, *p*-toluenesulfonyl chloride (1.3 g, 6.8 mmol) was added and the mixture was left at room temperature for 1 h. The mixture was then ice-cooled and H_2O (1 mL) was added. The organic solvent was removed and the resulting residue was purified by chromatography on silica haxane-EtOAc (4:1 v/v) to give **7** (0.77 g, 75%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, 2H, *J* 8.2, OTs), 7.32 (d, 2H, *J* 8.1, OTs), 4.00 (t, 2H, *J* 6.4, $TsOCH_2$), 3.63 (s, 3H, OMe), 2.43 (s, 3H, OTs), 2.22 (t, 2H, *J* 7.5, CH_2COOMe), 1.64 (m, 2H, $TsOCH_2CH_2$), 1.56 (m, 2H, CH_2CH_2COOMe), 1.33 (m, 2H, $CH_2(CH_2)_2COOMe$); High Res. ES-MS $C_{14}H_{20}O_5SNa^+$: 323.0924; found: 323.0934.

*5-(Methoxycarbonyl)pentyl 2,3,4,6-tetra-O-acetyl-1-thio-β-*D*-mannopyranoside* (**8**) and *5-(Methoxycarbonyl)pentyl 2,3,4,6-tetra-O-acetyl-1-thio-α-*D*-mannopyranoside* (**9**)

A solution of compound **2** (150 mg, 369 μmol) and tosylate **7** (140 mg, 503 μmol) in anhydrous DMF (2 mL) was cooled to -55 °C, diethylamine (200 μL) was added dropwise under argon, and the mixture was stirred for 48 h. The mixture was then diluted with EtOAc (30 mL) and the organic phase was washed with H_2O (1 × 20 mL), brine (1 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel using hexane – AcOEt (7:3 v/v) as eluent gave first compound **9** (39 mg, 21% yield), then the β-isomer **8** (115 mg, 64% yield). Data for β-isomer **8** - $[\alpha]_D^{22}$ –55.4° (*c* 3.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ5.48 (d, 1H, *J* 3.5, H-2), 5.23 (t, 1H, *J* 10.1, H-4), 5.04 (dd, 1H, *J* 10.3, H-3), 4.72 (s, 1H, H-1), 4.24 (dd, 1H, *J* 5.9, 12.3, H-6a), 4.12 (dd, 1H, *J* 2.4, H-6b), 3.67 (ddd, 1H, H-5), 3.64 (s, 3H, OMe), 2.68 (t, 2H, *J* 7.5, SCH_2), 2.28 (t, 2H, *J* 7.5, CH_2COOMe), 2.16 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.61 (m, 4H, $SCH_2CH_2CH_2CH_2$), 1.40 (m. 2H, $CH_2(CH_2)_2$COOMe); δ_C ($CDCl_3$, 600 MHz, from HMQC) 82.7 (C-1, $J_{C1,H1}$ 151.7), 76.8 (C-5), 72.0 (C-3), 70.4 (C-2), 66.0 (C-4), 62.9 (C-6), 51.8 (OMe), 34.0 (CH_2COOMe), 31.7 (SCH_2), 29.2 (CH_2CH_2COOMe), 28.1 ($CH_2(CH_2)_2$COOMe), 24.6 (SCH_2CH_2), 20.5 (4 × OAc); High Res. ES-MS $C_{21}H_{32}O_{11}SNa^+$: 515.1558; found: 515.1556. Anal. Calcd. for $C_{21}H_{32}O_{11}S$: C 51.2, H 6.6; found: C 50.8, H 6.7.

Data for α-isomer **9** - $[\alpha]_D^{22}$ +44.7° (*c* 3.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 5.31 (dd, 1H, *J* 3.3, H-2), 5.29 (t, 1H, *J* 9.9, H-4), 5.24 (dd, 1H, *J* 10.1, H-3), 5.23 (d, 1H, *J* 1.7, H-1), 4.35 (ddd, 1H, H-5), 4.29 (dd, 1H, *J* 5.3, 12.3, H-6a), 4.07 (dd, 1H, *J* 2.4, 12.3, H-6b), 3.65 (s, 3H, OMe), 2.60 (m, 2H, SCH_2), 2.29 (t, 2H, *J* 7.3,

CH_2COOMe), 2.14 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.62 (m, 4H, $SCH_2CH_2CH_2CH_2$), 1.39 (m. 2H, $S(CH_2)_2CH_2$); High Res. ES-MS $C_{21}H_{32}O_{11}SNa^+$: 515.1558; found: 515.1556. Anal. Calcd. for $C_{21}H_{32}O_{11}S$: C 51.2, H 6.55; found: C 51.2, H 6.5.

5-(Methoxycarbonyl)pentyl 1-thio-β-D-*mannopyranoside* (**10**)

Compound **8** (100 mg, 203 μmol) was deprotected by transesterification in anhydrous methanol (20 ml) containing a catalytic amount of NaOMe, and the product **10** was purified by reverse -phase chromatography using a MeOH - H_2O gradient as eluent (60 mg, 91%); $[\alpha]_D^{22}$ –73.1° (*c* 1.3, MeOH); ^{1}H NMR (D_2O, 600 MHz): δ4.74 (d, 1H, *J* 0.9, H-1), 3.96 (dd, 1H, *J* 3.5, H-2), 3.84 (dd, 1H, *J* 2.2, 12.3, H-6a), 3.66 (dd, 1H, *J* 6.2, H-6b), 3.63 (s, 3H, OMe), 3.60 (dd, 1H, *J* 9.7, H-3), 3.53 (t, 1H, *J* 9.9, H-4), 3.34 (ddd, 1H, H-5), 2.69 (m, 2H, SCH_2), 2.34 (t, 2H, *J* 7.3, CH_2COOMe), 1.58 (m, 4H, $SCH_2CH_2CH_2CH_2$), 1.35 (m, 2H, $S(CH_2)_2CH_2$); δ_C ($CDCl_3$, 600 MHz, from HMQC) 85.3 (C-1, $J_{C1,H1}$ 154.0), 79.0 (C-5), 74.8 (C-3), 73.2 (C-2), 67.5 (C-4), 62.0 (C-6), 53.0 (OMe), 34.7 (CH_2COOMe), 31.2 (SCH_2), 29.6 (CH_2CH_2COOMe), 28.2 ($S(CH_2)_2CH_2$). 24.6 (SCH_2CH_2); High Res. ES-MS $C_{13}H_{24}O_7SNa^+$: 347.1135; found: 347.1143. Anal. Calcd. for $C_{13}H_{24}O_7S$: C 48.1, H 7.5; Found: C 48.5, H 7.0.

Methyl 2,3-di-O-acetyl-4-O-benzoyl-6-S-(2,3,4,6-tetra-O-acetyl-β-D-*mannopyranosyl)-6-thio-α*-D-*glucopyranoside* (**12**)

A mixture of compound **2** (60 mg, 148 μmol) and methyl 6-bromo-4-*O*-benzoyl-6-deoxy-2,3-di-*O*-acetyl-α-*D*-glucopyranside (**11**) (100 mg, 225 μmol) in anhydrous

DMF (2 mL) was cooled to –15 °C under argon, diethylamine (300 μL) was added dropwise and the mixture was stirred for 24 h. The reaction was diluted with EtOAc (30 mL) and the organic phase was washed with H_2O (2 ×15 ml), dried over anhydrous Na_2SO_4 and concentrated. Compound **12** was obtained by chromatography on silica gel using toluene - AcOEt (7:3 v/v) as eluent (80 mg, 74%). This compound was contaminated with a trace amount of the α-isomer (~ 6%, judged from NMR); $[\alpha]_D^{22}$ +18.4° (*c* 5.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ7.97 – 7.42 (m, 5H, Bz), 5.63 (t, 1H, *J* 9.7, H-3), 5.50 (d, 1H, *J* 3.5, H-2'), 5.19 (t, 1H, *J* 10.1, H-4'), 5.12 (t, 1H, *J* 9.7, H-4), 5.00 (dd, 1H, J 10.1, H-3'), 4.98 (d, 1H, *J* 3.7, H-1), 4.92 (dd, 1H, *J* 10.1, H-2), 4.91 (s, 1H, H-1'), 4.19 (dd, 1H, *J* 6.2, 12.5, H-6a'), 4.08 – 4.05 (m, 2H, H-6b' + H-5), 3.63 (ddd, 1H, H-5'), 3.46 (s, 3H, OMe), 2.88 (dd, 1H, *J* 9.3, 14.3, H-6a), 2.83 (dd, 1H, *J* 2.8, H-6b), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 6H, 2 × OAc), 1.94 (s, 3H, OAc), 1.86 (s, 3H, OAc); δ_C ($CDCl_3$, 600 MHz, from HMQC) 133.8 – 128.2 (Bz), 96.5(C-1, $J_{C1,H1}$ 171.9), 83.6 (C-1', $J_{C1',H1'}$ 154.0), 76.6 (C-5'), 72.4 (C-4), 71.8 (C-3'), 70.8 (C-2), 70.2 (C-2' + C-5), 69.4 (C-3), 65.5 (C-4'), 62.8 (C-6'), 56.0 (Me), 33.9 (C-6), 20.7 (8 × OAc); High Res. ES-MS $C_{32}H_{40}O_{17}SNa^+$: 751.1884; found: 751.1874. Anal. Calcd. for $C_{32}H_{40}O_{17}S$: C 52.7, H 5.5, S 4.4; found: C 52.3, H 5.7, S 4.6.

*Methyl 6-S-(β-*D*-mannopyranosyl)-6-thio-α-*D*-glucopyranoside* (**13**)

Compound **12** (54 mg, 74.1 μmol) was transesterified and purified as described for **8** to yield **13** in pure form (25 mg, 92%); $[\alpha]_D^{22}$ +23.3° (*c* 3.0, MeOH); ^{1}H NMR (D_2O, 600 MHz): δ4.95 (d, 1H, *J* 0.7, H-1'), 4.79 (d, 1H, *J* 3.8, H-1), 4.07 (dd, 1H, *J* 3.5, H-2'), 3.92 (dd, 1H, *J* 2.2, 12.3, H-6a'), 3.82 (m, 1H, H-5), 3.75 (dd, 1H, *J* 6.1, H-6b'),

3.66 (dd, 1H, *J* 3.5, 9.5, H-3'), 3.64 (t, 1H, *J* 8.6, H-4'), 3.61 (t, 1H, *J* 9.7, H-3), 3.58 (dd, 1H, *J* 9.7, H-2), 3.45 (s, 3H, OMe), 3.41 (ddd, 1H, H-5'), 3.33 (t, 1H, *J* 9.2, H-4), 3.25 (dd, 1H, *J* 2.4, 14.1, H-6a), 2.93 (dd, 1H, *J* 8.6, H-6b); δ_C ($CDCl_3$, 600 MHz, from HMQC) 100.1 (C-1, $J_{C1,H1}$ 168.2), 86.5 (C-1', $J_{C'1,H'1}$ 154.1), 81.4 (C-5'), 74.7 (C-3'), 74.0 (C-4'), 73.7 (C-4), 73.0 (C-2'), 72.2 (C-5 + C-2), 67.5 (C-3), 62.0 (C-6'), 56.0 (Me), 33.9 (C-6); High Res. ES-MS $C_{13}H_{24}O_{10}SNa^+$: 395.0982; found: 395.0984. Anal. Calcd. for $C_{13}H_{24}O_{10}S{\cdot}H_2O$: C 40.0, H 6.7; found: C 40.2, H 6.5.

Methyl 2,3,6-tri-O-benzoyl-4-S-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-4-thio-β-D-glucopyranoside (**15**)

Under argon, diethylamine (500 μL) was added dropwise to a stirred solution of triflate (**14**) (202 mg, 316 μmol) and thioacetate **2** (117 mg, 288 μmol) in anhydrous DMF (4 mL) at –5 °C, and the reaction was continued for 12 h. EtOAc (100 mL) was added and the resulting solution was washed with H_2O (1 × 30 mL), brine (1 × 30 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica gel using toluene - EtOAc (7:3 v/v) as eluent to give compound **15** (196 mg, 80%). This compound was contaminated with the α-isomer (~ 5%, judged from NMR); $[\alpha]_D^{22}$ –3.4° (*c* 8.3, $CHCl_3$); ^{1}H NMR (C_6D_6, 600 MHz): δ8.20 – 6.86 (m, 15H, Bz),), 5.99 – 5.92 (m, 2H, H-3 + H-2), 5.82 (dd, 1H, *J* 3.5, H-2'), 5.50 (t, 1H, *J* 10.1, H-4'), 5.42 (dd, 1H, *J* 10.1, H-3'), 5.14 (d, 1H, *J* 1.1, H-1'), 5.09 (dd, 1H, *J* 2.4, 12.1, H-6a), 4.95 (dd, 1H, *J* 5.3, H-6b), 4.67 (d, 1H, *J* 7.5, H-1), 4.16 (dd, 1H, *J* 2.4, 12.3, H-6a'), 4.07 (dd, 1H, *J* 6.0, H-6b'), 4.00 (ddd, 1H, H-5), 3.26 (s, 3H, OMe), 3.21 (ddd, 1H, H-5'), 3.10 (t, 1H, *J* 10.6, H-4), 1.75 (s, 3H, OAc), 1.69 (s, 3H, OAc), 1.64 (s, 3H, OAc), 1.56

(s, 3H, OAc); δ_C ($CDCl_3$, 600 MHz, from HMQC) 132.5 – 129.5 (Bz), 102.0 (C-1, $J_{C1,H1}$ 157.7), 80.5 (C-1', $J_{C1',H1'}$ 150.5), 76.5 (C-5'), 74.3 (C-5), 73.3 (C-2), 71.6 (C-3'), 71.2 (C-3), 70.2 (C-2'), 65.8 (C-4'), 64.0 (C-6), 62.0 (C-6'), 55.3 (OMe), 46.6 (C-4), 20.0 (4 × OAc); High Res. ES-MS $C_{42}H_{44}O_{17}SNa^+$: 875.2197; found: 875.2199. Anal. Calcd. for $C_{42}H_{44}O_{17}S$: C 59.15, H 5.2; Found: C 58.9; H 5.3.

Methyl 4-S-(β-D-mannopyranosyl)-4-thio-β-D-glucopyranoside (**16**)

Compound **15** (50 mg, 58.6 μmol) was deprotected and purified as described for **8** to yield **16** in pure form (20 mg, 93%); $[\alpha]_D^{22}$ –40.2° (*c* 2.5, MeOH); ^{1}H NMR (D_2O, 600 MHz): δ4.95 (s, 1H, H-1'), 4.36 (d, 1H, *J* 8.1, H-1), 4.15 (dd, 1H, *J* 2.0, 12.3, H-6a), 4.07 (d, 1H, *J* 3.5, H-2'), 3.90 (dd, 1H, J 5.1, H-6b), 3.89 (dd, 1H, *J* 2.4, 12.4, H-6a'), 3.73 – 3.68 (m, 2H, H-6b' +H-5), 3.67 (dd, 1H, *J* 9.7, H-3'), 3.63 (dd, 1H, *J* 10.4, H-3), 3.59 (t, 1H, *J* 9.9, H-4'), 3.42 (ddd, 1H, *J* 2.2, 6.4, 9.3, H-5'), 3.3 (t, 1H, *J* 8.6, H-2), 2.82 (t, 1H, *J* 10.8, H-4); δ_C (D_2O, 600 MHz, from HMQC) 104.0 (C-1, $J_{C1,H1}$ 160.8), 86.0 (C-1', $J_{C1',H1'}$ 153.5), 81.1 (C-5'), 77.2 (C-5), 75.3 (C-2), 74.5 (C-3'), 73.5 (C-3), 73.0 (C-2'), 67.5 (C-4'), 62.0 (C-6 + C-6'), 57.9 (OMe), 48.5 (C-4); High Res. ES-MS $C_{13}H_{24}O_{10}SNa^+$: 395.0982; found: 395.0980. Anal. Calcd. for $C_{13}H_{24}O_{10}S{\cdot}2.5H_2O$: C 37.4, H 7.0; found: C 37.3, H 6.9.

Heptyl 2,4-di-O-benzoyl-1-thio-β-D-*mannopyranoside* (**19**), *heptyl 2,6-di-O-benzoyl-1-thio-β*-D-*mannopyranoside* (**20**) and *heptyl 2-O-benzoyl-1-thio-β*-D-*mannopyranoside* (**21**)

10-Camphor sulfonic acid (40 mg, 0.17 mmol) was added to a stirred suspension of **5** (300 mg, 1.02 mmol) and trimethyl orthobenzoate (700 μL, 4.1 mmol) in $CHCl_3$ (60 mL). After the mixture became clear, the solution was concentrated on a rotovapor to ~10 mL, $CHCl_3$ (50 mL) was added and the solution was concentrated again to ~10 mL volume. This process was repeated until TLC showed that all the starting material was consumed. Triethylamine (3 mL) was added to the reaction mixture and the solution was evaporated to dryness. A solution of 90% acetic acid – H_2O (10 mL) was added to the reaction flask and reaction was continued for 30 min. After evaporation, the mixture was purified by column chromatography (toluene - EtOAc 4:1 → 1:1 v/v) to give, first **19** (166 mg, 32%), then **20** (204 mg, 40%), and last **21** (51 mg, 13%). Data for **19**: $[\alpha]_D^{22}$ – 94.0° (*c* 5.7, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.06 – 7.44 (m, 10H, Bz), 5.75 (dd, 1H, *J* 3.6, H-2), 5.38 (t, 1H, *J* 9.5, H-4), 4.88 (d, 1H, *J* 0.7, H-1), 4.18 (dd, 1H, *J* 9.8, H-3), 3.86 – 3.68 (m, 3H, H-5 + H6a + H6b), 2.70 (t, 2H, *J* 7.5, SCH_2), 1.60 (m, 2H, SCH_2CH_2), 1.38 – 1.20 (m, 8H, $(CH_2)_4CH_3$), 0.85 (t, 3H, *J* 6.8, CH_3); δ_C ($CDCl_3$, 600 MHz, from HMQC) 133.8 – 128.6 (Bz), 83.3 (C-1, $J_{C1,H1}$ 149.2), 78.8 (C-5), 73.9 (C-2), 73.0 (C-3), 70.4 (C-4), 62.1 (C-6), 31.9 (SCH_2), 31.7 (SCH_2CH_2), 29.8 ($S(CH_2)_2CH_2$), 28.9 ($SCH_2)_3CH_2$), 22.6 ($CH_2CH_2CH_3$), 21.0 (CH_2CH_3), 14.1 (CH_3); High Res. ES-MS $C_{27}H_{34}O_7SNa^+$: 525.1932; found: 525.1932. Anal. Calcd. for $C_{27}H_{34}O_7S$: C 64.5, H 6.8; found: C 64.4, H 6.7.

Data for **20**: $[\alpha]_D^{22}$ –70.9° (*c* 3.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ8.11 – 7.24 (m, 10H, Bz), 5.63 (d, 1H, *J* 3.5, H-2), 4.83 (s, 1H, H-1), 4.79 (dd, 1H, *J* 4.8, 12.1, H-6a), 4.62 (dd, 1H, *J* 2.0, H-6b), 3.93 (dd, 1H, *J* 9.3, H-3), 3.82 (t, 1H, *J* 9.5, H-4), 3.67 (ddd, 1H, *J* 2.2, 4.8, 9.5, H-5), 2.69 (m, 2H, SCH_2), 1.61 - 1.20 (m, 10H, $SCH_2(CH_2)_5$), 0.83 (t, 3H, *J* 7.0, CH_3); δ_C ($CDCl_3$, 600 MHz, from HMQC) 130.0 – 128.2 (Bz), 83.0 (C-1, $J_{C1,H1}$ 153.7), 79.0 (C-5), 75.0 (C-3), 75.0 (C-3), 72.8 (C-2), 68.0 (C-4), 64.4 (C-6), 31.5 (SCH_2), 28.9 - 14.2 ($SCH_2(CH_2)_5$); High Res. ES-MS $C_{27}H_{34}O_7SCs^+$: 635.1074; found: 635.1070. Anal. Calcd. for $C_{27}H_{34}O_7S$: C 64.5, H 6.8; found: C 64.4, H 6.7.

Data for **21**: $[\alpha]_D^{22}$ –51.2° (*c* 4.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ8.08 – 7.42 (m, 5H, Bz), 5.60 (s, 1H, H-2), 4.83 (s, 1H, H-1), 3.97 (dd, 1H, *J* 3.7, 12.1, H-6a), 3.89 – 3.87 (m, 3H, H-3, H-4 + H-6b), 3.44 (m, 1H, H-5), 2.69 (t, 2H, *J* 7.5, SCH_2), 1.93 (broad, 3H, 3 × OH), 1.58 (m, 2H, SCH_2CH_2), 1.35 - 1.22 (m, 8H, $(CH_2)_4CH_3$), 0.85 (t, 3H, *J* 7.0, CH_3); δ_C ($CDCl_3$, 600 MHz, from HMQC) 167.1, 133.6, 130.2, 129.2, 128.6 (Bz), 83.2 (C-1), 79.8 (C-5), 77.5 (C-2), 74.1 (C-3), 68.6 (C-4), 62.8 (C-6), 31.9 (SCH_2), 31.7 (SCH_2CH_2), 29.9 ($S(CH_2)_2CH_2$), 28.9 ($S(CH_2)_3CH_2$), 28.8 ($CH_2CH_2CH_3$), 22.6 (CH_2CH_3), 14.1 (CH_3); High Res. ES-MS $C_{20}H_{30}O_6SNa^+$: 421.1655; found: 421.1658. Anal. Calcd. for $C_{20}H_{30}O_6S$: C 60.3, H 7.6, S 8.05; found: C 60.1, H 7.7, S 7.9.

*Heptyl 2,4-di-O-benzoyl-3,6-di-O-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-1-thio-β-*D*-mannopyranoside* (**23**) and *heptyl 2,4-di-O-benzoyl-6-O-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-1-thio-β-*D*-mannopyranoside* (**24**)

A solution of compound **19** (100 mg, 199 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl trichloroacetimidate (**22**) (262 mg, 531 μmol) in anhydrous CH_2Cl_2 (6

mL) was cooled to 0 °C under argon. Boron trifluoride etherate (40 μL, 316 μmol) was added dropwise to the solution and the reaction mixture was allowed to warm slowly and stirred for 5 h at room temperature. The reaction was quenched with triethylamine (2 mL) and the solvent was evaporated. The mixture was purified by chromatography on silica gel using hexane - EtOAc (7:3 v/v) as eluent to afford, first, disaccharide **24** (80 mg, 48%) and then the trisaccharide **23** (119 mg, 51%). Data for **23**: $[\alpha]_D^{22}$ –30.5° (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ 8.12 – 7.41 (m, 10H, Bz), 5.78 (dd, 1H, *J* 3.5, H-2), 5.51 (t, 1H, *J* 9.9, H-4), 5.28 (dd, 1H, *J* 10.1, H-3"), 5.24 (t, 1H, *J* 9.9, H-4"), 5.17 (dd, 1H, *J* 1.8, 3.3, H-2"), 5.12 (t, 1H, *J* 9.7, H-4'), 5.01 (dd, 1H, *J* 9.7, H-3'), 4.92 (d, 1H, *J* 2.6, H-1'), 4.89 (d, 1H, *J* 0.9, H-1), 4.83 (dd, 1H, *J* 3.5, H-2'), 4.77 (d, 1H, *J* 1.7, H-1"), 4.30 (dd, 1H, *J* 2.2, 4.8, H-5'), 4.26 (dd, 1H, *J* 12.3, H-6a'), 4.23 (dd, 1H, *J* 10.2, H-3), 4.21 (dd, 1H, *J* 4.8, 11.9, H-6a"), 4.17 (dd, 1H, *J* 2.0,H-6b'), 4.07 (ddd, 1H, H-5"), 4.03 (dd, 1H, *J* 2.4,H-6b"), 3.95 (dd, 1H, *J* 7.1, 10.8, H-6a), 3.85 (ddd, 1H, *J* 2.4, H-5), 3.60 (dd, 1H, H-6b), 2.72 (m, 2H, SCH_2), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.79 (s, 3H, OAc), 1.78 (s, 3H, OAc), 1.60 (m, 4H, $SCH_2(CH_2)_2$), 1.36 - 1.19 (m, 6H, $(CH_2)_3CH_3$), 0.85 (t, 3H, *J* 7.1, CH_3); δ_C ($CDCl_3$, 600 MHz, from HMQC) 132.3 – 128.5 (Bz), 99.4 (C-1', $J_{C1',H1'}$ 172.3), 97.0 (C-1", $J_{C1",H1"}$ 172.6), 82.7 (C-1, $J_{C1,H1}$ 150.5), 77.2 (C-5 + C-6"), 72.4 (C-2), 69.3 (C-4 + C-5'), 69.2 (C-2"), 69.0 (C-2'), 68.9 (C-4"), 68.2 (C-5"), 68.0 (C-3'), 67.4 (C-6), 65.7 (C-3" + C-4'), 62.4 (C-3 + C-6'), 31.4 –22.4 ($S(CH_2)_6$), 20.6 – 20.2 (8 × OAc), 13.8 (CH_3); High Res. ES-MS $C_{55}H_{70}O_{25}SNa^+$: 1185.3819; found: 1185.3810. Anal. Calcd. for $C_{55}H_{70}O_{25}S$: C 56.8, H 6.1; found: C 56.6, H 5.9.

Data for **24**: $[\alpha]_D^{22}$ –12.1° (*c* 6.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ8.14 – 7.42 (m, 10H, Bz), 5.75 (d, 1H, *J* 3.5, H-2), 5.38 (t, 1H, *J* 9.7, H-4), 5.31 – 5.23 (m, 3H, H-2', H-3' + H-4'), 4.88 (s, 1H, H-1), 4.81 (d, 1H, *J* 1.5, H-1'), 4.15 (dd, 1H, *J* 9.7, H-3), 4.13 (dd, 1H, *J* 4.8, 12.1, H-6a'), 3.99 –3.89 (m, 4H, H-5' + H-6a + H-6b' + H-5), 3.66 (dd, 1H, *J* 1.8 H, 10.6, H-6b), 2.72 (m, 2H, SCH_2), 2.11 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.64 – 1.20 (m, 10H, $(CH_2)_5CH_3$), 0.85 (t, 3H, *J* 7.1, CH_3); δ_C ($CDCl_3$, 600 MHz, from HMQC) 133.6 – 128.7 (Bz), 97.1 (C-1', $J_{C1',H1'}$ 170.2), 82.8 (C-1, $J_{C1,H1}$ 149.5), 76.8 (C-5), 73.7 (C-2), 73.0 (C-3), 70.5 (C-4), 69.0 (C-2' + C-3'), 68.3 (C-5'), 67.0 (C-6), 65.8 (C-4'), 62.0 (C-6'), 31.4 – 22.4 ($S(CH_2)_6$), 20.8 – 20.4 (4 × OAc); 13.8 (CH_3). High Res. ES-MS $C_{41}H_{52}O_{16}SNa^+$: 855.2868; found: 855.2873. Anal. Calcd. for $C_{41}H_{52}O_{16}S$: C 59.1, H 6.3; found: C 59.0, H 3.6.

*Heptyl 3,6-di-O-(α-*D*-mannopyranosyl)-1-thio-β-*D*-mannopyranoside* (**25**)

Compound **23** (110 mg, 95 μmol) was transesterified and the crude product was purified as described for **8** to yield **25** (53 mg, 91%); $[\alpha]_D^{22}$ +18.4° (*c* 2.6, MeOH); ^1H NM (D_2O,600 MHz): δ5.11 (d, 1H, *J* 1.6, H-1'), 4.90 (d, 1H, *J* 1.7, H-1"), 4.85 (s, 1H, H-1), 4.19 (d, 1H, *J* 3.5, H-2), 4.07 (dd, 1H, *J* 3.5, H-2'), 3.99 (dd, 1H, *J* 3.5, H-2"), 3.95 (dd, 1H, *J* 5.1, 11.4, H-6a), 3.92 – 3.87 (m, 3H, H-3' + H-6a' + H-6a"), 3.83 (dd, 1H, *J* 9.0, H-3"), 3.82 (t, 1H, *J* 9.7, H-4), 3.79 – 3.73 (m, 5H, H-3 + H-4' + H-6b + H-6b' + H-6b"), 3.72 – 3.65 (m, 3H, H-5" + H-5' + H-4"), 3.60 (ddd, 1H, *J* 1.8, H-5), 2.74 (m, 2H, SCH_2), 1.64 (m, 2H, SCH_2CH_2), 1.42 - 1.24 (m, 8H, $(CH_2)_4$), 0.87 (t, 3H, *J* 7.0, CH_3); δ_C (D_2O, 600 MHz, from HMQC) 103.2 (C-1', $J_{C1',H1'}$ 172.4), 100.2 (C-1", $J_{C1",H1"}$ 171.6), 85.9 (C-1, $J_{C1,H1}$ 154.1), 82.7 (C-4'), 79.2 (C-5), 74.2 (C-3), 73.4 (C-5"), 72.8 (C-2), 71.6

(C-4), 71.2 (C-3'), 71.0 (C-2'), 70.9 (C-2"), 67.6 (C-4" + C-5'), 66.6 (C-3"), 66.4 (C-6), 61.9 (C-6' + C-6"), 31.8 (SCH_2), 30.2 (SCH_2CH_2), 32.2 – 23.0 ($(CH_2)_4$), 14.4 (CH_3); High Res. ES-MS $C_{25}H_{46}O_{15}SNa^+$: 641.2450; found: 641.2446. Anal. Calcd. for $C_{25}H_{46}O_{15}S{\cdot}2H_2O$: C 45.85, H 7.7; found: C 45.55, H 7.5.

2,3,4-Tri-O-acetyl-1-S-acetyl-1-thio-β-L-rhamnopyranose (**27**)

A solution of thioacetic acid (3.85 mL, 53.9 mmol) in dry DMF (25 mL) was cooled to 0 °C under argon, and potassium *tert*-butoxide (5.17 g, 45.7 mol) was added by portions. After stirring for 15 minutes at room temperature, a dark red homogenous solution was obtained. A solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**26**, 9.5 g, 26.9 mmol) in anhydrous THF (15 mL) was added dropwise over 15 minutes and the reaction continued for 4 hours at room temperature. The mixture was diluted with EtOAc (1000 mL), the organic phase was successively washed with H_2O (3 × 500 mL), brine (1 × 500 mL), dried over anhydrous Na_2SO_4, decolorized with charcoal and filtered through a thin layer of celite pad. Compound **27** (6.18 g, yield 66%) was obtained by crystallization from EtOAc - hexane; mp 176-177° C (hexane – EtOAc); $[\alpha]_D^{22}$ +6.3° (*c* 6.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ5.45 (m, 2H, H-1 + H-2), 5.07 (dd, 1H, *J* 3.9 Hz, 10.1 Hz, H-3), 5.04 (t, 1H, *J* 10.1 Hz, H-4), 3.67 (dq, *J* 6.2 Hz, 1H, H-5), 2.34 (s, 3H, SAc), 2.16 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.24 (d, 1H, H-6); ^{13}C NMR (500 MHz, $CDCl_3$, from GHMQC): δ78.8 (C-1, $J_{C1,HI}$ 156.1), 74.8 (C-5), 71.5 (C-3), 71.0 (C-2), 70.0 (C-4), 30.4 (SAc), 20.4 (3 × OAc), 15.2 (C-6); m/z (High Res. ES-MS) 371.078122. Calcd for $C_{14}H_{20}O_8SNa^+$ 371.077659. Anal. Calcd for $C_{14}H_{20}O_8S$: C, 48.27; H, 5.79; S, 9.20. Found: C, 48.13; H, 5.79; S, 9.37

2,3,4-Tri-O-acetyl-1-S-phenyl-1-thio-β-L-rhamnopyranoside (**28**)

To an ice-cold solution of thiophenol (2.07 mL, 20 mmol) in anhydrous DMF (15 mL), was added *tert*-butoxide (2.13g, 18 mmol), and the mixture was stirred for 10 min. A solution of compound **26** (3.53g, 10 mmol) in anhydrous THF (20 mL) was added dropwise, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and the residue was diluted with EtOAc (250 mL), washed with brine (2 X 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel using EtOAc-hexane (7:3 v/v) afforded the β-thiorhamnopyrannoside **28** in pure form (3.57g, 94% yield). $[\alpha]_D^{22}$ +50.3° (*c* 3.1, $CHCl_3$); mp 89 - 90° C (hexane – EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ7.49 – 7.47 (m, 2H, Ph), 7.32 – 7.28 (m, 3H, Ph), 5.63 (d, 1H, *J* 1.2 Hz, 3.5 Hz, H-2), 5.10 (t, 1H, *J* 9.9 Hz, H-4), 4.99 (dd, 1H, H-3), 4.88 (d, 1H, H-1), 3.53 (dq, 1H, *J* 6.1 Hz, H-5), 2.18 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.30 (d, 1H, H-6); ^{13}C NMR (500 MHz, $CDCl_3$, from GHMQC) δ 132.0 –129.0 (Ph), 85.3 (C-1, $J_{C1,H1}$ 154.1), 74.7 (C-5), 71.8 (C-3), 71.0 (C-2), 70.5 (C-4), 20.4 (3 × OAc), 17.5 (C-6); m/z (High Res. ES-MS) 405.098909. Calcd for $C_{18}H_{22}O_7SNa^+$ requires 405.098395. Anal. Calcd for $C_{18}H_{22}O_7S$: C, 56.53; H, 5.80. Found: C, 56.25; H, 5.68.

Methyl 1-thio-β-L-rhamnopyranoside (**30**)

Compound **27** (100 mg, 287 μmol) was dissolved in anhydrous MeOH (3 mL), and a solution of dry NaOMe/MeOH (100 μL, 3.5 M) was added under argon. Iodomethane (28 μL, 615 μmol) was then added dropwise to the mixture. After 5 min, the mixture was neutralized with Dowex 50W (H^+) resin and concentrated. Coumpound

30 was purified by reverse-phase chromatography (54.7 mg, 98%); $[\alpha]_D^{22}$ +107.5° (*c* 2.0, MeOH) ^{1}H NMR (600 MHz, CD_3OD): δ4.58 (d, 1H, *J* 1.1 Hz, H-1), 3.88 (dd, 1H, *J* 3.5 Hz, H-2), 3.41 (dd, 1H, *J* 9.3 Hz, H-3), 3.32 (t, 1H, *J* 10.1 Hz, H-4), 3.24 (dq, 1H, *J* 6.2 Hz, H-5), 2.20 (s, 3H, OMe), 1.30 (d, 1H, H-6); ^{13}C NMR (600 MHz, D_2O, from GHMQC): δ86.5 (C-1, $J_{C1,H1}$ 154.0), 78.0 (C-5), 74.2 (C-3), 72.8 (C-2), 72.0 (C-4), 17.5 (C-6), 14.3 (OMe); m/z (High Res. ES-MS) 217.050868. Calcd for $C_7H_{14}O_4SNa^+$ 217.051051. Anal. Calcd for $C_7H_{14}O_5S{\cdot}H_2O$: C, 39.6; H, 7.6. Found: C, 39.85; H, 7.55.

*Heptyl 1-thio-β-*L*-rhamnopyranoside* (**31**)

Compound **27** (100 mg, 287 μmol) was dissolved in a anhydrous MeOH (3 mL), and a solution of dry NaOMe/MeOH (100 μL, 3.5 M) was added under argon. Liquid 1-iodoheptane (94 μL, 573 μmol) was then added dropwise to the mixture. After 30 min, the mixture was neutralized with Dowex 50W (H^+) resin and concentrated. Coumpound **31** was purified by reverse-phase chromatography (55 mg, 99%); $[\alpha]_D^{22}$ +83.0° (*c* 2.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ4.64 (d, 1H, *J* 0.9 Hz, H-1), 3.87 (dd, 1H, *J* 3.5 Hz, H-2), 3.41 (dd, 1H, *J* 9.3 Hz, H-3), 3.31 (t, 1H, *J* 9.3 Hz, H-4), 3.23 (dq, 1H, *J* 6.1 Hz, H-5), 2.67 (m, 2H, SCH_2), 1.62 (m, 2H, SCH_2CH_2), 1.39 (m, 2H, $S(CH_2)_2CH_2$), 1.34 – 1.29 (m, 6H, $(CH_2)_3CH_3$), 1.28 (d, 3H, H-6), 0.90 (t, 3H, *J* 6.9, CH_3); ^{13}C NMR (500 MHz, CD_3OD, from GHMQC): δ 86.1 (C-1, $J_{C1,H1}$ 151.2), 77.9 (C-5), 76.1 (C-3), 74.0 (C-2), 73.8 (C-4), 32.2 (SCH_2). 31.0 (SCH_2CH_2), 29.7 ($S(CH_2)_2CH_2$), 32.8 – 23.4 ($(CH_2)_3CH_3$), 18.2 (C-6), 14.4 (CH_3); m/z (High Res. ES-MS) 301.145129. Calcd for $C_{13}H_{26}O_4SNa^+$ requires 301.144851. Anal. Calcd for $C_{13}H_{26}O_4S{\cdot}0.5H_2O$ requires C, 54.32; H, 9.4. Found: C, 54.40; H, 9.12.

Methyl 2,3-di-O-acetyl-4-O-benzoyl-6-S-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-6-thio-α-D-glucopyranoside (**33**)

A mixture of compound **27** (50 mg, 144 μmol) and methyl 6-bromo-4-*O*-benzoyl-6-deoxy-2,3-di-*O*-acetyl-α-*D*-glucopyranside **32** (84 mg, 189 μmol) in anhydrous DMF (2 mL) was cooled to –10 °C under argon, diethylamine (70 μL) was added dropwise and the mixture was stirred for 48 h. The reaction was diluted with EtOAc (30 mL) and the organic phase was washed with H_2O (2 ×15 ml), dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel using toluene - EtOAc (3:1 v/v) as eluent gave first the α-anomer **33a** (6.4 mg, 7%) and β-anomer **33b** (76 mg, 79%). Data for β-anomer: $[\alpha]_D^{22}$ +89.4° (*c* 3.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 – 7.42 (m, 5H, Bz), 5.63 (t, 1H, *J* 9.7 Hz, H-3), 5.44 (dd, 1H, *J* 1.1 Hz, 3.3 Hz, H-2'), 5.17 (t, 1H, H-4), 4.98 (t, 1H, *J* 10.1 Hz, H-4'), 4.96 (overlapped, 1H, H-1), 4.95 (dd, 1H, H-3'), 4.93 (dd, 1H, *J* 3.7 Hz, H-2), 4.70 (d, 1H, H-1'), 4.04 (ddd, 1H, *J* 10.3 Hz, 2.8 Hz, 7.7 Hz, H-5), 3.45 (s, 3H, OMe), 3.37 (dq, 1H, *J* 6.2 Hz, H-5'), 2.94 (dd, 1H, *J* 14.3 Hz, H-6a), 2.81 (dd, 1H, H-6b), 2.12 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.88 (s, 3H, OAc), 1.08 (d, 3H, H-6'); ^{13}C NMR (500 MHz, $CDCl_3$, from GHMQC): δ 134.0 – 128.4 (Bz), 91.5(C-1, $J_{C1,H1}$ 170.8), 81.9 (C-1', $J_{C1',H1'}$ 152.8), 74.8 (C-5'), 71.9 (C-4), 71.0 (C-2), 70.8 (C-3'), 70.7 (C-2'), 70.2 (C-4'), 69.6 (C-3), 69.1 (C-5), 55.8 (OMe), 32.2 (C-6), 20.8 (5 × OAc), 17.6 (C-6'); m/z (High Res. ES-MS) 693.182490. Calcd for $C_{30}H_{38}O_{15}SNa^+$ 693.182913. Anal. Calcd for $C_{30}H_{38}O_{15}S$ C, 53.7; H, 5.7. Found: C, 53.5; H, 5.9. Data for α-anomer: $[\alpha]_D^{22}$ –19.8° (*c* 4.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 – 7.42 (m, 5H, Bz), 5.63 (t, 1H, *J* 9.7 Hz, H-3), 5.42 (d, 1H, *J*

1.5 Hz, H-1'), 5.32 (dd, 1H, *J* 3.3 Hz, H-2'), 5.13 (dd, 1H, *J* 10.1 Hz, H-3'), 5.11 (t, 1H, *J* 10.2 Hz, H-4), 5.06 (t, 1H, *J* 9.9 Hz, H-4'), 4.96 (d, 1H, *J* 3.7 Hz, H-2), 4.11 (dq, 1H, *J* 6.2 Hz, H-5'), 4.05 (ddd, 1H, *J* 9.9 Hz, 2.4 Hz, 9.9 Hz, H-5), 3.48 (s, 3H, OMe), 2.83 (dd, 1H, *J* 14.7 Hz, H-6a), 2.66 (dd, 1H, H-6b), 2.12 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.17 (d, 3H, H-6'); ^{13}C NMR (500 MHz, $CDCl_3$, from GHMQC): δ 134.0 – 128.4 (Bz), 96.2 (C-1, $J_{C1,H1}$ 174.7), 83.0 (C-1', $J_{C1',H1'}$ 170.4), 72.3 (C-4), 71.2 (C-2'), 71.0 (C-4' + C-5 + C-5'), 70.9 (C-2), 69.3 (C-3), 69 (C-3'), 55.1 (OMe), 31.4 (C-6), 20.5 (5 × OAc), 17.2 (C-6'); m/z (High Res. ES-MS) 693.183354. Calcd for $C_{30}H_{38}O_{15}SNa^+$ 693.182913. Anal. Calcd for $C_{30}H_{38}O_{15}S$: C, 53.7; H, 5.7. Found: C, 53.4; H, 5.8.

*Methyl 6-S-(β-*L*-rhamnopyranosyl)-6-thio-α-*D*-glucopyranoside* (**34**)

Compound **33** (48 mg, 71.6 μmol) was deprotected by transesterification in anhydrous methanol (4 mL) containing a prepared NaOMe/MeOH solution (243 μL, 3.5 M). The product was purified by reversed-phase chromatography using H_2O as eluent (25 mg, 98%). $[\alpha]_D^{22}$ +151.9° (*c* 1.6, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 4.87 (s, 1H, H-1'), 4.78 (d, 1H, *J* 3.7 Hz, H-1), 4.03 (d, 1H, *J* 3.5 Hz, H-2'), 3.78 (ddd, 1H, *J* 10.1 Hz, 2.6 Hz, 7.8 Hz, H-5), 3.65 (t, 1H, *J* 9.5 Hz, H-4'), 3.61 (dd, 1H, H-3'), 3.58 (dd, 1H, *J* 9.8 Hz, H-2), 3.46 – 3.35 (m, 6H, H-5 + OMe + H-4 + H-3), 3.22 (dd, 1H, *J* 14.4 Hz, H-6a), 3.87 (dd, 1H, H-6b), 1.30 (d, 1H, *J* 5.8 Hz, H-6'); ^{1}H NMR (600 MHz, $CDCl_3$, from GHMQC): δ 100.2 (C-1, $J_{C1,H1}$ 170.5), 85.1 (C-1', $J_{C'1,H'1}$ 155.8), 77.4 (C-5'), 74.7 (C-3'), 74.4 (C-4'), 73.7 (C-3'), 73.1 (C-2'), 72.8 (C-3 + C-4), 72.2 (C-2), 71.4 (C-5), 56.3 (OMe), 32.7 (C-6), 17.8 (C-6'); m/z (High Res. ES-MS) 379.103417. Calcd for

$C_{13}H_{24}O_9SNa^+$: 379.103874. Anal. Calcd for $C_{13}H_{24}O_{19}S{\cdot}3H_2O$: C, 38.0; H, 7.4. Found: C, 38.2; H, 6.4.

Methyl 2,3,6-tri-O-benzoyl-4-S-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-4-thio-β-D-glucopyranoside (**36**)

Under argon, diethylamine (70 μL) was added dropwise to a stirred solution of triflate **35** (120 mg, 188 μmol) and thioacetate **27** (50 mg, 144 μmol) in anhydrous DMF (1.2 mL) at 0 °C, and reaction was continued for 17 h. EtOAc (100 mL) was added and the resulting solution was washed with H_2O (1 × 30 mL), brine (1 × 30 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using toluene - EtOAc (7:3 v/v) as eluent to give compound **36** (89 mg, 78%) and the α-anomer (~ 5%, judged from NMR); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.08 – 7.12 (m, 15H, Bz), 5.89 (t, 1H, *J* 10.4 Hz, H-3), 5.19 (dd, 1H, *J* 3.5 Hz, H-2), 5.15 (d, 1H, H-1), 5.11 (d, 1H, *J* 3.7 Hz, H-2'), 4.92 (t, 1H, *J* 9.9 Hz, H-4'), 4.90 (dd, 1H, *J* 2.1 Hz, 12.1 Hz, H-6a), 4.78 (s, 1H, H-1'), 4.70 (m, 2H, H-6b + H-3'), 4.15 (ddd, 1H, *J* 11.0 Hz, 4.8 Hz, H-5), 3.41 (s, 3H, OMe), 3.36 (dq, 1H, *J* 6.0 Hz, H-5'), 3.30 (t, 1H, H-4), 2.07 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.14 (d, 3H, H-6'). ^{13}C NMR (500 MHz, $CDCl_3$, from GHMQC): δ 133.5 – 128.0 (Bz), 97.0 (C-1, $J_{C1,H1}$ 171.7), 82.5 (C-1', $J_{C1',H1'}$ 154.1), 74.5 (C-5'), 73.2 (C-2), 72.5 (C-3'), 70.8 (C-3), 70.5 (C-2'), 70.2 (C-4'), 69.2 (C-5), 55.5 (OMe), 47.5 (C-4), 20.8 (3 × OAc), 17.4 (C-6'); m/z (High Res. ES-MS) 817.214443. Calcd for $C_{40}H_{42}O_{15}SNa^+$: 817.214213.

Methyl 4-S-(β-L-rhamnopyranosyl)-4-thio-β-D-glucopyranoside (**37**)

Compound **37** (45 mg, 56.6 μmol) was deprotected and purified as described for **34** to yield **37** in pure form (19 mg, 94%); $[\alpha]_D^{22}$ +133.6° (*c* 2.5, MeOH); ^{1}H NMR (600 MHz, D_2O): δ 4.96 (s, 1H, H-1'), 4.87 (d, 1H, *J* 3.7 Hz, H-1), 4.08 (d, 1H, *J* 3.7 Hz, H-2'), 4.01 (dd, 1H, *J* 4.6 Hz, *J* 12.5 Hz, H-6a), 3.97 (dd, 1H, *J* 2.2 Hz, H-6b), 3.83 (ddd, 1H, *J* 11.4 Hz, H-5), 3.79 (t, 1H, *J* 9.9 Hz, H-3), 3.61 (dd, 1H, *J* 9.5 Hz, H-3'), 3.59 (dd, 1H, H-2), 3.45 (dq, 1H, *J* 9.5 Hz, 5.9 Hz, H-5'), 3.39 (t, 1H, H-4'), 2.81 (t, 1H, H-4), 1.32 (d, 3H, H-6'); ^{13}C NMR (600 MHz, D_2O, from GHMQC): δ 100.2 (C-1, $J_{C1,H1}$ 168.0), 84.9 (C-1', $J_{C1',H1'}$ 154.6), 77.2 (C-5'), 74.5 (C-3'), 73.1 (C-5 + C-2'), 72.9 (C-2), 72.4 (C-4'), 71.8 (C-3), 62.4 (C-6), 48.6 (C-4), 18.0 (C-6'); m/z (High Res. ES-MS) 379.104101. Calcd for $C_{13}H_{24}O_9SNa^+$: 379.103874. Anal. Calcd for $C_{13}H_{24}O_9S \cdot 1.5H_2O$: C, 40.7; H, 7.1. Found: C, 40.1; H, 7.1.

1,2,3-tri-O-acetyl-α/β-D-galactopyranose (**39**)

To a solution of 1,2,3-tri-*O*-acetyl-4,6-*O*-benzylidene-*D*-galactopyranose **38** (22) (1.25 g, 3.17 mmole) in methanol (50 mL), $Pd(OH)_2$-C (20% Pd, 0.3 g) was added. The mixture was hydrogenated for 18 h. The catalyst was filtered off and the filtrated was concentrated. Compound **39** (930 mg, 96%) was obtained by chromatography on silica using acetone - toluene (3:7 v/v) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$) for α-anomer: δ 6.37 (d, 1H, *J* 3.8 Hz, H-1), 5.46 (dd, 1H, *J* 10.7 Hz, H-2), 5.25 (dd, 1H, *J* 3.0 Hz, H-3), 4.31 (dd, 1H, *J* 1.2 Hz, H-4), 4.99 (ddd, 1H, *J* 4.3 Hz, H-5), 3.90 (overlapped, 2H, H-6), 2.12 (s, 3H, OAc), 2.10 (s, 3H, OAc), 1.99 (s, 3H, OAc). ^{1}H NMR (300 MHz, $CDCl_3$) for β-anomer:: δ 5.68 (d, 1H, *J* 8.2 Hz, H-1), 5.42 (dd, 1H, *J* 10.2 Hz, H-2), 4.98 (dd, 1H, *J*

3.1 Hz, H-3), 4.21 (dd, 1H, *J* 0.8 Hz, H-4), 3.90 (overlapped, 2H, H-6), 3.71 (ddd, 1H, *J* 5.1 Hz, 5.4 Hz, H-5), 2.12 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc).

1,2,3-tri-O-acetyl-6-O-benzoyl-D-*glucopyranose* (**40**)

Under argon, benzoyl chloride (150 μL, 1.34 mmole) was added dropwise to a stirred solution of 1, 2, 3-tri-*O*-acetyl-*D*-glucopyranose (**39**) (390 mg, 1.27 mmol) in anhyrous pyridine (10 mL) at -37 °C. The reaction was allowed to warm slowly to room temperature overnight. The mixture was concentrated and the resulting residue was purified by chromatography on silica using acetone - toluene (3:17 v/v) as eluent to give compound **40** as α/β mixture (510 mg, 80%, α/β ration is 3:1). ^{1}H NMR (500 MHz, $CDCl_3$) for α-anomer: δ 8.0 – 7.45 (m, 5H, Bz), 6.16 (d, 1H, *J* 3.7 Hz, H-1), 5.46 (dd, 1H, *J* 10.9 Hz, H-2), 5.31 (dd, 1H, *J* 3.1 Hz, H-3), 4.65 (dd, 1H, *J* 7.0 Hz, 11.5 Hz, H-6a), 4.38 (dd, 1H, *J* 6.0 Hz, H-6b), 4.29 (t, 1H, *J* 6.4 Hz, H-5), 4.20 (d, 1H, H-4), 2.51 (bs, 1H, 4-OH), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.00 (s, 3H, OAc). ^{1}H NMR (500 MHz, $CDCl_3$) for β-anomer: δ 8.0 – 7.45 (m, 5H, Bz),), 5.70 (d, 1H, *J* 8.3 Hz, H-1), 5.41 (dd, 1H, *J* 10.2 Hz, H-2), 5.04 (dd, 1H, *J* 3.2 Hz, H-3), 4.65 (dd, 1H, *J* 5.5 Hz, 11.6 Hz, H-6a), 4.44 (dd, 1H, *J* 6.1 Hz, H-6b), 4.12 (d, 1H, H-4'), 4.02 (t, 1H, *J* 6.7, H-5'), 2.51 (bs, 1H, 4'-OH), 2.10 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.02 (s, 3H, OAc). m/z (High Res. ES-MS) 433.110829. Calcd for $C_{19}H_{22}O_{10}Na^+$: 433.111067. Anal. Calcd for $C_{19}H_{22}O_{10}$ C, 55.6; H, 5.4. Found: C, 55.9; H, 5.8.

1,2,3-tri-O-acetyl-6-O-benzoyl-4-S-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-4-thio-D-glucopyranose (**42**)

Under argon, diethylamine (230 μL) was added dropwise to a stirred solution of triflate (**41**) (317mg, 584 μmol) and thioacetate **27** (146 mg, 419 μmol) in anhydrous DMF (3.5mL) at 0 °C, and reaction was continued for 16 h. EtOAc (200 mL) was added and the resulting solution was washed with H_2O (1 × 100 mL), brine (1 × 60 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using toluene - EtOAc (17:3 v/v) as eluent to give, first the desired β-anomer **42b** (7mg, 10%), which was contaminated with of the 2,3,4,tri-*O*-aectyl-1-thio-β-**L**-rhamnopyranose(~ 45%, judged from NMR); continued elution gave the desired α-anomer **42a** (192 mg, 66%). Data for α-anomer: mp 113-115° C ($CDCl_3$); $[\alpha]_D^{22}$ +46.8° (*c* 6.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ8.03 – 7.15 (m, 5H, Bz), 6.32 (d, 1H, *J* 3.7 Hz, H-1), 5.42 (dd, 1H, *J* 1.3 Hz, 3.1 Hz, H-2'), 5.33 (dd, 1H, *J* 10.1 Hz, 11.2 Hz, H-3), 5.07 (dd, 1H, H-2), 4.95 (t, 1H, *J* 9.9 Hz, H-4'), 4.83 (dd, 1H, H-3'), 4.72 (m, 2H, H-6), 4.69 (d, 1H, H-1'), 4.11 (ddd, 1H, *J* 11.2 Hz, 2.9 Hz, 5.9 Hz, H-5), 3.23 (t, 1H, H-4), 3.19 (dq, 1H, *J* 6.2 Hz, H-5'), 2.15 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.08 (d, 1H, H-6'). ^{1}H NMR (600 MHz, $CDCl_3$, from GHMQC): δ 133.4 – 128.5 (Bz), 89.4 (C-1, $J_{C1,H1}$ 179.9), 84.2 (C-1', $J_{C1',H1'}$ 152.7), 72.0 (C-5'), 71.2 (C-3'), 71.0 (C-2), 70.4 (C-2), 69.5 (C-4'), 69.3 (C-3), 63.7 (C-6), 48.7 (C-4), 20.6 – 20.3 (6 × OAc), 16.9 (C-6'); m/z (High Res. ES-MS) 721.177638. Calcd for $C_{31}H_{38}O_{16}SNa^+$: 721.177827. Anal. Calcd for $C_{31}H_{38}O_{16}S$ C, 53.29; H, 5.48. Found: C, 53.25; H, 5.86. Data for β-anomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ8.04 – 7.18 (m, 5H, Bz), 5.70 (d, 1H, *J* 7.8 Hz, H-1), 5.40 (dd, 1H, *J* 1.1 Hz,

3.5 Hz, H-2'), 5.07 (t, 1H, *J* 9.3 Hz, H-3), 5.02 (overlapped, 1H, H-2), 4.94 (t, 1H, *J* 9.8 Hz, H-4'), 4.82 (dd, *J* 3.5 Hz, 10.2 Hz, H-3'), 4.78 (dd, 1H, *J* 2.1 Hz, 12.4 Hz, H-6a), 4.69 (d, 1H, H-1'), 4.65 (dd, 1H, *J* 4.4 Hz, H-6b), 3.85 (ddd, 1H, *J* 11.0 Hz, H-5), 3.20 (t, 1H, H-4), 3.19 (dq, 1H, *J* 6.1 Hz, H-5'), 2.15 – 1.92 (overlapped, 18H, 6 X OAc), 1.08 (d, 1H, H-6'). Data for the 2,3,4,tri-*O*-aectyl-1-thio-β-L-rhamnopyranose: (500 MHz, $CDCl_3$): δ 5.56 (dd, 1H, *J* 1.1 Hz, *J* 3.2 Hz, H-2), 5.04 (overlapped, 1H, H-4), 4.98 (overlapped, 1H, H-3), 4.84 (d, 1H, H-1), 5.40), 3.57 (dq, 1H, *J* 9.3 Hz, 6.3 Hz, H-5), 2.15 – 1.92 (overlapped, 9H, 3 X OAc), 1.27 (d, 1H, H-6).

*2,3-tri-O-acetyl-6-O-benzoyl-4-S-(2,3,4-tri-O-acetyl-β-*L*-rhamnopyranosyl)-4-thio-*D*-glucopyranosyl bromide* (**43**)

Compound **42** (46 mg, 66 μmole) was dissolved in hydrobromic acid (30-32%) in acetic acid at 0 °C. The reaction was allowed to warm up to ambient temperature for 1 hour. The mixture was then concentrated with toluene and the residue solvent was removed under reduced pressure, quantitative yield of bromide **43** was obtained (judged by TLC). Proton NMR showed that the compound was pure enough to carry out next step without purification. Data for α-anomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 – 7.12 (m, 5H, Bz), 6.57 (d, 1H, *J* 3.9 Hz, H-1), 5.46 (dd, 1H, *J* 9.9 Hz, *J* 11.0 Hz, H-3), 5.42 (d, 1H, *J* 3.2 Hz, H-2'), 4.96 (t, 1H, *J* 9.9 Hz, H-4'), 4.86 (dd, 1H, H-3'), 4.81 (dd, 1H, H-2), 4.77 (m, 2H, H-6), 4.73 (s, 1H, H-1'), 4.33 (ddd, 1H, *J* 11.1 Hz, 3.1 Hz, 6.0 Hz, H-5), 3.25 (dq, 1H, *J* 6.2 Hz, H-5'), 3.19 (t, 1H, H-4), 2.14 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.12 (d, 1H, H-6').

2,3-di-O-acetyl-6-O-benzoyl-4-S-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-4-thio-D-*glucopyranose* (**46**)

Solid hydrazine acetate (17.5 mg, 190 μmole) was added to a stirred solution of compound **42** (102 mg, 146 μmol) in anhydrous DMF (8 mL) at room temperature. The reaction was allowed to proceed for 2 h. EtOAc (100 mL) was added and the resulting solution was washed with H_2O (1 × 80 mL), brine (1 × 50 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using toluene - EtOAc (3:2 v/v) as eluent to give compound **46** (82 mg, 86%). The α/β ratio is 3:1(judged from NMR). ^{1}H NMR (500 MHz, $CDCl_3$) for α-anomer: δ 8.03 – 7.41 (m, 5H, Bz), 5.42 – 5.38 (m, 3H, H-1 + H-3 +H-2'), 4.95 (t, 1H, *J* 10.1 Hz, H-4'), 4.90 (dd, 1H, *J* 3.5 Hz, 9.9 Hz, H-2), 4.85 (dd, 1H, *J* 3.5 Hz, H-3'), 4.77 (m, 3H, H-1 + H-6), 4.28 (ddd, 1H, *J* 11.2 Hz, 2.4 Hz, 3.7 Hz, H-5), 3.24 (dq, 1H, *J* 6.1 Hz, H-5'), 3.16 (t, 1H, *J* 11.2 Hz, H-4), 2.13 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.09 (d, 1H, H-6'). ^{13}C NMR (600 MHz, $CDCl_3$, from GHMQC): δ133.4 – 128.4 (Bz), 90.5 (C-1, $J_{C1,H1}$ 173.4), 84.0 (C-1', $J_{C1',H1'}$ 153.0), 75.0 (C-5'), 72.5 (C-3'), 71.0 (C-2'), 69.5 (C-5), 68.5 (C-3), 64.0 (C-6), 48.5 (C-4), 20.6 (5 × OAc), 17.0 (C-6'); ^{1}H NMR (500 MHz, $CDCl_3$) for β-anomer: δ_H 8.03 – 7.41 (m, 5H, Bz),), 5.42-5.38 (overlapped, 1H, H-2'), 5.07 (dd, 1H, *J* 9.5 Hz, 11.3 Hz, H-3), 4.95 (t, 1H, *J* 10.1 Hz, H-4'), 4.85 (dd, 1H, *J* 3.5 Hz, H-3'), 4.82 (overlapped, 1H, H-2), 4.75 (overlapped, 1H, H-1), 4.72 (overlapped, 1H, H-1'), 4.68 (m, 2H, H-6), 3.75 (ddd, 1H, *J* 11.0 Hz, 2.1 Hz, 4.7Hz, H-5), 3.24 (dq, 1H, *J* 6.1 Hz, H-5'), 3.16 (t, 1H, H-4), 2.12 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.92 (s, 3H, OAc),

1.11 (d, 1H, H-6'). m/z (High Res. ES-MS) 679.167879. Calcd for $C_{29}H_{36}O_{15}SNa^+$: 679.167263. Anal. Calcd for $C_{29}H_{36}O_{15}S$ requires C, 53.0; H, 5.5. Found: C, 53.3; H, 5.9.

*2,3-Di-O-acetyl-6-O-benzoyl-4-S-(2,3,4-tri-O-acetyl-β-*L*-rhamnopyranosyl)-4-thio-α-*D*-glucopyranosyl trichloroacetimidate* (**47**)

Under argon, trichloroacetonitrile (0.19 mL, 1.9 mmole) was added to a solution containing disaccharide **46** (70 mg, 106.7 μmole) in anhydrous dichloromethane (3.5 mL). The mixture was cooled to 0 °C. Liquid 1,8-diazabicyclo[5.4.0]undec-7-ene (3.5 μL, 23.4 μmole) was added. After 3.5 h, the organic solvent was evaporated and the resulting residue was purified by chromatography on silica using hexane - EtOAc (7:3 v/v) as eluent to give compound **47** as an α/β mixture (α/β ratio is 19:1, 78 mg, 91%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 – 7.42 (m, 5H, Bz), 6.53 (d, 1H, *J* 3.5 Hz, H-1), 5.46 (dd, 1H, *J* 9.9 Hz, 11.1 Hz, H-3), 4.43 (dd, 1H, *J* 1.3 Hz, 3.5 Hz, H-2'), 5.10 (dd, 1H, H-2), 4.96 (t, 1H, *J* 10.1 Hz, H-4'), 4.85 (dd, 1H, H-3'), 4.78 (dd, 1H, *J* 2.2, 12.3 Hz, H-6a), 4.73 (d, 1H, H-1'), 4.70 (dd, 1H, *J* 4.4 Hz, H-6b), 4.26 (ddd, 1H, *J* 11.4 Hz, H-5), 3.25 (dq, 1H, *J* 6.0 Hz, H-5'), 3.24 (t, 1H, H-4), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.12 (d, 1H, H-6'). ^{13}C NMR (600 MHz, $CDCl_3$, from GHMQC) δ 133.1 – 128.6 (Bz), 93.0 (C-1, $J_{C1,H1}$ 179.3), 83.6 (C-1', $J_{C1',H1'}$ 154.3), 75.0 (C-5'), 73.0 (C-5), 71.6 (C-3'), 70.8 (C-2 + C-2'), 69.5 (C-4), 69.0 (C-3), 63.7 (C-6), 47.9 (C-4), 20.6 (5 × OAc), 17.1 (C-6'); m/z (High Res. ES-MS) 822.076466. Calcd for $C_{31}H_{36}Cl_3NO_{15}SNa^+$: 822.076895.

Methyl 2,3,4-tri-O-acetyl-β-L-*rhamnopyranosyl--(1→4)-S-(2,3-di-O-acetyl-6-O-benzoyl-4-thio-β*-D-*glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β*-D-*galatopyranoside* (**45**)

Under argon, methyl 2,3,6-tri-*O*-benzoyl-β-D-galatopyranoside **44** (50 mg, 98.7 μmole) and trichloroacetimidate **47** (46 mg, 57.4 μmole) were dissolved in anhydrous dichloromethane (1.5 mL). The solution was cooled to –20 °C and liquid trimethylsilyl trifluoromethanesulfonate (2 μL, 10.2 μmole) was subsequently added. The reaction was warmed to room temperature and proceeded for 3 h. It was quenched with triethyl amine. The organic solvent was evaporated and the resulting residue was purified by chromatography on silica using toluene - EtOAc (4:1 v/v) as eluent to give compound **45** (50.4 mg, 77%). $[\alpha]_D^{22}$ +45.1° (*c* 5.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.08 – 7.32 (m, 20H, 4 × Bz), 5.54 (dd, 1H, *J* 7.9 Hz, 10.3 Hz, H-2), 5.47 (dd, 1H, *J* 2.9 Hz, H-3), 5.36 (dd, 1H, *J* 1.3 Hz, 3.5 Hz, H-2''), 5.05 (dd, 1H, *J* 8.1 Hz, 9.7 Hz, H-2'), 4.92 (t, 1H, *J* 9.5 Hz, H-4''), 4.91 (t, 1H, *J* 9.5 Hz, H-3'), 4.82 (dd, 1H, H-3''), 4.71 (d, 1H, H-1'), 4.67 (d, 1H, H-1''), 4.62 (dd, 1H, *J* 7.7 Hz, 11.5 Hz, H-6a), 4.57 (overlapped, 1H, H-1), 4.56 (overlapped, 2H, H-6'), 4.42 (d, 1H, *J* 6.8 Hz, H-6b), 4.31 (d, 1H, H-4), 4.01 (t, 1H, *J* 6.2 Hz, H-5), 3.48 – 3.45 (m, 4H, H-5' + OMe), 3.22 (dq, 1H, $J_{5'',6''}$ 6.0 Hz, H-5''), 3.15 (t, 1H, *J* 11.2 Hz, H-4'), 2.27 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.04 (d, 1H, H-6''). ^{13}C NMR (600 MHz, $CDCl_3$, from GHMQC): δ 133.7 – 128.4 (Bz), 102.0 (C-1, $J_{C1,H1}$ 161.4), 100.4 (C-1', $J_{C1',H1'}$ 163.5), 83.9 (C-1'', $J_{C1'',H1''}$ 152.7), 75.8 (C-5''), 74.4 (C-5'), 73.8 (C-4), 73.1 (C-3 + C-4''), 72.4 (C-2'), 72.2 (C-5), 71.4 (C-3''), 70.7 (C-2''), 69.5 (C-3'), 69.3 (C-2), 63.2 (C-6 + C-6'), 56.8 (OMe), 47.7 (C-4'), 20.4 – 20.2 (5 × OAc), 17.1 (C-6''); m/z (High Res. ES-MS)

1167.313865. Calcd for $C_{57}H_{60}O_{23}SNa^+$: 1167.314381. Anal. Calcd for $C_{57}H_{60}O_{23}S$ requires C, 59.78; H, 5.28. Found: C, 59.65; H, 5.52.

Methyl β-L-rhamnopyranosyl-(1→4)-S-(4-thio-β-D-glucopyranosyl)-(1→4)-β-D-galatopyranoside (**48**)

Compound **45** (30 mg, 26.2 μmol) was deprotected as described for **34,** and purified by reverse-phase HPLC using pure H_2O as eluent to yield **48** in pure form (11.8 mg, 87%). $[\alpha]_D^{22}$ +34.5° (*c* 2.9, H_2O); ^1H NMR (600 MHz, D_2O): δ4.96 (s, 1H, H-1"), 4.63 (d, 1H, *J* 7.9 Hz, H-1'), 4.35 (d, 1H, *J* 7.9 Hz, H-1), 4.16 (d, 1H, *J* 3.3 Hz, H-4), 4.09 (d, 1H, *J* 3.5 Hz, H-2"), 4.06 (dd, 1H, *J* 2.2 Hz, 12.6 Hz, H-6'a), 3.95 (dd, 1H, *J* 5.3 Hz, H-6'b), 3.84 (dd, 1H, *J* 5.7 Hz, 11.7 Hz, H-6a), 3.80 (dd, 1H, *J* 7.0 Hz, H-6b), 3.75 (dd, 1H, *J* 10.1 Hz, H-3), 3.73 (overlapped, 1H, H-5), 3.63 – 3.59 (m, 3H, H-5' + H-3' + H-3"), 3.58 (overlapped, 1H, H-2), 3.58 (s, 3H, OMe), 3.44 (dq, 1H, *J* 9.5 Hz, 6.1 Hz, H-5"), 3.39 (t, 1H, *J* 9.3 Hz, H-2'), 3.37 (t, 1H, *J* 8.1 Hz, H-4"), 2.80 (t, 1H, *J* 10.8 Hz, H-4'), 1.32 (d, 2H, H-6"). ^{13}C NMR (600 MHz, D_2O, from GHMQC): δ 104.6 (C-1, $J_{C1,H1}$ 161.8), 104.2 (C-1', $J_{C1',H1'}$ 160.4), 85.1 (C-1", $J_{C1",H1"}$ 154.6), 78.1 (C-4), 77.2 (C-5"), 76.7 (C-5'), 75.7 (C-3' + C-4"), 75.2 (C-5), 74.1 (C-3"), 74.0 (C-3), 73.0 (C-2"), 72.5 (C-2'), 72.0 (C-2), 62.6 (C-6'), 61.6 (C-6), 58.0 (OMe), 49.0 (C-4'), 18.0 (C-6"); m/z (High Res. ES-MS) 541.156671. Calcd for $C_{19}H_{34}O_{14}SNa^+$: 541.156698.

*Methyl 2,3,6-tri-O-benzoyl-4-S-(2,3,4,6-tetra-O-acetyl-β-*D*-mannopyranosyl)-4-thio-α-*D*-glucopyranoside* (**52**)

Under argon, diethylamine (500 μL) was added dropwise to a stirred solution of triflate (231 mg, 361 μmol) and thioacetate **2** (134 mg, 329 μmol) in anhydrous DMF (4 mL) at –5 °C, and the reaction mixture was stirred for 12 h. EtOAc (100 mL) was added and the resulting solution was washed with H_2O (1 × 30 mL), brine (1 × 30 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using toluene - EtOAc (7:3 v/v) as eluent to give compound **52** (231 mg, 82%). $[\alpha]_D^{22}$ +25.0° (*c* 2.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 – 7.33 (m, 15H, Bz),), 5.99 (dd, 1H, *J* 10.0, 10.9, H-3), 5.42 (dd, 1H, *J* 1.3, 2.9, H-2'), 5.30 (dd, 1H, *J* 3.6, H-2), 5.18 (m, 3H, H-1' + H-3' + H-4'), 5.16 (d, 1H, H-1), 4.86 (dd, 1H, *J* 3.9, 12.1, H-6a), 4.78 (dd, 1H, *J* 2.1, H-6b), 4.34 (ddd, 1H, *J* 11.0, H-5), 4.15 (m, 2H, H-6a), 3.76 (ddd, 1H, *J* 3.2, 5.3, 9.4, H-5'), 3.43 (s, 3H, OMe), 3.20 (t, 1H, H-4), 2.05 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.96 (s, 3H, OAc); ^{13}C (600 MHz, $CDCl_3$, from HMQC) δ 133.3 – 128.3 (Bz), 97.3 (C-1, $J_{C1,H1}$ 172.4), 79.5 (C-1', $J_{C1',H1'}$ 154.5), 76.2 (C-5'), 73.5 (C-2), 71.6 (C-3'), 70.1 (C-2'), 69.0 (C-5), 67.5 (C-4), 65.9 (C-4'), 64.0 (C-6), 62.7 (C-6'), 55.4 (OMe), 46.0 (C-4), 20.3 (4 × OAc); m/z (High Res. ES-MS) calcd $C_{42}H_{44}O_{17}SNa^+$: 875.21914, found: 875.21882. Anal. Calcd for $C_{42}H_{44}O_{17}S$: C, 59.15; H, 5.2. Found: C, 58.9; H, 5.1.

Methyl 4-S-(β-D-mannopyranosyl)-4-thio-α-D-glucopyranoside (**49**).

Compound **52** (200 mg, 235 μmol) was deprotected under standard Zemplen transesterification condition and the product **49** was purified by reverse-phase chromatography using H_2O-MeOH as eluent (83 mg, 95%). $[\alpha]_D^{22}$ +2.7° (*c* 2.8, H_2O); ^1H NMR (600 MHz, D_2O) δ4.96 (s, 1H, H-1'), 4.87 (d, 1H, *J* 3.7, H-1), 4.06 (m, 2H, H-2' + H-6a), 3.96 – 3.92 (m, 2H, H-6b + H-5), 3.89 (d, 1H, *J* 2.2, 12.3, H-6'a), 3.83 (dd, 1H, *J* 9.5, 10.3, H-3), 3.71 (dd, 1H, *J* 6.4, H-6'b), 3.69 (dd, 1H, *J* 3.5, 9.7, H-3'), 3.63 (dd, 1H, H-2), 3.59 (t, 1H, *J* 9.7, H-4'), 3.42 (s, 3H, OMe), 3.42 (ddd, 1H, H-5'), 2.83 (t, 1H, *J* 10.8, H-4); ^{13}C (600 MHz, D_2O, from HMQC) δ 100.2 (C-1, $J_{C1,H1}$ 170.2), 82.6 (C-1', $J_{C1',H1'}$ 154.8), 81.1 (C-5'), 74.4 (C-3'), 73.3 (C-2), 72.8 (C-2'), 72.5 (C-5), 70.2 (C-3), 67.5 (C-4'), 62.1 (C-6), 62.0 (C-6'), 55.9 (OMe), 47.9 (C-4); m/z (High Res. ES-MS) calcd $C_{13}H_{24}O_{10}SNa^+$ 395.09824; found: 395.09741. Anal. Calcd for $C_{13}H_{24}O_{10}S{\cdot}H_2O$: C, 40.0; H, 6.7. Found: C, 40.3; H, 6.5.

X-ray crystal data for *2,3,4,6-tetra-O-acetyl-1-S-acetyl-1-thio-β-D-mannopyranose* (**2**): $C_{16}H_{22}O_{10}S$, M = 406.40, monoclinic space group $P2_1$ (No. 4), a = 9.7403 (8) Å, b = 8.7488 (7) Å, c = 11.5396 (10) Å, β = 102.7060 (14)°, V = 959.28 (14) Å^3, Z = 2, D_c = 1.407 g cm^{-3}, μ(Mo Kα [0.71073 Å]) = 0.220 mm^{-1}. Final $R_1(F)$ = 0.0374 (for 2479 reflections with $F_o^2 \geq 2\sigma(F_o^2)$) and $wR_2(F^2)$ = 0.0661 (for all 3020 unique data) and 249 parameters varied.

X-ray crystal data for *Methyl 4-S-(β-D-mannopyranosyl)-4-thio-α-D-glucopyranoside* (**49**) : $C_{15}H_{32}O_{12}S$, M = 436.47, monoclinic space group $P2_1$ (No. 4), a = 10.232 (4) Å, b

= 9.095(3) Å, c = 11.254 (4) Å, β = 107.424 (7)°, V = 999.2 (6) Å^3, Z = 2, D_c = 1.451 g cm^{-3}, μ(Mo Kα [0.71073 Å]) = 0.223 mm^{-1}. Final $R_1(F)$ = 0.0628 (for 2790 reflections with $F_o^2 \geq 2\sigma(F_o^2)$) and $wR_2(F^2)$ = 0.0661 (for all 4027 unique data) and 264 parameters varied.

4-Pentenyl 2-acetamido-2-deoxy-4,6-O-benzylidene-β-D-galactopyranoside (**59**)

To a mixture of 4-pentenyl 2-acetamido-2-deoxy-β-D-galactopyranoside **68** (8 g, 27.7 mmol) in benzaldehyde (100 mL), dry $ZnCl_2$ power (10 g) was added. After stirring for 36 h, the mixture was treated with ice-cold H_2O-Hexane (H_2O 300 mL, Hexane 300 mL). The mixture was filtered. The filter cake was dissolved in dichloromethane (200 mL); and the mixture was washed with saturated $NaHCO_3$ solution (200 mL), H_2O (200 mL), brine, and dried over Na_2SO_4. Evaporation of the organic solvent gave **59** as a white powder in pure form. (9.9 g, yield 95%). $[\alpha]_D^{22}$ –63.0° (*c* 3.0, $CHCl_3$); ^{1}H NMR (CD_3COCD_3, 600 MHz): δ 7.49 – 7.34 (m, 5H, Ph), 7.21 (d, 1H, *J* 8.2 Hz, 2-NH), 5.82 (tdd, 1H, *J* 6.6 Hz, 10.3 Hz, 17.0 Hz, C*H*=CH$_2$), 5.60 (s, 1H, PhC*H*), 5.01 (ddd, 1H, *J* 1.7 Hz, 3.5 Hz, CH=C*Ha*), 4.93 (m, 1H, CH=C*Hb*), 4.68 (d, 1H, *J* 8.4 Hz, H-1), 4.59 (d, 1H, *J* 4.2 Hz, 3-OH), 4.23 (dd, 1H, *J* 5.1 Hz, 10.3 Hz, H-6a), 3.89 (m, 1H, H-3), 3.80 (td, 1H, *J* 6.2 Hz, 9.7 Hz, OCHa), 3.76 ('t', 1H, *J* 10.3 Hz, H-6b), 3.65 ('t'd, 1H, H-2), 3.50 ('t', 1H, *J* 9.3 Hz, H-4), 3.49 (td, 1H, *J* 6.2 Hz, OCHb), 3.40 (d't', 1H, H-5), 2.54 (s, 3H, NAc), 2.05 (m, 2H, C*H*$_2$CH=CH$_2$), 1.61 (m, 2H, OCH$_2$C*H*$_2$); High Res. ES-MS calcd. for $C_{20}H_{28}NO_6^+$: 378.19111; found: 378.19160. Anal. Calcd for $C_{20}H_{27}NO_6$: C 63.64, H 7.21 N 3.71; Found: C 63.69, H 7.35, N 3.65.

4-Pentenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-*ribo-hexopyranosid-3-ulose* (**69**)

A mixture of **59** (1.0 g, 3.5 mmol) and Ac_2O (40 mL) in freshly distilled DMSO (80 mL) was stirred overnight. The organic solvents were co-evaporated with toluene and evaporated to dryness to give compound **69** (100%, judged by TLC). Compound **69** was proceeded to next synthetic step without further purification. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ7.48 - 7.36 (m, 5H, Ph), 5.95 (d, 1H, *J* 8.8 Hz, 2-NH), 5.80 (tdd, 1H, *J* 6.6 Hz, 10.1 Hz, 17.0 Hz, C*H*=CH_2), 5.56 (s, 1H, PhC*H*), 5.00 (ddd, 1H, *J* 1.7 Hz, 3.5 Hz, CH=C*Ha*), 4.96 (m, 1H, CH=C*Hb*), 4.68 (d't', 1H, *J* 1.3 Hz, H-2), 4.54 (d, 1H, H-1), 4.45 (dd, 1H, *J* 4.9 Hz, 10.6 Hz, H-6a), 4.37 (dd, 1H, *J* 10.0 Hz, H-4), 3.91 ('t', 1H, *J* 10.3 Hz, H-6b), 3.87 (td, 1H, *J* 6.4 Hz, 9.7 Hz, OCHa), 3.59 (d't', 1H, H-5), 3.53 (td, 1H, *J* 6.6 Hz, OCHb), 2.10 (m, 2H, C*H*$_2$CH=CH_2), 2.0 (s, 3H, NAc), 1.65 (m, 2H, OCH_2CH_2); ^{13}C NMR (CD_2Cl_2, 600 MHz, from GHMQC): δ137.4 (*C*H=CH_2), 129.7 – 126.2 (Ph), 115.0 (CH=CH_2), 105.0 (C-1, $J_{C1,H1}$ 162.2 Hz), 102.0 (PhCH), 83.0 (C-4), 70.0 (OCH_2), 69.2 (C-6), 67.0 (C-5), 61.6 (C-2), 30.2 (*C*H_2CH=CH_2), 28.8 (OCH_2CH_2), 24.3 (NAc); High Res. ES-MS $C_{20}H_{25}NO_6Na^+$: 398.15741; found: 398.15775.

4-Pentenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-*allopyranoside* (**70**)

Compound **69** from the previous step was dissolved in 1:1 mixture of anhydrous THF and CH_2Cl_2 (120 mL). The mixture was cooled to –78 °C and to it was added L-Selectride (8 mL, 1.0 M in THF) dropwise. After stirring for 5 min, the dry-ice bath was removed, and the mixture was stirred for 1 h. The reaction was then quenched with MeOH (2 mL). The organic solvent was evaporated and the resulting residue was

dissolved in dichloromethane (100 mL). The mixture was washed with hydrogen peroxide (5%, 100 mL), NaOH (1M, 100 mL), sodium bisulfide (5%, 100 mL), brine, and dry over Na_2SO_4. Removal of the organic solvent gave compound **70**, which was used for the next synthetic step without further purification. ^{1}H NMR (CD_2Cl_2, 600 MHz): δ7.48 - 7.36 (m, 5H, Ph), 6.00 (d, 1H, *J* 9.2 Hz, 2-NH), 5.82 (tdd, 1H, *J* 6.8 Hz, 10.3 Hz, 17.0 Hz, C*H*=CH_2), 5.60 (s, 1H, PhC*H*), 5.02 (ddd, 1H, *J* 1.8 Hz, 3.7 Hz, CH=C*Ha*), 4.96 (m, 1H, CH=C*Hb*), 4.64 (d, 1H, *J* 8.4 Hz, H-1), 4.34 (dd, 1H, *J* 5.0 Hz, 10.3 Hz, H-6a), 4.24 (bs, 1H, H-3), 4.07 (d't', 1H, *J* 3.1 Hz, H-2), 3.95 (d't', 1H, *J* 9.9 Hz, H-5), 3.83 (td, 1H, *J* 6.2 Hz, 9.9 Hz, OCHa), 3.78 ('t', 1H, H-6b), 3.66 (dd, 1H, *J* 2.6 Hz, H-4), 3.48 (td, 1H, *J* 6.6 Hz, OCHb), 2.10 (m, 2H, C*H*$_2$CH=CH_2), 1.98 (s, 3H, NAc), 1.65 (m, 2H, OCH_2CH_2); ^{13}C NMR (CD_2Cl_2, 600 MHz, from GHMQC): δ137.4 (*C*H=CH_2), 129.8 – 126.4 (Ph), 115.0 (CH=*C*H$_2$), 102.0 (PhCH), 100.9 (C-1, $J_{C1,H1}$ 161.2 Hz), 79.1 (C-4), 69.5 (OCH_2 + C-6), 64.0 (C-5), 52.1 (C-2), 30.1 (*C*H$_2$CH=CH_2), 28.9 (OCH_2CH_2), 23.3 (NAc); High Res. ES-MS $C_{20}H_{28}NO_6^+$: 378.19111; found: 378.19041.

*4-Pentenyl 4,6-O-benzylidene-2-deoxy-β-*D*-allopyranoside 2,3-sulfamidate* (**71**)

To an ice-cold cold mixture of crude compound **70** in anhydrous DMF (20 mL) was added NaH (60%, 230 mg, 5.8 mmol). The mixture was then cooled to –40 °C. A solution of 1,1'-sulfonyl diimidazole (820 mg, 4.1 mmol) in DMF (2 mL) was added dropwise, and the mixture was stirred for 5 h. The reaction was then quenched with MeOH, and the solvents were removed at room temperature to give crude product **71**. Without further purification, compound **71** was used directly for the preparation of **72**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ7.48 - 7.36 (m, 5H, Ph), 5.79 (tdd, 1H, *J* 6.6 Hz, 10.1 Hz,

16.9 Hz, C*H*=CH_2), 5.55 (s, 1H, PhC*H*), 5.18 ('t', 1H, *J* 3.5 Hz, H-3), 5.04 (ddd, 1H, *J* 1.7 Hz, 3.3 Hz, CH=C*Ha*), 4.98 (m, 1H, CH=C*Hb*), 4.85 (d, 1H, *J* 7.5 Hz, H-1), 4.42 (dd, 1H, *J* 5.3 Hz, 10.6 Hz, H-6a), 3.96 (d't', 1H, *J* 9.9 Hz, H-5), 3.90 (td, 1H, *J* 6.6 Hz, 9.7 Hz, OCHa), 3.86 (dd, 1H, H-4), 3.76 ('t', 1H, H-6b), 3.70 (m, 1H, H-2), 3.59 (td, 1H, 6.6 Hz, OCHb), 2.12 (m, 2H, CH_2CH=CH_2), 1.70 (m, 2H, OCH_2CH_2); ^{13}C NMR ($CDCl_3$, 600 MHz, from GHMQC): δ137.5 (*C*H=CH_2), 129.5 – 126.1 (Ph), 115.0 (CH=*C*H_2), 102.5 (Ph*C*H), 101.4 (C-1, $J_{C1,H1}$ 162.8 Hz), 80.0 (C-3), 75.5 (C-4), 70.0 (OCH_2), 68.8 (C-6), 62.5 (C-5), 59.2 (C-2), 29.5 (*C*H_2CH=CH_2), 28.5 (OCH_2CH_2); High Res. ES-MS $C_{18}H_{23}NO_7SNa^+$: 420.10875; found: 420.10968.

*4-Pentenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D*-allopyranoside 2,3-sulfamidate* (**72**)

To a suspension of the crude compound **71** in anhydrous dichloromethane (25 mL) was added freshly distilled pyridine (850 µL, 10.5 mmol). After 5 min, AcCl (500 µL, 7.0 mmol) was added dropwise, and the mixture was stirred for 1 h. The mixture was then diluted with dichloromethane (100 mL), washed with H_2O (50 mL X 2), and concentrated. The resulting residue was purified by chromatography on silica using CH_2Cl_2-EtOAc (49:1 v/v) to give **72** (0.72 g, 47% from compound **59**). Mp 165-166 °C ($CDCl_3$); $[\alpha]_D^{22}$ –12.4° (*c* 2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ7.46 - 7.38 (m, 5H, Ph), 5.76 (tdd, 1H, *J* 6.6 Hz, 10.1 Hz, 16.9 Hz, C*H*=CH_2), 5.57 (s, 1H, PhC*H*), 5.20 (dd, 1H, *J* 3.1 Hz, 4.2 Hz, H-3), 5.04 (ddd, 1H, *J* 1.7 Hz, 3.5 Hz, CH=C*Ha*), 4.98 (m, 1H, CH=C*Hb*), 4.86 (d, 1H, *J* 7.0 Hz, H-1), 4.61 (bs, 1H, H-2), 4.43 (dd, 1H, *J* 4.9 Hz, 10.4 Hz, H-6a), 3.99 (d't', 1H, *J* 9.9 Hz, H-5), 3.92 (dd, 1H, H-4), 3.88 (td, 1H, *J* 6.4 Hz, 9.7

Hz, OCHa), 3.78 ('t', 1H, H-6b), 3.53 (td, 1H, 6.6 Hz, OCHb), 2.44 (s, 3H, NAc), 2.08 (m, 2H, $CH_2CH=CH_2$), 1.68 (m, 2H, OCH_2CH_2); Selected ^{13}C NMR data ($CDCl_3$, 600 MHz, from GHMQC): δ137.4 ($CH=CH$), (129.6 – 126.0 (Ph), 115.0 ($CH=CH_2$), 102.7 (PhCH), 101.5 (C-1, $J_{C1,H1}$ 162.8 Hz), 78.0 (C-3), 74.8 (C-4), 69.6 (OCH_2), 68.8 (C-6), 63.0 (C-5), 29.5 ($CH_2CH=CH_2$), 28.3 (OCH_2CH_2), 22.3 (NAc); High Res. ES-MS $C_{20}H_{25}NO_8SNa^+$: 462.11931; found: 462.11976. Anal. Calcd for $C_{20}H_{25}NO_8S$: C 54.66, H 5.73; Found: C 54.20, H 5.63.

4-Pentenyl 2-acetamido-3-S-acetyl-4,6-O-benzylidene-2-deoxy-β-D-allopyranoside (**73**)

To a suspension **72** (215 mg, 489 μmol) in anhydrous DMF (7 mL) was added potassium thioacetate (290 mg, 2.61 mmol). The mixture was stirred for 3 h until completion. The solvent was then evaporated and the resulting residue was suspended in THF (4.8 mL). The mixture was treated with 2.4 mL of prepared solution (220 μL H_2SO_4 (96%), 17 μL H_2O and 5 μL THF) for 30 min. The reaction mixture was then diluted with dichloromethane, washed with saturated $NaHCO_3$ (20 mL), H_2O (25 mL) and dried over Na_2SO_4. The solvent was evaporated and the resulting residue was purified by chromatography on silica using toluene-EtOAc (3:1 v/v) to give **73** (165 mg, 77%). $[\alpha]_D^{22}$ –76.3° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ7.43 - 7.32 (m, 5H, Ph), 5.76 (tdd, 1H, *J* 6.6 Hz, 10.1 Hz, 16.9 Hz, $CH=CH_2$), 5.63 (d, 1H, *J* 9.5 Hz, 2-NH), 5.48 (s, 1H, PhCH), 4.98 (ddd, 1H, *J* 1.6 Hz, 3.5 Hz, CH=C*Ha*), 4.94 (m, 1H, CH=C*Hb*), 4.48 (d, 1H, *J* 8.2 Hz, H-1), 4.32 (dd, 1H, *J* 3.8 Hz, 10.4 Hz, H-6a), 4.06 (ddd, 1H, *J* 11.7 Hz, H-2), 3.85 (td, 1H, *J* 6.0 Hz, 9.7 Hz, OCHa), 3.82 ('t', 1H, *J* 11.4 Hz, H-3), 3.74 (m, 1H, H-5), 3.52 (m, 2H, H-6b + H-4), 3.47 (td, 1H, 6.4 Hz, OCHb), 2.34 (s, 3H, SAc), 2.07

(m, 2H, $CH_2CH{=}CH_2$), 1.92 (s, 3H, NAc), 1.64 (m, 2H, OCH_2CH_2); High Res. ES-MS $C_{22}H_{30}NO_6S^+$: 436.17884; found: 436.17884. Anal. Calcd for $C_{22}H_{29}NO_6S$: C 60.67, H 6.71, N 3.22; Found: C 60.74, H 6.63, N 3.06.

*2-(Trimethylsilyl)ethyl 2,3,4-tri-O-acetyl-α-*L*-rhamnopyranoside* (**74**)

A mixture containing the peracetylated rhamnopyranosyl bromide **61** (20.0 g, 56.6 mmol), TMSETOH (12.4 ml, 84.9 mmol) and M.S. 4 Å (15 g) in anhydrous CH_3CN (170 ml) was stirred for 3 hrs at r.t.. The mixture was ice-cooled, and $Hg(CN)_2$ (24.3 g, 96.2 mmol) and $HgBr_2$ (2.0 g, 5.5 mmol) was added successively. The reaction was allowed to warm slowly to room temperature within 2 h and the stirring was continued for an additional hour. The mixture was diluted with CH_2Cl_2 (500 mL), and filtered off. The organic solution was washed with a 1:1 mixture of aqueous $NaHCO_3$ (sat) and 10% KI (2 x 250 ml), dried over anhydrous Na_2SO_4, and evaporated. The syrup mixture was purified by chromatography on silica gel using EtOAc-hexane (20% → 35%) as eluent to afford the desired glycoside **74** as a colorless syrup (18.1 g, 82% yield). $[\alpha]_D^{22}$ –58.6° (*c* 5.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ5.28 (dd, 1H, *J* 3.5 Hz, 10.1 Hz, H-3), 5.18 (dd, 1H, *J* 1.8 Hz, H-2), 5.04 ('t', 1H, *J* 9.9 Hz, H-4), 4.71 (d, 1H, H-1), 3.86 (dq, 1H, *J* 6.3 Hz, H-5), 3.76 (dt, 1H, *J* 6.0 Hz, 10.0 Hz, OCHa), 3.53 (dt, 1H, *J* 6.2 Hz, OCHb), 2.12 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.20 (d, 3H, H-6), 0.93 (m, 2H, CH_2Si), 0.01 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{17}H_{30}O_8SiNa^+$: 413.16022; found: 413.16013. Anal. Calcd for $C_{17}H_{30}O_8Si$: C 52.29, H 7.74; Found: C 52.30, H 7.71.

2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-α-L-rhamnopyranoside (**62**)

Camphorsulfonic acid (189 mg, 0.81 mmol) was added to a stirred suspension of **75** (3.2 g, 12.1 mmol) and trimethyl orthoacetate (6.2 mL, 48.7 mmol) in $CHCl_3$ (300 mL). The solution was concentrated on a rotovapor to ~10 mL at 25 °C for the during of 20 min. Triethylamine (3 mL) was added to the reaction mixture and the solution was evaporated to dryness. Pyridine (100 mL) was added and the mixture was cooled to 0° C and treated with benzoyl chloride (2.8 mL, 24.1 mmol) for 1 h at 0° C. The reaction was quenched with MeOH (5 mL) and co-rotavapped with toluene. The residue was treated with aqueous 90% AcOH (100 mL) for 30 min. The mixture was concentrated and the residue was partitioned between EtOAc and 20% $NaHCO_3$. The organic phase was concentrated the resulting residue was purified by chromatography on silica using toluene-EtOAc (9:1 v/v) to give **62** (4.6 g, 93%). $[\alpha]_D^{22}$ -38.6° (*c* 6.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ8.09 - 7.46 (m, 5H, Bz), 5.08 ('t', 1H, *J* 9.8 Hz, H-4), 5.08 (dd, 1H, *J* 1.7 Hz, 3.7 Hz, H-2), 4.83 (d, 1H, *J* 1.5 Hz, H-1), 4.38 (dd, 1H, H-3), 3.98 (dq, 1H, *J* 6.3 Hz, H-5), 3.79 (dt, 1H, *J* 6.1 Hz, 10.1 Hz, OCHa), 3.53 (dt, 1H, *J* 6.1 Hz, OCHb), 2.17 (s, 3H, OAc), 1.26 (d, 3H, H-6), 0.96 (m, 2H, CH_2Si), 0.03 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{20}H_{30}O_7SiNa^+$: 433.16530; found: 433.16583. Anal. Calcd for $C_{20}H_{30}O_7Si$: C 58.51, H 7.37; Found: C 58.80, H 7.32.

2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**76**)

A mixture **76** (500 mg, 1.22 mmol) and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyanosyl bromide **61** (860 mg, 2.44 mmol) in anhydrous MeCN (40 mL) with 4 Å

molecular sieves was cooled to 0 °C under argon, $Hg(CN)_2$ (620 mg , 2.45 mmol) and $HgBr_2$ (310 mg, 0.85 mmol) was added. The mixture was stirred at room temperature for 1.5 h. The reaction mixture was quenched with triethylamine (1 mL) and concentrated. The residue was partitioned between EtOAc (150 mL) and H_2O (150 mL). The organic phase was concentrated and the resulting residue was purified by chromatography on silica using toluene-EtOAc (17:3 v/v) to give **76** (806 mg, 97%). $[\alpha]_D^{22}$ –19.2° (*c* 8.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.01 - 7.40 (m, 5H, Bz), 5.34 ('t', 1H, *J* 9.9 Hz, H-4), 5.21 (dd, 1H, *J* 1.9 Hz, 3.7 Hz, H-2), 5.10 (dd, 1H, *J* 3.3 Hz, 9.9 Hz, H-3'), 4.92 ('t', 1H, *J* 9.9 Hz, H-4'), 4.89 (dd, 1H, *J* 1.9 Hz, H-2'), 4.82 (d, 1H, H-1'), 4.76 (d, 1H, H-1), 4.27 (dd, 1H, H-3), 3.91 (m, 2H, H-5' + H-5), 3.80 (dt, 1H, *J* 6.0 Hz, 9.9 Hz, OCHa), 3.53 (dt, 1H, *J* 6.2 Hz, OCHb), 2.22 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.82 (s, 3H, OAc), 1.24 (d, 3H, *J* 6.2 Hz, H-6), 1.16 (d, 3H, *J* 6.2 Hz, H-6'), 0.96 (m, 2H, CH_2Si), 0.03 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{32}H_{46}NO_{14}SiNa^+$: 705.25491; found: 705.25473. Anal. Calcd for $C_{32}H_{46}O_{14}Si$: C 56.29, H 6.79; Found: C 56.27, H 6.55.

*2-O-acetyl-4-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl)-α-*L*-rhamnopyranose* (**77**)

To a solution of **76** (246 mg, 361 μmol) in dichloromethane (2.5 mL) was added trifluoroacetic acid (5 mL) under argon. The mixture was stirred for 8 h. EtOAc (10 mL) and toluene (20 mL) were added and then removed at ca. 5 Torr. A second portion of toluene (15 mL) was added and removed, which gave compound **77** in sufficient purirty for the next synthetic step. ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.04 - 7.42 (m, 5H, Bz), 5.35 ('t', 1H, *J* 9.8 Hz, H-4), 5.28 (dd, 1H, *J* 1.9 Hz, 3.5 Hz, H-2), 5.20 (dd, 1H, *J* 3.9 Hz, H-

1), 5.10 (dd, 1H, *J* 3.5 Hz, 9.9 Hz, H-3'), 4.93 ('t', 1H, *J* 9.9 Hz, H-4'), 4.91 (dd, 1H, *J* 1.8 Hz, H-2'), 4.85 (d, 1H, H-1'), 4.35 (dd, 1H, H-3), 4.17 (dq, 1H, *J* 6.3 Hz, H-5), 3.91 (dq, 1H, *J* 6.2 Hz, H-5'), 2.76 (d, 1H, 1-OH), 2.22 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.83 (s, 3H, OAc), 1.25 (d, 3H, H-6), 1.18 (d, 3H, H-6');

*2-O-acetyl-4-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl)-α-*L*-rhamnopyranosyl trichloroacetimidate* (**57**)

Under argon, trichloroacetonitrile (0.7 mL, 7.0 mmole) was added to a solution containing crude disaccharide **77** in anhydrous dichloromethane (8 mL). Liquid 1,8-diazabicyclo[5.4.0]undec-7-ene (25 μL, 167 μmole) was added. After 2 h, the organic solvent was evaporated and the resulting residue was purified by chromatography on silica using hexane - EtOAc (4:1 v/v) as eluent to give compound **57** (224 mg, 85% from compound **76**). $[\alpha]_D^{22}$ –8.9° (*c* 3.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ8.74 (s, 1H, NH), 8.02 - 7.38 (m, 5H, Bz), 6.23 (d, 1H, *J* 1.9 Hz, H-1), 5.45 ('t', 1H, *J* 9.9 Hz, H-4), 5.41 (dd, 1H, *J* 3.4 Hz, H-2), 5.08 (dd, 1H, *J* 3.4 Hz, 9.9 Hz, H-3'), 4.95 (m, 2H, H-4' + H-2'), 4.85 (d, 1H, *J* 1.7 Hz, H-1'), 4.36 (dd, 1H, H-3), 4.12 (dq, 1H, *J* 6.0 Hz, H-5), 3.92 (dq, 1H, *J* 6.3 Hz, 9.6 Hz, H-5'), 2.25 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.81 (s, 3H, OAc), 1.28 (d, 3H, H-6), 1.14 (d, 3H, H-6'); High Res. ES-MS $C_{29}H_{34}O_{14}NCl_3Na^+$: 748.09246; found: 748.09425. Anal. Calcd for $C_{29}H_{34}Cl_3O_{14}$: C 47.91, H 4.71, N 1.93; Found: C 47.72, H 5.13, N 1.90.

2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-6-deoxy-α-L-arabino-hexopyranosid-3-ulose (**78**) and *2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-6-deoxy-α-L-ribo-hexopyranosid-3-ulose* (**79**)

Method A: A mixture of **62** (0.9 g, 2.2 mmol) and Ac_2O (25 mL) in freshly distilled DMSO (50 mL) was stirred overnight. The organic solvents were co-rotavapped with toluene at room temperature and evaporated to dryness to give inseparable compound **78** and **79** (100%, judged by TLC, **78/79** ratio is 1:1, judged by NMR).

Method B: Under argon, Dess Martin periodinane (2.5 g, 5.8 mmol) was added to a stirred mixture of **62** (2.0 g, 4.9 mmol) in anhydrous dichloromethane (50 mL). After stirring overnight at room temperature, the mixture was diluted with dichloromethane (250 mL), and subsequently washed with saturated aqueous $NaHCO_3$ (150 mL), $Na_2S_2O_3$ (10%, 150 mL), H_2O (150 mL) and dried over Na_2SO_4. The organic phase was concentrated and dried under vacuum to give **78** (100%, judged by TLC). Compound **78** was sufficiently pure to use for the next synthetic step; ^{1}H NMR ($CDCl_3$, 500 MHz): δ8.03 - 7.42 (m, 5H, Bz), 5.48 (d, 1H, *J* 9.8 Hz, H-4), 5.08 (d, 1H, *J* 1.8 Hz, H-1), 5.04 (d, 1H, H-2), 4.27 (dq, 1H, *J* 6.2 Hz, H-5), 3.80 (dt, 1H, *J* 6.0 Hz, 10.1 Hz, OCHa), 3.57 (dt, 1H, *J* 6.1 Hz, OCHb), 2.18 (s, 3H, OAc), 1.43 (d, 3H, H-6), 0.94 (m, 2H, CH_2Si), 0.01 (s, 9H, $SiMe_3$); ^{13}C NMR (600 MHz, $CDCl_3$, from GGHMQC): δ131.8 – 128.1 (Bz), 98.4 (C-1, $J_{C1,H1}$ 173.6), 77.0 (C-4), 76.3 (C-2), 66.5 (C-5), 65.7 (OCH_2), 20.4 (OAc), 18.0 (CH_2Si), -2.7 ($SiMe_3$); High Res. ES-MS $C_{20}H_{28}O_7SiNa^+$: 431.14965; found: 431.14904.

Selected data for **79**: ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.07 - 7.42 (m, 5H, Bz), 5.45 (dd, 1H, *J* 1.1 Hz, 4.3 Hz, H-2), 5.26 (d, 1H, H-1), 5.24 (dd, 1H, *J* 1.1 Hz, 9.9 Hz, H-4),

4.28 (overlapped, 1H, H-5), 3.85 (dt, 1H, *J* 5.2 Hz, 10.2 Hz, OCHa), 3.59 (overlapped, 1H, OCHb), 2.16 (s, 3H, OAc), 1.44 (d, 3H, *J* 6.2 Hz, H-6), 1.00 (m, 2H, CH_2Si), 0.02 (s, 9H, $SiMe_3$); ^{13}C NMR (600 MHz, $CDCl_3$, from GGHMQC): δ131.8 – 128.1 (Bz), 98.0 (C-1, $J_{C1,H1}$ 174.2), 77.7 (C-4), 74.7 (C-2), 66.5 (C-5), 65.7 (OCH_2), 20.0 (OAc), 18.0 (CH_2Si), -2.7 ($SiMe_3$).

*2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-6-deoxy-α-*L*-altropyranoside* (**80**) and *2-(Trimethylsilyl)ethyl 2-O-acetyl-3-O-benzoyl-6-deoxy-α-*L*-altropyranoside* (**81**)

Method A: To a stirred mixture of crude ketone **79** (50.0 mg, 122 μmol) in dichloromethane – methanol (1:1, 4 mL) was added sodium borohydride (7.0 mg, 185 μmol). The mixture was stirred at room temperature for 5 min; it was quenched with saturated NH_4Cl (0.3 mL), diluted with dichloromethane (50 mL), washed with H_2O (50 mL) and dried over Na_2SO_4. The organic phase was concentrated and the resulting residue was purified by chromatography on silica using hexane - EtOAc (9:1 v/v) as eluent to give first, compound **80** (25.5 mg, 51%), and then compound **81** (17.1 mg, 34%);

Method B: To a stirred mixture of crude ketone **79** (1.60 g, 3.9 mmol) in dichloromethane – methanol (1:1, 120 mL) at –78 °C was added sodium borohydride (220 mg, 5.8 mmol). The mixture was stirred at –78 °C for 5 min; it was quenched with saturated aqueous NH_4Cl (5 mL), diluted with dichloromethane (300 mL), washed with H_2O (200 mL) and dried over Na_2SO_4. The organic phase was concentrated and the resulting residue was purified by chromatography on silica using hexane - EtOAc (9:1 v/v) as eluent to give compound **80** (1.21 g, 75%); $[\alpha]_D^{22}$ –74.4° (*c* 3.6, $CHCl_3$); ^1H NMR

($CDCl_3$, 500 MHz): δ8.06 - 7.42 (m, 5H, Bz), 5.04 (dd, 1H, *J* 3.1 Hz, 10.1 Hz, H-4), 4.98 (dd, 1H, *J* 1.4 Hz, 3.7 Hz, H-2), 4.85 (s, 1H, H-1), 4.28 (dq, 1H, *J* 6.2 Hz, H-5), 4.16 (d't', 1H, *J* 10.2 Hz, H-3), 3.87 (ddd, 1H, *J* 5.4 Hz, 9.9 Hz, 11.4 Hz, OCHa), 3.56 (ddd, 1H, *J* 6.0 Hz, 9.9 Hz, OCHb), 3.48 (d, 1H, 3-OH), 2.14 (s, 3H, OAc), 1.30 (d, 3H, H-6), 1.00 (m, 2H, CH_2Si), 0.03 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{20}H_{30}O_7SiNa^+$: 433.16530; found: 433.16542. Anal. Calcd for $C_{20}H_{30}O_7Si$: C 58.51, H 7.37; Found: C 58.31, H 7.47.

Data for **81**: $[\alpha]_D^{22}$ –9.5° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ8.05 - 7.40 (m, 5H, Bz), 5.11 ('t', 1H, *J* 3.3 Hz, H-3), 5.07 (dd, 1H, *J* 1.3 Hz, H-2), 4.72 (s, 1H, H-1), 4.14 (dq, 1H, *J* 6.3 Hz, 9.1 Hz, H-5), 3.84 (ddd, 1H, *J* 3.5 Hz, 11.2 Hz, H-4), 3.78 (m, 1H, OCHa), 3.48 (ddd, 1H, *J* 6.2 Hz, 9.7 Hz, 10.9 Hz, OCHb), 2.12 (s, 3H, OAc), 2.06 (b, 1H, 4-OH), 1.35 (d, 3H, H-6), 0.95 (m, 2H, CH_2Si), -0.02 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{20}H_{30}O_7SiNa^+$: 433.16585; found: 433.16566. Anal. Calcd for $C_{29}H_{30}O_7Si$: C 58.51, H 7.37; Found: C 58.20, H 7.49.

*2-(Trimethylsilyl)ethyl 2-O-acetyl-3-S-acetyl-4-O-benzoyl-α-*L*-rhamnopyranoside* (**82**)

Compound **22** (90 mg, 219 μmol) was suspended in freshly distilled pyridine and anhydrous dichloromethane (1:2, 5 mL) at –20 °C. After stirring for 10 min, liquid triflluromethanesulfonic anhydride (57 μL, 339 μmol) was added. The reaction was continued for 3 h at room temperature. The mixture was diluted with EtOAc (50 mL), quenched with H_2O (0.1 mL), washed with aqueous HCl (2N, 40 mL), saturated $NaHCO_3$ (40 mL), H_2O (40 mL) and dried over $NaSO_4$. The organic phase was concentrated and dried under vacuum to give crude compound **63**. Compound **82** was then suspended in anhydrous DMF (3 mL) at 0 °C under argon. Potassium thioacetate (125 mg, 1.1 mmol)

was added and the reaction was continued for 20 h at 0 °C. EtOAc (50 mL) was added and the resulting solution was washed with H_2O (30 mL), brine (30 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using toluene - EtOAc (19:1 v/v) as eluent to give compound **82** (70 mg, 68% from **80**); $[\alpha]_D^{22}$ –14.5° (*c* 3.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ7.98 - 7.41 (m, 5H, Bz), 5.16 (dd, 1H, *J* 9.6 Hz, 11.4 Hz, H-4), 5.01 (dd, 1H, *J* 1.7 Hz, 3.1 Hz, H-2), 4.75 (d, 1H, H-1), 4.46 (dd, 1H, H-3), 4.09 (dq, 1H, *J* 6.3 Hz, H-5), 3.83 (dt, 1H, *J* 6.1 Hz, 10.0 Hz, OCHa), 3.63 (dt, 1H, *J* 6.2 Hz, 10.1 H, OCHb), 2.14 (s, 3H, SAc), 2.13 (s, 3H, OAc), 1.22 (d, 3H, H-6), 1.01 (m, 2H, CH_2Si), 0.05 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{22}H_{32}O_7SSiNa^+$: 491.15303; found: 491.15331. Anal. Calcd for $C_{22}H_{32}O_7SSi$: C 56.38, H 6.88; Found: C 56.80, H 6.42.

*2,3,4-Tri-*O*-acetyl-1-S-acetyl-1-thio-α-*L*-rhamnopyranose* (**64**)

Under argon, silver trifluoromethanesulfonate (875 mg, 3.4 mmole) was added to a solution containing 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide **61** (1.0 g, 2.8 mmole) and thioacetic acid (0.4 mL, 5.7 mmol) in anhydrous dichloromethane (30 mL) at –78 °C. The dry-ice bath was removed and the reaction mixture was stirred for 1 h at ambient temperature. After removal of the organic solvent, the residue was dissolved in EtOAc (200 mL). The mixture was washed with $NaHCO_3$ (10%, 100 mL), H_2O (100 mL) and dried over Na_2SO_4. The organic solvent was removed and the resulting residue was purified by chromatography on silica using hexane - EtOAc (4:1 v/v) as eluent to give compound **64** (875 mg, 90%); $[\alpha]_D^{22}$ –68.4° (*c* 4.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 5.87 (d, 1H, *J* 1.9 Hz, H-1), 5.30 (dd, 1H, *J* 3.3 Hz, H-2), 5.09 (t, 1H, *J* 10.0

Hz, H-4), 5.04 (dd, 1H, H-3), 3.77 (dq, 1H, *J* 6.1 Hz, H-5), 2.40 (s, 3H, SAc), 2.16 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.21 (d, 3H, H-6); ^{13}C NMR (500 MHz, $CDCl_3$, from GGHMQC): δ78.8 (C-1, $J_{C1,H1}$ 156.1), 74.8 (C-5), 71.5 (C-3), 71.0 (C-2), 70.0 (C-4), 30.4 (SAc), 20.4 (3 × OAc), 15.2 (C-6); High Res. ES-MS $C_{14}H_{20}O_8SNa^+$: 371.07711; found: 371.07650. Anal. Calcd for $C_{14}H_{20}O_8S$: C 48.27, H 5.79; Found: C 48.14, H 5.47.

2-(Trimethylsilyl)ethyl 2-O-acetyl-4-O-benzoyl-3-S-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3-thio-α-D-rhamnopyranoside (**83**)

Triflate **63** was prepared as described above using **80** (300mg, 731 μmole). Compound **63** and thioacetate **64** (230 mg, 660 μmol) were then suspended in anhydrous DMF (7 mL) at 0 °C under argon. Liquid diethylamine (300 μL) was added dropwise. The reaction was continued for 24 h at 0 °C and 5 h at room temperature. EtOAc (300 mL) was added and the resulting solution was washed with H_2O (50 mL), brine (50 mL) dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography on silica using acetone - toluene (1:49 v/v) as eluent to give compound **83** (211mg, 46%). $[\alpha]_D^{22}$ –120.0° (c 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ 8.05 - 7.41 (m, 5H, Bz), 5.27 (dd, 1H, J 9.6 Hz, 11.2 Hz, H-4), 5.20 (d, 1H, J 1.5 Hz, H-1'), 5.04 (dd, 1H, J 3.1 Hz, H-2'), 5.01 (dd, 1H, J 9.9 Hz, H-3'), 4.98 ('t', 1H, J 9.9 Hz, H-4'), 4.97 (dd, 1H, J 1.6 Hz, 3.2 Hz, H-2), 4.73 (d, 1H, H-1), 4.10 (dq, 1H, J 6.2 Hz, H-5'), 3.96 (dq, 1H, J 6.3 Hz, H-5), 3.79 (dt, 1H, J 6.2 Hz, 10.2 Hz, OCHa), 3.60 (dd, 1H, H-3), 3.53 (dt, 1H, J 6.2 Hz, 9.9 Hz, OCHb), 2.18 (s, 3H, OAc), 2.10 (s, 3H, OAc), 1.90 (s, 3H, OAc),

1.86 (s, 3H, OAc), 1.21 (d, 3H, H-6'), (d, 3H, H-6), 0.96 (m, 2H, CH_2Si), 0.03 (s, 9H, $SiMe_3$); High Res. ES-MS $C_{32}H_{46}O_{13}SSiNa^+$: 721.23261; found: 721.23263.

2-O-acetyl-4-O-benzoyl-3-S-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3-thio-α-D-rhamnopyranosyl trichloroacetimidate (**58**)

To a solution of **83** (80 mg, 117 μmol) in dichloromethane (1 mL) was added trifluoroacetic acid (2 mL) under argon. The mixture was stirred for 5 h. EtOAc (5 mL) and toluene (10 mL) were added and then removed at ca. 5 Torr. A second portion of toluene (8 mL) was added and removed. The resulting residue was dissolved in anhydrous dichloromethane (2.5 mL) under argon. Trichloroacetonitrile (240 μL, 2.4 mmole) and 1,8-diazabicyclo[5.4.0]undec-7-ene (13 μL, 87 μmole) were added. After 2 h, the organic solvent was evaporated and the resulting residue was purified by chromatography on silica using hexane - EtOAc (4:1 v/v) as eluent to give compound **58** (71 mg, 82%); $[\alpha]_D^{22}$ –21.9° (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.75 (s, 1H, NH), 8.02 - 7.42 (m, 5H, Bz), 6.20 (d, 1H, *J* 1.7 Hz, H-1), 5.40 (dd, 1H, *J* 9.7 Hz, 11.3 Hz, H-4), 5.22 (d, 1H, *J* 1.3 Hz, H-1'), 5.20 (dd, 1H, *J* 3.1 Hz, H-2), 5.05 (dd, 1H, *J* 3.1 Hz, H-2'), 5.02 (dd, 1H, *J* 9.8 Hz, H-3'), 5.00 ('t', 1H, *J* 9.9 Hz, H-4'), 4.12 (m, 2H, H-5 + H-5'), 3.64 (dd, 1H, H-3), 2.22 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.87 (s, 3H, OAc), 1.27 (d, 3H, *J* 6.3 Hz, H-6), 1.18 (d, 3H, *J* 6.2 Hz, H-6'); High Res. ES-MS $C_{29}H_{34}Cl_3NO_{13}SNa^+$: 764.07087; found: 764.07056. Anal. Calcd for $C_{29}H_{34}Cl_3NO_{13}S$: C 46.88, H 4.61, N 1.89; Found: C 47.12, H 4.46, N 1.44.

4-Pentenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-S-(2-O-acetyl-4-O-benzoyl-3-thio-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**84**)

Under argon, compound **59** (73 mg, 193 μmole) and trichloroacetimidate **58** (96 mg, 129 μmole) were dissolved in anhydrous dichloromethane (5 mL). The solution was cooled to –20 °C and liquid trimethylsilyl trifluoromethanesulfonate (3 μL, 15.3 μmole) was subsequently added. The reaction was warmed to room temperature and stirred for 3 h. It was quenched with triethylamine. The organic solvent was evaporated and the resulting residue was purified by chromatography on silica using toluene - EtOAc (5:1 v/v) as eluent to give compound **84** (49.5 mg, 40%). $[\alpha]_D^{22}$ –68.0° (*c* 4.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ7.91 - 7.15 (m, 10H, Bz + Ph), 5.78 (tdd, 1H, *J* 6.7 Hz, 10.0 Hz, 17.0 Hz, C*H*=CH_2), 5.73 (d, 1H, *J* 7.3 Hz, 2-NH), 5.55 (s, 1H, PhC*H*), 5.19 (d, 1H, *J* 1.4 Hz, H-1"), 5.12 (dd, 1H, *J* 10.0 Hz, 11.0 Hz, H-4'), 5.05 (dd, 1H, *J* 2.8 Hz, H-2"), 5.04 – 4.95 (m, 5H, H-3" + H-1 + H-4" + CH=CH_2), 4.88 (dd, 1H, *J* 1.6 Hz, 3.1 Hz, H-2'), 4.82 (d, 1H, H-1'), 4.50 ('t', 1H, *J* 9.7 Hz, H-3), 4.35 (dd, 1H, *J* 4.6 Hz, 10.6 Hz, H-6a), 4.15 (m, 2H, H-5' + H-5"), 3.85 (td, 1H, *J* 6.2 Hz, 9.7 Hz, OCHa), 3.76 ('t', 1H, *J* 10.0 Hz, H-6b), 3.60 (dd, 1H, H-3'), 3.56 (m, 2H, H-4 + H-5), 3.50 (td, 1H, *J* 6.8 Hz, 9.9 Hz, OCHb), 3.25 (m, 1H, H-2), 2.14 (s, 3H, OAc), 2.08 (m, 2H, CH_2CH=CH_2), 2.01 (s, 3H, NAc), 1.95 (s, 3H, OAc), 1.90 (s, 3H, OAc), 1.87 (s, 3H, OAc), 1.64 (m, 2H, OCH_2CH_2), 1.22 (d, 3H, *J* 6.4 Hz, H-6"), 0.57 (d, 3H, *J* 6.2 Hz, H-6'); Selected ^{13}C NMR data ($CDCl_3$, 600 MHz, from GHMQC): δ137.5 (*C*H=CH_2), 133.0 – 126.0 (Ph + Bz), 115.0 (CH=*C*H_2), 102.0 (Ph*C*H), 99.6 (C-1, $J_{C1,H1}$ 164.4 Hz), 96.3 (C-1', $J_{C1',H1'}$ 172.7 Hz), 83.6 (C-1", $J_{C1",H1"}$ 167.5 Hz), 80.0 (C-4), 76.6 (C-2'), 72.7 (C-4'), 72.2 (C-

4"), 70.5 (C-2"), 69.2 (OCH_2), 68.5 (C-6 + C-3"), 66.8 (C-3 + C-5' + C-5"), 66.1 (C-5), 47.4 (C-3'), 31.0 ($CH_2CH=CH_2$), 29.0 (OCH_2CH_2), 23.4 (NAc), 20.3 (4 X OAc), 16.7 (C-6"), 16.2 (C-6'); High Res. ES-MS $C_{47}H_{59}NO_{18}SNa^+$: 980.33506; found: 980.33488. Anal. Calcd for $C_{47}H_{59}NO_{18}S$: C 58.92, H 6.21 N 1.46; Found: C 58.75, H 6.05, N 1.30.

4-Pentenyl α-L-rhamnopyranosyl-(1→3)-S-(3-thio-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**54**)

Compound **27** (35 mg, 36.5 μmol) was suspended in acetic acid-H_2O solution (9:1 v/v, 10 mL). The mixture was stirred at 100 °C for 1 h. The organic solvent was removed under vaccum. MeOH (10 mL) and a catalytic amount of prepared NaOMe/MeOH (3 M) was added. The reaction was continued at 45 °C for 6 h. MeOH (30 mL) was added and the solution was neutralized by H^+ resin. The resin by removed by filtration and the organic solvent was evaporated. The resulting residue was purified by HPLC using a gradient of $H_2O \rightarrow$ MeOH – H_2O (4:6, v/v) as eluent to give compound **54** (18.5 mg, 84%); $[\alpha]_D^{22}$ –196.7° (*c* 1.5, H_2O); ^{1}H NMR (D_2O, 600 MHz): δ5.88 (tdd, 1H, *J* 6.7 Hz, 10.3 Hz, 17.0 Hz, C*H*=CH_2), 5.40 (s, 1H, H-1"), 5.06 (m, 1H, CH=C*Ha*), 5.02 (m, 1H, CH=C*Hb*), 4.80 (d, 1H, *J* 1.3 Hz, H-1'), 4.52 (d, 1H, *J* 8.7 Hz, H-1), 4.12 (dd, 1H, *J* 1.5 Hz, 3.5 Hz, H-2"), 4.08 (dq, 1H, *J* 6.2 Hz, 9.6 Hz, H-5'), 4.01 (dq, 1H, *J* 6.3 Hz, 9.6 Hz, H-5"), 3.93 (dd, 1H, *J* 2.3 Hz, 12.5 Hz, H-6a), 3.90 (dt, 1H, *J* 6.0 Hz, 10.2 Hz, OCHa), 3.81 ('t', 1H, *J* 9.6 Hz, H-2), 3.79 (dd, 1H, *J* 4.5 Hz, H-2'), 3.75 ('t', 1H, *J* 10.1 Hz, H-6b), 3.74 (dd, 1H, *J* 9.6, H-3"), 3.62 ('t', 1H, *J* 9.6 Hz, H-3), 3.61 (td, 1H, *J* 6.5 Hz, 9.9 Hz, OCHb), 3.53 ('t', 1H, *J* 9.8 Hz, H-4), 3.48 ('t', 1H, H-4"), 3.45 (m, 1H, H-5), 3.42 ('t', 1H, *J* 10.3 Hz, H-4'), 3.18 (dd, 1H, H-3'), 2.08 (m, 2H, C*H*$_2$CH=CH_2), 2.04 (s, 3H,

NAc), 1.65 (m, 2H, OCH_2CH_2), 1.31 (d, 3H, H-6"), 1.24 (d, 3H, H-6'); ^{13}C NMR ($CDCl_3$, 600 MHz, from GHMQC): δ139.7 ($CH=CH_2$), 115.8 ($CH=CH_2$), 101.7 (C-1, $J_{C1,H1}$ 160.1 Hz), 101.3 (C-1', $J_{C1',H1'}$ 169.0 Hz), 87.3 (C-1", $J_{C1",H1"}$ 167.2 Hz), 82.0 (C-3), 76.8 (C-5), 73.2 (C-4"), 72.8 (C-2'), 72.3 (C-2"), 72.2 (C-4'), 71.5 (C-3"), 70.4 (C-5'), 70.3 (OCH_2), 69.8 (C-5"), 69.3 (C-4), 61.6 (C-6), 56.2 (C-2), 51.0 (C-3'), 30.0 ($CH_2CH=CH_2$), 29.0 (OCH_2CH_2), 22.8 (NAc), 17.5 (C-6'), 17.2 (C-6"); High Res. ES-MS $C_{25}H_{43}O_{13}NSNa^+$: 620.23473; found: 620.23457.

*4-Pentenyl 2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl-(1→3)-(2-O-acetyl-4-O-benzoyl-α-*L*-rhamnopyranosyl)-(1→3)-S-2-acetamido-4,6-O-benzylidene-2-deoxy-3-thio-β-*D*-glucopyranoside* (**85**)

Under argon, prepared MeOH/NaOMe (3M, 140 μL) was added to a stirring solution of **73** (21 mg, 48 μmol) in anhydrous degassed MeOH (3 mL). After 1 min, anhydrous degassed MeOH (10 mL) was added and the mixture was neutralized with H^+ resin. Removal of the resin and solvent gave thiol **60** as a white powder. Compound **60** and trichloroacetimidate **57** (45 mg, 62 μmole) were then dissolved in anhydrous dichloromethane (3 mL) under argon. The solution was cooled to –20 °C and liquid trimethylsilyl trifluoromethanesulfonate (2 μL, 10.2 μmole) was subsequently added. The reaction was warmed to room temperature and continue for 2 h. It was quenched with triethylamine. The organic solvent was evaporated and the resulting residue was purified by chromatography on silica using toluene - EtOAc (7:3 v/v) as eluent to give compound **85** (25.5 mg, 55% from **13**); $[\alpha]_D^{22}$ –18.5° (*c* 3.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ7.93 - 7.14 (m, 10H, Bz + Ph), 5.78 (m, 2H, $CH=CH_2$ + 2-NH), 5.53 (s, 1H, PhCH),

5.24 ('t', 1H, *J* 9.9 Hz, H-4'), 5.23 (d, 1H, *J* 1.4, H-1'), 5.22 (dd, 1H, *J* 3.4 Hz, H-2'), 5.08 (dd, 1H, *J* 4.0 Hz, 10.0 Hz, H-3"), 5.0 (m, 1H, CH=C*Ha*), 4.99 (d, 1H, *J* 5.6 Hz, H-1), 4.96 (m, 1H, CH=C*Hb*), 4.91 ('t', 1H, *J* 9.9 Hz, H-4"), 4.85 (dd, 1H, *J* 1.9 Hz, H-2"), 4.77 (d, 1H, H-1"), 4.32 (dd, 1H, *J* 5.0 Hz, 10.5 Hz, H-6a), 4.23 (dq, 1H, *J* 6.1 Hz, H-5'), 4.13 (dd, 1H, H-3'), 3.84 (td, 1H, *J* 6.3 Hz, 9.7 Hz, OCHa), 3.80 (dq, 1H, *J* 6.3 Hz, H-5"), 3.71 (m, 2H, H-3 + H-6b), 3.58 (d't', 1H, *J* 9.3 Hz, H-5), 3.51 (td, 1H, *J* 6.7 Hz, 9.8 Hz, OCHb), 3.38 (dd, 1H, *J* 10.7 Hz, H-4), 3.27 (m, 1H, H-2), 2.17 (s, 3H, OAc), 2.09 (m, 2H, $CH_2CH=CH_2$), 2.02 (s, 3H, NAc), 1.99 (s, 3H, OAc), 1.85 (s, 6H, 2 X OAc), 1.65 (m, 2H, OCH_2CH_2), 1.13 (d, 3H, H-6'), 0.82 (d, 3H, H-6"); ^{13}C NMR ($CDCl_3$, 600 MHz, from GHMQC): δ138.0 ($CH=CH_2$), 133.6 – 126.1 (Ph + Bz), 115.0 ($CH=CH_2$), 101.8 (PhCH), 100.6 (C-1, $J_{C1,H1}$ 163.7 Hz), 98.8 (C-1", $J_{C1",H1"}$ 172.3 Hz), 81.4 (C-1', $J_{C1',H1'}$ 169.0 Hz), 79.4 (C-4), 74.7 (C-3'), 72.9 (C-4' + C-2'), 70.8 (C-4"), 69.5 (C-2"), 69.4 (C-5), 69.1 (OCH_2), 68.4 (C-6), 68.2 (C-3"), 67.5 (C-5'), 67.0 (C-5"), 58.9 (C-2), 45.3 (C-3), 29.4 ($CH_2CH=CH_2$), 28.5 (OCH_2CH_2), 23.2 (NAc), 20.5 (4 X OAc), 16.9 (C-6"), 16.2 (C-6'); High Res. ES-MS $C_{47}H_{59}NO_{18}SNa^+$: 980.33506; found: 980.33528. Anal. Calcd for $C_{47}H_{59}NO_{18}S$: C 58.92, H 6.21 N 1.46; Found: C 59.07, H 6.11, N 1.26.

4-Pentenyl α-L-rhamnopyranosyl-(1→3)-(α-L-rhamnopyranosyl)-(1→3)-S-2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside (**55**)

Compound **84** (17 mg, 17.7 μmol) was deprotected as described for **54** to give **55** 9 mg, 85%); $[\alpha]_D^{22}$ –127.9° (*c* 6.1, H_2O); ^1H NMR (D_2O, 600 MHz): δ5.90 (tdd, 1H, *J* 6.7 Hz, 10.3 Hz, 17.0 Hz, $CH=CH_2$), 5.20 (d, 1H, *J* 0.7, H-1'), 5.07 (m, 1H, CH=C*Ha*), 5.04 (m, 1H, CH=C*Hb*), 5.03 (d, 1H, *J* 0.9 Hz, H-1"), 4.56 (d, 1H, *J* 8.3 Hz, H-1), 4.14

(dq, 1H, *J* 6.1 Hz, 12.4 Hz, H-5'), 4.12 (dd, 1H, *J* 2.9 Hz, H-2'), 4.06 (dd, 1H, *J* 3.3 Hz, H-2"), 3.94 (dd, 1H, *J* 1.9 Hz, 12.4 Hz, H-6a), 3.91 (td, 1H, *J* 6.0 Hz, 10.5 Hz, OCHa), 3.82 (dd, 1H, *J* 9.8 Hz, H-3"), 3.77 (m, 3H, H-2 + H-5" +H-6b), 3.71 (dd, 1H, *J* 9.7 Hz, H-3'), 3.63 (td, 1H, *J* 6.4 Hz, 10.3 Hz, OCHb), 3.57 ('t', 1H, H-4'), 3.53 (ddd, 1H, *J* 5.7 Hz, 11.5 Hz, H-5), 3.45 ('t', 2H, *J* 9.7 Hz, H-4 + H-4"), 2.86 ('t', *J* 11.1 Hz, H-3), 2.10 (m, 2H, $CH_2CH{=}CH_2$), 2.06 (s, 3H, NAc), 1.66 (m, 2H, OCH_2CH_2), 1.30 (d, 6H, *J* 6.2 Hz, H-6' + H-6"); ^{13}C NMR (D_2O, 600 MHz, from GHMQC): δ 138.5 ($CH{=}CH_2$), 115.8 ($CH{=}CH_2$), 103.3 (C-1", $J_{C1",H1"}$ 171.6 Hz), 103.0 (C-1, $J_{C1,H1}$ 161.4 Hz), 86.5 (C-1', $J_{C1',H1'}$ 166.3 Hz), 79.3 (C-3' + C-5), 72.7 (C-4 + C-2'), 72.2 (C-4'), 71.2 (C-2), 70.9 (C-2"), 70.7 (OCH_2 + H-5"), 70.5 (C-5'), 68.4 (C-4"), 62.1 (C-6), 56.2 (C-3"), 54.5 (C-3), 30.0 ($CH_2CH{=}CH_2$), 28.8 (OCH_2CH_2), 22.9 (NAc), 17.2 (C-6" + C-6'); High Res. ES-MS $C_{25}H_{43}O_{13}NSNa^+$: 620.23473; found: 620.23378.

*4-Pentenyl 2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl-(1→3)-S-(2-O-acetyl-4-O-benzoyl-3-thio-α-*L*-rhamnopyranosyl)-(1→3)-S-2-acetamido-4,6-O-benzylidene-2-deoxy-3-thio-β-*D*-glucopyranoside* (**86**)

Compound **73** (30 mg, 69 μmol) was de-*S*-acetylated as described for **85** to give thiol **60**. Compound **60** and trichloroacetimidate **58** (75 mg, 103 μmole) were then dissolved in anhydrous dichloromethane (5 mL) under argon. The solution was cooled to –20 °C and liquid trimethylsilyl trifluoromethanesulfonate (6 μL, 31 μmole) was subsequently added. The reaction was warmed to room temperature and continue for 8 h. It was quenched with triethylamine. The organic solvent was evaporated and the resulting residue was purified by chromatography on silica using gradient $CHCl_3$ → MeOH -

$CHCl_3$ (1:99 v/v) as eluent to give compound **86** (8 mg, 12% from **73**); $[\alpha]_D^{22}$ –54.4° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ8.02 - 7.15 (m, 10H, Bz + Ph), 5.77 (m, 2H, C*H*=CH_2 + 2-NH), 5.53 (s, 1H, PhC*H*), 5.23 (s, 1H, H-1'), 5.19 ('t', 1H, *J* 9.6 Hz, H-4'), 5.14 (d, 1H, *J* 1.2, H-1"), 5.03 – 4.98 (m, 6H, H-2" + 2' + CH=C*Ha* + H-1 + H-3" + H-4"), 4.96 (m, 1H, CH=C*Hb*), 4.32 (dd, 1H, *J* 4.9 Hz, 10.6 Hz, H-6a), 4.25 (dq, 1H, *J* 6.0 Hz, 9.4 Hz, H-5'), 4.04 (m, 1H, H-5"), 3.84 (td, 1H, *J* 6.4 Hz, 9.8 Hz, OCHa), 3.73 ('t', 1H, *J* 10.3 Hz, H-6b), 3.71 ('t', 1H, *J* 11.3 Hz, H-3), 3.58 (d't', 1H, *J* 9.8 Hz, H-5), 3.51 (td, 1H, *J* 6.8 Hz, 9.8 Hz, OCHb), 3.47 (dd, 1H, *J* 3.0 Hz, H-3'), 3.37 (dd, 1H, H-4), 3.25 (m, 1H, H-2), 2.17 (s, 3H, OAc), 2.08 (m, 2H, C*H*$_2$CH=CH_2), 2.02 (s, 3H, NAc), 2.01 (s, 3H, OAc), 1.89 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.66 (m, 2H, OCH_2C*H*$_2$), 1.20 (d, 3H, *J* 6.3 Hz, H-6"), 0.78 (d, 3H, H-6'); Selected ^{13}C NMR data ($CDCl_3$, 600 MHz, from GHMQC): δ133.1 – 126.2 (Ph + Bz), 112.4 (CH=*C*H_2), 101.0 (Ph*C*H), 100.5 (C-1, $J_{C1,H1}$ 163.8 Hz), 83.3 (C-1", $J_{C1",H1"}$ 168.3 Hz), 81.5 (C-1', $J_{C1',H1'}$ 169.8 Hz), 78.4 (C-4), 75.3 (C-2'), 73.3 (C-4'), 73.2 (C-2"), 70.3 (C-3"), 69.2 (OCH_2), 69.4 (C-5), 69.5 (C-6), 68.7 (C-4"), 68.4 (C-6 + C-5"), 68.1 (C-5'), 46.3 (C-3), 45.9 (C-3'), 29.7 (*C*H_2CH=CH_2), 28.5 (OCH_2*C*H_2), 23.2 (NAc), 20.4 (4 X OAc), 16.7 (C-6" +C-6'); High Res. ES-MS $C_{47}H_{59}O_{17}NS_2Na^+$: 996.31221; found: 996.31297.

4-Pentenyl α-L-*rhamnopyranosyl-(1→3)-S-(3-thio-*α-L-*rhamnopyranosyl)-(1→3)-S-2-acetamido-2-deoxy-3-thio-*β-D-*glucopyranoside* (**56**)

Compound **86** (5 mg, 5 μmol) was deprotected as described for **54** to give **56** (2.5 mg, 79%); $[\alpha]_D^{22}$ –193.0° (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 600 MHz): δ5.89 (ddt, 1H, *J* 6.7

Hz, 10.3 Hz, 17.0 Hz, C*H*=CH_2), 5.41 (s, 1H, H-1"), 5.19 (s, 1H, H-1'), 5.07 (m, 1H, CH=C*Ha*), 5.02 (m, 1H, CH=C*Hb*), 4.51 (d, 1H, *J* 8.3 Hz, H-1), 4.25 (dq, 1H, *J* 6.0 Hz, 9.3 Hz, H-5'), 4.10 (d, *J* 1.3 Hz, H-2"), 4.04 (m, 1H, H-2'), 3.99 (dq, 1H, *J* 6.0 Hz, 9.3 Hz, H-5"), 3.93 (dd, 1H, *J* 2.2 Hz, 12.4 Hz, H-6a), 3.90 (td, 1H, *J* 6.0 Hz, 10.4 Hz, OCHa), 3.78 – 3.74 (m, 3H, H-2 + H-6b + H-3"), 3.62 (td, 1H, *J* 6.4 Hz, 10.2 Hz, OCHb), 3.52 (m, 1H, H-5), 3.50 ('t', 1H, *J* 9.3 Hz, H-4"), 3.48 ('t', 1H, *J* 9.7 Hz, H-4'), 3.46 ('t', 1H, *J* 9.9 Hz, H-4), 3.05 (dd, 1H, *J* 2.8 Hz, H-3'), 28.5 (t, 1H, *J* 11.0 Hz, H-3), 2.09 (m, 2H, CH_2CH=CH_2), 2.05 (s, 3H, NAc), 1.65 (m, 2H, OCH_2CH_2), 1.30 (d, 3H, H-6"), 0.78 (d, 3H, H-6'); ^{13}C NMR (D_2O, 600 MHz, from GHMQC): δ138.7 (*C*H=CH_2), 115.8 (CH=*C*H_2), 103.2 (C-1, $J_{C1,H1}$ 161.4 Hz), 87.0 (C-1" + C-1', $J_{C1",H1"}$ 168.8 Hz, $J_{C1',H1'}$ 166.5 Hz), 79.1 (C-4"), 74.4 (C-2'), 73.0 (C-4' + C-5), 72.5 (C-2"), 70.8 (C-3"), 70.6 (C-5'), 70.3 (OCH_2), 69.9 (C-5"), 68.3 (C-4), 62.0 (C-6), 56.0 (C-2), 54.4 (C-3), 50.8 (C-3'), 30.0 (*C*H_2CH=CH_2), 28.9 (OCH_2*C*H_2), 23.3 (NAc), 17.9 (C-6" + C-6'); High Res. ES-MS $C_{25}H_{43}O_{12}NS_2Na^+$: 636.21189; found: 636.21143.

*5-(2-Aminoethylthio)pentyl α-*L*-rhamnopyranosyl-(1→3)-S-(3-thio-α-*L*-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside, acetic acid salt* (**87**)

Compound **54** (6 mg, 8.6 μmol) and 2-aminoethanethiol hydrochloride (9.8 mg, 86 μmol) were dissolved in anhydrous degassed MeOH (1 mL). The solution was stirred under argon for 3 days with irradiation by UV light of 254 nm wavelength. The solvent was evaporated and the resulting residue was purified by reverse-phase HPLC using gradient MeOH - H_2O (containing 0.3% acetic acid) as eluent to give compound **87**

(5.5mg, 87%); $[\alpha]_D^{22}$ –9.6° (*c* 1.9, H_2O); ^1H NMR (D_2O, 600 MHz): δ5.39 (s, 1H, H-1"), 4.79 (d, 1H, *J* 1.4 Hz, H-1'), 4.51 (d, 1H, *J* 8.7 Hz, H-1), 4.11 (dd, 1H, *J* 1.1 Hz, 3.4 Hz, H-2"), 4.07 (dq, 1H, *J* 6.3 Hz, 9.9 Hz, H-5'), 4.00 (dq, 1H, *J* 6.3 Hz, 12.6 Hz, H-5"), 3.92 (dd, 1H, *J* 2.1 Hz, 12.3 Hz, H-6a), 3.90 (m, 1H, H-5), 3.79 (m, 2H, H-2 + H-2'), 3.76 – 3.68 (m, 4H, H-3" + H-6b + OCH_2CH_2), 3.61 ('t', 1H, *J* 9.1 Hz, H-3), 3.59 ('t', 1H, *J* 10.0 Hz, H-4), 3.47 ('t', 1H, *J* 9.3 Hz, H-4"), 3.64 ('t', 1H, *J* 9.5 Hz, H-4'), 3.21 (t, 2H, *J* 6.7 Hz, SCH_2), 3.15 (dd, 1H, *J* 2.8 Hz, H-3'), 2.84 (t, 2H, $CH_2NH_3^+OAc^-$), 2.59 (t, 2H, *J* 7.2 Hz, CH_2S), 2.04 (s, 3H, NAc), 2.03 (OAc^-) 1.63 – 1.54 (m, 4H, OCH_2CH_2 + CH_2CH_2S), 1.42 (m, 2H, $OCH_2CH_2CH_2$), 1.30 (d, 3H, H-6"), 1.23 (d, 3H, H-6'); ^{13}C NMR (D_2O, 600 MHz, from GHMQC): δ108.3 (C-1, $J_{C1,H1}$ 159.6 Hz), 101.2 (C-1', $J_{C1',H1'}$ 169.8 Hz), 87.2 (C-1", $J_{C1",H1"}$ 167.9 Hz), 82.2 (C-3), 73.3 (C-4"), 72.8 (C-2'), 72.6 (C-2"), 72.4 (C-4'), 71.7 (C-3"), 71.1 (C-5' + C-5 + C-4), 70.4 (OCH_2), 70.0 (C-5"), 61.6 (C-2), 51.0 (C-3'), 39.2 (SCH_2), 31.4 (CH_2S), 28.8 ($CH_2NH_3^+OAc^-$ + OCH_2CH_2 + CH_2CH_2S), 22.8 (NAc), 22.0 (OAc^-), 17.6 (C-6'), 17.4 (C-6"); High Res. ES-MS $C_{27}H_{51}O_{13}N_2S_2Na^+$: 675.28326; found: 675.28325.

*5-(2-Aminoethylthio)pentyl α-*L*-rhamnopyranosyl-(1→3)-(α-*L*-rhamnopyranosyl)-(1→3)-S-2-acetamido-2-deoxy-3-thio-β-*D*-glucopyranoside, acetic acid salt* (**88**)

Compound **55** (6 mg, 8.6 μmol) and 2-aminoethanethiol hydrochloride (9.8 mg, 86 μmol) were dissolved in anhydrous degassed MeOH (1 mL). The solution was stirred under argon for 3 days with irradiation by UV light of 254 nm wavelength. The solvent was evaporated and the resulting residue was purified by reverse-phase HPLC using gradient MeOH - H_2O (containing 0.3% acetic acid) as eluent to give compound **88**

(5.3mg, 84%); $[\alpha]_D^{22}$ +52.0° (*c* 3.6, H_2O); ^1H NMR (D_2O, 600 MHz): δ5.18 (d, 1H, *J* 1.6 Hz, H-1'), 5.02 (d, 1H, *J* 1.6 Hz, H-1"), 4.51 (d, 1H, *J* 8.3 Hz, H-1), 4.13 (dq, 1H, *J* 6.1 Hz, 9.3 Hz, H-5'), 4.11 (dd, 1H, *J* 3.2 Hz, H-2'), 4.05 (dd, 1H, *J* 3.5Hz, H-2"), 3.93 (dd, 1H, *J* 2.2 Hz, 12.3 Hz, H-6a), 3.91 (dt, 1H, *J* 6.0 Hz, 10.2 Hz, OCHa), 3.81 (dd, 1H, *J* 9.8 Hz, H-3"), 3.76 (m, 3H, H-2 + H-5" + H-6b), 3.70 (dd, 1H, *J* 9.6 Hz, H-3'), 3.60 (dt, 1H, *J* 6.0 Hz, 9.5 Hz, OCHb), 3.55 ('t', 1H, H-4'), 3.52 (ddd, 1H, *J* 5.8 Hz, 9.4 Hz, H-5), 3.46 ('t', 1H, *J* 9.6 Hz, H-4"), 3.45 ('t', 1H, *J* 9.8 Hz, H-4), 3.21 (t, 2H, *J* 6.7 Hz, SCH_2), 2.85 ('t', 2H, $CH_2NH_3^+OAc^-$), 2.84 ('t', 1H, *J* 9.8 Hz, H-3), 2.60 (t, 2H, *J* 7.4 Hz, CH_2S), 2.06 (s, 3H, NAc), 2.00 (OAc^-), 1.63 – 1.55 (m, 4H, OCH_2CH_2 + CH_2CH_2S), 1.41 (m, 2H, $OCH_2CH_2CH_2$), 1.28 (d, 6H, *J* 6.2 Hz, H-6' + H-6"); ^{13}C NMR (D_2O, 600 MHz, from GHMQC): δ103.0 (C-1", $J_{C1",H1"}$ 171.7 Hz), 102.8 (C-1, $J_{C1,H1}$ 160.9 Hz), 86.5 (C-1', $J_{C1',H1'}$ 166.2 Hz), 79.0 (C-3' + C-5), 72.7 (C-2' + C-4), 72.5 (C-4'), 71.2 (OCH_2), 70.8 (C-2" + C-3"), 70.2 (C-5'), 70.0 (C-5"), 68.2 (C-4"), 62.0 (C-6), 56.0 (C-2), 54.4 (C-3), 39.1 (SCH_2), 31.4 (CH_2S), 28.9 ($CH_2NH_3^+OAc^-$ + OCH_2CH_2 + CH_2CH_2S), 22.9 (NAc), 22.8 (OAc^-), 17.5 (C-6' + C-6"); High Res. ES-MS $C_{27}H_{51}O_{13}N_2S_2Na^+$: 675.28326; found: 675.28302.

8-palmitamido-6-thia-octyl α-L-*rhamnopyranosyl-(1→3)-S-(3-thio-*α-L-*rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-*β-D-*glucopyranoside* (**93**)

To a stirring solution of compound **87** (1.7 mg, 2.3 μmol) and $NaHCO_3$ (1N, 36.5 μL) in MeOH (0.3 mL) was added palmitoyl chloride (7 μL, 23 μmol). The solution was stirred for 2 h. The solvent was evaporated and the resulting residue was purified by reverse-phase column using gradient H_2O → CH_3CN - H_2O (8:2, v/v) as eluent to give

compound **93** (1.0 mg, 47%); Selected NMR data: ^{1}H NMR (CD_3OD, 500 MHz): δ5.45 (d, 1H, *J* 1.1 Hz, H-1"), 4.76 (d, 1H, *J* 1.6 Hz, H-1'), 4.41 (d, 1H, *J* 8.6 Hz, H-1), 4.02 (m, 1H, H-5'), 3.99 (dd, 1H, *J* 3.7 Hz, H-2"), 3.95 (m, 1H, H-5"), 3.87 (dd, 1H, *J* 2.1 Hz, 12.0 Hz, H-6a), 3.70 (m, 2H, H-2 + H-6b), 3.60 (dd, 1H, *J* 9.6 Hz, H-3"), 3.45 ('t', 1H, *J* 9.5 Hz, H-4'), 3.40 ('t', 1H, *J* 9.6 Hz, H-4"), 3.34 (overlapped, 2H, SCH_2), 3.14 (dd, 1H, *J* 2.9 Hz, 10.7 Hz, H-3'), 2.60 (t, 2H, *J* 7.0 Hz, CH_2NH), 2.54 (t, 2H, *J* 7.3 Hz, CH_2S), 2.17 (t, 2H, *J* 7.6 Hz, $NC(=O)CH_2$), 1.97 (s, 3H, NAc), 1.61 – 1.26 (m, 33H, $OCH2(CH_2)_3CH2S$ + $NC(=O)CH_2(CH_2)_{12}$ + H-6"), 1.23 (d, 3H, *J* 6.2 Hz, H-6'); High Res. ES-MS $C_{43}H_{80}O_{14}N_2S_2Na^+$: 935.49487; found: 935.49425.

8-palmitamido-6-thia-octyl α-L-*rhamnopyranosyl-(1→3)-(*α-L-*rhamnopyranosyl)-(1→3)-S-2-acetamido-2-deoxy-3-thio-*β-D-*glucopyranoside* (**94**)

As described for **93**, compound **88** (1.0 mg, 1.4 μmol) was used to prepare **94** (0.9 mg, 73%); Selected NMR data: ^{1}H NMR (CD_3OD, 600 MHz): δ5.21 (d, 1H, *J* 1.6 Hz, H-1'), 4.99 (d, 1H, *J* 1.7 Hz, H-1"), 4.42 (d, 1H, *J* 8.2 Hz, H-1), 4.10 (dq, *J* 6.3 Hz, 9.4 Hz, 1H, H-5'), 4.03 (dd, 1H, *J* 3.2 Hz, H-2'), 3.94 (dd, 1H, *J* 3.4 Hz, H-2"), 3.87 (m, 2H, $OCHaCH_2$ + H-6a), 3.74 – 3.64 (m, 6H, H-3" + H-5" + H-6b + H-5 + H-3' + H-2), 3.54 ('t', 1H, *J* 9.6 Hz, H-4'), 3.47 (m, 1H, ('t', 1H, *J* 9.6 Hz, H-4"), 3.34 (overlapped, 2H, SCH_2), 3.14 (dd, 1H, *J* 2.9 Hz, $OCHbCH_2$), 3.37 ('t', 2H, *J* 9.5 Hz, H-4 + H-4"), 2.93 ('t', 1H, *J* 9.6 Hz, H-3), 2.60 (t, 2H, *J* 7.0 Hz, CH_2NH), 2.54 (t, 2H, *J* 7.3 Hz, CH_2S), 2.17 (t, 2H, *J* 7.6 Hz, $NC(=O)CH_2$), 1.70 (s, 3H, NAc), 1.61 – 1.26 (m, 33H, $OCH2(CH_2)_3CH2S$ + $NC(=O)CH_2(CH_2)_{12}$ + H-6'), 1.24 (d, 3H, *J* 6.2 Hz, H-6"); High Res. ES-MS $C_{43}H_{80}O_{14}N_2S_2Na^+$: 935.49487; found: 935.49457.

X-ray crystal data for *4-Pentenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-allopyranoside 2,3-sulfamidate* (**12**): $C_{20}H_{25}NO_8S$, M = 439.47, orthorhombic space group $P2_12_12_1$ (No. 19) a = 9.5466 (14) Å, b = 9.6865 (14) Å, c = 22.711 (3) Å, V = 2100.1 (5) Å^3, Z = 4, D_c = 1.390 g cm^{-3}, μ(Mo Kα [0.71073 Å]) = 0.201 mm^{-1}. Final $R_1(F)$ = 0.0391 (for 3684 reflections with $F_o^2 \geq 2\sigma(F_o^2)$) and $wR_2(F^2)$ = 0.0917 (for all 4261 unique data) and 272 parameters varied.

Glycoconjugate **91**

Amine **87** (5.0 mg) was dissolved in absolute ethanol (1 mL) and a stock solution containing 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol (126 µL, 0.8% v/v, 0.95 eq) was added. The mixture was stirred overnight at ambient temperature. Removal of the solvent gave the crude product **89**. Compound **89** was then added with stirring to solution containing BSA (28.6 mg, 1/15 eq) in borate buffer (5 mL, Na_2BO_4 0.07M, $KHCO_3$ 0.035M, pH 9.5). The reaction mixture was stirred overnight. It was then diluted with deionized water and dialysed against deionized water (2 L x 3) at 4 °C. Concentration of the mixture and lyophilization gave glycoconjugate **91** as a white solid. MALDI mass spectrometry showed a mass of 71005, indicating incorporation of 6 ligands per BSA molecule.

Glycoconjugate **92**

Amine **88** (2.6 mg) was dissolved in absolute ethanol (0.8 mL) and a stock solution containing 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol (0.5 µL, 0.5% v/v,

0.95 eq) was added. The mixture was stirred overnight at ambient temperature. Removal of the solvent gave the crude product **90**. Compound **90** was then added with stirring to a solution containing BSA (15.7 mg, 1/15 eq) in borate buffer (4 mL, Na_2BO_4 0.07M, $KHCO_3$ 0.035M, pH 9.5). The reaction mixture was stirred overnight and processed as discribed above. MALDI mass spectrometry showed a mass of 71005, indicating incorporation of 7 ligands per one BSA molecule.

Immunization of mice with glycoconjugate **91** and **92**.

Groups of 5 Balb/C mice, 10 week old were immunized with glycoconjugates **91** and **92**. A stock solution of the glycoconjugate antigen was prepared in phosphate buffered saline (PBS) at a concentration of 1 mg/mL. This stock solution was then diluted to 50μg/mL in PBS and mixed 2:1:1 with Freunds complete and incomplete adjuvant, or 1:1 with incomplete Freunds adjuvant. Injections of emulsified antigen/adjuvant mixture 200 μL containing 5 ug of antigen were given on days 0, 30, 60 and 95. The first injection used antigen suspended in complete Freund's adjuvant and subsequent injections used only incomplete adjuvants. Trial bleeds were made on day 70 and mice were exsanguinated on day 105 and sera were collected

Inhibitory activity of thio-oligosaccharides **54** and **56**

A stock solution of mouse monoclonal SYA/J6 antibody (~ 6 mg/mL) was diluted with 0.01 M sodium phosphate, 0.15 M NaCl (PBS) buffer solution to obtain a 0.005 μg/mL solution. A 96-well Nunc-Immuno ELISA plate (MaxiSorp F96) was coated with 100 μL of the antibody solution and allowed to sit at 4 °C overnight. Excess solution was

discarded, and the plate was washed 4 times with PBST, a PBS buffer that also contained 0.05 % polyoxyethylene-sorbitan monolaurate detergent (Tween-20). A 2.5 % milk (Difco) solution in PBS buffer (100 μL) was added and the plate was left to stand at room temperature for 1 h to block empty hydrophobic sites. The milk solution was discarded from the plate and serially-diluted solutions of synthetic ligands **54** and **55** (50 μL) containing 0.04 μg/mL solution of the biotinylated lipopolysaccharide antigen[151] (50 μL) in PBST were added in triplicate to the wells. The plate was allowed to stand at room temperature overnight. The plate was washed 4 times with PBST. A solution of streptavidin/horseradish peroxidase complex in PBST (100 μL, 25 ng/mL) was added to each well and allowed to equilibrate for 1 h. The plate was washed 4 times with PBST and a 3, 3', 5, 5' tetramethylbenzidine (TMB) solution (100 μL) was added to each well and the colorimetric reaction was allowed to proceed for 1 min. A 1 M phosphoric acid solution (100 μL) was added to each to quench the reaction and the plate was placed into a Dynatech MR5000 ELISA plate reader and read at 450 nm. Inhibition data were calculated from the absorbance readings.

The glycolipid conjugates **93-95** were used to coat 96 well ELISA plates (100 μL, 18 h at 4 °C). Stock solutions of **93** and **95** containing glycolipid 1 mg/mL in carbonate buffer (0.05 M, pH 9.8) were diluted in PBS to a final concentration of 10 μg/mL. Stock solutions of **94** containing glycolipid 1 mg/mL in methnol:water (1:1) was diluted in PBS to a final concentration of 10 μg/mL. Plates were washed five times with PBST (PBS containing Tween 20, 0.05% v/v) and blocked for 1 h at room temperature (2.5% milk (DiFco) PBS). Serially diluted mouse sera from mice immunized with glycoconjugates **91** and **92** were added to the coated microtitre plate in triplicate and incubated at room

temperature for 2 h. The plate was washed with PBST (4 x), and goat anti-mouse (IgG or IgM) antibody conjugated to horseradish peroxidase (diluted 1:2000, Kirkegaard and Perry Lab) in PBST (100 μL) was added and incubated for 1 h at room temperature. The plate was washed with PBST (5x), 3,3',5,5'-tetramethylbenzidine (TMB, 100 μL, Kirkegaard and Perry Lab) was added, and after 30 minutes the colour reaction was stopped by the addition of 1M phosphoric acid (100 μL). Absorbance was read at 450 nm. Antibody titres were measured at the sera dilution giving an OD 0.2 above background.

Chapter 8

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Appendix A. Crystallographic experimental details for **27** and **42a**

A. Crystal Data		
compound	**27**	**42a**
formula	$C_{14}H_{20}O_8S$	$C_{31}H_{38}O_{16}S$
formula weight	348.36	698.67
crystal dimensions (mm)	0.54 × 0.41 × 0.37	0.38 × 0.17 × 0.14
crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$ (No. 19)	$P2_12_12_1$ (No. 19)
unit cell parameters		
a (Å)	8.6991 (6)[a]	11.9295 (11)[b]
b (Å)	11.2959 (8)	13.3445 (12)
c (Å)	16.8456 (11)	23.775 (2)
V (Å^3)	1655.3 (2)	3784.8 (6)
Z	4	4
ρ_{calcd} (g cm^{-3})	1.398	1.226
μ (mm^{-1})	0.233	0.151
B. Data Collection and Refinement Conditions		
diffractometer	Bruker PLATFORM/SMART 1000 CCD[c]	
radiation (λ [Å])	graphite-monochromated Mo Kα (0.71073)	
temperature (°C)	–80	–80
scan type	ω scans (0.2°) (20 s exposures)	ω scans (0.2°) (25 s exposures)
data collection 2θ limit (deg)	52.74	50.00
total data collected	8648 (-10 ≤ h ≤ 10, -13 ≤ k ≤ 14, -21 ≤ l ≤ 19)	19675 (-14 ≤ h ≤ 14, -15 ≤ k ≤ 15, -26 ≤ l ≤ 28)
independent reflns	3372 (R_{int} = 0.0199)	6667 (R_{int} = 0.0741)
observed reflns (*NO*) [$F_o^2 \geq 2\sigma(F_o^2)$]	3261	4305
structure solution method	direct methods (*SHELXS–86*[d])	
refinement method	full-matrix least-squares on F^2 (*SHELXL–93*[e])	
absorption correction method	empirical (*SADABS*)	empirical (*SADABS*)
range of transmission factors	0.9186–0.8844	0.9791–0.9447
data/restraints/parameters	3372 / 0 / 212	6667 / 0 / 430
Flack absolute structure parameter[f]	0.02 (6)	0.1 (2)
goodness-of-fit (S) [$F_o^2 \geq -3\sigma(F_o^2)$][g]	1.045	1.055
final R indices[h]		
R_1 [$F_o^2 \geq 2\sigma(F_o^2)$]	0.0289	0.0853
wR_2 [$F_o^2 \geq -3\sigma(F_o^2)$]	0.0785	0.2470
largest difference peak and hole (e Å^{-3})	0.273 and –0.189	0.911 and –0.289

[a]Obtained from least-squares refinement of 6805 reflections with $4.58° < 2\theta < 49.59°$.

[b]Obtained from least-squares refinement of 4307 reflections with $5.27° < 2\theta < 52.67°$.

[c]Programs for diffractometer operation, data collection, data reduction and absorption correction were those supplied by Bruker.

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[g]$S = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$ (n = number of data; p = number of parameters varied; $w = [\sigma^2(F_o^2) + (a_0P)^2 + a_1P]^{-1}$ where $P = [\mathrm{Max}(F_o^2, 0) + 2F_c^2]/3$; for **2**, $a_0 = 0.0524$, $a_1 = 0.2577$; for **4a**, $a_0 = 0.1395$, $a_1 = 1.7221$).

[h]$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$.

Appendix B. Atomic coordinates and equivalent isotropic displacement parameters for compound **27**

Atom	*x*	*y*	*z*	U_{eq}, Å^2
S	-0.01230(4)	-0.09690(3)	0.08756(2)	0.02599(10)*
O1	0.00543(12)	-0.25059(9)	-0.02944(5)	0.0246(2)*
O2	0.05629(12)	-0.35302(10)	0.12028(6)	0.0249(2)*
O3	-0.14229(12)	-0.54004(9)	0.09767(6)	0.0254(2)*
O4	-0.14228(12)	-0.54949(10)	-0.07056(6)	0.0276(2)*
O10	-0.18042(15)	-0.00063(11)	-0.02624(7)	0.0362(3)*
O20	-0.03057(15)	-0.39562(12)	0.24317(6)	0.0383(3)*
O30	-0.36158(16)	-0.49382(14)	0.16195(9)	0.0504(4)*
O40	0.08271(14)	-0.63900(12)	-0.09858(9)	0.0454(3)*
C1	-0.09056(17)	-0.22396(13)	0.03674(8)	0.0221(3)*
C2	-0.09873(16)	-0.32806(13)	0.09348(9)	0.0229(3)*
C3	-0.15852(17)	-0.43611(13)	0.04871(8)	0.0224(3)*
C4	-0.06474(17)	-0.45746(13)	-0.02617(8)	0.0230(3)*
C5	-0.05995(17)	-0.34443(13)	-0.07617(9)	0.0244(3)*
C6	0.04046(19)	-0.35546(16)	-0.14979(9)	0.0315(3)*
C10	-0.09264(18)	0.01861(14)	0.02691(9)	0.0265(3)*
C11	-0.0354(2)	0.13868(15)	0.05087(11)	0.0369(4)*
C20	0.07247(19)	-0.39489(14)	0.19548(9)	0.0289(3)*
C21	0.2319(2)	-0.43970(18)	0.20889(11)	0.0417(4)*
C30	-0.25013(19)	-0.55512(15)	0.15541(9)	0.0307(3)*
C31	-0.2070(2)	-0.65557(16)	0.20818(11)	0.0373(4)*
C40	-0.0551(2)	-0.63581(15)	-0.10330(9)	0.0305(3)*
C41	-0.1526(2)	-0.72412(17)	-0.14556(11)	0.0418(4)*

Anisotropically-refined atoms are marked with an asterisk (*). The form of the anisotropic displacement parameter is: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$.

Appendix C. Atomic coordinates and equivalent isotropic displacement parameters for compound **42a**

Atom	*x*	*y*	*z*	U_{eq}, Å^2
S	-0.14993(17)	0.04351(13)	0.00614(7)	0.0502(5)*
O1	0.1980(4)	0.1280(3)	-0.07465(18)	0.0521(13)*
O2	0.1470(4)	-0.0416(3)	-0.14058(16)	0.0391(10)*
O3	-0.0623(4)	-0.0812(4)	-0.09457(16)	0.0447(11)*
O5	0.1908(4)	0.0318(3)	0.00503(18)	0.0488(12)*
O6	0.0783(4)	-0.0460(3)	0.10076(16)	0.0499(12)*
O11	0.3464(6)	0.1078(5)	-0.1317(3)	0.0849(19)*
O12	0.2583(4)	-0.1749(4)	-0.1300(2)	0.0556(13)*
O13	-0.1324(7)	0.0291(8)	-0.1544(4)	0.143(4)*
O14	0.0866(5)	0.0031(4)	0.19042(19)	0.0627(14)*
O2'	-0.3808(4)	0.0375(3)	0.04214(19)	0.0525(13)*
O3'	-0.5037(4)	-0.1293(3)	0.07925(16)	0.0428(11)*
O4'	-0.4126(4)	-0.1387(4)	0.18740(17)	0.0497(12)*
O5'	-0.1914(4)	-0.0064(3)	0.11165(16)	0.0415(11)*
O11'	-0.5015(6)	-0.0080(4)	-0.0262(2)	0.0847(19)*
O12'	-0.4724(5)	-0.2466(3)	0.01326(18)	0.0514(13)*
O13'	-0.4958(8)	-0.0041(8)	0.2236(3)	0.134(3)*
C1	0.2119(6)	0.0280(5)	-0.0526(3)	0.0445(16)*
C2	0.1292(5)	-0.0409(5)	-0.0805(2)	0.0350(14)*
C3	0.0105(5)	-0.0093(5)	-0.0699(2)	0.0376(15)*
C4	-0.0094(6)	-0.0053(5)	-0.0073(2)	0.0397(15)*
C5	0.0792(7)	0.0614(5)	0.0216(2)	0.0466(18)*
C6	0.0759(8)	0.0572(5)	0.0834(3)	0.059(2)*
C11	0.2727(9)	0.1610(6)	-0.1138(3)	0.063(2)*
C12	0.2476(10)	0.2639(6)	-0.1307(4)	0.081(3)*
C13	0.2137(5)	-0.1155(5)	-0.1604(3)	0.0389(15)*
C14	0.2238(7)	-0.1095(7)	-0.2222(3)	0.063(2)*
C15	-0.1260(7)	-0.0525(9)	-0.1384(3)	0.077(3)*
C16	-0.1773(9)	-0.1478(11)	-0.1655(4)	0.117(5)*
C20	0.0780(6)	-0.0634(6)	0.1567(2)	0.0475(18)*
C21	0.0607(6)	-0.1709(5)	0.1701(3)	0.0433(16)*
C22	0.0429(6)	-0.2413(5)	0.1279(3)	0.0415(16)*
C23	0.0194(6)	-0.3398(5)	0.1432(3)	0.0534(18)*
C24	0.0163(7)	-0.3662(6)	0.1988(3)	0.059(2)*
C25	0.0346(7)	-0.2979(7)	0.2384(3)	0.064(2)*
C26	0.0579(6)	-0.1989(6)	0.2257(3)	0.0534(19)*
C1'	-0.2071(6)	-0.0456(5)	0.0569(2)	0.0414(16)*
C2'	-0.3295(6)	-0.0600(5)	0.0431(3)	0.0425(17)*

Appendix C. Atomic coordinates and displacement parameters for **42a** (continued)

Atom	*x*	*y*	*z*	U_{eq}, Å^2
C3'	-0.3844(6)	-0.1236(5)	0.0874(2)	0.0379(15)*
C4'	-0.3634(6)	-0.0761(5)	0.1455(2)	0.0389(15)*
C5'	-0.2387(6)	-0.0696(5)	0.1545(2)	0.0378(15)*
C6'	-0.2084(6)	-0.0224(5)	0.2107(2)	0.0502(19)*
C11A[a,b]	-0.4764(9)	0.0528(7)	0.0088(4)	0.040(2)
C12A[a]	-0.5207(12)	0.1571(9)	0.0183(6)	0.076(4)
C11B[b,c]	-0.422(2)	0.0536(16)	-0.0091(9)	0.040(2)
C12B[c]	-0.448(3)	0.164(2)	-0.0172(14)	0.081(9)
C13'	-0.5397(7)	-0.1928(5)	0.0390(3)	0.0475(19)*
C14'	-0.6614(7)	-0.1882(6)	0.0305(3)	0.063(2)*
C15'	-0.4810(9)	-0.0952(10)	0.2237(4)	0.081(3)*
C16'	-0.5291(10)	-0.1648(10)	0.2652(4)	0.113(4)*

Anisotropically-refined atoms are marked with an asterisk (*). The form of the anisotropic displacement parameter is: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$. [a]Refined with an occupancy factor of 0.7. [b]The C11A and C11B atoms were refined with a common isotropic displacement parameter. [c]Refined with an occupancy factor of 0.3.