STUDIES RELATED TO HETEROANALOGUES OF OLIGOSACCHARIDES

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ABSTRACT

The consequences of substituting the ring oxygen of carbohydrates with sulfur are presented. The synthesis, configurational and/or conformational analysis, and enzyme inhibitory activity of glucopyranosylamines, and the synthesis of galactofuranose-containing oligosaccharides and their heteroatom analogues are described.

The synthesis of a series of *N*-(4-Y-substituted-phenyl) peracetylated-5-thio-D-glucopyranosylarylamines (Y = OMe, H, CF_3, NO_2) by reaction of 5-thio-D-glucopyranose pentaacetate with the corresponding arylamine using mercuric chloride as a catalyst is reported. The tetraacetylated products are deprotected to give α/β mixtures of the glycosylamines which are evaluated as inhibitors of the hydrolysis of maltose by glucoamylase G2.

The configurational (α:β) equilibria of neutral and protonated *N*-(4-Y-substituted-phenyl) peracetylated-5-thio-D-glucopyranosylarylamines (Y = OMe, H, CF_3, NO_2) and *N*-(4-Y-substituted-phenyl) peracetylated-D-glucopyranosylarylamines (Y = OMe, H, NO_2) are investigated by ^{1}H NMR spectroscopy. The results are used to probe the existence of a generalized reverse anomeric effect in molecules containing X-C-N^+ fragments. The configurational equilibria are determined by direct integration of the resonances of the individual isomers in the ^{1}H NMR spectra after equilibration of both the α- and β-isomers. The substituent and solvent effects on the equilibria are discussed in terms of steric and electrostatic effects, and orbital interactions associated with the endo-anomeric effect. No evidence is obtained to support the existence of a generalized reverse anomeric effect. The data suggest an enhanced endo-anomeric effect upon protonation. The trends in the values of the C1-H1 coupling constants as a function of

substituent and α- or β-configuration are discussed in terms of the Perlin effect and the interplay of the endo- and exo- anomeric effects.

The oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-(β-D-Gal*f*-(1→3))-α-D-Man*p*-(1→2)-α-D-Man*p* corresponds to the terminus of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease. Syntheses of methyl or ethylthio glycosides of the terminal disaccharide, trisaccharide, tetrasaccharide, and pentasaccharide corresponding to this structure are described. The syntheses of heteroatom analogues of the di-, tri-, and tetra-saccharides, containing a 4-thiogalactofuranosyl (4-thio-Gal*f*) residue, are achieved using a new glycosyl donor, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-1-selenogalactofuranoside.

The syntheses of three novel disaccharides containing a 4-thio-Gal*f* residue as the non-reducing unit and a nitrogen in the interglycosidic linkage are achieved by reaction of 4-thiogalactofuranose with the corresponding glycosylamine using acetic acid as a catalyst.

The preliminary analysis of the conformations of β-D-Gal*f*-(1→3)-α-D-Man*p*, the 4-thio-Gal*f* analogue and the 4-thio-Gal*f* analogue containing a nitrogen atom in the interglycosidic linkage is presented.

DEDICATION

This thesis is dedicated to Shawn, Mom, Dad, and Chad. Without your support and encouragement this dream would never have been realized.

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I would also like to thank all my labmates, both past and present, for creating an interesting work environment. I am grateful for all the encouragement that was offered and for putting up with me day in and day out! Special thanks to Margaret Johnson for all her help with the molecular modeling of the compounds in Chapter 7 and for reading Chapter 1 at such short notice. I thank Seema Mehta for all her input on Chapter 1 as well. Also, many thanks to Ernest Lee and Kristi Zychowka for making starting materials.

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LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	Acetic acid
AE	Anomeric Effect
AgOTf	Silver trifluoromethanesulfonate
AIDS	Acquired Immunodeficiency Syndrome
Ala	Alanine
All	Allyl
Arg	Arginine
Asn	Aspargine
$BF_3{\cdot}Et_2O$	Boron trifluoride etherate
Bn	Benzyl
bs	Broad singlet
BSA	Bovine Serum Albumin
Bz	Benzoyl
CHARMm	Chemistry at Harvard Macromolecular mechanics
ConA	Concanavalin A
COSY	Correlated spectroscopy
Cr	*p*-Methylphenyl
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
DEPT	Distortionless enhancement by polarization transfer

DGA	Dihydro-*gluco*-acarbose
DMAP	4-(Dimethylamino)pyridine
DMF	*N, N*-Dimethylformamide
DMSO-d_6	Dimethyl-d_6 sulfoxide
DNA	Deoxyribonucleic acid
E	Envelope
ER	Endoplasmic reticulum
Et	Ethyl
EtOAc	Ethyl Acetate
GA	Glucoamylase
Gal	Galactopyranose
Gal*f*	Galactofuranose
GIPL	Glycosylinositolphospholipids
Glc	Glucose
Glc*N*	Glucopyranosylamine
Glc*N*Ac	*N*-acetylglucopyranosylamine
GPI	Glycosylphosphatidylinositol anchors
Glu	Glutamate
HEWL	Hen egg white lysozyme
HIV	Human Immunodeficiency Virus
J	Coupling Constant in Hz
Leu	Leucine
Lys	Lysine

m	Multiplet
Mp	Melting point
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
Phe	Phenylalanine
PPDol	Dolichol pyrophosphate
Pr	Propyl
RAE	Reverse Anomeric Effect
s	Singlet
TESOTf	Triethylsilyl trifluoromethanesulfonate
TfOH, Triflic acid	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
4-ThioGal*f*	4-thiogalactofuranose
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOCSY	Total correlation spectroscopy
TPPI	Time-proportional phase incrementation
TrNOE	Transferred Nuclear Overhauser Effect
TrNOESY	Transferred Nuclear Overhauser Effect Spectroscopy
Trp	Tryptophan

Tyr	Tyrosine
UDP	Uridine diphosphate

CHAPTER 1: INTRODUCTION

1.1 General Introduction to Carbohydrates

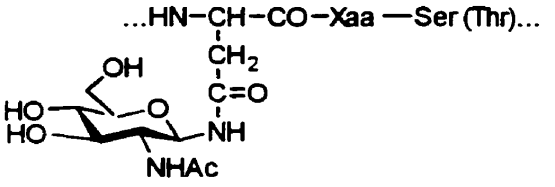
Carbohydrates have long been known to be an energy source in food (glycogen and starch), components of RNA and DNA and structural materials (cellulose and chitin). Over the past 40 years carbohydrates have also been shown to play vital roles in a vast number of biological recognition events. It is because of their complex structures that they were not studied extensively, and yet, it is this complexity that enables carbohydrates to be involved in such a wide range of processes. In contrast to amino acids or nucleic acids, which generally only form one type of linkage, two identical monosaccharide units can bond to form 11 different disaccharides. Four nucleotides can make only 256 distinct tetranucleotides, but four different monosaccharides can make 35, 560 unique tetrasaccharides.[1a]

Two new fields that deal with the roles of carbohydrates in these biological events have emerged and are called glycobiology and glycochemistry;[1b] they bring together the disciplines of organic chemistry, medicinal chemistry, molecular biology and biochemistry. Carbohydrates are found linked to proteins (glycoproteins and proteoglycans) and lipids (glycolipids) on the surface of cells. There are an immense number of these structures (known collectively as glycoconjugates) and they vary during cell development, differentiation and disease states. It has been determined that carbohydrates on the cell surface are key participants in biological recognition events.[2,3] The biological functions of glycoconjugates include cell adhesion, blood clotting, hormonal regulation, immunological protection and structural support.[4] These structures

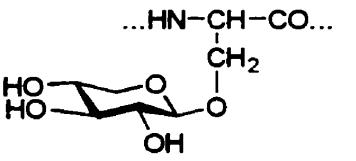
serve as receptors for other cells, bacteria, viruses, toxins and hormones.[3,5] They are also involved in cancer, where alteration of glycosylation or expression of lectins has an influence on tumour behaviour.[6]

The glycoproteins are either *N*-linked, usually by an *N*-acetylglucosamine residue β-linked to the amide side chain of an asparagine residue **1.1**,[7] or *O*-linked, through a β-glycosidic bond between D-xylose and a serine or threonine residue, *e.g.* **1.2** (Figure 1.1).[8] These carbohydrate chains are complex and are constructed using a variety of enzymes, both glycosyltransferases, which synthesize glycosidic bonds, and glycosidases, which hydrolyze glycosidic bonds. The processing of *N*-linked glycoproteins and the study of these interconversions by way of carbohydrate-based enzyme inhibitors will be discussed in the following sections.

Figure 1.1 Examples of *N*-Linked and *O*-Linked Glycoproteins



1.1

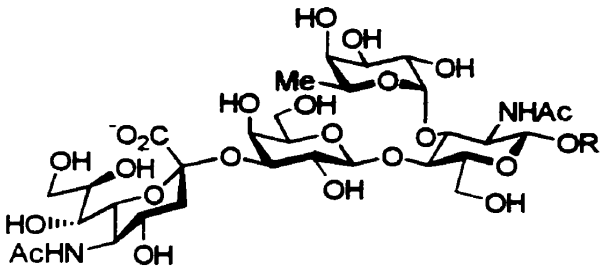


1.2

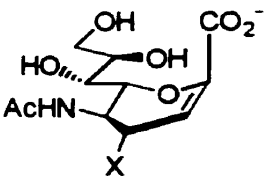
The use of oligosaccharides as inhibitors of biological processes, such as sialyl Lewis^X **1.3**, which has relevance in inflammation processes,[9] and **1.4** and **1.5** as viral neuraminidase inhibitors to prevent infection by the influenza virus,[10] or as potential drug candidates, such as acarbose[11] **1.6**, which has been approved in the USA for the treatment

of diabetes, has sparked more interest in carbohydrate chemistry (Figure 1.2). The fact that glycosylation may influence disease states also provides encouragement that altering glycosylation pathways or glycoproteins themselves may result in therapeutic agents.

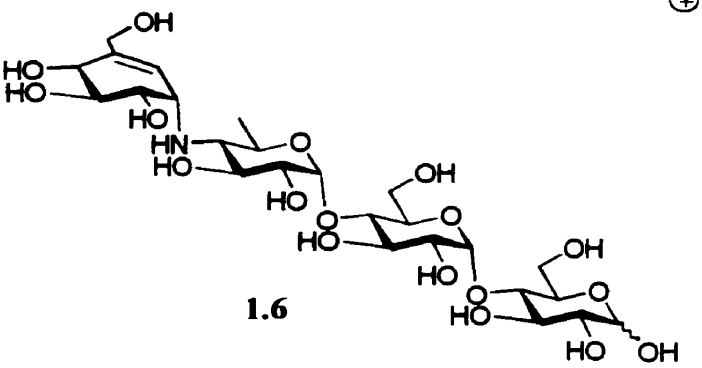
Figure 1.2 Examples of Oligosaccharides as Inhibitors of Biological Processes



1.3



1.4 X=NH_2
1.5 X= $HN{=}C(NH_2)(NH_2^{+})$



1.6

PART I

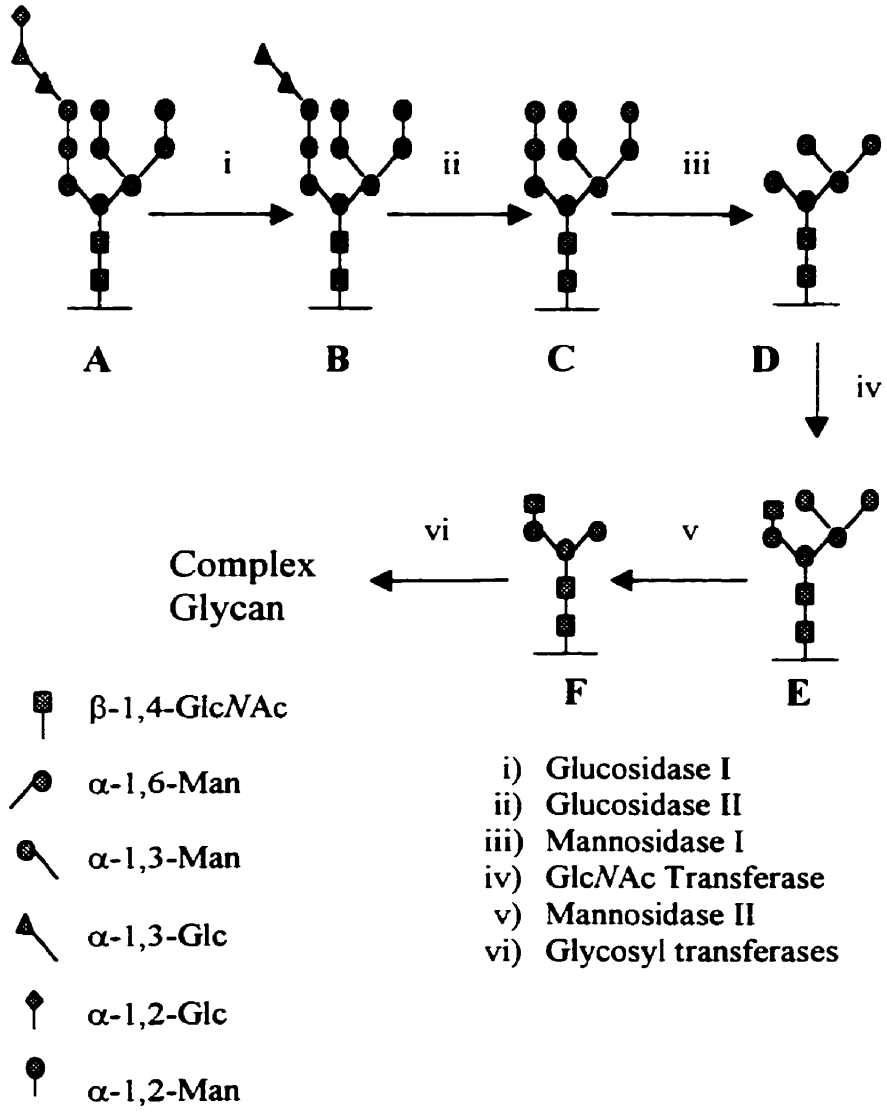
Part I of the introduction describes aspects related mainly to Chapters 2 and 3.

1.2 Glycosidases and Glycosyl Transferases

The enzymes utilized by Nature to create the glycosidic linkages found in glycoconjugates have been extensively studied. Enzymes are used in these oligosaccharide processing pathways to synthesize glycosidic bonds (glycosyl transferases) and to cleave glycosidic bonds (glycosidases). The maturation of glycoproteins[12,13] occurs through a sequence of enzyme-controlled transformations within the endoplasmic reticulum (ER) and Golgi apparatus. The glycoprotein processing sequence and the structures of the resulting glycoproteins are remarkably conserved throughout eukaryotes, providing additional evidence for the premise that oligosaccharides are biologically important. There are three main classes of *N*-glycoproteins: high mannose-, hybrid- and complex-type. All share a common core structure that is formed during the maturation process. The maturation process is initiated from dolichol pyrophosphate within the ER. A series of glycosyl transfers mediated by specific transferase enzymes converts dolichol pyrophosphate (PPDol) to a lipid-linked glycan precursor, $Glc_3Man_9GlcNAc_2PPDol$, in what is termed the dolichol pathway. This precursor is then transferred to specific sites on nascent proteins by oligosaccharyl transferase in the ER to give the high mannose type glycoprotein (Figure 1.3 **A**) which is immediately processed by removal of the three glucose residues by specific trimming glucosidases. The hydrolysis of the terminal α-(1$\rightarrow$2) linked glucose

residue is performed by glucosidase I (Figure 1.3 **B**), followed by hydrolysis of the two α-(1→3) linked glucose residues by glucosidase II. This results in the high mannose-type glycoprotein, $Man_9GlcNAc_2$ (Figure 1.3 **C**).

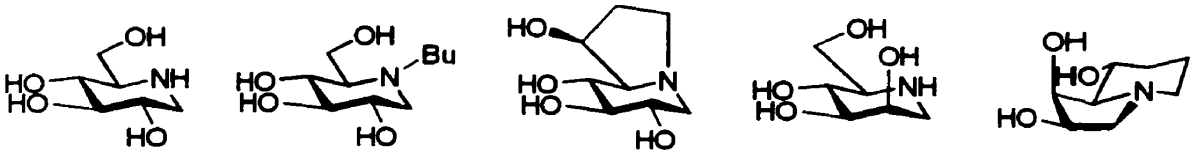
Figure 1.3 ***N*-Linked Glycan Biosynthesis**



Further processing by ER- and Golgi- located mannosidases permits the removal of the four α-(1→2) linked mannose residues to yield $Man_5GlcNAc_2$ (Figure 1.3 **D**). The action of Glc*N*Ac transferase I adds a branch point in the newly formed glycoprotein (Figure 1.3 **E**) and this structure can be processed further by various transferases such as sialyltransferase, fucosyltransferase, galactosyltransferase, and *N*-acetylglucosaminyl-transferase, to give mature complex and hybrid-type glycoproteins which are based on structures **E** and **F** (Figure 1.3).

The correct processing of these glycoproteins is vital for their function. It has been shown that interfering with trimming reactions through the use of enzyme inhibitors blocks subsequent maturation of oligosaccharides to complex structures.[14] Also, for some glycoproteins, inhibition of glucosidases in the ER interferes with normal protein folding. This stops the glycoprotein from being transported out of the ER. One reason for targeting these enzymes is to produce modified glycoproteins. The effects of such modifications can then be investigated and provide insight into the role of oligosaccharides in glycoprotein function. Some glucosidase I and II inhibitors[15] include 1-deoxynojirimycin **1.7**, *N*-butyl-1-deoxynojirimycin **1.8,** and castanospermine **1.9**, all having K_i values in the micromolar range. 1-Deoxymannojirimycin **1.10** and swainsonine **1.11** are known to inhibit mannosidase I and II respectively.[15]

Figure 1.4 Examples of Inhibitors of the *N*-Linked Glycoprotein Processing Enzymes



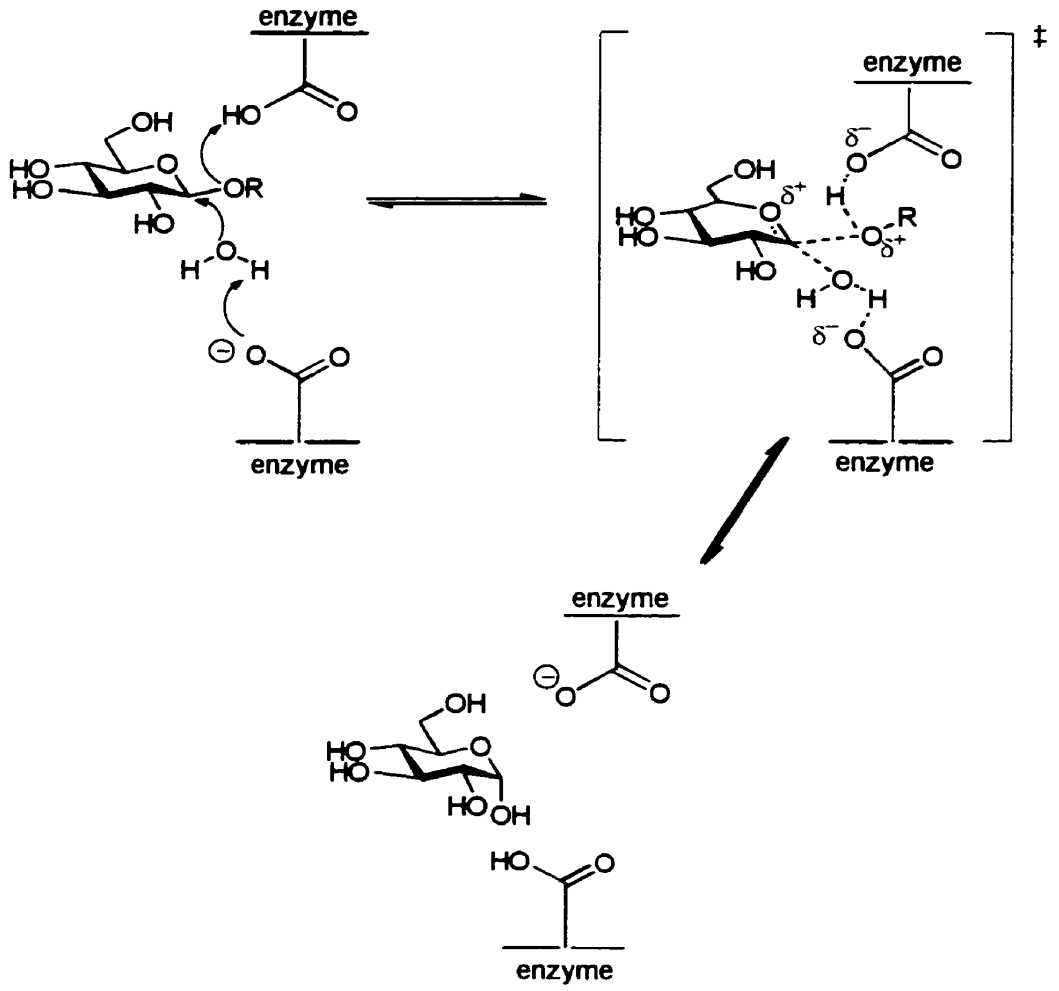
1.7 **1.8** **1.9** **1.10** **1.11**

These enzyme inhibitors have also been found to have therapeutic potential as anti-viral agents, for example, in HIV[16] and influenza[10] treatment, as anticancer[17] and antibacterial[18] agents, and in the treatment of metabolic disorders such as diabetes.[15]

1.2.1 Glycosidase Inhibition: Mechanisms of Glycosidases

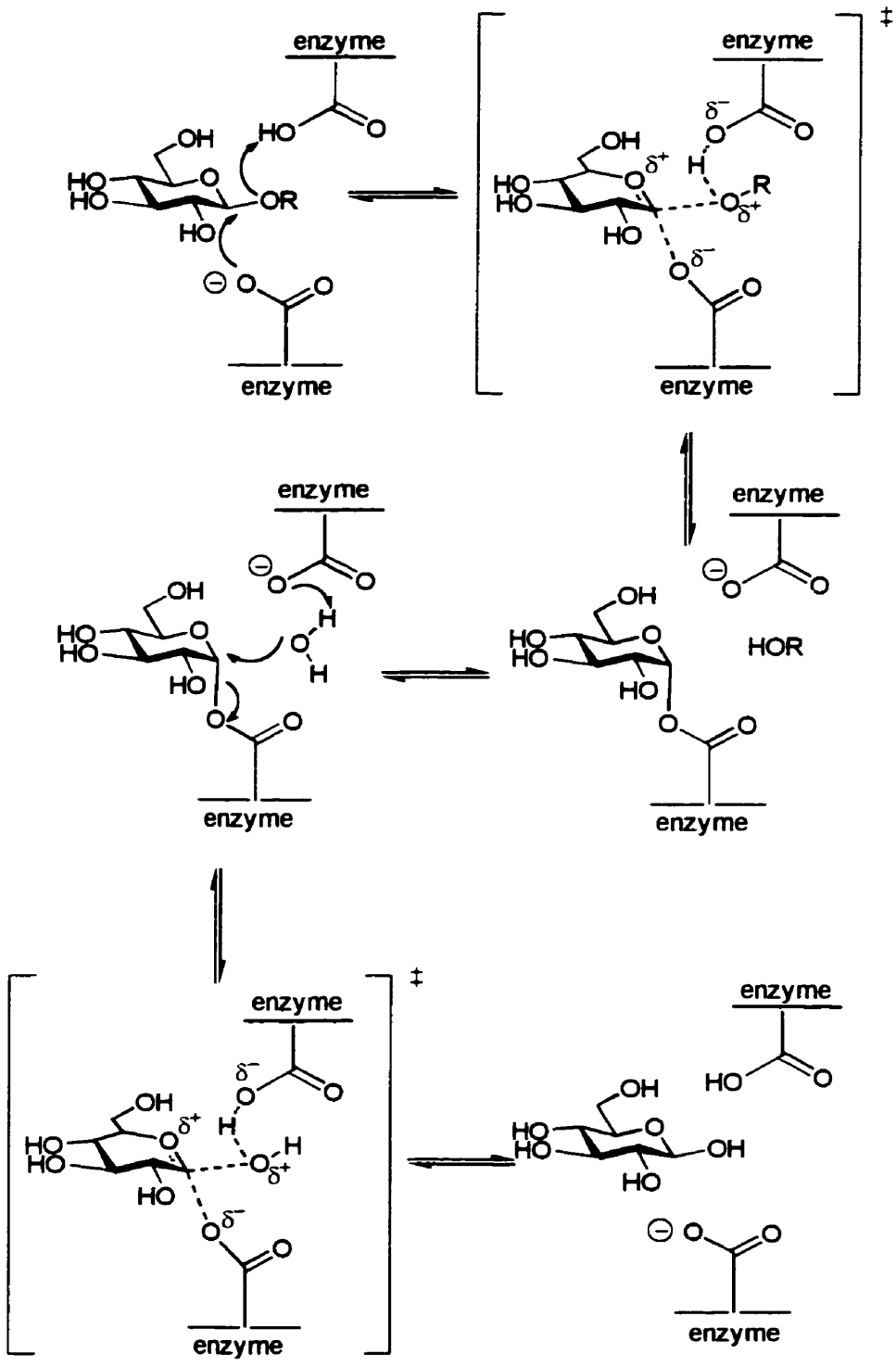
In order to design inhibitors for glycosidases and glycosyl transferases it is necessary to understand the mechanisms by which these enzymes act. It has been proposed that inhibition of these enzymes may lead to potential therapeutic agents, and also contribute to understanding of the structure-function relationships of enzyme specificity. The hydrolysis of the glycosidic linkage can proceed in two ways, with net retention or net inversion of configuration at the anomeric center. In 1953, Koshland[19] first proposed the mechanisms for these two classes of enzymes, and the general features of these proposals are still widely accepted.[20,21,22,23,24] The inverting glycosidases use a direct displacement mechanism, where two carboxylic acids in the active site are involved in the catalysis. One group acts as a general base to remove a proton from the attacking water molecule, while the other acts as a general acid by donating a proton to the aglycon (Figure 1.5).[20-24]

Figure 1.5 **Inverting Mechanism of Glycosidases**



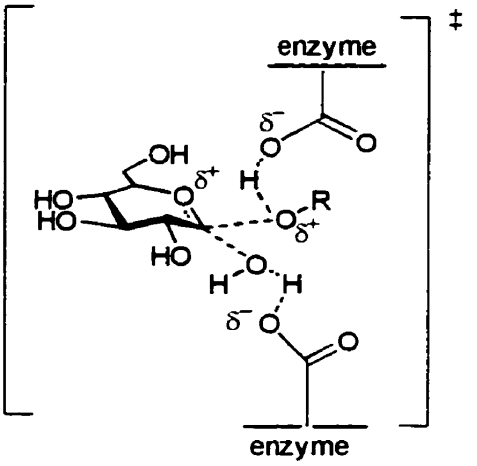
The hydrolysis of a glycosidic linkage with net retention proceeds via a double-displacement mechanism (Figure 1.6).[20-24] This mechanism has two different covalent glycosyl-enzyme intermediates, which are formed and hydrolyzed, respectively via oxacarbenium ion-like transition states. It is believed that two carboxyl residues suitably placed in the active site are responsible for this transformation. One of the residues acts as a general acid in the first step (glycosylation) and then as a general base in the second step (deglycosylation); the other residue acts as a nucleophile in the first step.

Figure 1.6 **Retaining Mechanism for Glycosidases**



Both mechanisms involve a transition-state with significant positive charge, a half-chair-like conformation and a trigonal bipyramidal anomeric center (Figure 1.7). The significant difference in the two mechanisms is the distance between the active site residues. In the retaining enzyme, the nucleophile-carboxylic acid pair is closer, 4.5-5.5 Å apart, compared to the acid-base pair in the inverting enzymes, which is approximately 9 Å apart.

Figure 1.7 **Putative Transition-State Structure**



The first crystal structure analysis of an enzyme-inhibitor complex aided in the understanding of these mechanisms. The retaining β-glycosidase hen egg white lysozyme (HEWL) was crystallized with chitotriose[25,26,27] and the structure of the enzyme-inhibitor complex was determined. This structure revealed the locations of two carboxyl groups in the active site of the enzyme that were essential to the hydrolysis mechanism. One residue (Glu-35) donated a proton to the glycosidic oxygen atom, while the other (Asp-52) stabilized the positive charge that resulted from the departure of the

protonated aglycon. Many more studies on protein-ligand crystal structures have been completed more recently and contribute to the understanding of these mechanisms.[28] The retaining enzymes generally have a glutamate or an aspartate as the catalytic nucleophile, which in most cases functions as the general acid/base. In a recent study by Davies *et al.*,[29] "snapshots" along the reaction pathway were obtained using time-resolved X-ray crystallography. The structures included the native enzyme, the Michaelis complex, a covalent intermediate, and the product complex, and were found to be compatible with the proposed double displacement mechanism. An interesting result in the enzyme-substrate complex is the distortion of the substrate sugar to a $^{1}S_{3}$ skew boat. This conformation places the leaving group at the anomeric center in a pseudo-axial orientation. The skew boat conformation should help to form the planar transition state structure. Other studies by the Withers group[30] involved isolation of the covalent glycosyl-enzyme intermediate using 2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride or 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-D-glucopyranoside as mechanism-based inactivators of β-glucosidases. The activated leaving group, a fluoride ion or a 2,4-dinitrophenylate, was displaced in the glycosylation step. However, the deglycosylation step was found to be very slow because of destabilization of the positive charge in the transition-state by the 2-fluoro substituent; thus, accumulation of the covalent glycosyl-enzyme intermediate occurred.

The 2-fluoro inactivators are very effective with β-glycosidases, but are relatively ineffective inactivators of α-glycosidases. The use of 2-deoxy-2,2-difluoro glycosides with reactive leaving groups such as trinitrophenyl did allow trapping of the glycosyl-enzyme intermediates of an α-glycosidase, as well as an α-amylase.[31] Another

approach involved repositioning the fluorine from the 2-position to the 5-position and again the intermediate was trapped. These compounds were also synthesized as the β-anomers and were inactivators of the β-glycosidases as well.[32,33]

Other studies on the mechanism of action of a glycosidase have involved mutagenesis of the enzyme itself.[34,35] The study by Kuroki *et al.*[35] used a T4 lysozyme with a mutation of threonine for the glutamic acid residue in the active site, which produced a covalently attached enzyme-substrate intermediate. The crystal structure indicated substantial distortion of the sugar ring. The most recent study involved mutations of a non-catalytic residue (Glu257Gln) and the active site-nucleophile (Asp229Asn) in cyclodextrin glycosyltransferase. The X-ray crystallographic structure gave evidence of an enzyme-substrate complex with a highly distorted sugar residue.[34]

The hydrolysis mechanisms described above all involve exocyclic bond cleavage as the crucial step. Alternative mechanisms involving endocyclic bond cleavage giving rise to enzyme-bound acyclic intermediates have been suggested.[36,37,38] These have been dismissed by Sinnott as being unsubstantiated due to contradictory evidence.[39]

Although the β-retaining glycosidases are the most widely studied among the different enzymes, the α-inverting enzyme glucoamylase (GA) from *Aspergillus* spp. (1,4-α-D-glucan glycohydrolase, EC 3.2.1.3) has also been studied extensively. It is an exo-acting hydrolase that catalyzes the release of β-D-glucose from the non-reducing ends of starch and related oligo- and polysaccharides. The enzyme is used in industry for the conversion of starch to glucose syrups. The glucose is then used in the production of fructose sweeteners, in the production of ethanol by fermentation and in the brewing of light beer.[40] GA tolerates a broad range of aglycon moieties, cleaving α-1,6-glucosidic

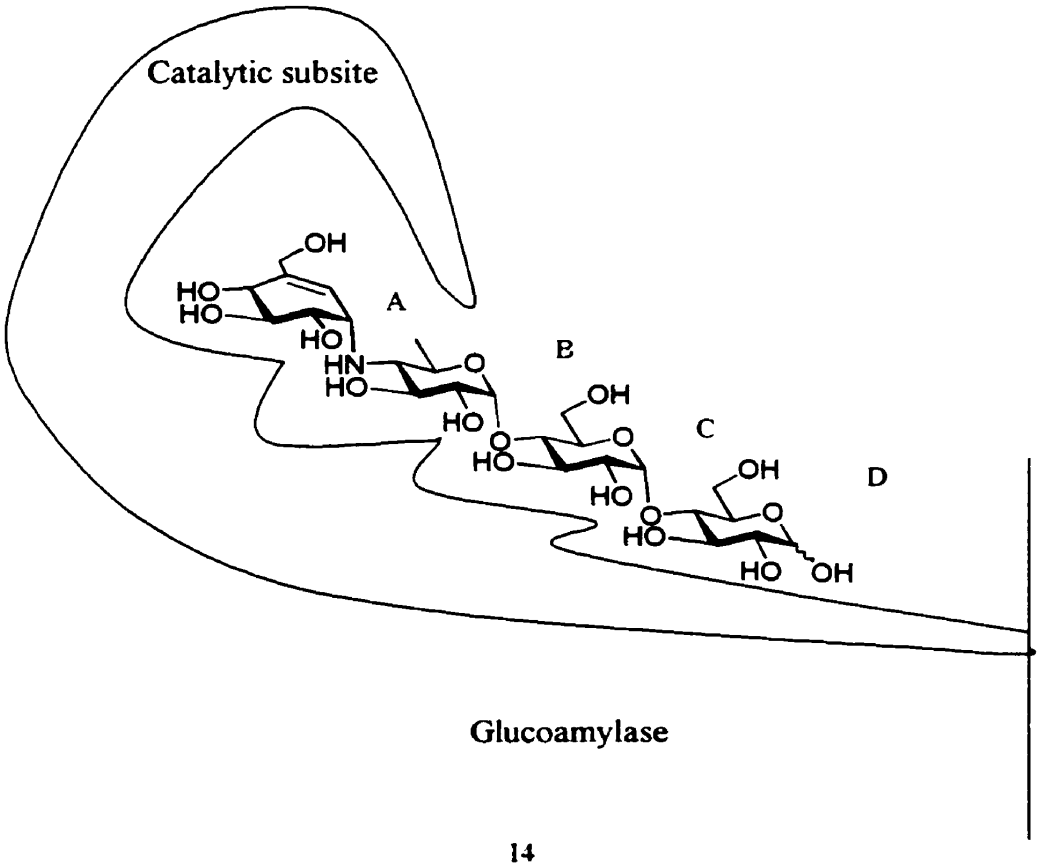
bonds and aryl glucosides with 500- and 50-fold lower activity, respectively, than α–(1→4)-linked oligosaccharides.[41,42,43] Several species of *Aspergillus* produce at least two major forms[44] of glucoamylase: G1 containing a catalytic domain, a highly *O*-glycosylated region, and a starch binding domain (82.7 kDa, residues 1-616 in GA from *A. niger*);[45] and G2 a smaller form lacking the starch binding domain (72 kDa, residues 1-512 in GA from *A. niger*).[46] Only GA G1 can hydrolyze raw starch granules.[47] The catalytic domain (residues 1-471) folds into an $(\alpha/\alpha)_6$ barrel where six highly conserved α→α loop regions connect segments of an inner and an outer α_6 barrel.[48] These conserved segments create the funnel-shaped active site. The architecture and details of the residues involved in catalysis, substrate binding and transition state stablilization are described in the following sections through use of crystallography, site-directed mutagenesis, inhibitor binding thermodynamics and molecular recognition of substrate analogues.

The complex of 1-deoxynojirimycin **1.7** (a strong inhibitor of glucoamylase with a K_i = 96 μM[15]) with GA G2 from *A. awamori* was studied using X-ray crystallography.[49] The structure of the inhibitor-complex confirms that the inhibitor is bound deep in the enzyme pocket in the innermost set of helices of the $(\alpha/\alpha)_6$ barrel. It was found that there is one water molecule (out of seven found in the native enzyme) still present in the active site when the inhibitor is bound. It is hydrogen bonded to Glu400 and the 6-OH of the inhibitor. It appears that the water is the nucleophile in a general base-catalyzed mechanism, and that Glu400 is the catalytic base and Glu179[50] the catalytic acid residue in the enzyme. The water molecules in the native enzyme occupy similar positions to the 2-, 3-, 4- and 6-hydroxyls, C-6 and the absent 1-OH of the inhibitor. There are two

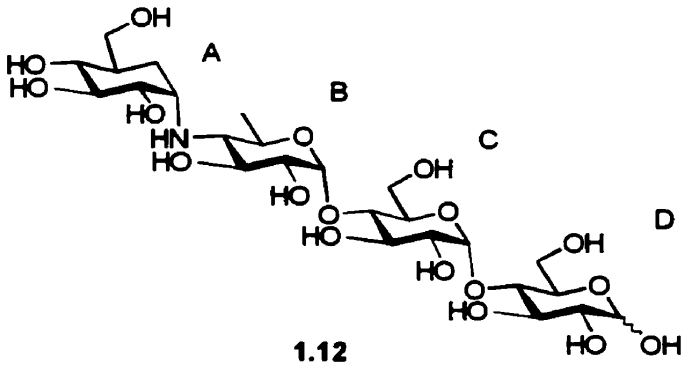
molecules of **1.7** bound to the enzyme, the second molecule apparently bound in a low-affinity site.

A study of the acarbose (**1.6**, $K_d = 10^{-12}$ M[51])-GA complex reveals that there is extended binding of this pseudo-sugar (see Figure 1.8).[52] The second residue (B) occupies the same site as the second molecule of 1-deoxynojirimycin **1.7**. The interactions between residue A and the enzyme are numerous and are similar to those seen for **1.7**. The catalytic acid Glu179 forms a salt-bridge with the amino linkage between residues A and B. This salt-link may contribute to the unusually tight association of acarbose with GA. The third residue C has a hydrophobic contact with the side chain of Trp120 which distorts the angle of the glucosidic linkage between B and C.

Figure 1.8 A Schematic of the Enzyme Catalytic Site with Acarbose Bound



A study of the complex between D-*gluco*-dihydroacarbose (**1.12**, $K_d = 10^{-8}$ M[53]) and GA was undertaken to rationalize the difference in the dissociation constants between acarbose **1.6** and **1.12**.[54,55] The data taken at pH 4 suggest that **1.6** and **1.12** bind in nearly identical positions. The reduced affinity of **1.12** may be due to a weakening of hydrogen bonds of the catalytic water to the enzyme. Steric contacts between the non-reducing end of **1.12** and the catalytic water disturb its location and the hydrogen bonding is no longer optimal. The 6-OH of both molecules is shifted towards a more axial position, which causes residue A to have a half-chair conformation. This is consistent with the generation of a glucopyranosyl cation in the transition-state, which leads to favorable electrostatic interactions between the proposed cation intermediate and the active site. The estimate of the magnitude of the stereochemical distortion observed for the substrate (2 kcal/mol) suggests that electrostatic interactions between the uncompensated negative charge in the active site and the partial positive charge on the carbocation are important factors in the mechanism. The role of electrostatic interactions in the mechanism of glucoamylase-catalyzed hydrolysis is substantiated by site-directed mutagenesis studies.[56]



1.12

Other studies involving mutagenesis include Leu177His, Trp178Arg, and Asn182Ala mutants.[57] These residues are highly conserved throughout the fungal glucoamylases and are located near three acidic groups, Glu179, Asp176, and Glu180. Substitutions of Leu177 and Trp178 cause significant decreases in k_{cat} with the substrates tested, whereas substitution of Asn182 does not affect binding or the catalytic parameters. This suggests that Leu177 (residing in subsite -1) provides a transition state contact with the 3-OH of the non-reducing ring. In subsite +1, Trp178 probably interacts with either the 3-OH or the ring oxygen in the transition state complex.

It has been found that Asp55 is important for catalytic activity, while Asp309 is important for hydrolyzing α-(1→6)-linked substrates.[58] This is important for industry because an unwanted side reaction involves hydrolysis of α-(1→6) bonds. To overcome this problem a mutant which does not have a high turnover rate for α-(1→6) linkages, but still maintains a high activity for α-(1→4) linkages is desirable.

The mutation of Trp120 established that this residue is involved in transition-state stabilization through hydrogen bonding to the general acid catalyst Glu179, and by direct stacking towards the sugar, directing conformational changes in subsites +1 and +2.[59] Many other studies have been undertaken to determine the role of different residues and the mechanism of action.[60,61,62,63,64]

The thermodynamics of binding was studied using wild-type and mutant enzymes as well as a range of inhibitors. It was found that binding to wild-type and most mutants occurs with favorable enthalpy and entropy.[53,65] Both hydrogen bonding and dehydration of the protein-oligosaccharide surfaces drive the formation of the complex.

The use of substrate analogues to determine the mechanism of hydrolysis for glucoamylase has been achieved using maltose, isomaltose and the mono-deoxygenated and mono-*O*-methylated analogues of isomaltose.[66,67,68] It was established that OH-4', OH-6', and OH-4 are critical groups for isomaltose hydrolysis, while the OH-2 and OH-3 of maltose account for high binding affinity in subsite +1 of GA.

In summary, much work has been performed to sort out the mechanism of action of GA and to determine the critical residues needed for optimizing the hydrolysis of α-(1→4)-linked glycosides.

1.3 Enzyme Inhibitors

There have been many different approaches taken in designing inhibitors of glycosidases. As shown above, glycosidases, both inverting and retaining, catalyze reactions through a transition state that has substantial oxocarbenium ion character.[20,22,69] Thus, the various classes of enzymes are inhibited by substrate analogues which mimic the charge and/or the shape of the transition state. This can be achieved by having a half-chair conformation or by sugars containing a basic nitrogen either in the ring or adjacent to the C-1' atom of disaccharides or the C-1 of monosaccharides.[21,70] Thiosugars are another class of substrate analogues and/or inhibitors.

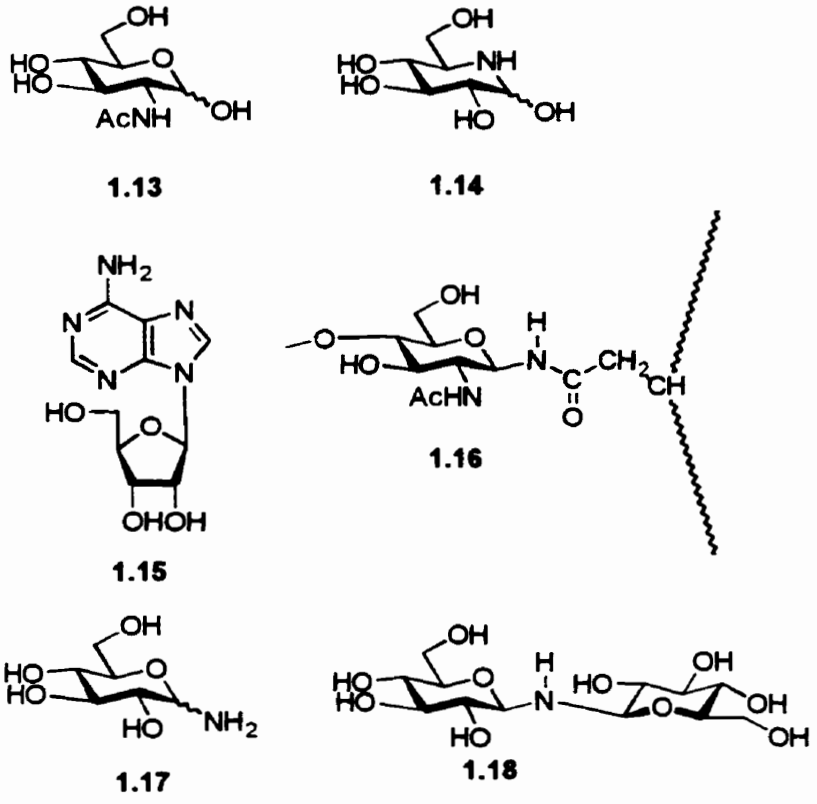
1.3.1 Nitrogen Containing Analogues

Carbohydrates in which one of the oxygen atoms has been replaced by a nitrogen are representatives of a large class of compounds. They can be subdivided into several classes: 1) amino sugars, 2) nitrogen as a replacement for oxygen in the ring, and 3)

nitrogen as a replacement for oxygen in the interglycosidic linkage (Figure 1.9). The amino sugars contain free or *N*-substituted amino groups, which replace one or more of the hydroxyl groups. These compounds are abundant in Nature, the most common being 2-acetamido-2-deoxy-D-glucopyranose **1.13**. The second class includes naturally occurring compounds such as nojirimycin **1.14** (a 5-amino-5-deoxy-hexose), which possesses antibiotic activity and potent glycosidase inhibitory activity.

The third class of compounds, in which the interglycosidic linkage is replaced by nitrogen can be subdivided into several different types. The nucleosides, for example **1.15**, in which a furanose sugar is β-linked to nitrogen in a heterocyclic base, are part of every living cell as components of nucleic acids. *N*-linked glycoproteins, for example **1.16**, as described in section 1.1, are found in all eukaryotic organisms. Disaccharides containing nitrogen in the interglycosidic linkage may arise as side products during the synthesis of glycosylamines. The treatment of a monosaccharide with a concentrated alcoholic solution of ammonia over a long period of time, produces the crystalline glycosylamine **1.17** in equilibrium with the diglycosylamine **1.18**.[71,72] Glycosylamines, such as *N*-butyl D-glucopyranosylamine, are prepared by reaction of D-glucopyranose with the corresponding amine.[73] The product is very reactive and tends to hydrolyze rapidly. The *N*-aryl glycosylamines are far more resistant to hydrolysis. They are prepared by reaction of the free sugar with the aromatic amine in hot alcoholic[74] or aqueous[75] solutions.

Figure 1.9 **Examples of Common Azasugars Found in Nature**



Disaccharides containing NH in the interglycosidic linkage have been reported by Barker *et al.*[76] in 1961 and Micheel *et al.* in 1965,[77] but to the best of our knowledge these compounds have not been reinvestigated since the original reports and, therefore, no proof of structure has ever been obtained by modern NMR methods. These compounds were not tested as enzyme inhibitors. The *O,N*-acetals are easily hydrolyzed and thus far, the synthesis of azapyranosyl disaccharides[78] has remained elusive.

Glycosylamines were first reported as enzyme inhibitors in 1973 by Lai and Axelrod.[79] Glycosylamines derived from D-glucose, D-galactose and D-mannose were found to be specific competitive inhibitors of α- and β-glucosidase, α- and β-galactosidase and α-mannosidase, respectively. The K_i values ranged from 2 μM to 0.23

mM. This represented considerable improvement in inhibition over the free sugars. The researchers speculated that the strong affinity of the glycosylamines for glycosidases originated not only from electrostatic stabilization of the oxocarbenium ion, but also through the basic glycosidic amine at the acidic site in the catalytic center.

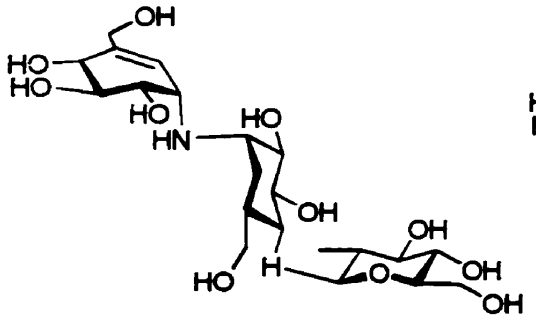
It was found that β-D-glucosylamine was a much stronger inhibitor for β-glucosidases from bitter and sweet almonds than its neutral parent compound β-D-glucose.[80] It was concluded that protonation of the inhibitor at the glycosyl nitrogen was a prerequisite for strong binding. Structurally similar compounds which were not able to be protonated at the glycosyl nitrogen were found to be weak inhibitors. A study using cationic nitrogen analogues showed that the inhibitors bearing a permanent positive charge (*e.g.* β-D-glucopyranosylpyridinium ion) were not bound as tightly as the neutral analogues.[81] The fact that different enzymes have different active site structures and charge requirements is demonstrated in the results obtained with β-D-glucosidase from *Escherichia coli*, in which the cationic inhibitors were bound no more tightly than the neutral analogues,[78] whereas β-D-glucosidase from *A. wentii* bound the cationic species (*e.g.* β-D-glucopyranosylpyridinium ion) much more tightly than the neutral analogues.[82] In all cases, the basic inhibitors were bound more tightly than their cationic and oxygenated counterparts. These results demonstrate that the presence of an amino group at the anomeric center of the sugar ring that can be protonated in the active site is a requirement for tight binding.

1.3.2 Pseudosugar Analogues

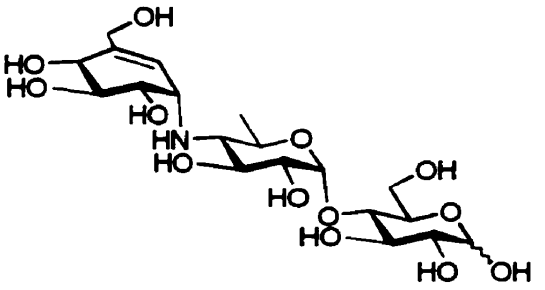
The inhibitor acarbose **1.6** is an example of a class of compounds known as pseudo-oligosaccharides. These compounds, which were isolated from different strains of *Streptomyces* during the 1970's and 80's primarily by the Bayer AG group[83] and others,[84] were found to be remarkable enzyme inhibitors. The compounds isolated include validamycin A[85] **1.19**, acarbose[86] **1.6**, amylostatin[86] **1.20**, adiposin[87] **1.21**, and trestatin B[87] **1.22** (Figure 1.10). These compounds all contain a cyclitol unit and a 4-amino-4-deoxy sugar (or pseudo-sugar in the case of validamycin) that is essential for their inhibitory activity. The substituents of the cyclitol unit are oriented in an analogous manner to glucose. A number of other pseudo-disaccharides have been isolated and/or synthesized and include the validoxylamines A **1.23**, and B **1.24**,[88] and acarviosin[89] **1.25** (Figure 1.11).

All of these compounds are biologically relevant. As previously stated, acarbose **1.6** is a very strong inhibitor of glucosidases such as α-amylase, glucoamylase and sucrase. Validamycin A **1.19** is a potent antibiotic.[85] Amylostatin XG **1.20** exhibits α-glucosidase activity,[90] while adiposin **1.21** exhibits α-glucosidase as well as antimicrobial activity. These compounds are thought to be good inhibitors because of the flattened half-chair conformation of the unsaturated cyclitol ring which resembles the transition-state structure of the sugar residue in the enzyme active site, and because of the complementarity of the basic nitrogen with the active site carboxylate group.

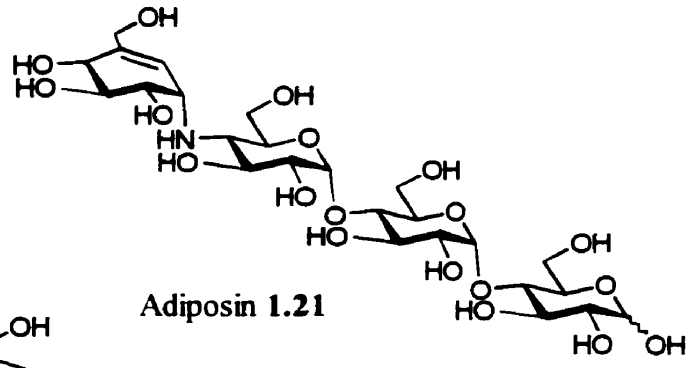
Figure 1.10 **Some Examples of Pseudo-Oligosaccharides**



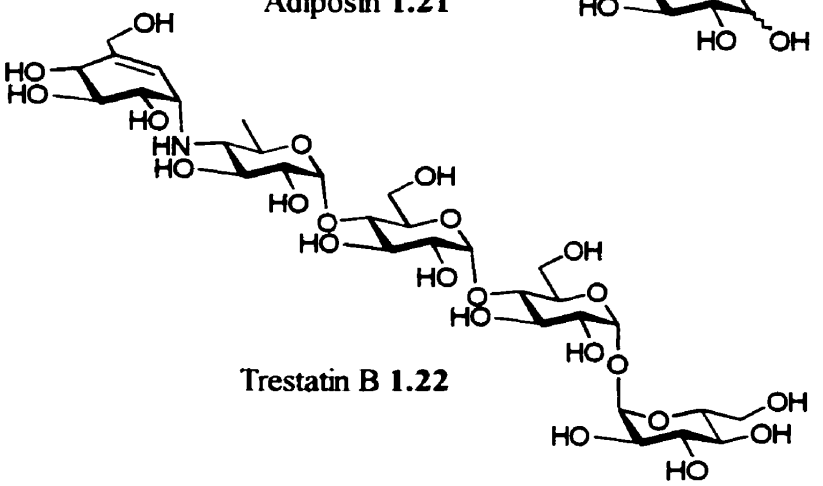
Validamycin A **1.19**



Amylostatin **1.20**

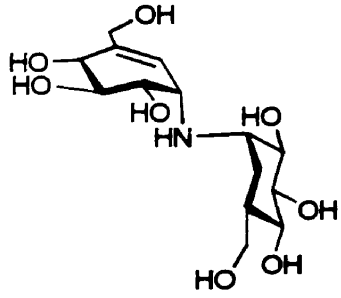


Adiposin **1.21**

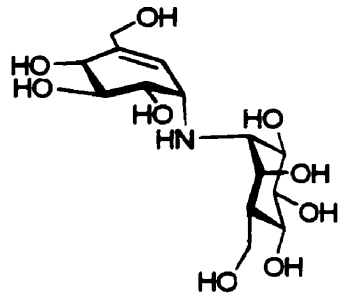


Trestatin B **1.22**

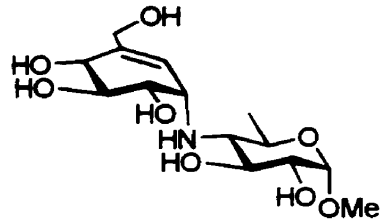
Figure 1.11 Biologically Active Pseudo-Disaccharides



Validoxylamine A **1.23**



Validoxylamine B **1.24**



Acarviosin **1.25**

The saturated analogue of acarbose **1.6**, D-*gluco*-dihydroacarbose **1.12**, is a good inhibitor of glucoamylase, but is not bound as tightly as **1.6**.[53] These results suggest that the basic nitrogen in the linkage is in part responsible for the tight binding of the inhibitor to the enzyme active site. It has also been shown that acarviosin **1.25**, is a much weaker inhibitor,[53] corroborating the fact that additional glucose units on **1.6** or **1.12** are interacting with the enzyme in a favourable way.

The argument of shape versus charge for the design of better inhibitors still continues. The results reviewed here show that both are important for good inhibition and tight binding.

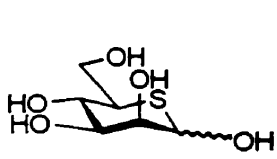
1.3.3 Sulfur Containing Analogues

The replacement of oxygen with sulfur is well studied in the literature and these compounds show interesting biological properties. These compounds are rare in Nature. Thiosugars may be divided into three classes: 1) those containing a thiol in place of one

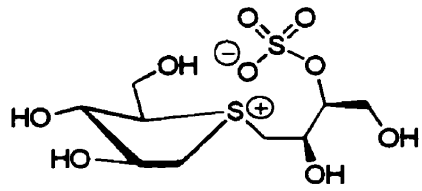
or more of the hydroxyl groups, 2) those in which a sulfur replaces the oxygen in the interglycosidic linkage, and 3) those in which the ring oxygen is replaced with sulfur.[91]

The first naturally occurring thiosugar isolated was 5-thio-D-mannose **1.26** from the marine sponge *Clathria pyramida*.[92a] 5-Thio-D-mannose is a potential mannosidase inhibitor. It was synthesized in 1989 by Yuasa *et al.*[92b] Other naturally occurring compounds recently isolated from *Salacia reticulata*, a herb used in Indian traditional medicine for treatment of diabetes, are salacinol **1.27**[93] and kotalanol **1.28**.[94] These compounds were found to be stronger inhibitors of α-glucosidases than acarbose.[94]

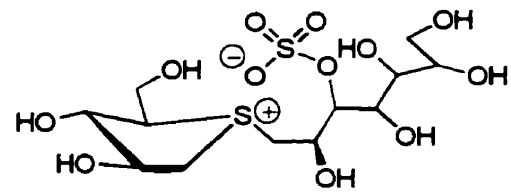
Figure 1.12 **Naturally Occurring Thiosugars**



1.26



1.27



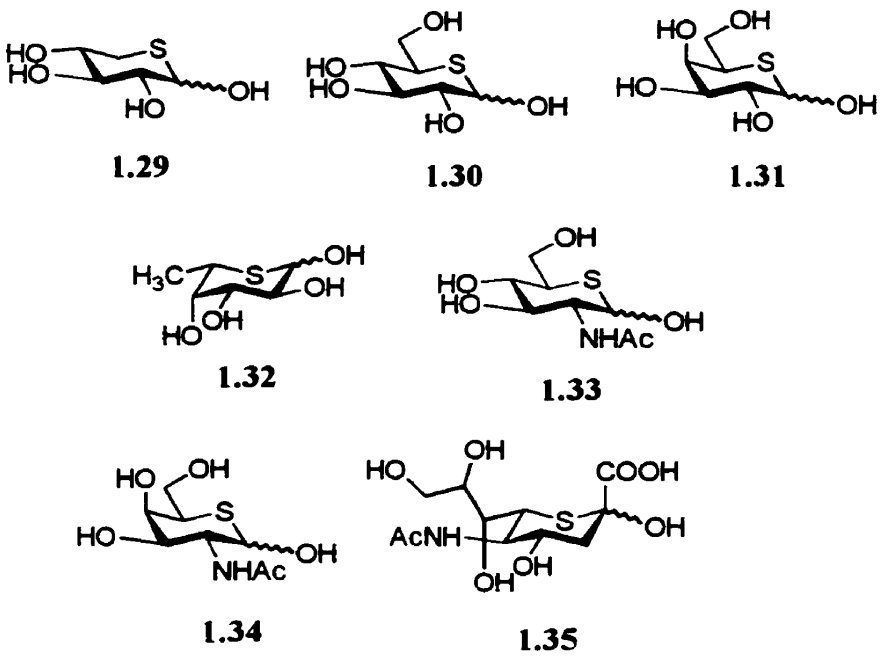
1.28

1.3.3.1 Monosaccharides Containing Sulfur in the Ring

The synthesis of 5-thioxylose **1.29** was achieved in 1961.[95] In 1962, Feather and Whistler[96] synthesized 5-thioglucose **1.30**, which is now commercially available and has been used in many biological studies. It was found to be an inhibitor of the transport of D-glucose in many tissues,[97] an inhibitor of the release of insulin,[98] and has exhibited cytotoxicity against hypoxic tumor cells.[99] It has an antispermatogenic effect in rats,[100] making it a good candidate for a male contraceptive. Other sugars containing sulfur in the ring have been synthesized, *e.g.*, 5-thiogalactose **1.31**,[101] 5-thiofucose **1.32**,[102] *N*-

acetyl-5-thioglucosamine **1.33**,[103] *N*-acetyl-5-thiogalactosamine **1.34**,[104] and 6-thiosialic acid **1.35**.[105] Thiosugars containing a sulfur atom in the ring have been classified as substrate analogues rather than transition-state analogues, due to the inability of the ring sulfur to be protonated in the manner analogous to the nitrogen inhibitors, and the fact that they do not mimic the putative transition-state of the substrate-active site complex. It is still of interest to study thiosugars in order to assess enzyme mechanisms and for mapping the active-site topography to determine structure-function relationships. Some thiosugar analogues are found to bind tighter to the enzyme active site and to be competitive inhibitors of the hydrolysis of the natural substrate by the enzyme due to the physical similarity of the compounds. There have been reports of thiosugars being strong enzyme inhibitors: 5-thio-α-D-glucopyranose **1.30** was reported to be a potent competitive inhibitor of α-glucosidase (Brewer's yeast) with a K_i of 7.5×10^{-4} M;[106] while 5-thio-L-fucose **1.32** was shown to inhibit fucosidases from bovine epidydimis and kidney with K_i values of 4.2×10^{-5} M and 8.4×10^{-5} M, respectively.[102]

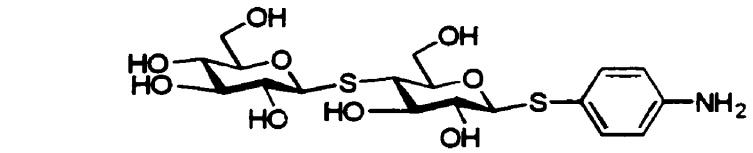
Figure 1.13 Synthetic 5-Thiosugars



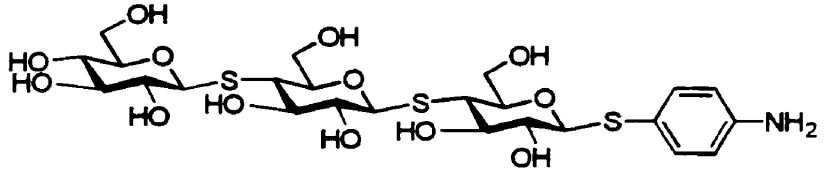
1.3.3.2 Compounds Containing Sulfur in the Interglycosidic Linkage

Compounds containing sulfur in the interglycosidic linkage have been synthesized and found to be inhibitors of various enzymes. Thioglycosides are hydrolyzed more slowly than their oxygen counterparts.[107] The thio analogues of β-cellobioside **1.36** and cellotrioside **1.37** were found to be competitive inhibitors of the hydrolysis of the substrate, 4-methylumbelliferyl β-lactoside, by cellobiohydrolases.[108] In a study by Hashimoto *et al.*[109] a series of thio-linked α-L-fucopyranosyl disaccharides **1.38-1.41** were synthesized for the purpose of characterizing α-L-fucosidases. The most potent competitive inhibitor against bovine kidney α-L-fucosidase was **1.40** with a K_i of 0.65 mM; this is substantially less potent than **1.32**.

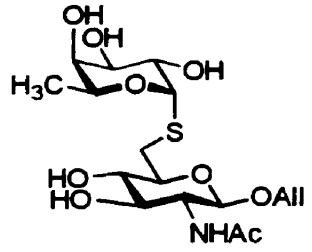
Figure 1.14 Compounds Containing Sulfur in the Interglycosidic Linkage



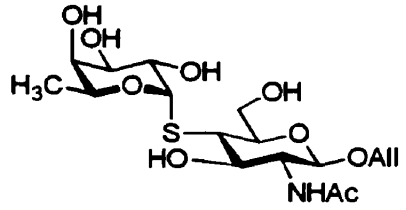
1.36



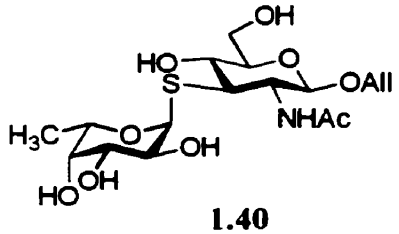
1.37



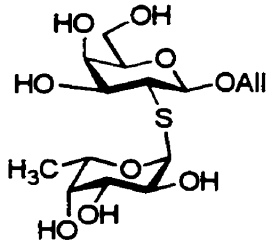
1.38



1.39



1.40

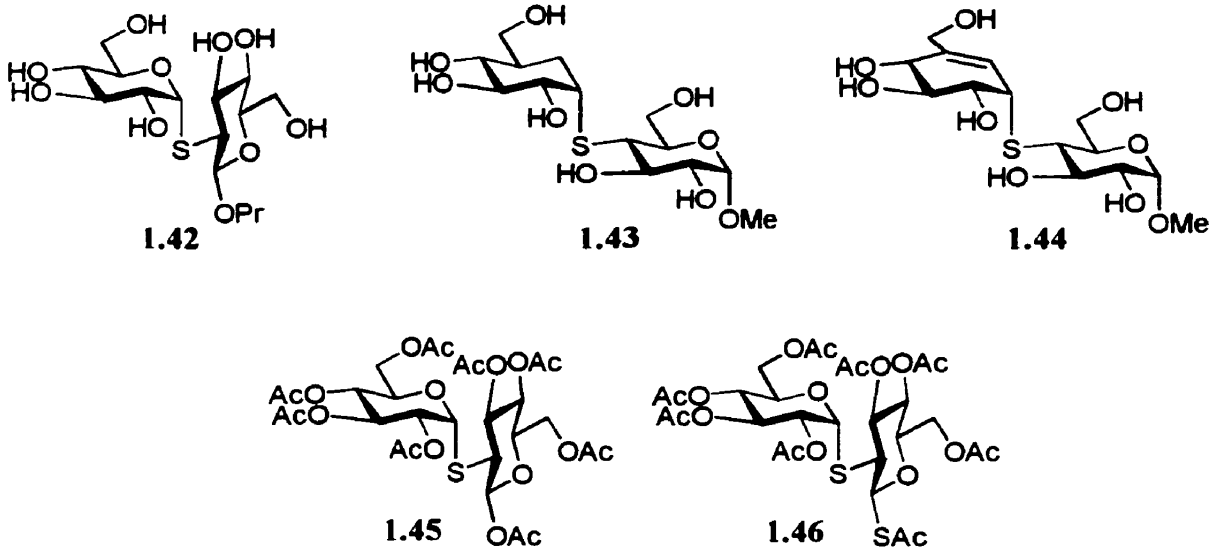


1.41

The synthesis of the kojibioside analogue **1.42** was achieved in our laboratory. [110] This compound was shown to be a competitive inhibitor of glucosidase I and glucosidase II with *Ki* values of >100 mM and 1.0 mM, respectively.[111] The synthesis of the compounds **1.43** and **1.44** led to a new class of pseudosugars, but these did not show significant inhibitory activity against α-glucosidase from Baker's yeast.[112] Interestingly,

two protected α-linked thio-disaccharides **1.45** and **1.46** were reported to have anti-HIV activity, while the deprotected compounds were inactive.[113]

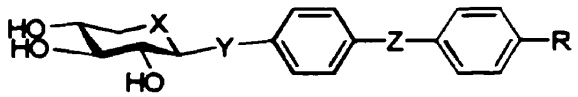
Figure 1.15 **Disaccharides and Pseudodisaccharides Containing Sulfur in the Interglycosidic Linkage**



1.3.3.3 Heteroanalogues Containing Sulfur in the Ring and Various Heteroatoms in the Interglycosidic Linkage

A series of monosaccharide heteroanalogues were synthesized and evaluated for their antithrombotic activity in rats (Figure 1.16).[114] Compound **1.58** (naroparcil) was selected for further development as a potential drug candidate. Present anticoagulant therapies are administered either orally or by injection, but both heparin and antivitamin K compounds have secondary effects which may include bleeding. It is hoped that this new type of drug can be taken orally and have a reduced risk of bleeding.

Figure 1.16 Antithrombotic Compounds

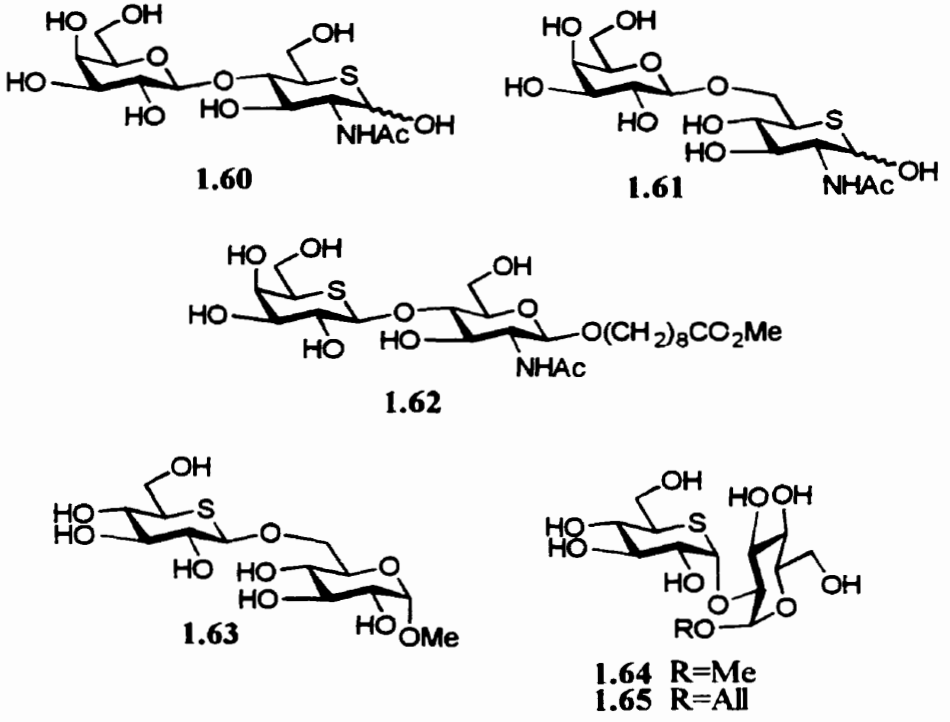


Compound	X	Y	Z	R
1.47	O	S	CO	NO_2
1.48	O	S	CHOH	NO_2
1.49	O	S	CH_2	NO_2
1.50	S	O	CO	NO_2
1.51	S	O	CHOH	NO_2
1.52	S	O	CH_2	NO_2
1.53	S	S	CO	NO_2
1.54	S	S	CHOH	NO_2
1.55	S	S	CH_2	NO_2
1.56	S	S	CO	Cl
1.57	S	S	CHOH	Cl
1.58	S	S	CO	CN
1.59	S	S	CHOH	CN

Oligosaccharides containing 5-thiosugars were first synthesized in the 1990's. Compounds containing sulfur in the reducing ring **1.60** and **1.61** were synthesized by Wong *et al.*[115,116] using an enzymatic approach. These compounds were used as acceptors in reactions with nucleotide sugar donors and glycosyltransferase enzymes.[117] The first synthesis of a disaccharide containing sulfur in the non-reducing ring was achieved by Yuasa *et al.*[118] The 5-thio-*N*-acetyllactosamine **1.62** derivative was synthesized by a chemo-enzymatic method.[118] The synthesis of methyl 5'-thio-α-isomaltoside **1.63** *via* an acyclic precursor was then reported by Hashimoto *et al.*[119] Compound **1.63** was not hydrolyzed by glucoamylase from *Rhizopus niveus*. The K_d value was determined to be 39 mM, which is comparable to that for isomaltose. A direct chemical synthesis of this type of disaccharide was first carried out in our laboratory at

about the same time by Mehta *et al.*[120] The syntheses of **1.63** and methyl and allyl 5'-thio-α-kojibioside **1.64** and **1.65** were achieved using the novel trichloroacetimidate of 5-thio-α-D-glucopyranose as the glycosyl donor. The allyl derivative **1.65** was found to be a poor inhibitor of glucosidase II, but a competitive inhibitor of glucosidase I with a K_i of 2.0 mM.[111]

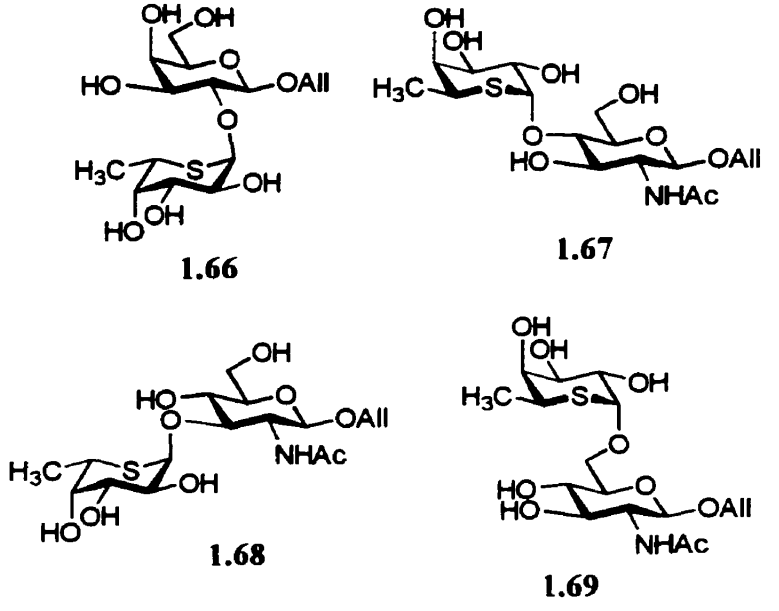
Figure 1.17 **The First Thio-Disaccharide Analogues**



The synthesis of allyl 2-*O*-(5'-thio-α-L-fucopyranosyl)-β-D-galactopyranoside **1.66** using a trichloroacetimidate glycosyl donor was reported and the compound had a K_i of 30 μM against α-L-fucosidase,[121] which is far more effective than the analogue **1.41** containing sulfur in the interglycosidic linkage. This work was extended to include

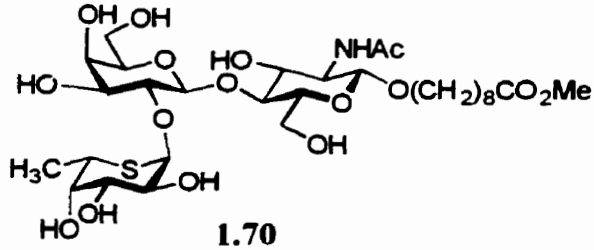
compounds **1.67, 1.68,** and **1.69**, the 5'-thio-analogues of **1.38-1.40**. The 5-thio-compounds were much more potent inhibitors of bovine epididymis α-L-fucosidase, having K_i values of 30-91 μM.

Figure 1.18 **α-L-Fucosidase Inhibitors Containing Sulfur in the Non-Reducing Ring**

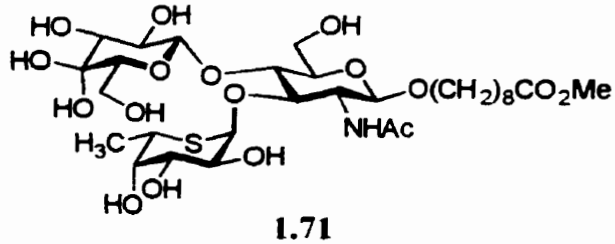


The syntheses of 5-thio-α-L-fucose-containing H-type 2 blood group antigens **1.70** and Lewis X **1.71** were reported, and **1.70** showed strong inhibitory activity against hemagglutination reactions with *Ulex europaeus* lectin I and the H-type 2 trisaccharide-specific monoclonal antibody.[122]

Figure 1.19 **Trisaccharide Analogues of the H-type 2 Blood Group Antigens**



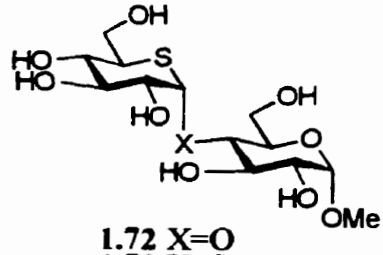
1.70



1.71

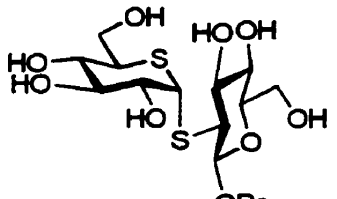
The syntheses of novel methyl maltoside analogues containing sulfur in the non-reducing ring and either oxygen **1.72**, sulfur **1.73** or selenium **1.74** in the interglycosidic linkage were reported and were found to be competitive inhibitors of glucoamylase G2, with K_i values of 1.34, 2.04 and 0.80 mM, respectively.[123]

Figure 1.20 **Novel Heteroanalogues of Methyl Maltoside**



1.72 X=O
1.73 X=S
1.74 X=Se

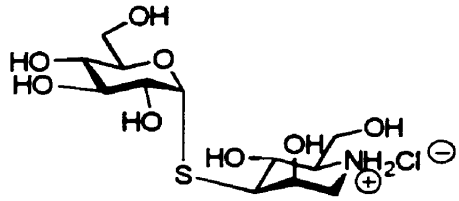
The synthesis of the dithio analogue of *n*-propyl kojibioside **1.75** as a potential competitive inhibitor of glucosidase I was also achieved in our laboratory.[124]



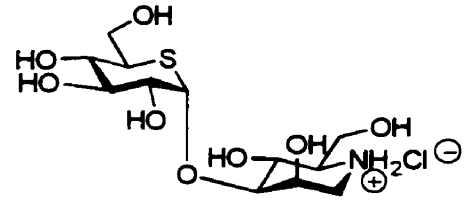
1.75

The syntheses of 1-deoxy-3-*S*-(1-thio-α-D-glucopyranosyl)-mannojirimycin **1.76** and 1-deoxy-3-*O*-(5-thio-α-D-glucopyranosyl)-mannojirimycin **1.77** were reported as potential *endo*-α-D-mannosidase inhibitors.[125] Another study by Izumi *et al.* reported the synthesis of 1-deoxy-3-*O*-(5-thio-α-D-glucopyranosyl)-mannojirimycin **1.78**, methyl 3-*O*-(5-thio-α-D-glucopyranosyl)-5-thio-α-D-mannopyranoside **1.79**, and methyl 3-*O*-(5-thio-α-D-glucopyranosyl)-α-D-mannopyranoside **1.80**.[126] The disaccharide analogues of α-D-Man-(1→2)-α-D-Man-OMe **1.81-1.83**, containing sulfur in the ring and/or interglycosidic linkage, as potential mannosidase Class I enzymes were reported by Johnston and Pinto.[127]

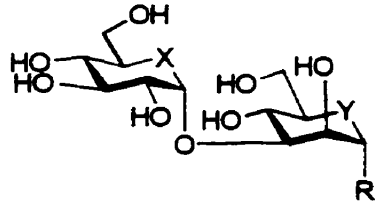
Figure 1.21 Potential Mannosidase Inhibitors



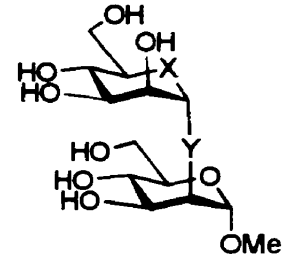
1.76



1.77



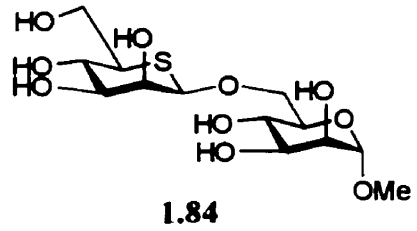
1.78 X=S, Y=NH, R=H
1.79 X=S, Y=S, R=OMe
1.80 X=S, Y=O, R=OMe



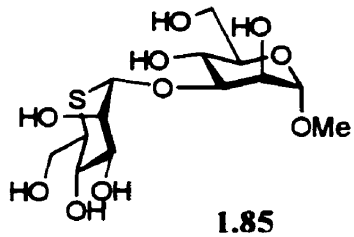
1.81 X=S, Y=S
1.82 X=S, Y=O
1.83 X=O, Y=S

A series 5-thiomannose-containing oligosaccharides **1.84-1.88** were synthesized and their ability to bind to Concanavalin A were determined.[128] The results showed that replacement of the ring oxygen atom with a sulfur atom caused a decrease in binding affinity for ConA.

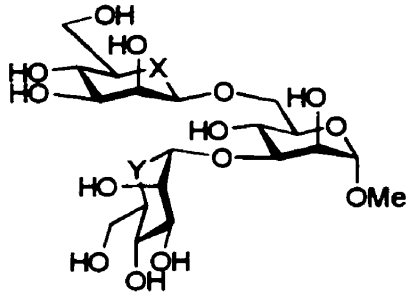
Figure 1.22 5-Thiomannose-Containing Oligosaccharides



1.84



1.85



1.86 X=S, Y=O
1.87 X=O, Y=S
1.88 X=S, Y=S

In summary, 1-thioglycosides and 5-thioglycosides have been shown to be effective glycosidase inhibitors.

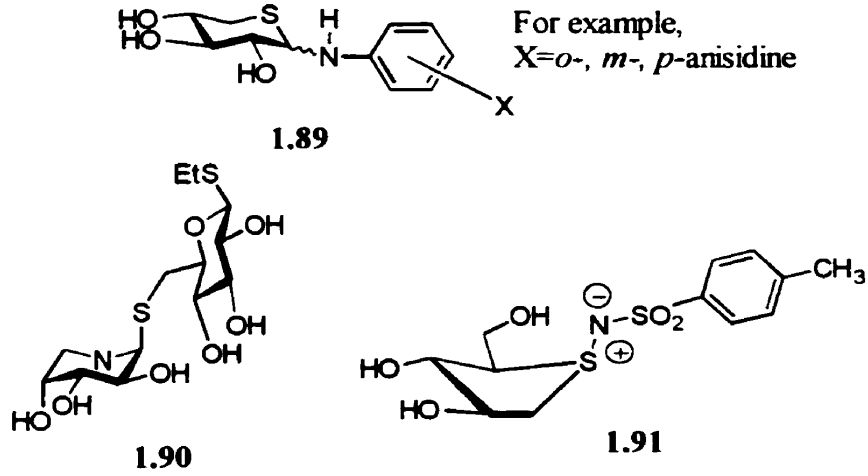
1.3.4 Analogues with Sulfur in the Ring and Nitrogen in the Interglycosidic Linkage

Although the synthesis of *O,N*-acetals has remained elusive, the syntheses of compounds containing sulfur in the ring and nitrogen in the interglycosidic linkage have been reported. Various aryl 5-thio-D-xylopyranosylamines **1.89** (see Figure 1.23) have been obtained from the acid catalyzed reaction of 5-thio-D-xylopyranose and the appropriate arylamine.[129] It was reported that these compounds were stable at room temperature. No assignment of the stereochemistry at the anomeric center was reported, but all showed large negative optical rotations, which suggested the presence of the β-anomer.

The synthesis of an azapyranosyl disaccharide with a 1,2-*cis*-linked thioglycosidic linkage **1.90** was reported.[130] This compound was found to be stable at low pH, but to hydrolyze rapidly at pH > 5.

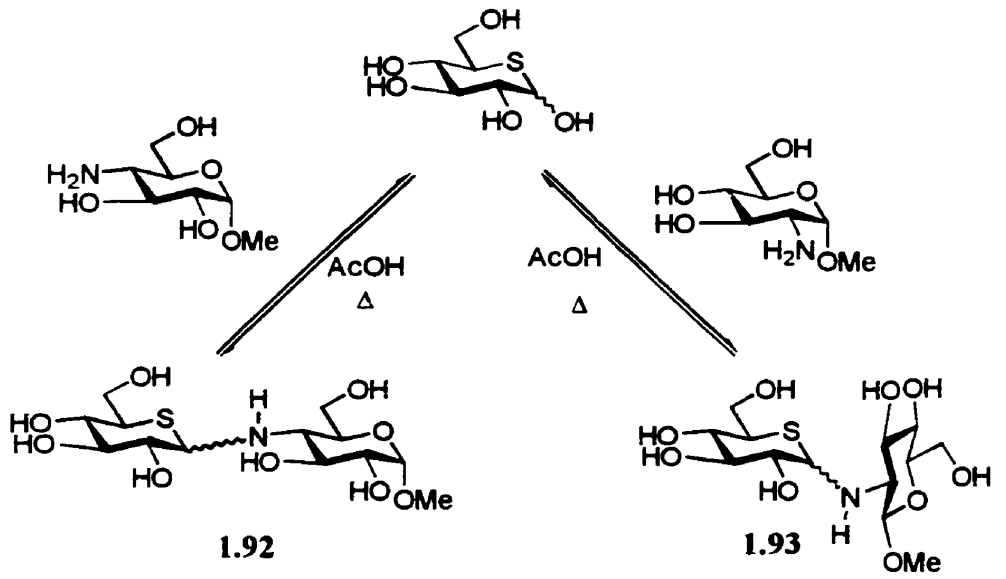
The synthesis of an iminothiasugar **1.91** was carried out with the expectation that this compound would be a transition-state analogue.[131] The five-membered ring would serve as a mimic of the half-chair conformation of the transition-state, while the charge separation of the S-N bond would mimic the transition-state of the glycosidic bond cleavage. It was thought that the sulfur atom would be near the anomeric carbon of a pyranoside because of the longer C-S bond (1.8 Å) and the C-S-C angle (95°) and that the aromatic ring would mimic the hydrophobic face of the aglycon sugar in the transition-state. Unfortunately, the compound was not a good inhibitor of β-glucosidase and showed no inhibition when tested with other enzymes.

Figure 1.23 **Various Heteroanalogues Containing Sulfur and Nitrogen**



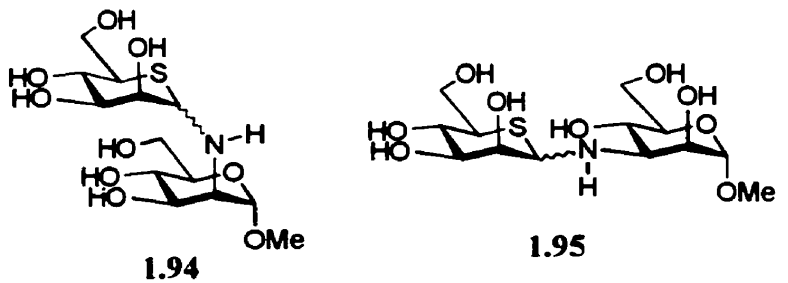
The compounds methyl 5'-thio-4-*N*-α-maltoside **1.92α**, methyl 5'-thio-4-*N*-α-cellobioside **1.92β**, methyl 5'-thio-2-*N*-β-kojibioside **1.93α**, and methyl 5'-thio-2-*N*-β-sophoroside **1.93β** were synthesized in our laboratory (Scheme 1.1).[132] The acetic acid-catalyzed condensations of the free amine and the free sugar in refluxing methanol proceeded in good yields. The α:β ratios were 1:2.5 for both **1.92** and **1.93** after equilibration for three days in aqueous solution. Compound **1.92α** was tested as an inhibitor of GA and was found to be a competitive inhibitor of maltose binding by GA 2, with a K_i of 4 μM,[132] making a it better inhibitor than any of the series of α-(1→4)-linked 5-thiopyranosyl analogues (**1.72-1.74**). Compound **1.92α** was also shown to be an effective competitive inhibitor of barley malt high pI α-glucosidase with a K_i of 0.9 μM.[133] Compound **1.93α** was tested as an inhibitor of glucose release from the structure α-D-Glc-(1→2)-α-D-Glc-(1→3)-α-D-Glc-O$(CH_2)_8CO_2CH_3$ by glucosidase I and II. Compound **1.93α** was determined to be a weak competitive inhibitor of glucosidase I, with a K_i of 3 mM, and a potent competitive inhibitor of glucosidase II, with a K_i of 30 μM.[111] These results reinforce the importance of a basic substituent at C-1' for effective binding in the enzyme active site.

Scheme 1.1



Another study in our laboratory involved the synthesis of methyl 2-amino-2-deoxy-2-*N*-(5-thio-α/β-D-mannopyranosyl)-α-D-mannopyranoside **1.94** and methyl 3-amino-3-deoxy-2-*N*-(5-thio-α/β-D-mannopyranosyl)-α-D-mannopyranoside **1.95** (Figure 1.24).[134] The compounds were synthesized using mercuric chloride catalysis in refluxing methanol. These compounds were synthesized as potential inhibitors of the processing mannosidase class I and class II enzymes.

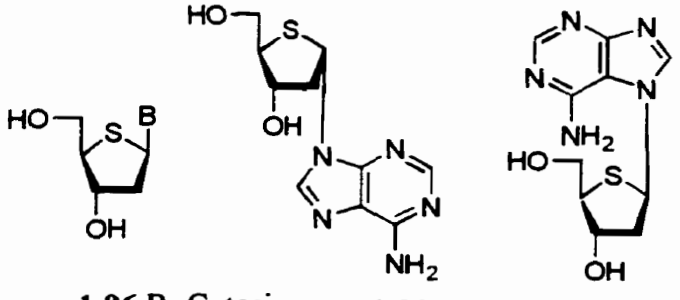
Figure 1.24 ***S,N*-Disaccharides Containing 5-Thiomannose**



In summary, the heteroanalogues of disaccharides containing sulfur in the ring and nitrogen in the interglycosidic linkage appear to be promising candidates as inhibitors of glucosidases.

The 4'-thio analogues of 2'-deoxynucleosides have been extensively investigated as potential antiviral agents.[135,136,137,138] Several of these compounds, including **1.96**, were found to have anti-viral activity against herpes simplex 1 and human cytomegalovirus in cell culture.[136] Compounds A recent study compared several 4'-thio analogues **1.96-1.100** with their oxygen counterparts.[139] It was found that the 4'thionucleosides were more stable towards acidic hydrolysis than the corresponding oxygen compounds.

Figure 1.25 Analogues of Nucleosides



1.96 B=Cytosine
1.97 B=Guanine
1.98 B=Adenine

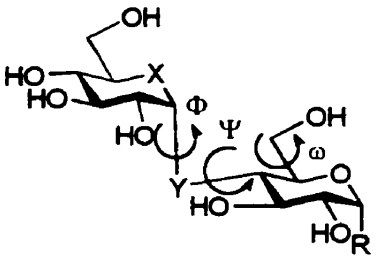
1.99

1.100

1.4 Conformational Analysis of Oligosaccharides Containing 5-Thiosugars

An understanding of the interactions between proteins and carbohydrates is dependent on the conformations of oligosaccharides.[140] The conformational analysis of oligosaccharides can be based on data from X-ray (or neutron) diffraction studies, chiroptical methods or NMR spectroscopic data. The use of diffraction studies is limited by the fact that many oligosaccharides are difficult to crystallize and that the conformation that the molecule adopts in the solid phase may not be the conformation selected by the protein in solution. Chiroptical methods result in information about the molecule in solution, but these methods work best when the compounds are simple repeating oligo- or polysaccharides. The use of NMR data enables the analysis of the conformation of complex oligosaccharides.[140,141] Information obtained from ^{1}H NMR data includes chemical shifts, coupling constants, spin lattice relaxation rates and nuclear Overhauser enhancements. The use of 2D experiments including COSY, TOCSY, and ^{13}C-^{1}H correlations enables the complete assignment of the ^{1}H and ^{13}C NMR signals. The coupling constants can be used to confirm the individual ring conformations, while the use of 1D and 2D NOESY experiments give information about through space connectivity to determine which protons are close in space. The pyranose forms of sugars normally exist in well-defined chair conformations in solution, but still have flexibility about the glycosidic linkages (Φ, Ψ) and the exocyclic C-C bond (torsion angle ω).[141]

Figure 1.26 Relevant Torsion Angles in Pyranose Rings

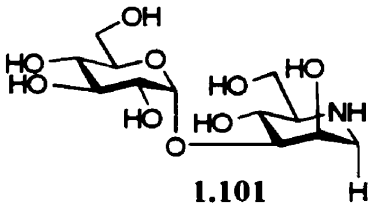


The NOE contacts obtained from NMR data help to determine the conformations about the glycosidic linkages, as well as the positioning of the individual monosaccharides in relation to each other. The data obtained through NMR experiments can be enhanced through the use of theoretical calculations.[141] The use of transferred NOE (trNOE) experiments has emerged as a valuable tool for studying carbohydrate-protein complexes.[142,143] The conformation of a ligand bound to a protein receptor can be determined using trNOE experiments. This technique relies upon fast exchange (fast on the time scale of spin-lattice relaxation) of free and bound ligand molecules. A carbohydrate ligand bound to the protein receptor will have a slower relaxation rate than a free ligand in solution because relaxation is governed by the protein's tumbling time τ_c, resulting in strong negative NOEs, so called trNOEs, and these reflect the bound conformation. The conformation of the protein is not able to be determined using this techinique because the molecular weights of these receptors are usually outside the limits of NMR spectroscopic analysis (ca. 30 kDa).[141]

The conformational analysis of the kojibioside analogue **1.65** (Figure 1.17) using NOE spectroscopy, in conjuction with molecular mechanics calculations, indicated that the disaccharide populated two conformations, the global minimum structure to the extent

of 90% and a local mimimum to the extent of 6%.[120b] An X-ray crystal structure indicated that the solid state conformation was similar to the global minimum found in solution.[120b]

A molecular modeling study of **1.78** (Figure 1.21) and its oxygen congener **1.101** found that the compounds existed in two and three clusters on the potential energy maps, respectively, with the largest cluster for each compound, 87% for **1.101** and 96% for **1.78**, containing the global minimum.[126] The global minimum structures for both compounds were superimposed using only the functional groups essential for inhibitory activity[144] and the results suggested that both disaccharides exist with these functional groups in similar positions.



1.101

The conformational analysis of the three methyl maltoside heteroanalogues **1.72, 1.73,** and **1.74** (Figure 1.20), was performed using high quality NOE data and molecular mechanics calculations.[145] The theoretical NOE data were compared with the experimental data. The gross conformational features of all three compounds were similar and the compounds were found to mainly populate two conformational regions of the potential energy maps. These regions were equivalent to those found for the parent maltose. The experimental NOE data and the theoretical energy differences and population distributions showed that the substitution of oxygen with sulfur or selenium

results in an increase in the flexibility about the interglycosidic linkage in the latter compounds.

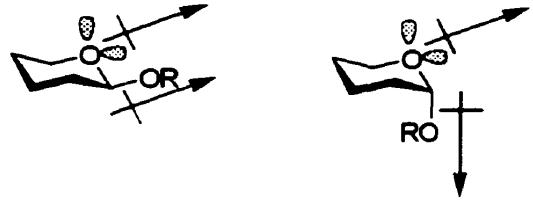
The complexes of glucoamylase with maltoside heteroanalogues **1.73** and **1.92α** were studied using trNOE experiments and molecular modeling.[146] A preliminary study on the binding of **1.92** to glucoamylase showed that only the α-isomer was bound.[132] It also indicated that the bound conformation was similar to the global minimum energy conformation. In a later study,[146] the NMR data suggested that, although each of the free ligands **1.73** and **1.92α** populated two conformational regions, both heteroanalogues were bound by the enzyme in conformations in the area of the global minimum. These conformations were used as initial points for docking the ligand into the active site of the enzyme taken from an X-ray crystal structure of D-*gluco*-dihydroacarbose **1.12** and GA.[54,55] The structures were then minimized and were found to be in good agreement with the experimental NOE results. The presence of a charged hydrogen bond between the carboxylate function of Glu179 and the hydrogen atom of the interglycosidic nitrogen atom in **1.92** accounted for the greater inhibitory potency of the S,N compound **1.92** as compared to the S,S analogue **1.73**.

In summary, the conformational analysis of free and bound ligands is important for the understanding of protein-ligand interactions. The data obtained from both theoretical and experimental methods are useful for the rational design of inhibitors. The results of the study by Weimar *et al.*[146] indicate that the combined use of transferred NOE spectroscopy and molecular modeling based on X-ray crystal structures of complexes of similar ligands will be useful for the rapid analysis of ligand-receptor pairs.

1.5 Anomeric Effect

The preference for electronegative substituents to adopt the axial orientation at the anomeric carbon of a pyranose ring is in marked contrast to the steric effects that cause substituents to be equatorial in cyclohexane derivatives. This anomalous behaviour was first noted by Edward[147] during his study of the relative stability of methyl-α- and β-glycopyranosides to acid hydrolysis. It was subsequently defined as the anomeric effect by Lemieux and Chü[148] as a result of their investigations of the anomeric equilibria of peracetylated pento- and hexo-pyranoses. Since then, extensive literature has appeared on studies of the anomeric effect.[149] The explanation originally offered by Edward and Lemieux involves destabilizing dipole interactions of the unpaired electrons of the ring oxygen and the C1-O polar bond when the electronegative substituent is equatorial as opposed to axial (see Figure 1.27), thus making the equatorial isomer less stable.

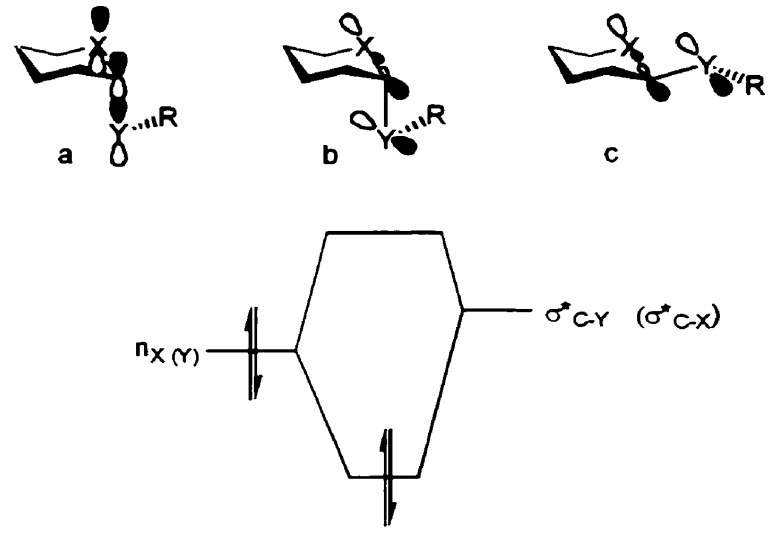
Figure 1.27 Dipole-Dipole Interactions in the Axial and Equatorial Conformers



The development of molecular orbital theory led to a stereoelectronic explanation of the anomeric effect. The widely accepted explanation is that a stabilizing interaction between the p-type lone pair (n_p) on the ring heteroatom and the σ* orbital of the exocyclic C-Y bond ($n_p \rightarrow \sigma^*_{C-Y}$) occurs in an X-C-Y fragment (Figure 1.28 a).[150] This

explains the observed shortening of the C1-X bond and lengthening of the C1-Y bond when the Y substituent is axially oriented. This effect has been termed the endo-anomeric effect.[151] The exo-anomeric effect refers to the preference for the gauche conformation around the X-C-Y-R aglyconic bond of glycopyranosides that permits interaction of the p-type orbital of the exocyclic oxygen with the σ* orbital of the C1-X bond ($n_p \rightarrow \sigma^*_{C\text{-}X}$, see Figure 1.28 b,c).[152]

Figure 1.28 Endo- and Exo-Anomeric Effect

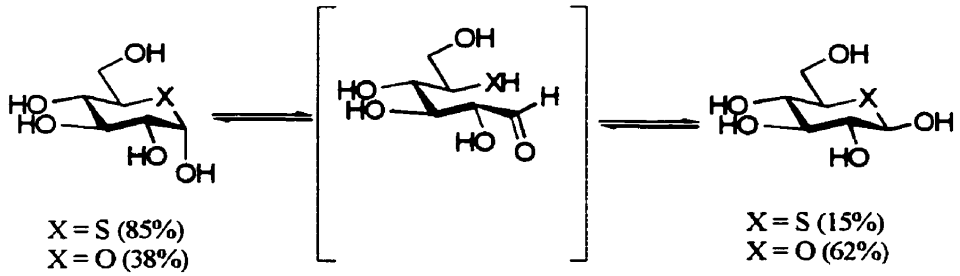


The endo- and exo-anomeric effects were found to be general effects operating in W-X-A-Y segments, where X and Y are heteroatoms or halogens (*e.g.* O, N, S, Se, Te, Cl, F, Br, I), and W and A are atoms of intermediate electronegativity.[153a-h] The bond length and bond angle variations in these types of compounds are also adequately explained within the framework of these conformational effects.[153i,j]

1.5.1 Anomeric Effect in Thiosugars

The anomeric effect in thiosugars as compared to the ring oxygen counterparts has been the subject of some concern. For example, observations of the equilibria of 5-thioglucopyranose vs. glucopyranose indicated that the α-isomer of 5-thioglucopyranose is preferred in aqueous solution (85%, Scheme 1.2), whereas the β-isomer of glucopyranose is preferred in aqueous solution (α-isomer only 38%, Scheme 1.2).[154] However, the steric effects in the axial conformer of thiosugars should be less than in the oxygen counterparts due to the longer C-S bond, and the increased proportion of the α-isomer in the 5-thiosugar could be due to the lesser steric effect.[155] The A values for 2-Me-tetrahydropyran[156] and 2-Me-tetrahydrothiapyran[157] are 2.86 ± 0.20 kcal mol^{-1} and 1.42 ± 0.07 kcal mol^{-1}, respectively.

Scheme 1.2



In order to address this question, Pinto and Leung[158] studied the conformational equilibria of 2-methoxytetrahydrothiapyran **1.102** compared to 2-methoxy-tetrahydropyran **1.103**. The conformational free energy differences (ΔG°) of the equilibria shown in Figure 1.29 were assessed experimentally and theoretically. The

$-\Delta G^{\circ}$ values are 1.53 kcal mol^{-1} and 0.46 kcal mol^{-1}, for **1.102** and **1.103**, respectively, showing that the axial conformers of both **1.102ax** and **1.103ax** are favoured. The individual steric ($\Delta G^{\circ}_{steric}$), electrostatic ($\Delta G^{\circ}_{elect}$), and orbital interaction (ΔG°_{orb}) components of the composite conformational effects in **1.102** and **1.103** (Table 1.1) were calculated using a combination of experimental and theoretical approaches.

Figure 1.29 Equilibrium of 2-Methoxytetrahydrothiapyran 1.102 and 2-Methoxytetrahydropyran 1.103

1.102ax X = S
1.103ax X = O

1.102eq X = S
1.103eq X = O

Table 1.1 Component Analysis of Conformational Effects

Compound	$\Delta G^{\circ}_{steric}$ [a]	ΔG°_{elect} [a]	ΔG°_{orb} [a,b]
1.102	0.35	-0.63	-1.25
1.103	0.77	-0.81	-0.42

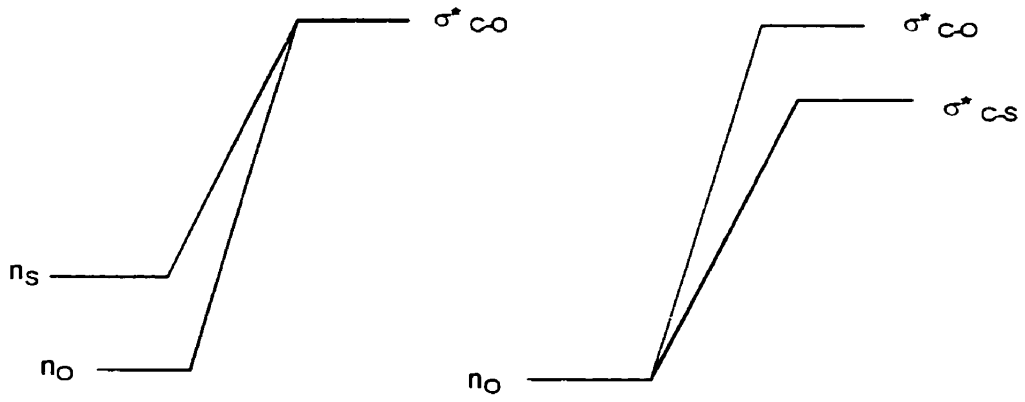
[a] in kcal mol^{-1}

[b] $\Delta G^{\circ}_{orb} = \Delta G^{\circ}_{Total} - (\Delta G^{\circ}_{steric} + \Delta G^{\circ}_{elect})$

It was found that the ΔG°_{elect} and ΔG°_{orb} favored the axial conformer in both compounds. The authors concluded that the sum of the endo $n_X \rightarrow \sigma^*_{CO}$ and exo $n_O \rightarrow \sigma^*_{CX}$ interaction in the axial conformer was more stabilizing than the exo anomeric $n_O \rightarrow \sigma^*_{CX}$ interaction in the equatorial conformer. There was a greater orbital interaction component (endo- and exo-anomeric effect) in **1.102ax** than in **1.103ax**. The larger

ΔG°_{orb} in **1.102ax** could result from a greater endo $n_S \rightarrow \sigma^*_{CO}$ interaction due to the higher lying S lone pair (n_S) orbital than n_O orbital; however, this might be offset by the lesser overlap between n_S and σ^*_{CO} orbitals. Alternatively, the more stabilizing ΔG°_{orb} in **1.102ax** could result because of the lower lying σ^*_{CS} orbital which leads to a greater exo $n_O \rightarrow \sigma^*_{CS}$ interaction (Figure 1.30). The latter explanation is the more likely one given the relative magnitudes of anomeric effects with first-row *vs.* second row elements.[153]

Figure 1.30 A Schematic Orbital Interaction Diagram of the Endo- and Exo-Anomeric Effects in 1.102 and 1.103

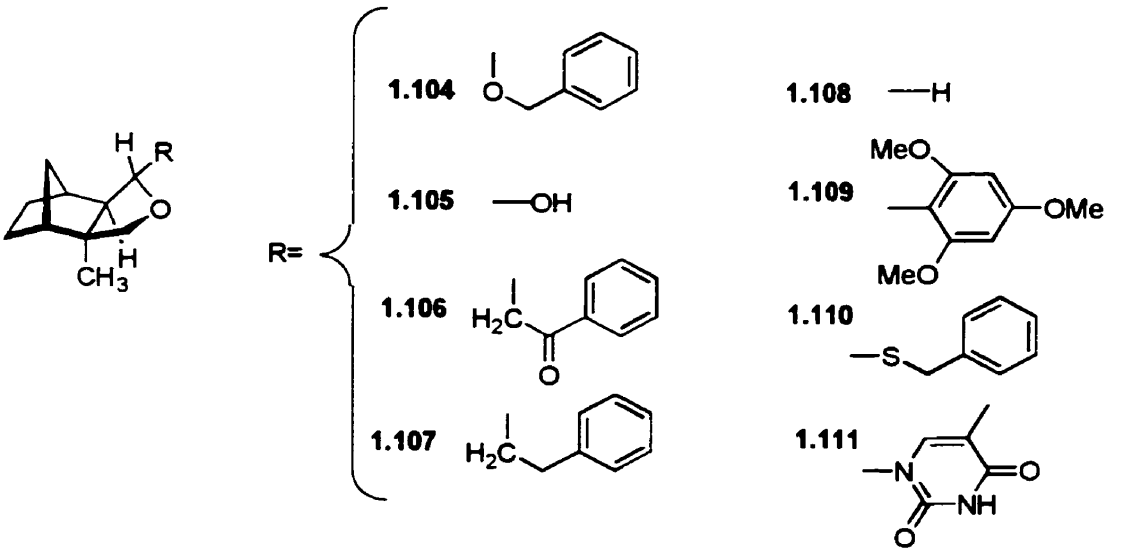


1.5.2 Anomeric Effect in Furanose Sugars

The anomeric effect in furanose sugars has not been studied as widely as in the pyranose systems, but evidence does suggest the presence of an anomeric effect.[159] Due to the inherent flexibility in the furanosyl residues, the consequences of the AE are much less dramatic. A number of studies on furanosides involve nucleosides and nucleotides,[160] where there is good evidence from conformational preferences and patterns of bond lengths that have been attributed to the anomeric effect. A recent report by Ellervik and Magnusson[161] using conformationally restricted *O-*, *C-*, *S-*, and *N-*

"furanosides" (see Figure 1.31) found that an anomeric effect is operating in the *O*- (**1.104**) and *S*- (**1.110**) furanosides. The furanoside carbons are kept in the same plane by the norbornane skeleton; the two conformations dictate pseudoaxial and pseudoequatorial positions for the R group. These equilibria were investigated by various NMR methods, X-ray crystallography, and molecular mechanics calculations. The most relevant observation is that **1.104** preferred the pseudoaxial position, while the *C*-"glycoside" **1.107** preferred the pseudoequatorial position.

Figure 1.31 Conformationally Restricted "Furanosides"

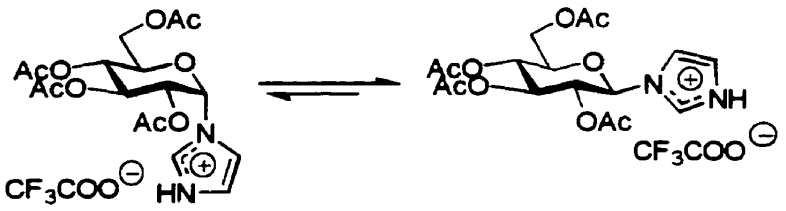


1.6 The Reverse Anomeric Effect (RAE)

In recent years, another conformational effect, the reverse anomeric effect (RAE), has also gained notoriety and has provoked some debate wherein even its very existence

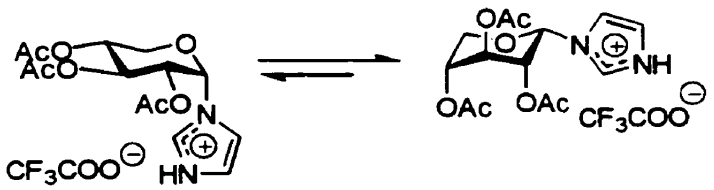
has been questioned. This effect was defined by Lemieux and Morgan[162] as the tendency of an aglycon bearing a positive charge in a sugar ring to adopt the equatorial orientation; the systems under study contained quaternary nitrogen aromatic substituents such as pyridinium and imidazolium (e.g. Scheme 1.3).

Scheme 1.3



The work was later extended by Paulsen *et al.*[163] to the study of the conformational equilibria in peracetylated pentopyranosyl imidazoles and the corresponding protonated species (e.g. Scheme 1.4), and by Finch and Nagpurkar[164] to the neutral and positively charged *N*-(hexopyranosyl)imidazoles and their tetraacetates. It has also been suggested that neutral and protonated amino and alkylamino substituents would show a RAE,[165] but this has been questioned.[158,166]

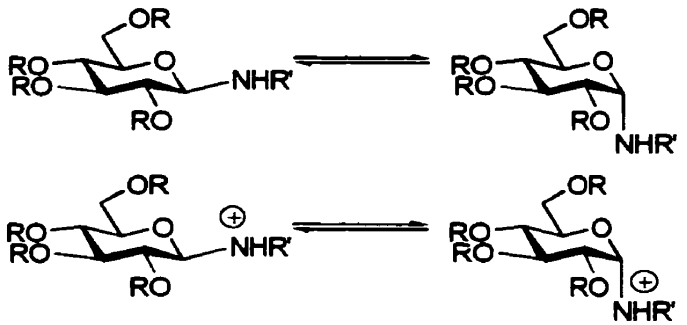
Scheme 1.4



1.6.1 Experimental and Theoretical Results Concerning the RAE

The greater equatorial preferences have been attributed to accentuated steric effects, although the conclusions are based on data from highly biased equilibria.[158,166a] Perrin and Armstrong studied the equilibria of a series of glucopyranosylammonium ions by ^{1}H NMR in a variety of solvents, including acidic media. It was found that the proportion of axial conformer was slightly larger upon protonation (Scheme 1.5), indicating an enhanced anomeric effect.

Scheme 1.5



R = H, R' = H, Me, Et, Bu
R = Ac, R' = H, Me, Et, Bu

A recent study with a more sterically balanced, 2,2'-substituted-1,3-dioxane system, confirmed these conclusions.[166f] The general picture that is emerging from recent studies is that protonation of either an alkylamino substituent or an imidazole substituent results in a stronger anomeric effect,[166] and that the equatorial preference has its origin in favorable electrostatic interactions.[167] Nevertheless, the conclusions were not universal and appeared to be system-dependent. Thus, whereas Perrin and coworkers[166a,b] claimed an absence of a RAE with protonated *N*-(glucopyranosyl)imidazoles (Scheme 1.3), by

examination of configurational equilibria using an NMR titration method, the Queen's group[167] claimed the existence of such an effect in protonated *N*-(xylopyranosyl)imidazoles (Scheme 1.4), by examination of conformational equilibria using an approximate average coupling-constant method, which they attributed to intramolecular electrostatic attraction in the equatorial isomers. Such subtle differences indicate that the magnitude of the measured effect is small.

The matter has been resolved very recently by use of an NMR titration method to estimate the RAE in protonated *N*-(xylopyranosyl)imidazoles (Scheme 1.4).[166g] An α:β mixture of the *N*-(xylopyranosyl)imidazole was dissolved in different NMR solvents and aliquots of acid were added until there was no further change in the ^{1}H NMR chemical shifts. The H-1 and H-2' signals of the sugar residue and imidazole, respectively, underwent large shifts upon protonation. The extent of protonation of each isomer was monitored using these signals. The results showed that the $\Delta\Delta G^{\circ}_{\beta\rightarrow\alpha}$ was generally <1, corresponding to a greater preference of the protonated substituent for the axial position, than in the neutral compounds. The authors concluded that no RAE exists in these compounds and that the previous conclusions[163,164,167b] resulted because of the approximations inherent in the average coupling-constant method.[166g]

PART II

Part II of the introduction describes aspects related mainly to Chapters 4-7.

1.7 The Biological Roles, Biosynthesis and Chemical Synthesis of Oligosaccharides Containing Furanose Sugars

Many bacteria,[168] fungi,[169] and protozoa[170] synthesize oligosaccharide chains that contain furanosyl residues. Glycoconjugates containing the furanosyl residues are generally found in the cell wall and play critical roles in the survival and pathogenicity of microorganisms in diseases such as tuberculosis and leprosy.[168] The protozoa of the family Trypanosomatidae are responsible for several infectious diseases, including Chagas disease,[170] while the protozoa from the genus *Leishmania* are the causative agents of leishmaniasis.[171]

1.7.1 Glycosylphosphatidylinositol Anchors (GPIs) and Glycosylinositolphospholipids (GIPLs)

Many glycoconjugates have been found to be anchored to the plasma membrane *via* glycosylphosphatidylinositol (GPI) anchors.[172] These structures are found in organisms ranging from yeast to man, but are much more prominent in lower eukaryotes, such as protozoa.[173, 174] All of the GPI anchors which have been characterized to date contain an identical ethanolamine-phosphate-α-Man-(1→2)-α-Man-(1→6)-α-Man-(1→4)-α-Glc*N*-(1→6)-*myo*inositol backbone (Figure 1.32), suggesting that this sequence is likely to be conserved in all GPI anchors. In protozoa, GPI anchors have been widely studied in their role as anchors for cell surface proteins. In the parasitic protozoa most of the major cell-surface proteins are GPI-anchored.

The protozoa of the family Trypanosomatidae are divided into nine genera based on the developmental stages in their life cycles (Table 1.2). The developmental stages are defined according to body shape, emergence of the flagellum, and relative positions of the kinetoplast and nucleus.[175] These forms are: promastigotes, opisthomastigotes, epimastigotes, choanomastigotes, trypomastigotes and amastigotes. The characterization of cell surface glycoconjugates in the various developmental stages of these parasites is important because there is evidence that some of these glycoconjugates are essential for the survival and infectivity of the parasite. Some of these structures are known to be involved in parasite protection[174,176] or specific host-parasite interactions.[177,178]

Figure 1.32 Comparison of GPI Structures

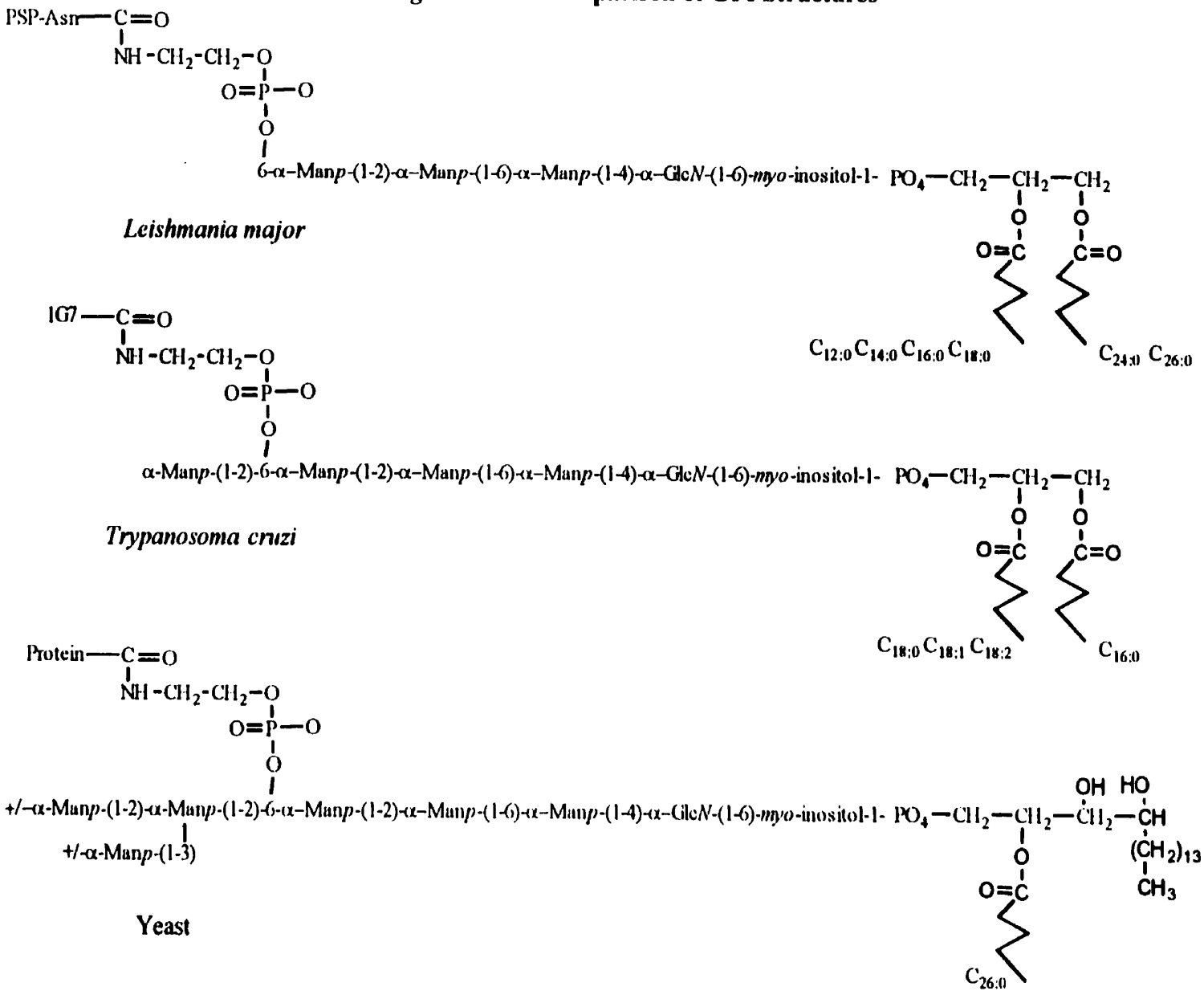
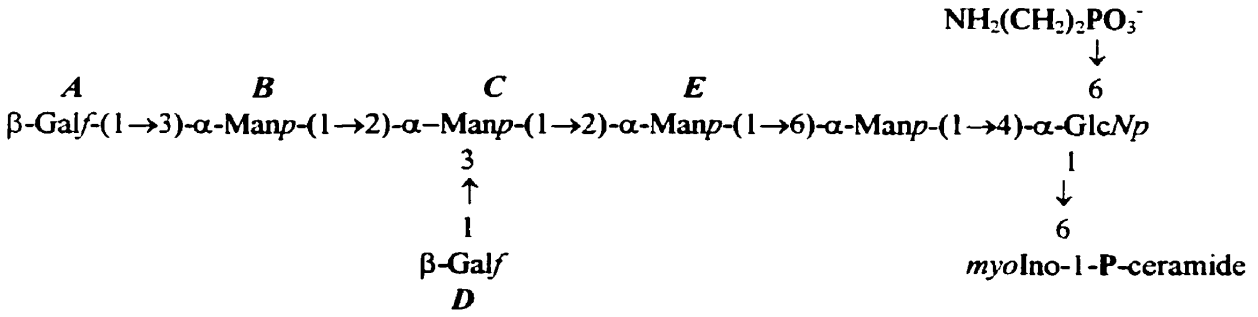


Table 1.2 The Nine Genera of Trypanosomatids

Type	Genus	Host (vector)	Developmental Stages
1	*Trypanosoma*	Vertebrates (insects)	trypo-, epi- and amastigote
	Leishmania	Vertebrates (insects)	pro- and amastigote
	Endotrypanum	Vertebrates (insects)	pro- and epimastigote
2	*Phytomonas*	Plants (insects)	promastigote
3	*Herpetomonas*	Insects	pro- and opisthomastigote
4	*Crithidia*	Insects	choanomastigote
5	*Blastocritidia*	Insects	epimastigote
	Leptomonas	Insects and protozoa	promastigote
6	*Rhynchoidomon*	Insects	trypomastigotes

Several protozoa also synthesize unique GPI derivatives which are not covalently linked to protein or modified by additional glycoconjugates. These low molecular weight structures, referred to as glycosylinositolphospholipids (GIPLs) are included as members of the GPI family by virtue of the core sequence α-Man-(1→4)-α-Glc*N*-(1→6)-*myo*inositol.[173] Galactofuranose (Gal*f*) is present as a constituent of these GIPLs in protozoa,[179] bacteria,[180,181] and fungi.[182] These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection.[183] It is known that Gal*f* is part of the oligosaccharide core of the GIPL from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[184] The GIPL structure of *T. cruzi* contains the same tetrasaccharide core sequence as the protein-bound GPI anchors, but diverges from the protein anchors beyond this sequence. The GIPL contains up to two additional β-Gal*f* residues and there is a 2-aminoethylphosphonic acid group located at the C-6 position of the glucosamine residue (Figure 1.33). The lipid moiety is a ceramide containing sphinganine and *N*-linked lignoceric ($C_{24:0}$) acid instead of the alkylglycerol found in the protein anchors.[170,173,185]

Figure 1.33 The Protein-Free GIPL of *T. cruzi*



This structure is the most abundant cell surface glycoconjugate present in the insect dwelling epimastigote stage of the *T. cruzi* life cycle.[179] The glycoconjugates on the cell surface during the infectious stage of *T. cruzi* are not modified with galactofuranose; however, it has been shown that the β-D-Gal*f* moiety is recognized by antibodies that inhibit *T. cruzi* internalization into mammalian cells.[183] Recently, it was demonstrated that *T. cruzi* GIPLs were able to block T-lymphocyte activation.[186] Thus, interaction between host cellular defense mechanisms and the GIPLs of *T. cruzi* may play a role in establishment and maintenance of chronic infection.[187]

Galactofuranose has also been isolated from another class of GIPLs found in *Leishmania*.[188] These GIPLs differ from those of *T. cruzi* by having a glycerolipid instead of a ceramide and the β-D-Gal*f*-(1→3)-Man*p* moiety as an internal unit in the oligosaccharide core. Glycoconjugates containing galactofuranose have also been found in the GIPLs of *Leptomonas samueli*[189] and *Endotrypanum schaudinni*,[190] both of which contain a ceramide lipid. The Gal*f* moiety is the terminal non-reducing sugar in *L. samueli* and it is internal in *E. schaudinni*. In all of the above examples, the

galactofuranose is linked β-(1→3) to α-Man*p*. This specificity suggests that a β-(1→3)-galactofuranosyltransferase might be involved in the biosynthesis, although a sugar donor has not been identified.[170]

Figure 1.34 GIPLs of Other Protozoa

A)

R-3)-β–Gal*f*-(1→3)-α-Man*p*-(1→3)-α-Man*p*-(1→4)-α-Glc*Np*(1→6)-*myo*Ino-1-P-OCH_2
|
CHOAcyl
|
CH_2Alkyl

R	GIPL	Parasite
H	GIPL-1	*L. major*
α-Gal-(1-	GIPL-2	*L. major and L. mexicana*
α-Gal-(1-6)-α-Gal-(1-	GIPL-3	*L. major and L. mexicana*
β-Gal-(1-3)-α-Gal-(1-	GIPL-A	*L. major*

B)

R_2PO R_2PO
| |
6 6
R_1-β–Gal*f*-(1→3)-α-Man*p*-(1→3)-α-Man*p*-(1→4)-α-Glc*Np*(1→6)-*myo*Ino-1-P-ceramide

Leptomonas samueli R_1=H R_2=H_2N-CH_2-CH_2-

Endotrypanum schaudinni R_1=α-Ara*p*-(1→2)-β-Gal*f*-(1→3)-β-Gal*f*-(1→3)-

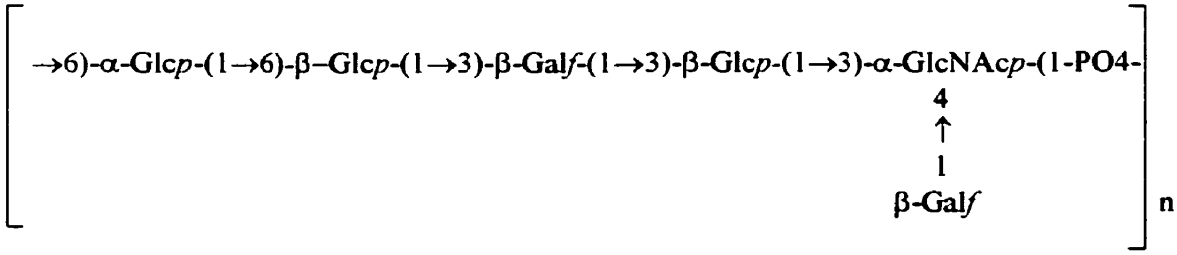
R_2=H_2N-CH_2-CH_2-O-

The acidic glycolipid, purified from *Paracoccidioides brasiliensis*, containing a terminal Gal*f*, has been shown to be reactive with sera of patients infected with paracoccidioidomycosis.[191] The structure of the glycan is believed to be β-Gal*f*-(1→6)-α-Man*p*-(1→3)-β-Man*p*-(1→2)-*myo*inositol. Removal of the β-Gal*f* decreases the reactivity of the sera, suggesting that this residue is immunodominant.

Other polysaccharides isolated from *Penicillium*[169a,c,182] and *Aspergillus*[182,192] contain a side chain composed of β-Gal*f*-(1→5)-β-Gal*f*. A series of Gal*f* disaccharides and oligosaccharides were tested for immunological activity. It was found that β-Gal*f*-(1→5)-β-Gal*f* gave the highest inhibition of the reaction between the polysaccharide antigens and antibodies raised in rabbits. It was also found that the longer the chain of (1→5)-β-Gal*f* residues, the more inhibition was obtained.

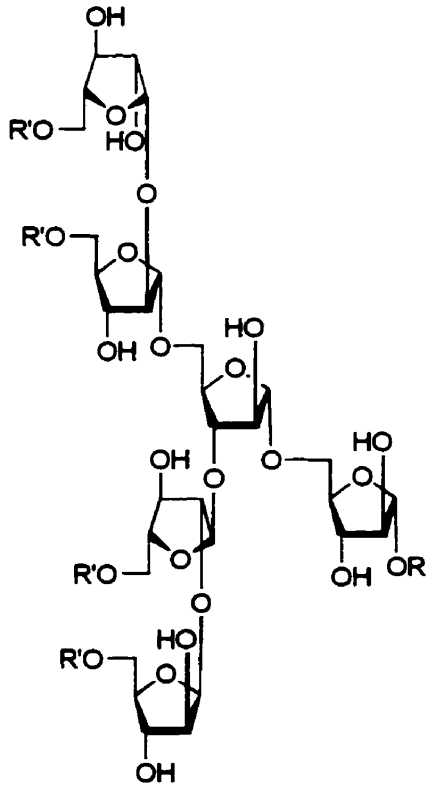
The specific capsular polysaccharide produced by *Streptococcus pneumoniae* type 20 contains both an internal Gal*f* and a branched Gal*f* moiety.[193] The polysaccharide shown in Figure 1.35 is a repeating unit and has been shown to be an effective antigen which can induce an antibody response in humans. This response can provide protection against pneumococcal infections.

Figure 1.35 Repeating Unit of *Streptococcus pneumoniae* Capsular Polysaccharide



The cell-wall arabinogalactans of *Mycobacterium leprae* and *Mycobacterium tuberculosis* contain arabinofuranosyl and galactofuranosyl residues.[180,194] The arabinan region of the cell wall of *Mycobacterium tuberculosis* is shown in Figure 1.36. Mycobacterial diseases are health threats world-wide. They have produced drug resistant strains[195] and new strains, such as *Mycobacterium avium*, are common in AIDS patients.[196] Identification of new antibiotics for these mycobacterial diseases is an area of research that is of current interest.

Figure 1.36 A Hexasaccharide Found at the Non-reducing End of the Arabinogalactan and Lipoarabinomannan of *Mycobacterium tuberculosis*



R = arabinogalactan, R' = mycolic acids

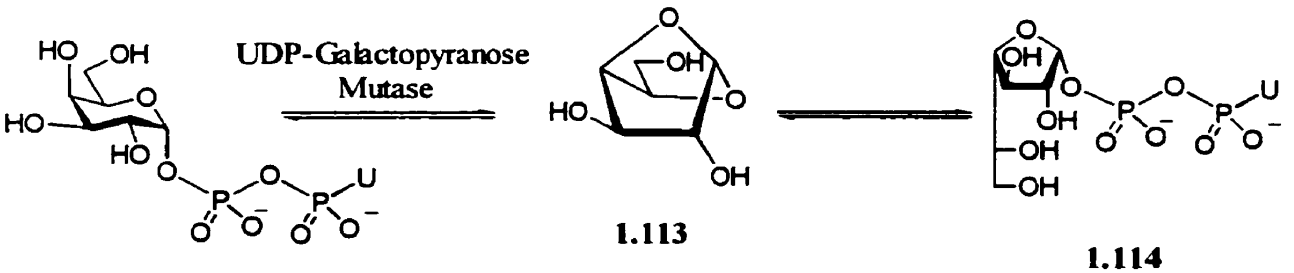
The reason for the use of furanosyl residues by microorganisms is not completely understood, but one postulate is that the flexibility of the furanose rings as compared to pyranose rings enables the side chains to pack together in tight arrays. This may create a near-crystalline structure of side-by-side lipids (attached to these sugars) which is thick and hard to penetrate,[197] but this hypothesis has not yet been tested. The synthesis of oligosaccharides containing furanosyl residues (*e.g.* Gal*f*) may therefore be useful for understanding the role that furanosyl residues play in microorganisms and for studying the biosynthesis of furanosyl-containing glycoconjugates. Compounds containing Gal*f* (or other furanosyl residues) may also be used as inhibitors to probe the development of infections, to develop diagnostic methods, or as vaccines.

1.7.2 Biosynthesis of Gal*f* Containing Glycoconjugates

The biosynthesis of the protozoan GPIs has been studied extensively.[172,183] However, the metabolic pathways by which Gal*f* is incorporated into the polysaccharide chains of GIPLs or removed from these compounds have not been elucidated.[170] Recently, the enzyme UDP-galactopyranose mutase (UDP-galactopyranose furanomutase; EC 5.4.99.9) was cloned by Duncan and co-workers,[198] from *E. coli* K12. This enzyme catalyzes a novel isomerization reaction: the conversion of the glycoside uridine diphosphate galactose from the pyranose form to the furanose form (see Scheme 1.6). This is the first example of this type of conversion occurring in Nature. A mechanistic study by Barlow *et al.*[199] demonstrated that the anomeric C-O bond is broken

during UDP-Gal*p*UDP-Gal*f* interconversion, which leads to the α-UDP-Gal*f*. The putative intermediate of this reaction is the 1,4-anhydrogalactose **1.113**.

Scheme 1.6



1.112 **1.113** **1.114**

U = Uridine

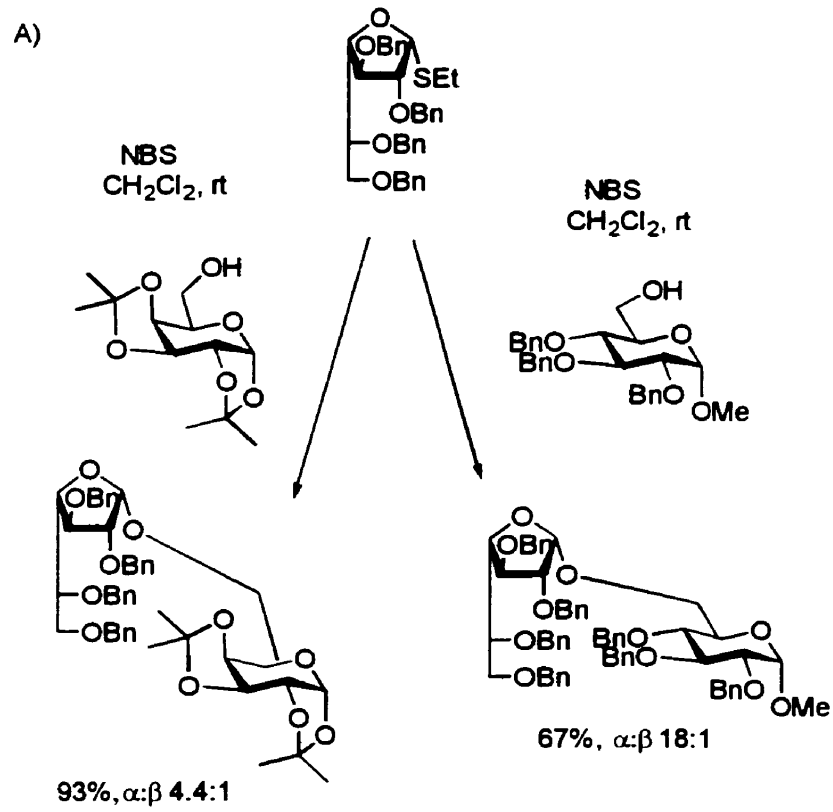
The rational design of inhibitors will be possible with the knowledge of the mechanism of this reaction. Two other mutases UDP-Gal*p* and UDP-Ara*p* from *Klebsiella pneumoniae*[200] and *Mycobacterium tuberculosis*,[201] respectively, have been cloned. The latter may help in deciphering the biosynthesis of the mycobacterial arabinan. It is known that arabinofuranosyl residues are derived from UDP-Ara*p* in grasses.[202]

1.7.3 Synthesis of Oligosaccharides Containing Furanosyl Moieties

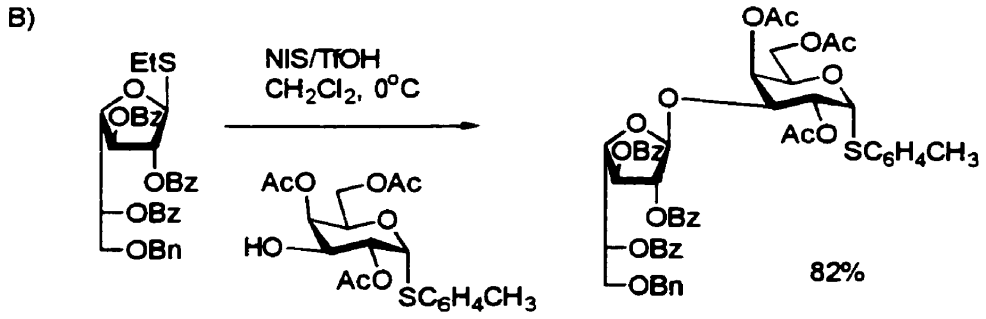
Oligosaccharide synthesis involving furanosyl glycosyl donors has not been studied to the same extent as with pyranosyl donors, but methods have been developed that employ thioglycosides (Scheme 1.7),[203] *n*-pentenyl glycosides (Scheme 1.8),[204,205] anomeric benzoates (Scheme 1.9),[206] anomeric xanthates (Scheme 1.10),[207] trichloroacetimidates (Scheme 1.11),[208,209] and selenoglycosides (Scheme 1.12).[210,211] In

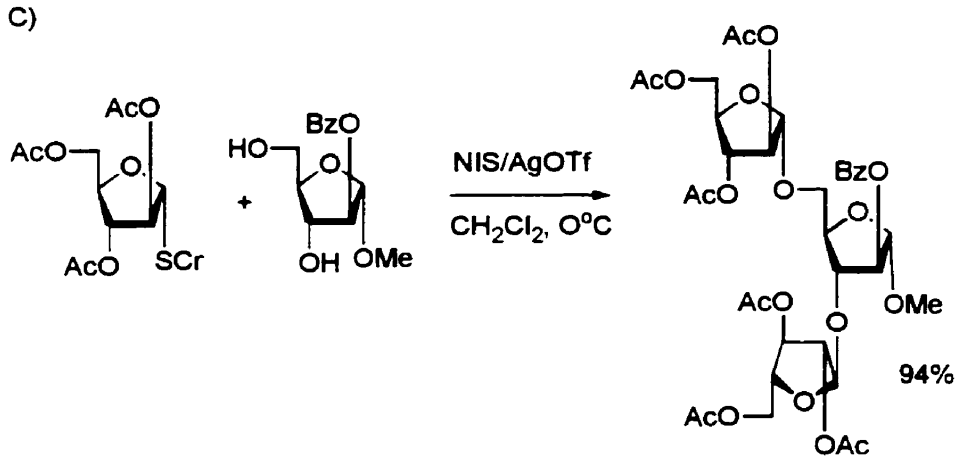
addition, an indirect approach to galactofuranosyl-containing disaccharides involving acyclic glycosyl donors has recently been reported.[212] The syntheses of α-D-galactofuranose-containing oligosaccharides have not been as widely investigated as those of the β-anomers, but have been achieved in high yields, using ethyl 2,3,5,6-tetra-*O*-benzyl-α-D-thiogalactofuranoside and *N*-bromosuccinimide (Scheme 1.7, A).[203a]

Scheme 1.7

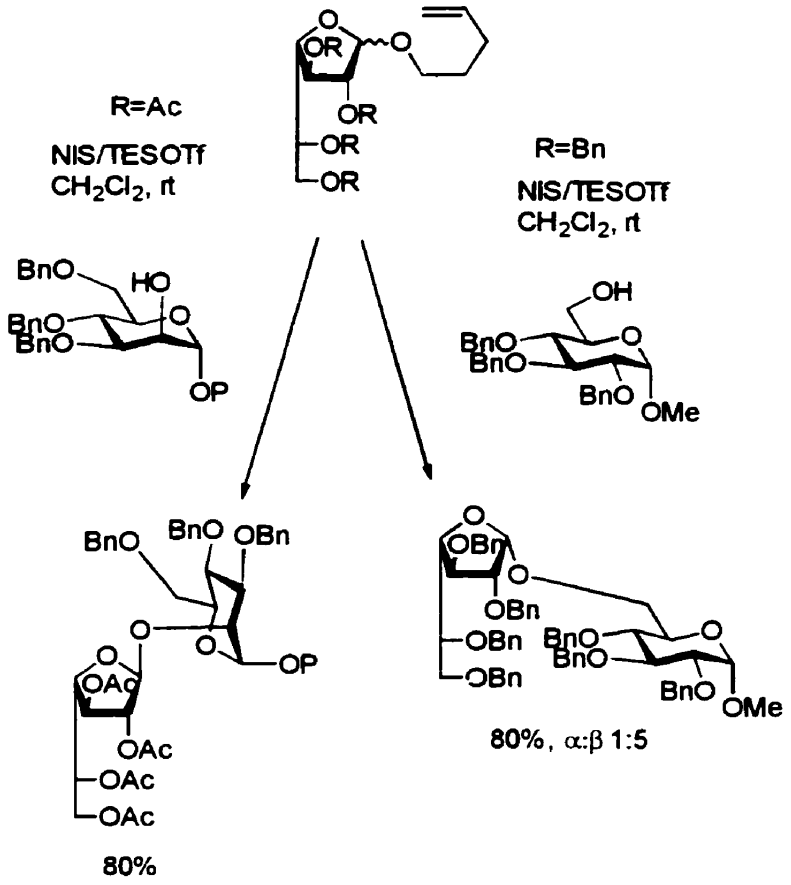


Scheme 1.7 cont.

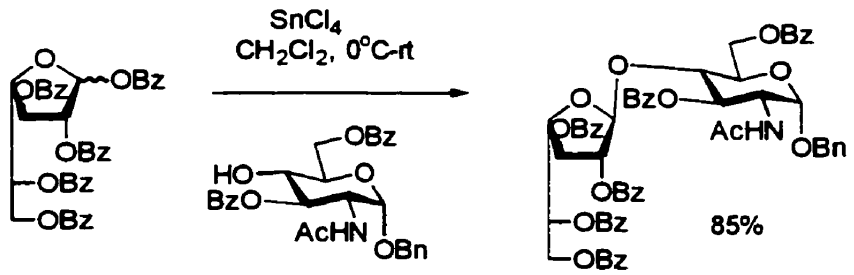




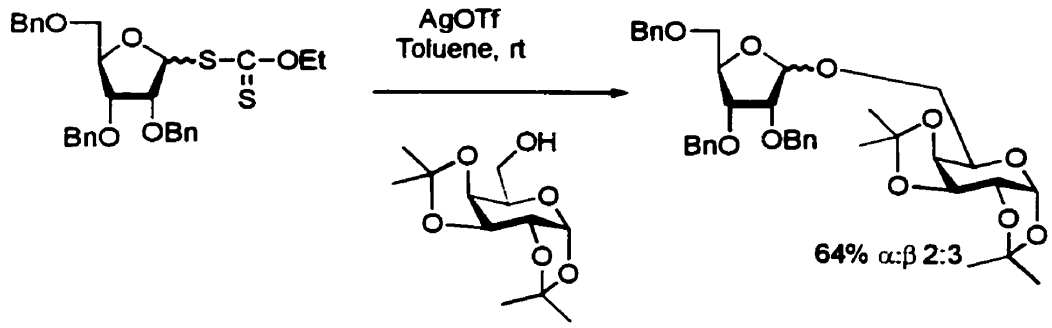
Scheme 1.8



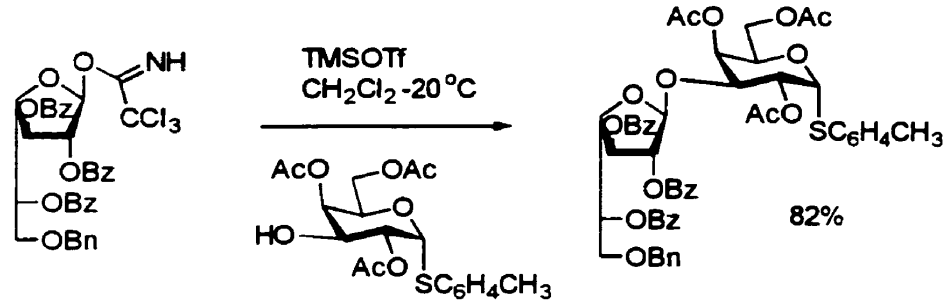
Scheme 1.9



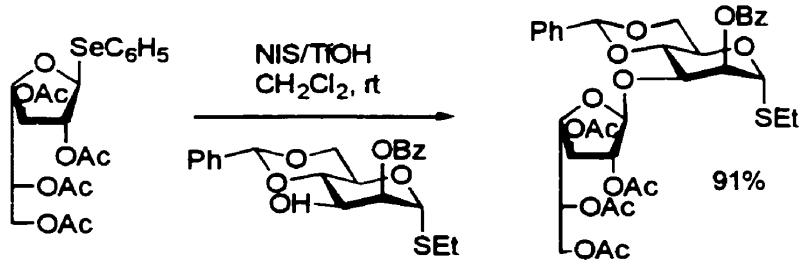
Scheme 1.10



Scheme 1.11

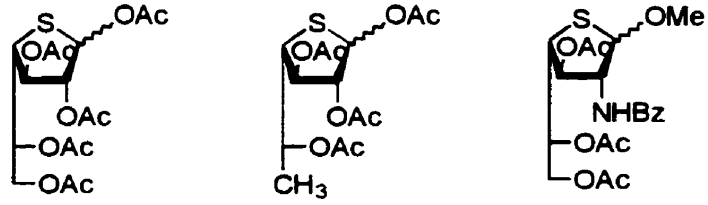


Scheme 1.12



The syntheses of furanose sugars containing 4-thio-Gal*f* are of interest for understanding the role Gal*f* plays in microorganisms, for studying the biosynthesis of furanosyl-containing glycoconjugates, and for their use as inhibitors to probe the development of infections. There are limited reports of compounds containing 4-thio-Gal*f*. A convenient synthesis of 4-thio-Gal*f* (**1.115**) was published in 1989 by Varela *et al.*[213] Subsequently, this group has synthesized 6-deoxy-4-thio-Gal*f* derivatives (**1.116**). Fernández-Bolaños *et al.*[214] have since reported the synthesis of 4-thiofuranoside derivatives of D-galactosamine (**1.117**).

Figure 1.37 Known 4-Thio-Gal*f* Monosaccharides



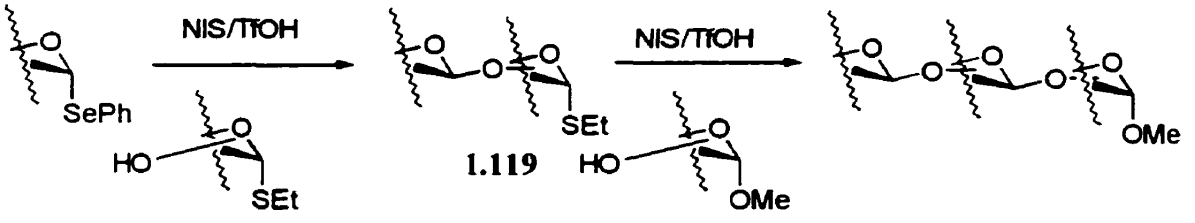
1.115 **1.116** **1.117**

1.7.4 Selective Activation in Glycosylation Reactions

The convergent block synthesis of oligosaccharides is more desirable and more efficient than the linear, stepwise approach. The use of protecting groups and manipulations of the anomeric center are reduced in a convergent synthesis. The selective activation strategy of activating a phenyl selenoglycoside over an ethyl

thioglycoside (Figure 1.38) can provide monosaccharide blocks that can be used as both a glycosyl acceptor (**1.118**) in the first glycosylation reaction and a glycosyl donor (**1.119**) in the second step. This is an extension of other studies of the selective activation of selenoglycoside pyranosyl donors in the presence of thioglycoside acceptors,[120c, 215, 216] A previous report from our laboratory described the viability of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside (Scheme 1.12) as a glycosyl donor.[211]

Figure 1.38 Selective Activation of Phenyl Selenoglycoside Donors over Ethyl Thioglycoside Acceptors



1.8 Thesis Overview

As seen in Chapter 1, carbohydrates are important molecules that mediate several biological recognition events. The study of heteroanalogues as enzyme inhibitors and as potential drug candidates is of importance.

Part I

Chapter 2 presents a manuscript [Randell, K. D.; Frandsen, T. P.; Stoffer, B.; Johnson, M. A.; Svensson, B.; Pinto, B. M. *Carbohydr. Res.* **1999**, *321*, 143-156.] that describes the synthesis, enzyme inhibitory activity, and molecular modeling of a series of 5-thio-D-glucopyranosylarylamines. It was thought that these compounds would be good inhibitors because of the ability of the nitrogen in the glycosidic linkage to be protonated. These compounds were evaluated as inhibitors of glucoamylase G2 from *Aspergillus niger*. Molecular modeling of these compounds led to an explanation for the differences in the K_i values obtained. I synthesized the compounds and performed some of the enzyme inhibition studies reported in this paper.

Chapter 3 presents a manuscript [Randell, K. D.; Johnston, B. D.; Green, D. F.; Pinto, B. M. *J. Org. Chem.* **1999**, in press.] that describes the acid catalyzed configurational equilibria of the series of 5-thio-D-glucopyranosylarylamines and a series of glucopyranosylarylamines and their protonated counterparts. The question under investigation was whether a generalized reverse anomeric effect exists. I performed the work on the 5-thio compounds.

Part II

In Chapter 4, the focus of the thesis changes to the synthesis of oligosaccharides containing galactofuranose (Gal*f*) [Randell, K. D.; Johnston, B. D.; Brown, P. N.; Pinto, B. M. *Carbohydr. Res.* **2000**, in press.]. The oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-(β-D-Gal*f*-(1→3))-α-D-Man*p*-(1→2)-α-D-Man*p* corresponds to the terminus of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease. Syntheses of methyl or ethylthio glycosides of the terminal disaccharide, trisaccharide, tetrasaccharide, and pentasaccharide corresponding to this structure are described. These compounds were synthesized from monosaccharide precursors. The known building blocks were synthesized following literature procedures. Novel building blocks were synthesized using known protecting group strategies. The glycosylation reactions employed the selective activation of a phenyl 1-selenogalactofuranoside or a phenyl 1-selenomannopyranoside donor over ethyl 1-thioglycoside acceptors with NIS/TfOH. The final compounds were obtained through deprotection steps. I synthesized compounds **4.1**, **4.3**, **4.4**, **4.8-4.15**, **4.17**, and **4.18**.

Chapter 5 presents a manuscript [Randell, K. D.; Johnston, B. D.; Lee, E. E.; Pinto, B. M. *Tetrahedron: Asymmetry* **1999**, in press.] that describes the synthesis of heteroatom analogues (containing 4-thio-Gal*f*) of the disaccharide, trisaccharide, and tetrasaccharide synthesized in Chapter 4. These syntheses employed a new glycosyl donor, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-1-selenogalactofuranoside. The compounds are the first examples of oligosaccharides containing 4-thio-Gal*f*. I synthesized compounds **5.1-5.3**, and **5.6-5.14.**

The syntheses of three novel disaccharides are described in Chapter 6 [Randell, K. D.; Johnston, B. D.; Pinto, B. M. *Carbohydr. Res.* **1999**, submitted.]. The disaccharides contain 4-thio-galactofuranose in the non-reducing ring and nitrogen in the interglycosidic linkage and represent a new class of comopunds. These disaccharides were synthesized without the use of protecting groups. The glycosylamines were reacted with 4-thio-Gal*f* using acetic acid as a catalyst. These reactions produced α:β mixtures of the desired compounds. Again, it is of interest to compare the inhibitory activity of these *S,N*-acetals to the parent compounds **4.1** and **5.1**. I synthesized compounds **6.1-6.3**.

In Chapter 7, the conformational analysis of β-D-Gal*f*-(1→3)-α-D-Man*p* (**4.1**), the 4-thio-Gal*f* analogue (**5.1**), and the 4-thio-Gal*f* analogue containing a nitrogen in the interglycosidic linkage (**6.1**) is presented. The effects of heteroatom substitution are probed. These structures need to be subjected to rigorous molecular dynamics calculations together with an analysis of full NOE build-up curves before the results can be considered suitable for publication.

1.9 References

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CHAPTER 2: SYNTHESIS AND GLYCOSIDASE INHIBITORY ACTIVITY OF 5-THIOGLUCOPYRANOSYLARYLAMINES. MOLECULAR MODELING OF COMPLEXES WITH GLUCOAMYLASE

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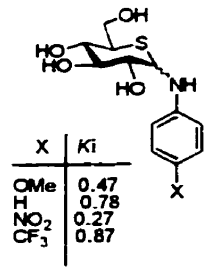
2.1 Graphical Abstract

Synthesis and glycosidase inhibitory activity of 5-thioglucopyranosylamines. Molecular modeling of complexes with glucoamylase.



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Keywords: Glucoamylase; α-Glucosidase; 5-Thio-D-glucopyranosylamines; Inhibitors; Molecular modeling; Transferred NOE NMR.

2.2 Abstract

The synthesis of a series of 5-thio-D-glucopyranosylarylamines by reaction of 5-thio-D-glucopyranose pentaacetate with the corresponding arylamine and mercuric chloride catalysis is reported. The products are obtained as anomeric mixtures of the tetraacetates which can be separated and crystallized. The tetraacetates are deprotected to give α/β mixtures of the parent compounds which are evaluated as inhibitors of the hydrolysis of maltose by glucoamylase G2 (GA). A transferred NOE NMR experiment with an α/β mixture of **2.7** in the presence of GA shows that only the α-isomer is bound by the enzyme. The K_i values, calculated on the basis of specific binding of the α-isomers, are 0.47 mM for *p*-methoxy-*N*-phenyl-5-thio-D-glucopyranosylamine **2.7**, 0.78 mM for *N*-phenyl-5-thio-D-glucopyranosylamine **2.8**, 0.27 mM for *p*-nitro-*N*-phenyl-5-thio-D-glucopyranosylamine **2.9** and 0.87 mM for *p*-trifluoromethyl- *N*-phenyl-5-thio-D-glucopyranosylamine **2.10**, and the K_m values for the substrates maltose and *p*-nitrophenyl α-D-glucopyranoside are 1.2 and 3.7 mM, respectively. Methyl 4-amino-4-deoxy-4-*N*-(5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside **2.11** is a competitive inhibitor of GA wild-type (K_i 4 μM) and the active site mutant Trp120→Phe GA (K_i 0.12 mM). Compounds **2.7**, **2.8**, and **2.11** are also competitive inhibitors of α-glucosidase from brewer's yeast, with K_i values of 1.05 mM, >10 mM, and 0.5 mM, respectively. Molecular modeling of the inhibitors in the catalytic site of GA is used to probe the ligand-enzyme complementary interactions and to offer insight into the differences in inhibitory potencies of the ligands.

2.3 Introduction

Glucoamylase (GA) (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3) from *Aspergillus niger* is an exo-acting and inverting carbohydrase, catalyzing the release of β-D-glucopyranose from non-reducing ends of starch and related poly- and oligo-saccharides. GA tolerates a broad range of aglycon moieties, cleaving α-1,6-glucosidic bonds and aryl glucosides with 500- and 50-fold lower activity, respectively, than for α-1,4-linked oligosaccharides.[1-3] GA from *Aspergillus niger* is secreted as two glycosylated forms:[4] G1 containing a catalytic domain, a highly *O*-glycosylated region, and a starch binding domain (82.7 kDa, residues 1-616);[5] and G2 a smaller form lacking the starch binding domain (72 kDa, residues 1-512).[6] Crystallographic structure determination of the 94% identical GA from *Aspergillus awamori* var. *X100* shows that the catalytic domain (residues 1-471) folds into an $(\alpha/\alpha)_6$-barrel, where six highly conserved α→α loop regions connect segments of an inner and an outer α6 barrel.[7] These conserved segments whose architecture creates the funnel-shaped active site and details of residues involved in catalysis, substrate binding, and transition-state stabilization are described by crystallography in several GA-inhibitor complexes,[8-10] using site-directed mutagenesis,[2,11-15] inhibitor binding thermodynamics,[16,17] and by molecular recognition of substrate analogues.[12,18-20]

α-Glucosidases (α-D-glucoside glucohydrolase, EC 3.2.1.20) are also exo-acting carbohydrases, catalyzing the release of D-glucopyranose from non-reducing ends of various substrates having an α-glucosidic linkage, such as disaccharides, oligosaccharides, aryl glucosides and even starch.[21] α-Glucosidases are widespread in

nature and have been isolated from fungi, animals, and higher plants.[21] They generally display broad substrate specificity, and are conventionally classified into three types dependent on distinct properties; *i.e.* class I, typical α-glucosidases hydrolyzing heterogeneous substrates such as aryl glucosides, class II, maltases hydrolyzing homogeneous substrates like maltose and class III, like the maltases, but capable of also attacking α-glucans.[21] α-Glucosidase from brewer's yeast is a class I enzyme, having a 120-fold preference, based on k_{cat}/K_M, for *p*-nitrophenyl α-D-glycopyranoside compared to maltose.[22] In contrast to GA, which acts by inversion of configuration, the exact mechanism of hydrolysis by brewer's yeast α-glucosidase is currently unknown; also details concerning residues involved in catalysis and substrate binding are lacking.

Glucosidases, inverting and retaining, catalyze reactions proceeding through transition-states having substantial oxo-carbenium ion character.[23-25] Thus, the various classes of enzymes are efficiently inhibited by substrate analogs mimicking either the charge and/or the shape of the transition state; thus glucosidases are often inhibited by analogues having a half-chair conformation or by sugars bearing a basic nitrogen adjacent to the C-1' atom of disaccharides or the C-1 atom of monosaccharides.[26,27] Electrostatic interactions between protonated inhibitors and negatively charged side-chains in the active site cleft thus might contribute significantly to the mechanism and strengths of inhibition. We have recently reported that heteroanalogues of methyl maltosides containing sulfur and selenium were inhibitors of *A. niger* with K_i-values in the millimolar range,[28] whereas an analogue containing sulfur in the non-reducing ring and nitrogen in the glycosidic linkage showed much stronger inhibition with a K_i of 4 μM.[29] It was of interest, therefore, to examine the general class of compounds containing S/N

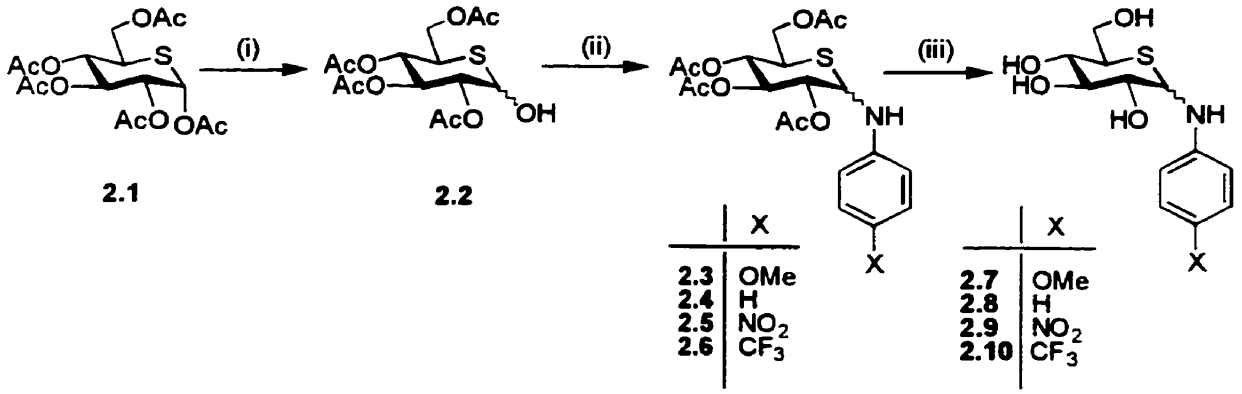
acetal functions as potential inhibitors of GA from *A. niger* and brewer's yeast α-glucosidase. In the present study we report the synthesis and enzyme inhibitory activity of a series of α-D-glucopyranosyl amines containing simple arylamines as the aglycons.

2.4 Results and Discussion

2.4.1 Synthesis

1,2,3,4,6-Penta-*O*-acetyl-5-thio-D-glucopyranose **2.1**[30,31] was treated with hydrazine acetate[32] to afford **2.2**, in 90% yield. The tetraacetate **2.2** was then reacted with the respective arylamine. The reactions were catalyzed by 10% $HgCl_2$ (Scheme 2.1) and presumably proceed via stabilization of an acyclic intermediate by complexation of Hg^{2+} to the sulfur atom. It is significant that reactions catalyzed by protic acids such as acetic acid did not proceed smoothly. Reaction conditions varied slightly for **2.3-2.6**, but all reactions were completed at 50°C or less and in less than 20 hours. The resulting anomeric mixtures were separated and purified by chromatography and the pure isomers were obtained by recrystallization.

Scheme 2.1



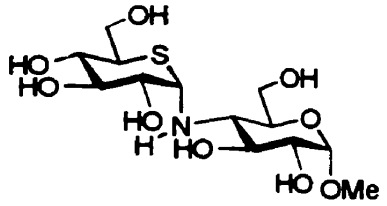
(i) $H_2NNH_2{\cdot}AcOH$, DMF; (ii) *p*-X-$C_6H_4NH_2$, 10% $HgCl_2$; (iii) 5:1:1 MeOH-H_2O-Et_3N.

The tetraacetates **2.3-2.6** were deprotected with MeOH:H_2O:Et_3N (5:1:1)[29] to give **2.7-2.10**, respectively. The deprotected sugars were obtained as anomeric mixtures which could not be separated. The percentages of the α-isomers in equilibrated samples in D_2O were assessed by ^1H NMR spectroscopy and were used in the calculation of the K_i values, as discussed below. The α:β ratios for **2.7-2.10** were 1:2.2, 1:2, 1:0.9, and 1:2.4, respectively.

2.4.2 Preferential Binding of the α-Isomers

As was the case in our earlier study of glucoamylase binding of the maltoside heteroanalogue **2.11**, containing sulfur in the non-reducing ring and nitrogen in the interglycosidic linkage,[29] NMR studies with an α/β mixture of compound **2.7** in the presence of GA indicated significant line-broadening effects for the signals of only the α-isomer. Thus, when glucoamylase was added to the sample containing **2.7**, selective broadening (2.4 Hz to 3.9 Hz) of the H-1 resonance of the α-isomer was observed. Other

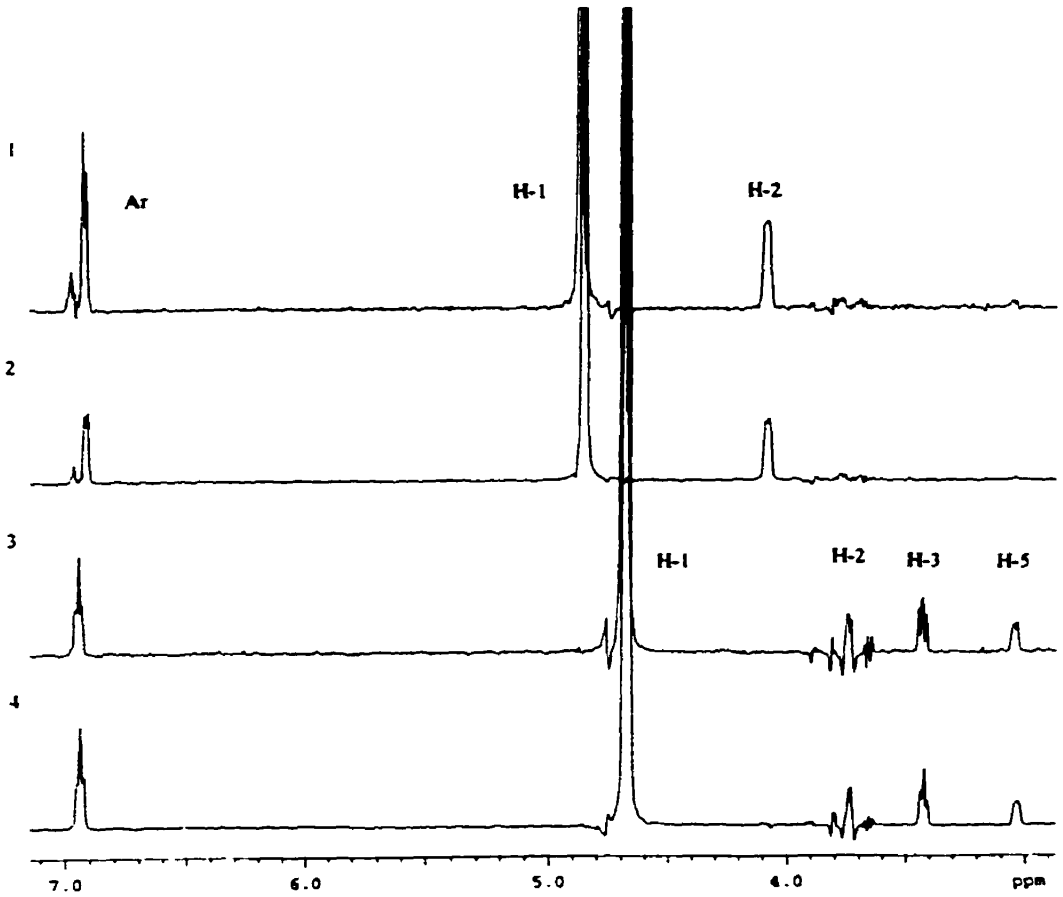
resonances of the α-isomer were also broadened such that $\Delta\nu_{1/2}$ was not measurable. Linewidths for the resonances of the β-isomer did not change.



2.11

In order to confirm that the α-isomer was preferentially bound, transferred NOE experiments[33] were performed. A control NOE experiment, in which the viscosity of the protein environment was simulated with BSA, showed negative NOE effects from H-1 of both α- and β-isomers of the free ligand **2.7.** A further negative enhancement due to the transferred NOE was observed at both mixing times by comparing peak intensities to those of the control sample. The normalized peak intensity for the α anomer increased up to 100% in the sample containing enzyme relative to the sample containing BSA, indicating the presence of intensity due to transferred NOE. In contrast, β peak intensities decreased slightly or did not change. For example, at a mixing time of 205 ms, the α H-1 - H-2 contact peak intensity increased from 0.070 in the control to 0.098 with glucoamylase (relative to the H-1 diagonal peak), an increase of 40%. In contrast, the β H-1 - H-3 contact peak intensities were 0.035 and 0.034 in the control and with glucoamylase, respectively. For the H-1 - aromatic contact, α peak intensity increased 95% from 0.073 to 0.142, while β intensity decreased from 0.068 to 0.050. (see Figure 2.1). This evidence suggests[33] that GA binds only the α-isomer.

Figure 2.1 **Traces from TRNOESY Spectra (mixing time 205 ms) of Compound 2.7. (1 and 2): at α H-1 resonance; (1) with Glucoamylase, and (2) with BSA. (3 and 4): at β H-1 resonance; (3) with Glucoamylase, and (4) with BSA**



2.4.3 Enzyme Inhibition

The K_i-values for inhibition of GA by compounds **2.7-2.11** are shown in Table 2.1. In addition, compounds **2.7** and **2.8** were chosen as representative of the first set of compounds and their inhibition of α-glucosidase from brewer's yeast was compared to the inhibitory effect of **2.11** (Table 2.1). Because **2.11** was a much better inhibitor of GA

Table 2.1 Inhibition of Glucoamylase and α-Glucosidase by (2.7-2.11)

Inhibitor	K_i (mM) GA[a]	K_i (mM) α-Glucosidase
p-Methoxy-*N*-phenyl 5-thio-D-glucopyranosylamine **(2.7)**	0.47 ± 0.12[b]	1.05 ± 0.18
N-Phenyl 5-thio-D-glucopyranosylamine **(2.8)**	0.78 ± 0.27	>10
p-Nitro-*N*-phenyl 5-thio-D-glucopyranosylamine **(2.9)**	0.27 ± 0.02	[c]
p-Trifluoromethyl-*N*-phenyl 5-thio-D-glucopyranosylamine **(2.10)**	0.87 ± 0.12	[c]
Methyl 4-amino-4-deoxy-4-*N*-(5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside**(2.11)**[29]	0.0044 ± 0.003	0.5 ± 0.15

[a]Adjusted for the proportion of the α-isomer
[b]Standard deviation
[c]Not determined

than **2.7** or **2.8** it was of interest to examine whether this trend would hold for a related enzyme. These values were determined by assuming that only the α-isomers in the anomeric mixtures would be accepted as inhibitors of glucoamylase. GA is inhibited by compounds **2.7-2.11**, all with K_i values under 1 mM (Table 2.1). The K_m values for the substrates maltose and *p*-nitrophenyl α-D-glucopyranoside are 1.2 and 3.7 mM, respectively. The differences in K_i may reflect the influence of the substituents on the

pK_a-value, and thus the ionization state of the nitrogen atom. The pK_a-values for the isolated aglycon differ significantly: 4.60 for aniline, 5.31 for *p*-anisidine, 2.45 for *p*-trifluoromethylaniline and 1.00 for *p*-nitroaniline.[34] Protonation of the inhibitor may take place and this is favorable for binding in two ways, the formation of an ion pair with an active site carboxylate and also introduction of positive charge in the active site is not accompanied by a thermodynamic penalty associated with burying uncompensated negative charge.[9] One might have expected that the K_i values would increase with decreasing pK_a-values.

Thermodynamic analysis of the complexation between GA and various inhibitors has shown several interesting features of the mechanism and strength of inhibition.[16] The binding process is both enthalpically and entropically driven, thus both hydrogen-bonding and dehydration of protein-oligosaccharide surfaces drives the complex formation.[16] pH-dependence studies of the complex formation between GA and 1-deoxynojirimycin and acarbose suggested that the complex formation was most efficient at pH-values close to the pK_a of the inhibitor nitrogen.[16] Binding of acarbose and 1-deoxynojirimycin at pH 4.5 is accompanied by the release of 0.5 ± 0.1 proton, probably due to partial protonation of both inhibitor and general acid catalyst in the active site, whereas 0.7 ± 0.1 proton is taken up at pH 7.5.[16] Formation of an ion pair in the active site, consisting of a protonated inhibitor and an anionic residue can in principle take place in two ways; a neutral form of the inhibitor could be bound by the enzyme, and subsequently protonated by the general acid catalyst; alternatively, a protonated form of the inhibitor could form an ion pair with an active site carboxylate. Thus, although the glycosidic nitrogen atom in the *p*-nitro analogues might be unprotonated, it may still be

involved in a strong ion pair with the general acid catalyst, accounting for the low K_i value.

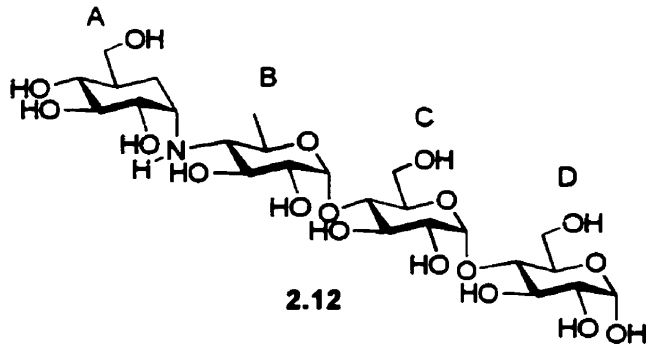
The relatively higher K_i values for **2.7-2.10** compared to **2.11** can be rationalized based on the analysis of complementary interactions between ligands and enzyme at subsites -1 and +1. It is of note that analysis of recognition of deoxygenated maltoside analogues by GA revealed key polar group interactions of OH-4', -6', and -3 at substrate binding subsites -1 and +1, respectively.[19] Elimination of the OH-3 interacting with NH-1 of Arg305[9] resulted in loss of transition-state stabilization of 11.3 kJ/mol.[19] We speculate, therefore, that the higher K_i values for **2.7-2.10** compared to **2.11** are due in part to the loss of hydrogen-bonding from Arg305 to the aglycon at the binding subsite +1. Alternatively, the greater inhibition by **2.11** could result from its higher basicity relative to **2.7-2.10**. In contrast to glucoamylase, brewer's yeast α-glucosidase has no key polar group interactions to the substrate aglycon at binding subsite +1, and binding thus strongly depends on charged hydrogen-bonds to all OH-groups of the non-reducing sugar ring.[35] Compound **2.7** which lacks possible OH-group partners is therefore essentially as good an inhibitor of brewer's yeast α-glucosidase as is compound **2.11**. The significant difference in K_i values between compounds **2.7** and **2.8** for brewer's yeast α-glucosidase (Table 2.1) may, in this case, reflect the influence of the substituent on the pK_a value of the nitrogen atom. Alternatively, a hydrogen bond to the methoxy substituent may be formed at the periphery of binding subsite +1.

Mutational and structural analysis of GA suggested Trp120 to be involved in transition-state stabilization through hydrogen-bonding to the general acid catalyst Glu179, and by direct stacking towards the sugar in subsite +2.[2,9,10,36] Mutation of

Trp120 to Phe thus decreased activity 80-fold compared to wild-type GA using maltose as substrate, accompanied, however, by no changes in K_m.[2] In the present study, compound **2.11** showed competitive inhibition towards Trp120→Phe GA with a K_i value of 0.12 mM. Although K_m on maltose for wild-type and Trp120→Phe GA are similar, K_i for **2.11** is 30-fold higher for Trp120→Phe than for the wild-type enzyme. The effect of Trp120→Phe was previously shown, using stopped-flow fluorescence spectroscopy to be substrate length dependent; thus the mutation is less severe on small substrates like maltose compared to longer oligosaccharides.[36] Assuming that the mutation using a short inhibitor, like **2.11**, only affects the hydrogen-bond to the general acid catalyst Glu179, the present study reinforces the importance for efficient inhibition of a salt-linkage from Glu179 to the basic nitrogen, stabilized in the wild-type enzyme by Trp120, but lost in the mutant. The area surrounding Glu179 is probably extremely sensitive to mutation and the actual ionization state of Glu179 has in fact been questioned by crystallographic studies of the GA-acarbose complex.[9] In addition, acarbose binding was affected in Trp120→Phe GA. The association constant for acarbose is thus decreased markedly (10^{-5}-fold) in Trp120→Phe GA compared to wild-type GA,[17] a combination probably of loss of hydrophobic stacking against the sugar, and of the hydrogen-bond to Glu179. Significantly, a pH-activity dependence study for Trp120→Phe has shown that the pKa of Glu179 is not changed in the substrate bound form.[37] It is therefore likely that the elimination of the Trp120 to Glu179 H-bond in the mutant enzyme leads to an unfavourable orientation of the catalytic acid Glu179 and a higher K_i value for **2.11**.

2.4.4 Molecular Modeling

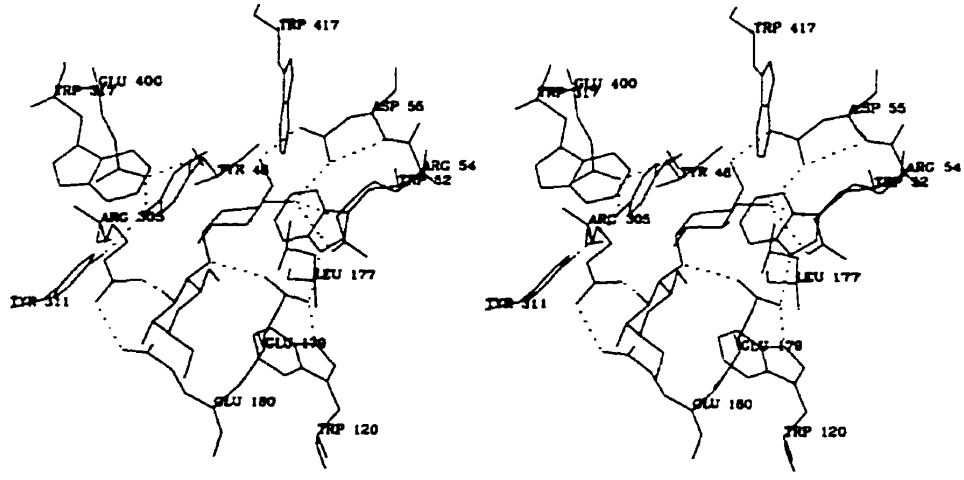
Compounds **2.7-2.9** and **2.11** were used in molecular modeling studies to investigate the ligand-enzyme interactions and to offer insight into the differences in inhibitory potencies of the ligands. The compounds were introduced into the active site of the D-*gluco*-dihydroacarbose (DGA, **2.12**)-glucoamylase complex.[38]



2.12

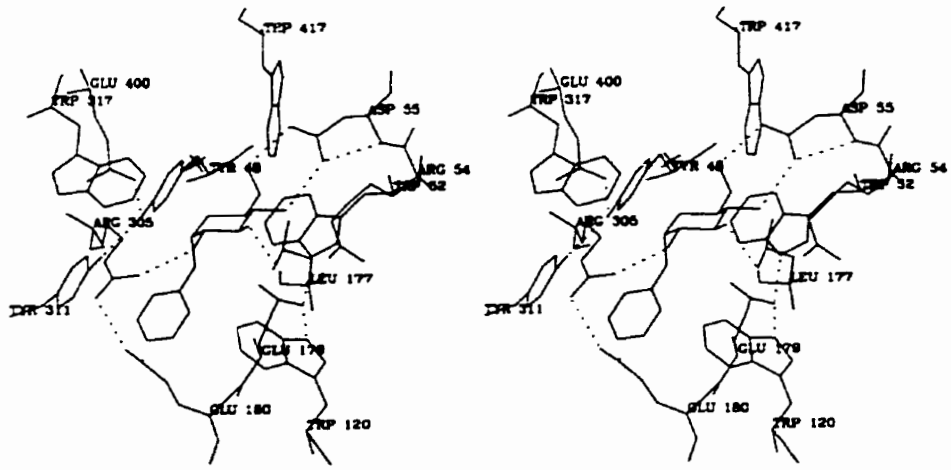
Overall the models have the same hydrogen bond pattern for the non-reducing ends as seen for the DGA-glucoamylase complex both before and after minimization. Furthermore, before minimization the reducing end of **2.11** shows the same hydrogen bonds as seen for ring B in **2.12**. The only exception is a hydrogen bond contact between OH-6 and Lys108 through a water molecule. After minimization (Figure 2.2) the hydrogen bond from OH-2 to Glu180 is changed from 2.84 to 3.38 Å and the hydrogen bond from OH-2' to Arg305 NH-1 is changed from 3.00 to 3.30 Å. All other hydrogen bond interactions are approximately unchanged upon minimization. D-*Gluco*-dihydroacarbose **2.12** is the second strongest inhibitor known for glucoamylase, with a $K_i=10^{-8}$ M. The similarity in hydrogen bonding and conformational patterns between **2.11** and **2.12** explains why it is also a good inhibitor.

Figure 2.2 **Stereoview of the Active Site of Glucoamylase with 2.11 Bound. Hydrogen bonds less than 3Å are represented by dashed lines. The catalytic water molecule is represented by an X.**



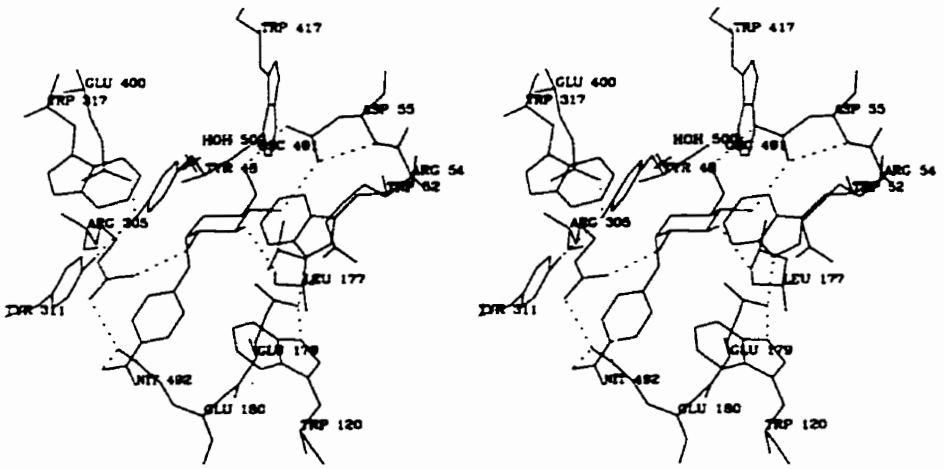
As compared to **2.11**, the aniline analogue **2.8** has lost three hydrogen bond interactions due to substitution of the reducing end residue with an aromatic moiety (Figure 2.3). The hydrogen bond distance of OH-4' to Arg54 NH-1 is lengthened from 2.88 to 3.02 Å. The aromatic ring of **2.8** stacks with the side chain of Tyr311 with an angle of 36°. The increase in K_i of this compound as compared to **2.11** is probably due to the loss of the three hydrogen bonds in the active site.

Figure 2.3 **Stereoview of the Active Site of Glucoamylase with 2.8 Bound. Hydrogen bonds less than 3Å are represented by dashed lines. The catalytic water molecule is represented by an X.**



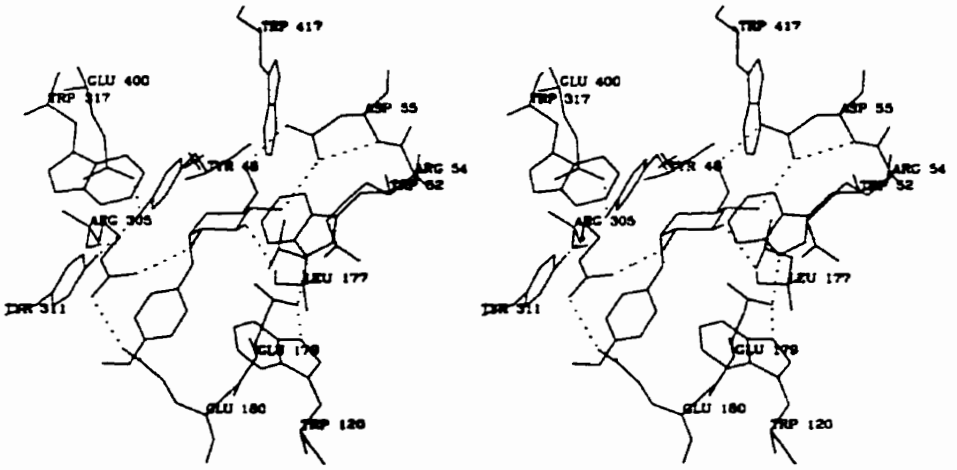
In compound **2.9**, the aromatic ring again stacks with Tyr311, with an angle of 33°. In addition, a hydrogen bond between Glu180 OE-2 and the nitro functionality (Figure 2.4) was detected. This hydrogen bond is 2.97 Å and it does not change upon minimization although we note that the hydrogen bond is not in the σ-plane of the aromatic ring. It is not clear, therefore, whether the decrease in K_i relative to that of **2.8** could be attributed to this additional hydrogen bond.

Figure 2.4 **Stereoview of the Active Site of Glucoamylase with 2.9 bound. Hydrogen bonds less than 3Å are represented by dashed lines. The catalytic water molecule is represented by an X.**



Compound **2.7** was also found to have the same hydrogen bond to the Arg54 NH-1 as in **2.8**, but the *O*-methyl function has an additional weak hydrogen bond with Glu180 OE-2 of 3.47 Å (Figure 2.5). The aromatic ring stacks with Tyr311 with an angle of 33°. The decrease and increase in K_i values as compared to those of **2.8** and **2.9**, respectively can be attributed to this weak H-bond of the *O*-methyl function.

Figure 2.5 **Stereoview of the Active Site of Glucoamylase with 2.7 bound. Hydrogen bonds less than 3Å are represented by dashed lines. The catalytic water molecule is represented by an X.**



2.5 Experimental

2.5.1 General Methods

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively, unless otherwise stated. The spectra were recorded in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from TMS for those measured in deuterochloroform and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in deuterium oxide. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated at 150°C. All compounds were purified by flash column chromatography on Kieselgel 60 (230-400 mesh). Solvents were distilled before use and were dried as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40°C.

2.5.2 Enzymes

Recombinant *Aspergillus niger* wild-type (AnMT 833) and Trp120→Phe GA were obtained as culture filtrates from Novo-Nordisk (Bagsvaerd, Denmark) and purified on individual acarbose-Sepharose columns,[39] followed by ion-exchange chromatography

using Hiload Q-Sepharose to separate G1 and G2.[40] G2 was used throughout the present study. Brewer's yeast α-glucosidase (Type VI, EC 3.2.1.20) was obtained as a lyophilized powder from Sigma (G-6136; Lot nr. 21F8105). After resuspension in 0.05 M phosphate, pH 6.8 and dialysis overnight at 4°C against 0.05 M phosphate, pH 6.8, the enzyme (360 U/mL) was used for inhibition studies without further purification.

2.5.3 Enzyme Inhibition Assays

The initial rates of GA G2 catalyzed hydrolysis of maltose (10 different substrate concentrations in the range 0.19-22.7 mM) were followed in the absence and in the presence of the different inhibitors (5 different concentrations in the range 0.3-8 mM) in 0.1 M sodium acetate, pH 4.5 at 45°C and a final enzyme concentration in the range 15-90 nM. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL.[4,14,41,42] Absorbances were read at 450 nm or 490 nm after 1 hour incubation at room temperature and quantitated using D-glucose as a standard.

The initial rates of α-glucosidase-catalyzed hydrolysis of *p*-nitrophenyl α-D-glucopyranoside were followed in 0.05 M phosphate, pH 6.8 and at 30°C at ten different substrate concentrations in the range 0.049-3.92 mM, in the absence and presence of inhibitor. The *p*-nitrophenol released was analyzed in aliquots removed at appropriate time intervals and quenched in 0.1 M sodium borate, pH 9.4. Absorbances were read at 405 nm and quantitated using *p*-nitrophenol as a standard.

The inhibitors were all competitive, and the constants of inhibition were calculated from $K_{m'} = K_m(1 + ([I]/K_i))$, where $K_{m'}$ and K_m are the Michaelis-Menten constants determined in the presence and the absence of inhibitor, using the software ENZFITTER,[43] and [I] is the concentration of inhibitor. V_{max} and K_m, in the absence and presence of inhibitor, were obtained by fitting the velocities as a function of substrate concentration to the Michaelis-Menten equation. k_{cat} was derived from $k_{cat} = V_{max}/[E_o]$ where V_{max} is the maximum velocity and $[E_o]$ the molecular enzyme concentration. With 5-thio-D-glucose, the glucose oxidase had ≤1% of the activity toward D-glucose and neither 5-thio-D-glucose nor the glucoamylase inhibitors tested were inhibitors of the glucose oxidase.

2.5.4 Molecular Modeling

Docking and minimization procedures are outlined below. The 5-thio-D-glucopyranosyl amines were introduced into the active site of D-*gluco*-dihydroacarbose (DGA **2.12**)-glucoamylase complex, determined at 1.7 Å resolution,[38] using the following procedure. First, the DGA complex was prepared for docking of ligands and minimization by removing all the hydrogen atoms and all residues of the low occupancy conformation.[38] The ligand **2.11** was constructed using standard procedures in INSIGHT II V 97.0 (Molecular Simulations Inc, San Diego, CA) from two molecules of α-D-glucose linked by an α-(1-4) bond followed by replacing the glucosidic oxygen with a nitrogen and the endocyclic oxygen of the non-reducing ring with a sulfur. Finally, an α-*O*-methyl group was added to the reducing end of the glucose unit. The C2, C3 and C4 atoms of the non-reducing ring of **2.11** were superimposed upon C2, C3 and C4 of ring A

of DGA **2.12**. The model of **2.11** was then adjusted to minimize close Van der Waals contacts. The adjustment placed the reducing end residue of **2.11** in an equivalent position as seen for ring B of DGA **2.12**.

The model of **2.8** was constructed by superimposing the nitrogen and six carbons of the aniline group from 1eld.pdb[44] on the N-4, C-4, C-3, C-2, C-1, O-5 and C-5 atoms of the reducing end residue of **2.11**. The non-reducing ring and the superimposed aniline group were then linked together, resulting in a model for **2.8**. This model complex was then adjusted to minimize close Van der Waals contacts.

The model of **2.9** was constructed by superimposing the *p*-nitroaniline group from 1pip.pdb[45] on the aniline moiety of **2.8**. The non-reducing ring of **2.11** and the superimposed *p*-nitroaniline group were then linked together, resulting in a model of **2.9**; minimization was then performed. Furthermore, after the adjustment, an oxygen atom from the nitro functionality was positioned within hydrogen bonding distance to OE2 of Glu180; the aromatic portion of the molecule was stacked with the aromatic moiety of Tyr311 in a 33-36° angle.

The model of **2.7** was constructed from **2.9**, by replacing the nitro functionality with the *O*-methyl group. The complex was minimized, bringing the methyl group within hydrophobic contact distance to Tyr311.

After initial construction of the 5-thio-D-glucopyranosylarylamine-glucoamylase models, hydrogens were added using X-PLOR v3.851[46] and the CHARMM 22 parameter and topology parameter set, assuming the γ-carboxylate function of Glu180 was protonated. Finally, to fill out cavities created upon deletion of residues from the original DGA-GA complex and to give a uniform surface, the models were soaked in a water

shell of 2.5 Å. Parameters for standard groups were generated from existing parameters of equivalent groups.

The models were subjected to 100 cycles of conventional Powell minimization using X-PLOR v3.851. The positions of the amino acids in the active site, the atoms of the ligands, and the water shell molecules were all optimized. The gradients changed from about 50 to about 10 after 100 cycles of optimization. Further refinement of the models may be obtained by molecular dynamics simulations but the present models were considered to be sufficient for the purposes of the present work. Inspection and adjusting of the models were done using TURBO_FRODO version OpenGL.1 (CNRS-AFMB, Marseille, France).

2.5.5 Transferred NOE Experiments

Spectra were acquired on a Bruker AMX600 spectrometer at 600 MHz. One-dimensional spectra were recorded spinning at 300 K; 32K data points were acquired over a spectral width of 6 ppm. For one-dimensional spectra, 1 mg of an α/β mixture of compound **2.7** was dissolved in 0.6 mL of phosphate buffered saline/D_2O (pH 7.5) to 5.4 mM (1.8 mM α, 3.6 mM β). For each spectrum, 64 scans preceded by 16 dummy scans were acquired, with a relaxation delay of 3.5 s. For 2D TRNOESY spectra, 1 mg of an α/β mixture of compound **2.7** was dissolved in 0.6 mL of phosphate buffered saline/D_2O (pH 7.5) along with 5.5 mg glucoamylase G2 (0.12 mM), giving a ratio of approximately 15:1 α : enzyme. A control sample was prepared by dissolving 1 mg of the α/β mixture of compound **2.7** in 0.6 ml of phosphate buffered saline/D_2O (pH 7.5) along with 5.4 mg bovine serum albumin (0.14 mM). 2D TRNOESY spectra were acquired nonspinning at

298 K, in phase-sensitive mode using TPPI.[47] The spectral width was 8 ppm. Spectra were acquired under identical conditions for both samples. One set of spectra used a mixing time of 125 ms and 48 scans per increment; the other used a mixing time of 205 ms and 96 scans per increment. All experiments were preceded by 16 dummy scans. Water suppression was achieved by presaturation during the relaxation delay (2 s) and a 180° pulse in the middle of the mixing time followed by a 5 ms homospoil pulse. An 18 ms $T_{1\rho}$ filter[48] was applied after the excitation pulse to relax protein resonances.

Processing of data was performed with standard UXNMR and XWINNMR (Bruker) software. Zero-filling of the acquired data (512 t_1 values and 2K data points in t_2) led to a final data matrix of 1K x 2K (F_1 x F_2) data points. Chemical shifts were referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The baseline was corrected in F_1 and F_2 with a fifth-order polynomial prior to integration of peak volumes.

2.5.6 Synthesis

***p*-Methoxy-*N*-phenyl 2,3,4,6-tetra-*O*-acetyl-α/β-5-thio-D-glucopyranosylamine (2.3α, 2.3β).** To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2.2**) (470mg, 1.16mmol) in MeOH (10mL) was added anisidine (1.16mmol, 143mg) and $HgCl_2$ (34mg, 0.123mmol). The reaction mixture was stirred at room temperature under nitrogen and a precipitate formed immediately. The solvent was then evaporated, the residue was dissolved in CH_2Cl_2 (50mL) and washed with $NaHCO_3$ (10mL) and H_2O (10mL), and dried over Na_2SO_4. The solvent was evaporated to yield an orange foam. The α isomer was crystallized from 95% ethanol, as fine needles. The remaining mixture was separated by column chromatography, using toluene:ethyl acetate

4:1 as the eluant. Both **2.3α** and **2.3β** were then recrystallized from 95% ethanol (56%, **2.3α**:**2.3β** 8.8:1).

2.3α: M.p. 198-200°C; $[\alpha]_D^{22}$ 326° (c 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 3.47 (ddd, 1 H, $J_{4,5}$ 10.8 Hz, $J_{5,6B}$ 3.0, $J_{5,6A}$ 4.1 Hz, H-5), 3.76 (s, 3 H, OCH_3), 3.98 (dd, 1 H, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.10 (d, 1 H, $J_{NH,1}$ 4.9 Hz, NH), 4.44 (dd, 1 H, H-6a), 4.79 (dd, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-4), 5.40 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 5.47 (dd, 1 H, H-3), 6.75-6.87 (dd, 4 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.76-20.88 (4-$OCOCH_3$), 38.79 (C-5), 56.01 (OCH_3), 58.38 (C-1, J_{C1-H1} 153.85 Hz), 61.83 (C-6), 71.49, 72.65, 74.13 (C-2, C-3, or C-4), 115.19 (C-*m*), 116.81 (C-*o*), 138.90 (C-*i*), 154.23 (C-*p*), 169.51-170.71 (C=O); Anal. Calcd for $C_{21}H_{27}NO_9S$: C, 53.71; H, 5.81; N, 2.98. Found: C, 53.75; H, 5.68; N, 2.80%.

2.3β: M.p. 203-204°C; $[\alpha]_D^{22}$ -66° (c 0.52, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.98 (s, 3 H, $OCOCH_3$), 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 3.37 (ddd, 1 H, $J_{4,5}$ 10.7, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.0 Hz, H-5), 3.69 (d, 1 H, $J_{NH,1}$ 10.9 Hz, NH), 3.74 (s, 3 H, OCH_3), 4.08 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.32 (dd, 1 H, H-6A), 4.69 (dd, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.18 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-4), 5.32 (dd, 1 H, H-2), 6.67-6.8 (dd, 4 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.48-20.66 (4-$OCOCH_3$), 41.77 (C-5), 55.73 (OCH_3) 60.62 (C-1, J_{C1-H1} 150.3 Hz.), 61.52 (C-6), 72.26, 73.50, 74.63 (C-2, C-3, or C-4), 114.92 (C-*m*), 115.81 (C-*o*), 138.35 (C-*i*), 153.69 (C-*p*), 169.45-170.99 (C=O); Anal. Calcd for $C_{21}H_{27}NO_9S$: C, 53.71; H, 5.81; N, 2.98. Found: C, 53.69; H, 5.72; N, 2.76%.

***N*-Phenyl 2,3,4,6-tetra-*O*-acetyl-α/β-5-thio-D-glucopyranosylamine (2.4α, 2.4β).** To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2.2**) (700mg, 1.72mmol) in MeOH (10mL) was added aniline (1.72mmol, 156 mL) and $HgCl_2$ (0.172mmol, 47mg). The reaction mixture was stirred at room temperature under nitrogen for 0.5 h. The solvent was evaporated, the product was dissolved in CH_2Cl_2 (50mL) and washed with $NaHCO_3$ (10mL) and H_2O (10mL), and dried over Na_2SO_4. The solvent was evaporated to yield an orange foam. The α isomer was crystallized from 95% ethanol, as a cottony fiber. The remaining mixture was separated by column chromatography, using toluene:ethyl acetate 4:1 as the eluant. Both **2.4α** and the **2.4β** were then recrystallized from 95% ethanol (58%, **2.4α**:**2.4β** 4.8:1).

2.4α: M.p. 207-209°C; $[\alpha]_D^{22}$ 400° (c 0.52, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.01 (s, 3 H, OCOC*H*$_3$), 2.03 (s, 3 H, OCOC*H*$_3$), 2.04 (s, 3 H, OCOC*H*$_3$), 2.05 (s, 3 H, OCOC*H*$_3$), 3.44 (ddd, 1 H, $J_{4,5}$ 10.9, $J_{5,6B}$ 3.2, $J_{5,6A}$ 4.2 Hz, H-5), 3.98 (dd, 1 H, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.28 (d, 1 H, $J_{NH,1}$ 3.8 Hz, NH), 4.43 (dd, 1 H, H-6a), 4.88 (dd, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 5.41 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 5.47 (dd, 1 H, H-3), 6.7-7.3 (m, 5 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.51-20.61 (4-OCOCH_3), 38.69 (C-5), 57.18 (C-1, J_{C1-H1} 154.52 Hz), 61.29 (C-6), 71.22, 72.49, 73.71 (C-2, C-3, or C-4), 114.88 (C-*o*), 119.89 (C-*p*), 129.30 (C-*m*), 144.82 (C-*i*), 169.25-170.43 (C=O). Anal. Calcd for $C_{20}H_{25}NO_8S$: C, 54.65; H, 5.74; N, 3.19. Found: C, 54.75; H, 5.60; N, 3.06%.

2.4β: M.p. 146-148°C; $[\alpha]_D^{22}$ -71° (c 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.98 (s, 3 H, OCOC*H*$_3$), 2.03 (s, 3 H, OCOC*H*$_3$), 2.04 (s, 3 H, OCOC*H*$_3$), 2.06 (s, 3 H, OCOC*H*$_3$), 3.28 (ddd, 1 H, $J_{4,5}$ 10.6, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.0 Hz, H-5), 4.03 (d, 1 H, $J_{NH,1}$ 10.0 Hz, NH), 4.10 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.33 (dd, 1 H, H-6A), 4.78 (dd, 1 H, $J_{1,2}$ 10.0 Hz, H-

1), 5.19 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-4), 5.33 (dd 1 H, , H-2), 6.0-7.7 (m, 5 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.48-20.63 (4-OCO*C*H$_3$), 41.77 (C-5), 59.31 (C-1, $J_{C1\text{-}H1}$ 151.70 Hz), 61.48 (C-6), 72.23, 73.45, 74.62 (C-2, C-3, or C-4), 114.06 (C-*o*), 119.65 (C-*p*), 129.38 (C-*m*), 144.47 (C-*i*), 169.46-171.15 (C=O); Anal. Calcd for $C_{20}H_{25}NO_8S$: C, 54.65; H, 5.74; N, 3.19. Found: C, 54.67; H, 5.61; N, 3.04%.

***p*-Nitro-*N*-phenyl 2,3,4,6-tetra-*O*-acetyl-α/β-5-thio-D-glucopyranosylamine** (**2.5α**, **2.5β**). To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2.2**) (1.0g, 2.46mmol) in MeOH (20mL) was added *p*-nitroaniline (340mg, 2.46mmol) and $HgCl_2$ (134mg, 0.492mmol). The reaction mixture was stirred at 50°C, under nitrogen for 18 h. The solvent was then evaporated, the product was dissolved in CH_2Cl_2 (50mL) and washed with $NaHCO_3$ (10mL) and H_2O (10mL), and dried over Na_2SO_4. The solvent was evaporated to yield a yellow/brown foam. The mixture was separated by column chromatography, using toluene:ethyl acetate 4:1 as the eluant. Both **2.5α** and **2.5β** were then recrystallized from 95% ethanol (8.4%, **2.5α**:**2.5β** 1:1).

2.5α: M.p. 215-217°C; $[\alpha]_D^{22}$ 353° (c 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3 H, OCOC*H*$_3$), 2.04 (s, 3 H, OCOC*H*$_3$), 2.045 (s, 3 H, OCOC*H*$_3$), 2.07 (s, 3 H, OCOC*H*$_3$), 3.38 (ddd, 1 H, $J_{4,5}$ 10.8, $J_{5,6B}$ 3.2, $J_{5,6A}$ 4.5 Hz, H-5), 4.0 (dd, 1 H, $J_{6A,6B}$ 12.2 Hz, H-6B), 4.41 (dd, 1 H, H-6a), 4.96 (dd, 1 H, $J_{1,2}$ 4.4 Hz, H-1), 5.21 (d, 1 H, $J_{NH,1}$ 4.4 Hz, NH), 5.35 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-4), 5.40 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 5.45 (dd, 1 H, H-3), 6.7-7.3 (dd, 4 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.71-20.80 (4-OCO*C*H$_3$), 39.36 (C-5), 56.27 (C-1, $J_{C1\text{-}H1}$ 154.9 Hz), 61.24 (C-6), 70.98, 72.44, 73.48 (C-2, C-3, or C-4), 113.01 (C-*o*), 126.10 (C-*m*), 140.80 (C-*p*), 150.28 (C-*i*), 169.43-170.59 (C=O); Anal.

Calcd for $C_{20}H_{24}N_2O_{10}S$: C, 49.57; H, 5.00; N, 5.78. Found: C, 49.68; H, 5.14; N, 5.58%.

2.5β: M.p. 183-185°C; $[\alpha]_D^{22}$ -22° (c 0.51, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.01 (s, 3 H, OCOC*H*$_3$), 2.03 (s, 3 H, OCOC*H*$_3$), 2.05 (s, 3 H, OCOC*H*$_3$), 2.06 (s, 3 H, OCOC*H*$_3$), 3.34 (ddd, 1 H, $J_{4,5}$ 10.6, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.3 Hz, H-5), 4.13 (dd, 1 H, $J_{6A,6B}$ 12.0 Hz, H-6B), 4.34 (dd, 1 H, H-6A), 4.78 (dd, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 4.94 (d, 1 H, $J_{NH,1}$ 9.3 Hz, NH), 5.21 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, H-4), 5.32 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 6.7-8.0 (dd, 4 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.60-20.76 (4-OCO*C*H$_3$), 42.34 (C-5), 58.50 (C-1, J_{C1-H1} 154.5 Hz), 61.49 (C-6), 72.30, 73.35, 74.94 (C-2, C-3, or C-4), 113.01(C-*o*), 126.33 (C-*m*), 140.57 (C-*p*), 150.15 (C-*i*), 169.59-171.74 (C=O); Anal. Calcd for $C_{20}H_{24}N_2O_{10}S$: C, 49.57; H, 5.00; N, 5.78. Found: C, 49.68; H, 5.07; N, 5.62%.

***p*-Trifluoromethyl-*N*-phenyl 2,3,4,6-tetra-*O*-acetyl-α/β-5-thio-D-glucopyranosylamine (2.6α, 2.6β)**. To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2.2**) (500mg, 1.23mmol) in dry MeOH (10mL) was added *p*-trifluoromethylaniline (170mL, 1.35mmol) and $HgCl_2$ (34mg, 0.123mmol). The reaction mixture was stirred at 50°C, under nitrogen for 1 h, during which a white precipitate formed. The solvent was then evaporated, the product was dissolved in CH_2Cl_2 (50mL) and washed with $NaHCO_3$ (10mL) and H_2O (10mL), and dried over Na_2SO_4. The solvent was evaporated to yield a light brown foam. The α isomer was crystallized from 95% EtOH, to yield a cottony fiber. The remaining mixture was separated by column

chromatography, using toluene:ethyl acetate 4:1 as the eluant. Both **2.6α** and **2.6β** were then recrystallized from 95% ethanol (44%; **2.6α**:**2.6β** 8:1).

2.6α: M.p. 231-234°C; $[\alpha]_D^{22}$ 261° (c 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.02 (s, 3 H, OCOC*H*$_3$), 2.03 (s, 3 H, OCOC*H*$_3$), 2.04 (s, 3 H, OCOC*H*$_3$), 2.05 (s, 3 H, OCOC*H*$_3$), 3.39 (ddd, 1 H, $J_{4,5}$ 10.7 Hz, H-5), 3.99 (dd, 1 H, $J_{5,6B}$ 3.1, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.43 (dd, 1 H, $J_{5,6A}$ 4.4 Hz, H-6a), 4.68 (d, 1 H, $J_{NH,1}$ 4.3 Hz, NH), 4.90 (dd, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 5.33 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 5.41 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 5.45 (dd, 1 H, H-3), 6.84 (d, 2 H, Aromatic), 7.5 (d, 2 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.46-20.54 (4-OCO*C*H$_3$), 38.89 (C-5), 56.46 (C-1, J_{C1-H1} 154.43 Hz), 61.10 (C-6), 70.93, 72.32, 73.45 (C-2, C-3, or C-4), 114.21 (C-*o*), 126.5(C-*m*), 147.40 (C-*i*), 169.19-170.37 (C=O); Anal. Calcd for $C_{21}H_{24}F_3NO_8S$: C, 49.70; H, 4.77; N, 2.76. Found: C, 49.71; H, 4.81; N, 2.74%.

2.6β: M.p. 189-192°C; $[\alpha]_D^{22}$ -24° (c 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.00 (s, 3 H, OCOC*H*$_3$), 2.03 (s, 3 H, OCOC*H*$_3$), 2.05 (s, 3 H, OCOC*H*$_3$), 2.07 (s, 3 H, OCOC*H*$_3$), 3.30 (ddd, 1 H, H-5), 4.12 (dd, 1 H, $J_{5,6B}$ 3.4, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.33 (dd, 1 H, $J_{5,6A}$ 5.2 Hz, H-6A), 4.45 (d, 1 H, $J_{NH,1}$ 9.5 Hz, NH), 4.78 (dd, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 5.20 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 5.32 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 6.73 (d, 2 H, Aromatic), 7.45 (d, 2 H, Aromatic); ^{13}C NMR ($CDCl_3$): δ 20.36-20.50 (4-OCO*C*H$_3$), 41.10 (C-5), 58.65 (C-1, J_{C1-H1} 155.9 Hz), 61.37 (C-6), 72.20, 73.29, 74.69 (C-2, C-3, C-4), 113.31 (C-*o*), 114.22 (C-*p*), 126.70 (C-*m*), 147.20 (C-*i*), 169.3-171.3 (C=O); Anal. Calcd for $C_{21}H_{24}F_3NO_8S$: C, 49.70; H, 4.77; N, 2.76. Found: C, 49.88; H, 4.80; N, 2.58%.

p-Methoxy-*N*-phenyl α/β-5-thio-D-glucopyranosylamine (2.7). The tetraacetate **2.3** (300 mg, 0.64 mmol) was dissolved in a mixture of MeOH:H_2O:Et_3N (5:1:1, 21 mL) and the mixture was stirred at room temperature overnight. The solvent was removed and the residue was purified by flash column chromatography using EtOAc:MeOH (10:1) as the eluant to give a white solid. (155 mg, 80% α:β 1:1.7). ^{1}H NMR (D_2O): δ 2.98 (m, 2 H, H-5 α/β), 3.38 (dd, 1 H, $J_{2,3}$, $J_{3,4}$ 9.1 Hz, H-3β), 3.62 (m, 2 H, H-4 α/β), 3.70 (m, 2 H, H-2β/H-3α), 3.76 (s, 6 H, OCH_3), 3.85 (m, 3 H, H-6Aβ, H-6Bα, H-6Bβ), 3.91 (dd, 1 H, $J_{5,6A}$ 5.2, $J_{6,6}$ 11.9 Hz, H-6Aα), 4.13 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2α), 4.63 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.81 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1α), 6.90 (m, 8 H, Aromatic); ^{13}C NMR (D_2O): δ 45.91 (C-5α), 48.79 (C-5β), 58.93 (OCH_3), 62.93 (C-1α, $J_{C1\text{-}H1}$ 152.81 Hz), 63.12 (C-6α), 63.22 (C-1β, $J_{C1\text{-}H1}$ 156.38 Hz), 63.30 (C-6β), 76.20 (C-4β), 76.64 (C-4α), 76.92 (2C, C-2α, C-3α), 79.04 (C-2β), 80.54 (C-3β), 118.18 (2C, C-*m* α/β), 119.40 (C-*o* β), 119.77 (C-*o* α), 142.90 (2C, C-*i* α/β), 155.49 (2C, C-*p* α/β); Anal. Calcd for $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.35; N, 4.65. Found: C, 51.66; H, 6.18; N, 4.81%.

***N*-Phenyl α/β-5-thio-D-glucopyranosylamine (2.8).** The tetraacetate **2.4** (200 mg, 0.46 mmol) was dissolved in a mixture of MeOH:H_2O:Et_3N (5:1:1, 14 mL) and the mixture was stirred at room temperature overnight. The solvent was removed and the residue was purified by flash column chromatography using EtOAc:MeOH (10:1) as the eluant to give a white solid. (105 mg, 85%, α:β 1:1.6). ^{1}H NMR (D_2O): δ 3.02 (m, 2 H, H-5 α/β), 3.39 (dd, 1 H, $J_{2,3}$, $J_{3,4}$ 9.1 Hz, H-3β), 3.62 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4β), 3.66

(dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.5 Hz, H-4α), 3.72 (m, 2 H, H-2β, H-3α), 3.77 (dd, 1 H, $J_{5,6B}$ 5.9 Hz, H-6Bβ), 3.81 (dd, 1 H, $J_{5,6B}$ 5.3 Hz, H-6Bα), 3.85 (m, 1 H, H-6Aβ), 3.91 (dd, 1 H, $J_{5,6A}$ 5.2 Hz, $J_{6A,6B}$ 12.0 Hz, H-6Aα), 4.05 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.73 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.91 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1α), 6.88 (m, 6 H, aromatic), 7.27 (m, 4 H, aromatic). ^{13}C NMR (D_2O): δ 46.01 (C-5α), 48.79 (C-5β), 61.62 (C-1α, $J_{C1\text{-}H1}$ 152.9 Hz), 62.02 (C-1β, $J_{C1\text{-}H1}$ 149.5 Hz), 63.01 (C-6α), 63.19 (C-6β), 76.19 (C-4β), 76.63 (C-4α), 76.83 (C-2α), 76.94 (C-3α), 78.98 (C-2β), 80.52 (C-3β), 117.47 (C-*o* β), 117.48 (C-*o* α), 122.18 (C-*p* α), 122.24 (C-*p* β), 132.39 (C-*m* α), 132.44 (C-*m* β), 148.66 (2C, C-*i* α/β); Anal. Calcd for $C_{12}H_{17}NO_4S$: C, 53.12; H, 6.32; N, 5.16. Found: C, 53.15; H, 6.18; N, 5.02%.

***p*-Nitro-*N*-phenyl α/β-5-thio-D-glucopyranosylamine (2.9).** The tetraacetate **2.5** (100 mg, 0.206 mmol) was dissolved in a mixture of MeOH:H_2O:Et_3N (5:1:1, 7 mL) and the mixture was stirred at room temperature overnight. The solvent was removed and the residue was purified by flash column chromatography using EtOAc:MeOH (10:1) as the eluant to give a yellow solid. (45 mg, 69%, α:β 1:1.75). ^{1}H NMR (D_2O): δ 3.03 (ddd, 1 H, $J_{5,6}$ 3.2, $J_{5,6}$ 5.4 Hz, H-5 α), 3.10 (ddd, 1 H, $J_{5,6}$ 3.5, $J_{5,6}$ 5.7 Hz, H-5 β), 3.40 (dd, 1 H, $J_{2,3}$, $J_{3,4}$ 9.1 Hz, H-3β), 3.63 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4β), 3.68 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.5 Hz, H-4α), 3.77 (m, 3 H, H-2β, H-3α, H-6Bα), 3.84 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6Bβ), 3.90 (m, 2 H, H-6Aα, H-6Aβ), 4.09 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.84 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 5.01 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1α), 6.90 (m, 4 H, aromatic), 8.1 (m, 4 H, aromatic). ^{13}C NMR (CD_3OD): δ 45.57 (C-5α), 48.00 (C-5β), 58.85 (C-1α, $J_{C1\text{-}H1}$

152.0), 59.60 (C-1β, $J_{C1\text{-}H\text{-}1}$ 152.1 Hz), 62.39 (2C, C-6α/β), 75.48 (C-4β), 75.62, 75.76 (3C, C-4α, C-2α, C-3α), 77.62 (C-2β), 79.74 (C-3β), 113.45 (C-*o* β), 114.06 (C-*o* α), 126.60 (C-*p* α), 126.87 (C-*p* β), 139.49 (2C, C-*m* α/β), 154.30 (2C, C-*i* α/β); Anal. Calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86. Found: C, 45.31; H, 5.15; N, 8.90%.

***p*-Trifluoromethyl-*N*-phenyl α/β-5-thio-D-glucopyranosylamine (2.10).** The tetraacetate **2.6** (250 mg, 0.49 mmol) was dissolved in a mixture of MeOH:H_2O:Et_3N (5:1:1, 21 mL) and the mixture was stirred at room temperature overnight. The solvent was removed and the residue was purified by flash column chromatography using EtOAc:MeOH (10:1) as the eluant to give a white solid. (128 mg, 77%, α:β 1:2.24). ^{1}H NMR (D_2O): δ 3.00 (ddd, 1 H, $J_{5,6}$ 3.1 Hz, $J_{5,6}$ 5.2 Hz, H-5 α), 3.05 (ddd, 1 H, $J_{5,6}$ 3.4 Hz, $J_{5,6}$ 5.4 Hz, H-5 β), 3.39 (dd, 1 H, $J_{2,3}$, $J_{3,4}$ 9.1 Hz, H-3β), 3.62 (dd, 1 H, $J_{4,5}$ 10.3 Hz, H-4β), 3.66 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4α), 3.75 (m, 3 H, H-2β, H-3α, H-6B), 3.83 (dd, 1 H, $J_{6A,6B}$ 12.0 Hz, H-6B), 3.89 (dd, 1 H, $J_{6,6}$ 12.3 Hz, H-6Aβ), 3.91 (dd, 1 H, H-6Aα), 4.08 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.80 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.95 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1α), 6.98 (m, 4 H, aromatic), 7.50 (m, 4 H, aromatic). ^{13}C NMR (CD_3OD): δ 45.36 (C-5α), 47.89 (C-5β), 59.43 (C-1α, $J_{C1\text{-}H1}$ 151.0 Hz), 60.25 (C-1β, $J_{C1\text{-}H1}$ 151.2 Hz), 62.60 (2C, C-6α/β), 75.70 (C-4β), 75.77, 75.83, 76.01 (C-4α, C-2α, C-3α), 77.80 (C-2β), 79.86 (C-3β), 114.16 (C-*o* β), 114.78 (C-*o* α), 126.97, 127.14, 127.17, 127.74 (C-*p* α/β, C-*m* α/β), 151.13 (2C, C-*i* α/β); Anal. Calcd for $C_{13}H_{16}F_3NO_4S$: C, 46.02; H, 4.75; N, 4.13. Found: C, 45.78; H, 4.90; N, 3.95%.

2.6 Acknowledgements

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CHAPTER 3: IS THERE A GENERALIZED REVERSE ANOMERIC EFFECT? SUBSTITUENT AND SOLVENT EFFECTS ON THE CONFIGURATIONAL EQUILIBRIA OF NEUTRAL AND PROTONATED *N*-ARYL-GLUCOPYRANOSYLAMINES AND *N*-ARYL-5-THIOGLUCOPYRANOSYLAMINES[#]

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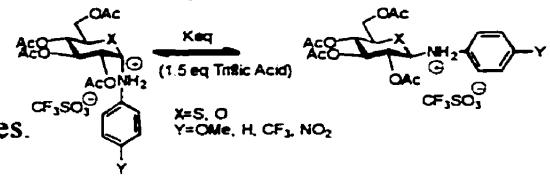
This work is dedicated, with respect, to the memory of J. T. Edward.

*To whom correspondence may be addressed.

3.1 Graphical Abstract

Is There a Generalized Reverse Anomeric Effect? Substituent and Solvent Effects on the Configurational Equilibria of Neutral and Protonated *N*-Aryl-Glucopyranosylamines and *N*-Aryl-5-Thioglucopyranosylamines.



Karla D. Randell, Blair D. Johnston, David F. Green and B. Mario Pinto

3.2 Abstract

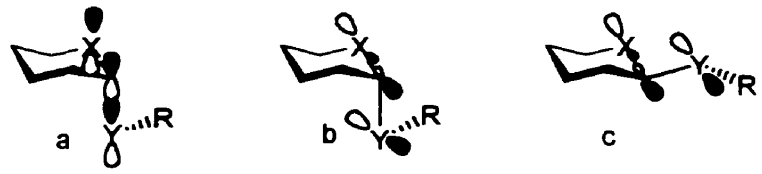
The effects of substitution and solvent on the configurational equilibria of neutral and protonated *N*-(4-Y-substituted-phenyl) peracetylated-5-thioglucopyranosylamines (Y= OMe, H, CF_3, NO_2) **3.1-3.4** and *N*-(4-Y-substituted-phenyl) peracetylated-glucopyranosylamines (Y= OMe, H, NO_2) **3.9-3.11** are described. The configurational equilibria were determined by direct integration of the resonances of the individual isomers in the ^1H NMR spectra after equilibration of both α- and β-isomers. The equilibrations of the neutral compounds **3.1-3.4** in CD_3OD, CD_3NO_2, and $(CD_3)_2CO$ were achieved by $HgCl_2$ catalysis and those of the neutral compounds **3.9-3.11** in CD_2Cl_2 and CD_3OD by triflic acid catalysis. The equilibrations of the protonated compounds in both the sulfur series (solvents: CD_3OD, CD_3NO_2, $(CD_3)_2CO$, $CDCl_3$ and CD_2Cl_2) and oxygen series (solvents: CD_2Cl_2 and CD_3OD) were achieved with triflic acid. The substituent and solvent effects on the equilibria are discussed in terms of steric and electrostatic effects, and orbital interactions associated with the endo-anomeric effect. A generalized reverse anomeric effect does not exist in neutral or protonated *N*-aryl-5-thioglucopyranosylamines and *N*-aryl-glucopyranosylamines. The anomeric effect ranges from 0.85 kcal mol^{-1} in **3.2** to 1.54 kcal mol^{-1} in **3.10**. The compounds **3.1-3.4** and **3.9-3.11** show an enhanced endo-anomeric effect upon protonation, ranging from 1.73 kcal mol^{-1} in **3.2** to 2.57 kcal mol^{-1} in **3.10**. We estimate the increase in the anomeric effect upon protonation of **3.10** to be approximately 1.0 kcal mol^{-1}. However, this effect is offset by steric effects due to the associated counterion which we estimate to be approximately 1.2 kcal mol^{-1}. The values of K_{eq}(axial-equatorial) in protonated **3.1-3.4**

increase in the order OMe<H<CF_3<NO_2, in agreement with the dominance of steric effects (due to the counterion) over the endo-anomeric effect. The values of K_{eq}(axial-equatorial) in protonated **3.9-3.11** show the trend OMe>H<NO_2 that is explained by the balance of the endo-anomeric effect and steric effects in the individual compounds. The trends in the values of the C_1-H_1 coupling constants for **3.1-3.4**, and the corresponding deacetylated compounds **3.5-3.8,** as a function of substituent and α- or β-configuration are discussed in terms of the Perlin effect and the interplay of the endo- and exo-anomeric effects.

3.3 Introduction

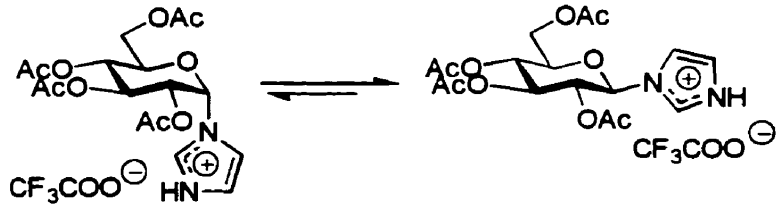
The preference for the axial orientation of electronegative substituents at the anomeric carbon of a pyranose ring is in marked contrast to expectations based solely on the consideration of steric interactions. This anomalous behavior was first noted by Edward[1] in his investigation of the relative stability of methyl α- and β- glycopyranosides to acid hydrolysis and was clearly defined as the anomeric effect by Lemieux and Chü[2] as a result of their investigations of the anomeric equilibria of peracetylated pento- and hexo-pyranoses. Since that time, the anomeric effect seems to have taken on a life of its own and has engaged the imagination and efforts of both experimental and theoretical chemists in their attempts to define its nature and assign its origin.[3] The effect is stereoelectronic in nature and has since been shown to be a general effect operating in X-A-Y segments. The effect has been classified further in terms of the endo- and exo-anomeric effect. The endo-anomeric effect[4] refers to the preference of electronegative groups attached to the anomeric carbon for the axial orientation. The preference is dictated partly by stabilizing $n_X \rightarrow \sigma^*_{C\text{-}Y}$ orbital interactions (Chart 3.1, a).[5] The exo-anomeric effect[6] is the preference for the gauche conformation around the C_1-aglyconic carbon bond of glycopyranosides that permits expression of an $n_Y \rightarrow \sigma^*_{C\text{-}X}$ stabilizing orbital interaction (Chart 3.1, b and c).[5]

Chart 3.1



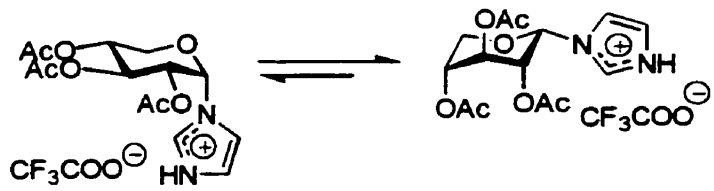
In recent years, another phenomenological conformational effect, the reverse anomeric effect (RAE), has also gained notoriety and has provoked some debate wherein even its very existence has been questioned. The latter effect was defined by Lemieux and Morgan[7] as the tendency of an aglycon bearing a positive charge in a sugar ring to adopt the equatorial orientation; the systems under study contained quaternary nitrogen aromatic substituents such as pyridinium and imidazolium (e.g. Scheme 3.1). The work

Scheme 3.1



was later extended by Paulsen *et al.*[8] to the study of the conformational equilibria in peracetylated pentopyranosyl imidazoles and the corresponding protonated species (e.g. Scheme 3.2), and by Finch and Nagpurkar[9] to the neutral and positively charged *N*-(hexopyranosyl)imidazoles and their tetraacetates. It has also been suggested that

Scheme 3.2

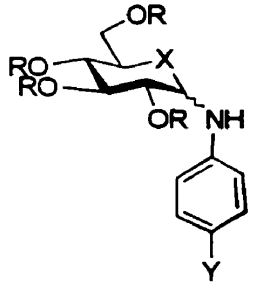


neutral and protonated amino and alkylamino substituents would show a RAE,[10] but this has been questioned.[5,11] The greater equatorial preferences have now been attributed to accentuated steric effects, although the conclusions are based on data from highly biased equilibria.[5,11a] A recent study with a more sterically balanced, 2,2'-substituted-1,3-dioxane system, confirmed these conclusions.[11f] The general picture that is emerging from recent studies is that protonation of either an alkylamino substituent or an imidazole substituent results in a stronger anomeric effect,[11a-g] and that the equatorial preference has its origin in favorable electrostatic interactions.[12] Nevertheless, the conclusions were not universal and appeared to be system-dependent. Thus, whereas Perrin and coworkers[11a,b] claimed an absence of a RAE with protonated *N*-(glucopyranosyl)imidazoles (Scheme 3.1), by examination of configurational equilibria using an NMR titration method, the Queen's group[12] claimed the existence of such an effect in protonated *N*-(xylopyranosyl)imidazoles (Scheme 3.2), by examination of conformational equilibria using an approximate average coupling-constant method, which they attributed to intramolecular electrostatic attraction in the equatorial isomers. Such subtle differences indicate that the magnitude of the measured effect is small. The matter has been resolved very recently by use of an NMR titration method to estimate the RAE in protonated *N*-(xylopyranosyl)imidazoles.[11g] The authors conclude that no RAE

exists in these compounds and that the previous conclusions[8,9,12b] resulted because of the approximations inherent in the average coupling-constant method.[11g]

We chose to address the question of the existence of a *generalized* RAE by systematic examination of substituent and solvent effects on the configurational equilibria of *N*-aryl-5-thioglucopyranosylamines **3.1-3.4** and *N*-aryl-glucopyranosylamines **3.9-3.11** and the corresponding protonated species. The 5-thio compounds **3.1-3.4** are more stable than their oxygen congeners **3.9-3.11** and are readily amenable to analysis. Both series also display equilibria that are not highly biased and differ from systems studied to date; they constitute, therefore, a valuable test system. The question of a generalized RAE is of interest for an understanding of the enzyme inhibitory activity of glucopyranosyl amines and their 5-thio-analogues.[13]

Chart 3.2



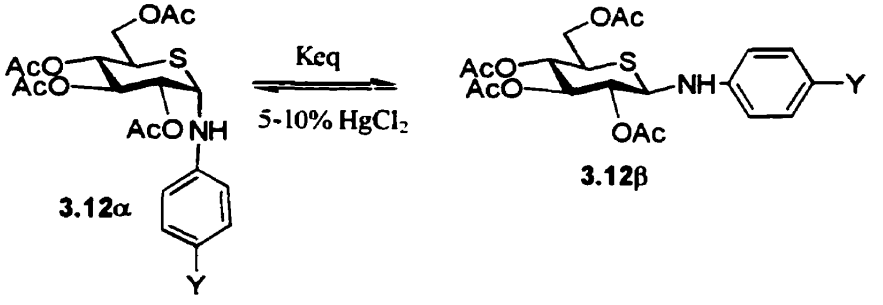
Compound	R	X	Y
3.1	Ac	S	OMe
3.2	Ac	S	H
3.3	Ac	S	CF_3
3.4	Ac	S	NO_2
3.5	H	S	OMe
3.6	H	S	H
3.7	H	S	CF_3
3.8	H	S	NO_2
3.9	Ac	O	OMe
3.10	Ac	O	H
3.11	Ac	O	NO_2

3.4 Results and Discussion

3.4.1 Configurational Analysis

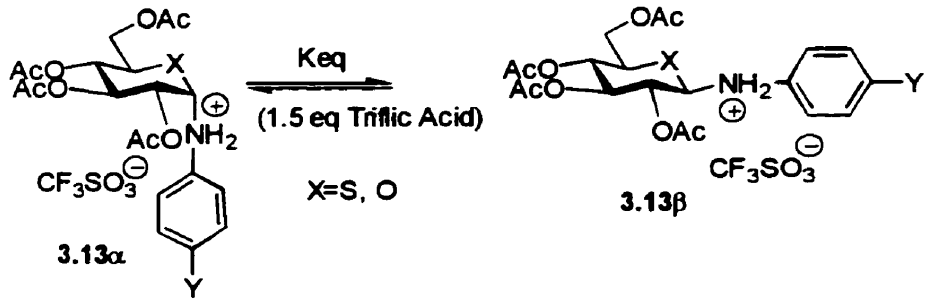
The equilibrium populations of the 5-thio-compounds **3.1-3.4** and their protonated derivatives were assessed by ^{1}H NMR spectroscopy at 294 K. Equilibration of the neutral species (**3.12**, Scheme 3.3) was achieved by the $HgCl_2$ catalysis of the individual

Scheme 3.3



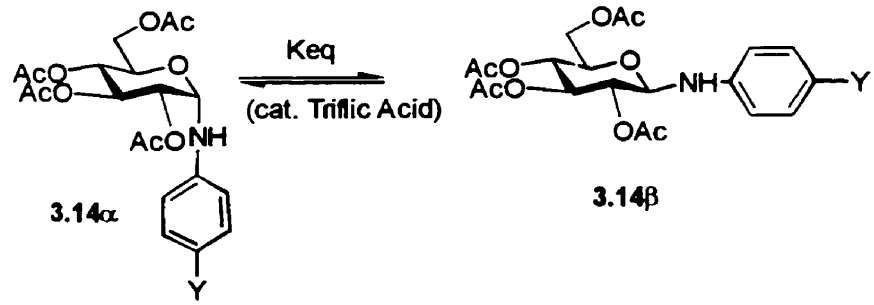
isomers in the polar solvents CD_3OD, CD_3NO_2, and $(CD_3)_2CO$ only, owing to the limited solubility of $HgCl_2$ in non-polar solvents. The equilibria were approached from both directions, *i.e.* starting from pure α- or pure β-anomers, to ensure that a true equilibrium had been reached. The corresponding equilibrations of the protonated species (**3.13**, Scheme 3.4) were studied in the presence of 1.5 equivalents of triflic acid, in polar and non-polar solvents. The addition of 1.5 equivalents of triflic acid would ensure complete protonation of the amines since the pK_a of triflic acid is -5.9[14] while the pK_as of the isolated aglycons are 5.31 for *p*-anisidine, 4.60 for aniline, 2.45 for *p*-trifluoromethylaniline, and 1.00 for the weakest base, *p*-nitroaniline.[15] The equilibration

Scheme 3.4



of the oxygen congeners **3.9-3.11** was performed at 230 K only in CD_2Cl_2 and CD_3OD. In this series of compounds, we were restricted in our choice of solvents because of the instability of the compounds or line broadening effects in the spectra, which did not permit unambiguous assignment of signals or their accurate integration. The equilibrations of the neutral species (**3.14**, Scheme 3.5) were achieved by addition of

Scheme 3.5



catalytic amounts of triflic acid to solutions of the individual anomers, and those of the protonated species (**3.13**, Scheme 3.4) were achieved by addition of excess triflic acid, as described above. The equilibrium constants and free energy values for **3.1-3.4** are listed in Table 3.1 and those for **3.9-3.11** in Table 3.2.

Table 3.1 Effects of Protonation, Solvent, and Substituent on K_{eq} and ΔG for the Neutral Compounds in which X=S

Solvent	Y	K_{eq} (error) Neutral	ΔG^a (error) Neutral	K_{eq} (error) Protonated	ΔG^a (error) Protonated
CD_3NO_2	**3.1** OMe	1.31 (0.15)	-0.16 (0.07)	0.32 (0.07)	0.67 (0.10)
	3.2 H	1.26 (0.08)	-0.14 (0.04)	0.45 (0.15)	0.47 (0.20)
	3.3 CF_3	1.15 (0.13)	-0.08 (0.07)	1.05 (0.08)	-0.03 (0.04)
	3.4 NO_2	1.51 (0.10)	-0.24 (0.04)	1.27 (0.07)	-0.14 (0.03)
CD_3OD	**3.1** OMe	1.37 (0.10)	-0.18 (0.04)	b	b
	3.2 H	1.22 (0.08)	-0.12 (0.03)	b	b
	3.3 CF_3	1.24 (0.19)	-0.13 (0.09)	0.97 (0.09)[c]	0.02 (0.05)[c]
	3.4 NO_2	1.34 (0.16)	-0.17 (0.01)	b	b
$(CD_3)_2CO$	**3.1** OMe	1.15 (0.08)	-0.08 (0.04)	0.38 (0.11)	0.56 (0.17)
	3.2 H	1.15 (0.06)	-0.08 (0.03)	0.81 (0.13)	0.12 (0.09)
	3.3 CF_3	0.98 (0.07)	0.01 (0.04)	1.07 (0.08)	-0.04 (0.04)
	3.4 NO_2	0.98 (0.08)	0.01 (0.05)	b	b
$CDCl_3$	**3.1** OMe	d	d	0.48 (0.13)	0.43 (0.10)
	3.2 H	d	d	0.81 (0.08)	0.13 (0.06)
	3.3 CF_3	d	d	1.69 (0.09)	-0.31 (0.02)
	3.4 NO_2	d	d	1.93 (0.14)	-0.39 (0.04)
CD_2Cl_2	**3.1** OMe	d	d	0.32 (0.03)	0.68 (0.05)
	3.2 H	d	d	0.65 (0.03)	0.26 (0.03)
	3.3 CF_3	d	d	1.40 (0.12)	-0.20 (0.05)
	3.4 NO_2	d	d	1.71 (0.07)	-0.31 (0.02)

[a] in kcal mol^{-1} at 294 K

[b] decomposition of samples did not permit accurate determination of K_{eq}

[c] only equilibrated from $\alpha \rightarrow \beta$ (3 days)

[d] equilibration of the neutral species in these solvents was not possible owing to the insolubility of $HgCl_2$

Table 3.2 Effects of Protonation, Solvent, and Substituent on K_{eq} and ΔG for the Neutral Compounds in which X=O

Solvent	Y	K_{eq} (error) Neutral	ΔG^a (error) Neutral	K_{eq} (error) Protonated	ΔG^a (error) Protonated
CD_2Cl_2	**3.9** OMe	2.65 (0.03)	-0.45 (0.01)	6.00 (0.09)	-0.82 (0.01)
	3.10 H	2.74 (0.07)	-0.46 (0.01)	4.24 (0.07)	-0.66 (0.01)
	3.11 NO_2	3.18 (0.03)	-0.53 (0.01)	6.58 (0.03)	-0.82 (0.01)
CD_3OD	**3.9** OMe	4.04 (0.11)	-0.64 (0.02)	6.87 (0.25)	-0.88 (0.03)
	3.10 H	3.95 (0.06)	-0.63 (0.01)	3.87 (0.06)	-0.62 (0.01)
	3.11 NO_2	b	b	10.9 (0.20)	-1.09 (0.03)

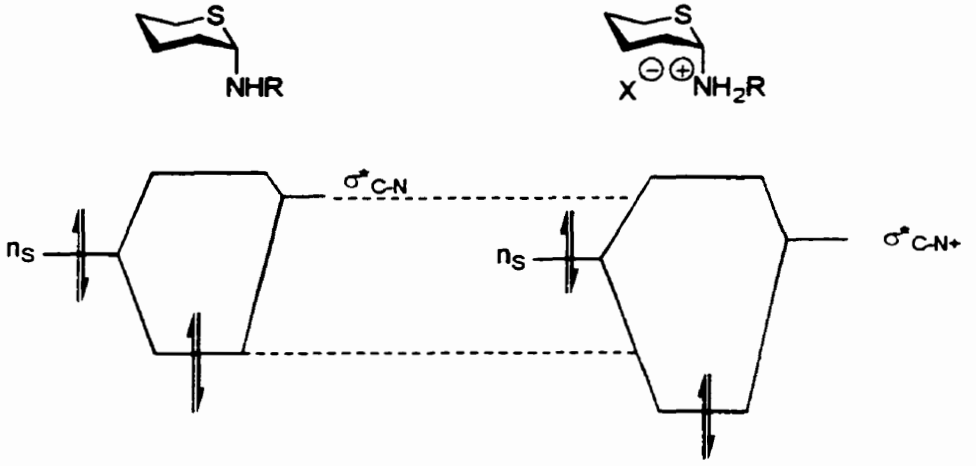
[a] in kcal mol^{-1} at 230 K

[b] no observable α-isomer

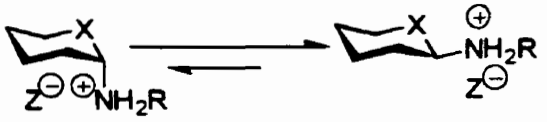
3.4.2 Equilibration of the 5-Thio-Compounds 3.1-3.4

Examination of the equilibria of the neutral species **3.1-3.4** in polar solvents indicates that there is no marked substituent or solvent effect. Interestingly, the effects of protonation on the equilibria are a function of the substituent. When Y= OMe (**3.1**) or H (**3.2**), the proportion of the axial isomer (**3.13α**, Scheme 3.4) increases upon protonation. This can be attributed to an increase in the endo-anomeric effect because the strength of the n-σ* interactions increases due to a smaller energy gap between interacting fragment orbitals (see Scheme 3.6).[10] When Y=CF_3 (**3.3**) or NO_2 (**3.4**), there is no significant change in K_{eq} upon protonation. The positive charge on nitrogen must enhance the endo-anomeric effect as above. However, since the nitrogen atom bears a greater positive charge because of the inductive and field effects of these substituents, we suggest that the counterion is bound more tightly and that there is an *increased* steric effect that offsets the increased anomeric effect (see Scheme 3.7). Corroboration of this hypothesis derives from the effects of substituents and solvent on the equilibria of the protonated species. Thus, when Y=OMe (**3.1**) or H (**3.2**), the axial isomer (**3.13α**, Scheme 3.4) dominates for

Scheme 3.6



Scheme 3.7



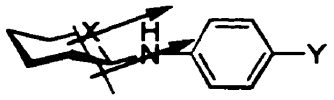
all solvents and the endo- anomeric effect is pronounced. One predicts that the *p*-OMe substituent will cause the nitrogen to be less positive, and therefore, the endo-anomeric effect will not be as strong as in the parent aniline derivative **3.2**.[16] One also predicts that the counterion will not be as tightly bound and that steric effects will be less pronounced. The observation of a greater proportion of the axial isomer for **3.1** (Y=OMe) than **3.2** (Y=H) suggests that the steric effects dominate. When Y=CF_3 (**3.3**) or NO_2 (**3.4**), the β-isomer (**3.13β**, Scheme 3.4) is favored more than in the compounds in which Y=OMe (**3.1**) or H (**3.2**). The observation supports our argument that the electron-withdrawing substituents cause the nitrogen atom to have a greater positive charge, a tightly bound counterion, and therefore, a greater steric effect (see Scheme 3.7); these increased steric

effects favor the equatorial isomer (**3.13β**, Scheme 3.4). In non-polar solvents, the steric effect of the associated counterion should be even more significant because the ion-pair is not solvated effectively. In accord with this hypothesis, when Y=CF_3 (**3.3**) or NO_2 (**3.4**), there is a greater proportion of the β-isomer in non-polar solvents than in polar solvents; this effect is more pronounced than for compounds in which Y= OMe (**3.1**) or H (**3.2**). In **3.3** and **3.4**, the counterion is bound more tightly and these equilibria are more sensitive to the effects of solvent. The values of K_{eq} increase in the order OMe<H<CF_3<NO_2, in agreement with the dominance of steric effects (due to the counterion) over the endo-anomeric effect.

3.4.3 Equilibration of the Oxygen Congeners 3.9-3.11

The percentages of the β-isomers in compounds **3.9-3.11** are greater than in the corresponding sulfur analogues, in accord with the greater steric effects in the former series. As was the case with the 5-thio-compounds **3.1-3.4**, there is no marked substituent effect on the equilibria of the neutral species. However, in this series we were able to examine the equilibria in both a non-polar and polar solvent and there is a notable solvent effect: the proportion of the α-anomer is greater in CD_2Cl_2 compared to CD_3OD. We attribute this effect to the lesser ability of the non-polar solvent CD_2Cl_2 to solvate the dipole-dipole interactions in the β-anomers that are normally associated with the endo-anomeric effect (see Scheme 3.8). There was also a greater proportion of the β-isomer for **3.9-3.11** in CD_3NO_2 than in CD_2Cl_2 (data not shown). The greater effect of dipolar interactions in the oxygen series as compared to the sulfur series is expected based on the greater electronegativity of oxygen than sulfur.

Scheme 3.8



The effects of protonation and the effects of solvent on the equilibria of the protonated species reveal some interesting trends. Unfortunately, the comparison is only possible for the equilibrations in CD_3OD and CD_2Cl_2 since attempts to extend these studies to other solvents, e.g. CD_3NO_2, were frustrated by problems of line broadening that made assignment of the signals ambiguous and integrations imprecise. When Y=H (**3.10**), there is no effect upon protonation in CD_3OD, but in CD_2Cl_2 more of the β-isomer is observed. We attribute this to the solvation or damping by the polar solvent of the favorable dipole-monopole interaction between the resultant dipole of the lone pairs on the ring oxygen and the positive monopole on the protonated nitrogen atom in the β-isomer. When Y=OMe (**3.9**), there is an increase in the proportion of the β-isomer upon protonation in both solvents. When Y= NO_2 (**3.11**), the α-isomer is barely detectable in the neutral compound, but in CD_3OD, the α-isomer is present in the protonated form. The positive charge must enhance the endo-anomeric effect, as no α-isomer is present in the unprotonated species. The more pronounced α-preference in CD_3OD relative to CD_2Cl_2 likely results from better solvation of the dipole-monopole interactions in the β-isomer. Since the nitrogen atom is more positive in this compound because of the substituent, we propose that the counterion is more tightly bound and that there is an increased steric effect that offsets the increased endo-anomeric effect (see Scheme 3.7).

It is instructive to compare the effects of substituents on the equilibria of the protonated species, as with the sulfur series of compounds. When Y=H (**3.10**), there is a greater proportion of the α-isomer present as compared to the compounds in which Y=OMe (**3.9**) or NO_2 (**3.11**). With regard to the *p*-OMe compound **3.9**, one predicts again that the substituent will cause the nitrogen to be less positive, and therefore, the anomeric effect will not be as strong as in the parent aniline derivative **3.10**.[16] One also predicts that the counterion will not be as tightly bound and that steric effects will be less pronounced. The observation of a greater proportion of the α-isomer for **3.10** (Y=H) than **3.9** (Y=OMe) suggests that the endo-anomeric effect dominates, in contrast to the situation with the sulfur series. With regard to the *p*-NO_2 compound **3.11**, the β- isomer (**3.13β**, Scheme 3.4) is favored more than in the compounds in which Y=OMe (**3.9**) or H (**3.10**). As with the sulfur series, the observation supports our argument that the electron-withdrawing substituents cause the nitrogen atom to have a greater positive charge, a tightly bound counterion, and therefore, a greater steric effect (see Scheme 3.7); these increased steric effects favor the equatorial isomer (**3.13β**, Scheme 3.4).

The results presented in the foregoing sections suggest that there is no generalized reverse anomeric effect operating in X-C-N or X-C-N^+ fragments. In fact, protonation leads to a greater endo-anomeric effect than in the corresponding neutral X-C-N fragments. The effects of substitution on the equilibria of the protonated species can be interpreted in terms of the dominance of steric effects (due to the counterion) over the endo-anomeric effect. For compounds **3.1-3.4**, in non-polar solvents, ion pair separation is not as effective and the accentuated steric effects are more pronounced when X=CF_3 or NO_2 than when X=H or OMe.

3.4.4 The Anomeric Effect

In order to estimate the magnitudes of the anomeric effects in **3.1-3.4** and **3.9-3.11**, cis-4-methyl-1-*N*-phenyl-cyclohexylamine **3.15** was used as a model compound in order to approximate the steric component. The methyl group was used as a counterpoise group to give a more balanced conformational equilibrium, and the conformational equilibria of **3.15** and the corresponding protonated species **3.16** (Scheme 3.9) were analyzed at 160 K in a mixture of CD_2Cl_2:$CFCl_3$ (85:15) (see Table 3.3).

Scheme 3.9

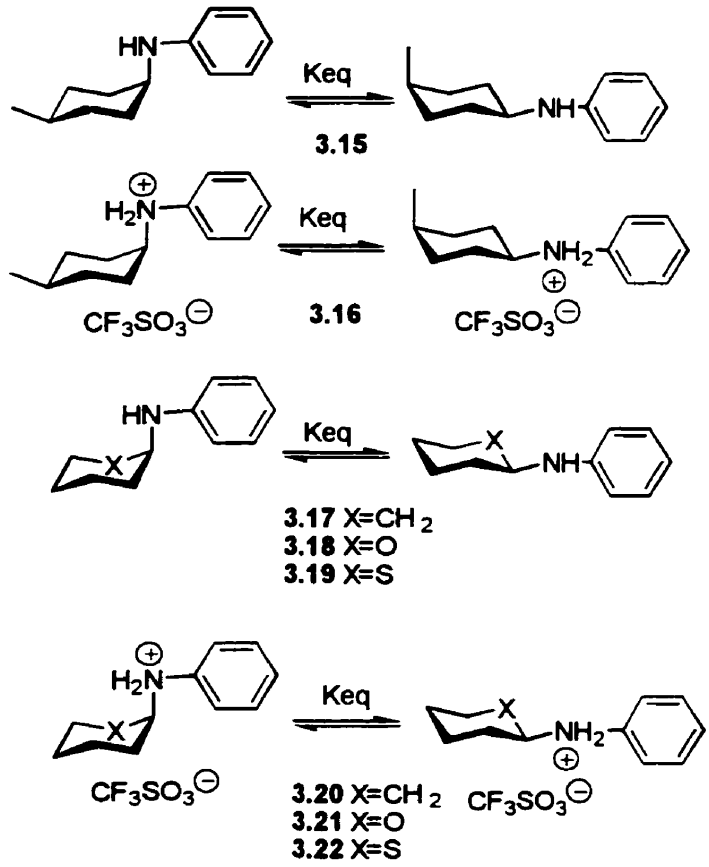


Table 3.3 Equilibrium Data for 3.15 and 3.16[a]

Compound	K_{eq} (error)	ΔG (error)[b]
3.15	0.18 (0.01)[c]	0.54 (0.01)
3.16	2.03 (0.01)[d]	-0.23 (0.01)

[a] in $CD_2Cl_2/CFCl_3$ (85:15)
[b] in kcal mol^{-1} at 160 K
[c] derived from integration of signals for $H1_{axial}$ (δ 3.20); NH (δ 3.60) in the minor conformer and $H1_{equatorial}$ (δ 3.70); NH (δ 3.94) in the major conformer.
[d] derived from integration of signals for $H1_{axial}$ (δ 3.17) in the major conformer and $H1_{equatorial}$ (δ 3.68) in the minor conformer.

The A-value of the methyl group (1.80 ± 0.02 kcal mol^{-1}, 157 K)[17] was then used to give an estimate of the conformational free energy for *N*-phenyl-cyclohexylamine **3.17** and its protonated counterpart **3.20**. These data (Table 3.4) give an estimate of approximately

Table 3.4 Steric Component of ΔG in 3.17-3.22[a]

Compound	-ΔG
3.17	1.26 (0.10)
3.18	2.00 (0.14)
3.19	0.99 (0.05)
3.20	2.03 (0.02)
3.21	3.23 (0.23)
3.22	1.60 (0.08)

[a] in kcal mol^{-1}

0.8 kcal mol^{-1} for the steric effect upon protonation that can be used as a rough measure of the steric effect of the associated counterion. The steric components of the conformational free energies for *N*-phenyl-tetrahydropyranylamine **3.18**, and its protonated counterpart **3.21**, *N*-phenyl-tetrahydrothiapyranylamine **3.19**, and its protonated counterpart **3.22** were then calculated (see Table 3.4) by taking into account the ratios of the respective conformational free energies for the axial-equatorial equilibria

in 2-Me-tetrahydropyran (2.86 ± 0.20 kcal mol^{-1}),[18] 2-Me-tetrahydrothiapyran (1.42 ± 0.07 kcal mol^{-1}),[19] and methylcyclohexane (1.80 ± 0.02 kcal mol^{-1}).[17] Finally, estimates of the anomeric effects operating in the neutral and protonated compounds **3.2** and **3.10** were obtained by adding the steric components to the conformational free energies (Tables 3.1, 3.2) for neutral and protonated **3.2** and **3.10** (see Table 3.5). We realize that different temperatures were used to obtain the different ΔG values described above, but feel that the treatment will provide a rough estimate of the magnitude of the anomeric

Table 3.5 Anomeric Effect in Compounds 3.2 and 3.10[a]

Compound	Solvent	Neutral	Protonated
3.2	CD_3NO_2	0.85 (0.06)	b
3.2	CD_3OD	0.87 (0.06)	b
3.2	$(CD_3)_2CO$	0.91 (0.06)	b
3.2	$CDCl_3$	c	1.73 (0.08)
3.2	CD_2Cl_2	c	1.86 (0.08)
3.10	CD_3OD	1.37 (0.14)	b
3.10	CD_2Cl_2	1.54 (0.14)	2.57 (0.23)

[a] in kcal mol^{-1}
[b] not appropriate (see text)
[c] experiments not performed because of limited solubility of $HgCl_2$

effect. For the neutral compounds, the anomeric effect ranges from 0.85 kcal mol^{-1} in **3.2** to 1.54 kcal mol^{-1} in **3.10**. We have shown that in the equilibria of the protonated species, the associated counterion is critical in determining the steric effect, and that the solvent and substituent play a crucial role in the solvation of ion pairs and hence the steric effect. Therefore, since we have only assessed the steric component for the protonated, unsubstituted compound **3.16** in a non-polar solvent, we have only estimated the magnitudes of the anomeric effect for the protonated, unsubstituted compounds **3.2** and **3.10** in the non polar solvents $CDCl_3$ and CD_2Cl_2. The anomeric effect in the protonated

derivatives ranges from 1.73 kcal mol^{-1} in **3.2** to 2.57 kcal mol^{-1} in **3.10**. In the case of **3.10**, the anomeric effect increases on protonation by approximately 1.0 kcal mol^{-1}. This is offset by a steric effect of the associated counterion of about 1.2 kcal mol^{-1}. Unfortunately, in the case of **3.2**, the equilibrium data for the neutral species in CD_2Cl_2 are not available, and an estimate of the magnitude of the differential anomeric effect on protonation is not possible. Nevertheless, the results indicate that the reverse anomeric effect does not exist in both neutral and protonated *N*-(phenyl)glucopyranosylamine **3.10**, and in protonated *N*-phenyl-5-thioglucopyranosylamine **3.2**; rather, the anomeric effect exists.

3.4.5 Perlin Effect

As a final point of interest, we comment here on the $J_{C1\text{-}H1}$ coupling constants in compounds **3.1-3.8** and their relationship to the Perlin effect which correlates larger $J_{C1\text{-}H1}$ values with greater C-H bond strengths.[20] The data are summarized in Table 3.6. The

Table 3.6 Coupling Constants $J_{C1\text{-}H1}$ (Hz) for Compounds 3.1-3.8

Compound (Y;R)	α $J_{C1\text{-}H1}$	β $J_{C1\text{-}H1}$
3.1 (OMe; Ac)	153.9	156.2
3.2 (H; Ac)	154.5	151.7
3.3 (CF_3; Ac)	154.4	155.9
3.4 (NO_2; Ac)	154.9	154.5
3.5 (OMe; H)	152.8	156.4
3.6 (H; H)	152.9	149.5
3.7 (CF_3; H)	151.0	151.2
3.8 (NO_2; H)	152.0	152.1

trends are the same for the acetylated and free sugars. The *p*-CF_3- and *p*-NO_2-derivatives (**3.3, 3.4, 3.7, 3.8**) have similar coupling constants for both the α- and the β-isomers. The

p-OMe-derivatives (**3.1, 3.5**) have larger coupling constants for the β-isomer, whereas the aniline derivatives (**3.2, 3.6**) have larger coupling constants for the α-isomer. Compounds **3.2** and **3.6** follow the normal trend in that an $n_S \rightarrow \sigma^*_{C1\text{-}H1}$ orbital interaction in the β-isomers results in weaker C-H bonds and smaller coupling constants.[20]

We propose that in the case of the *p*-OMe derivatives β-**3.1** and β-**3.5**, a greater exo-anomeric effect ($n_{N\rightarrow}\sigma^*_{C\text{-}S}$) relative to that in the unsubstituted compounds **3.2** and **3.6**, respectively,[16] leads to greater s-character in the C-H bond and to a reversal in C_1-H_1 bond strengths. This effect outweighs the opposing bond lengthening effect caused by the Perlin effect.

Within the series of the acetylated α-isomers the $J_{C1\text{-}H1}$ values are very similar, in contrast to those for the β-isomers which increase in **3.1**, **3.3**, and **3.4** relative to the value for the unsubstituted compound **3.2**, the greatest difference being observed for the *p*-OMe derivative **3.1**. Similar trends are observed for the α- and β- isomers in the deprotected compounds **3.5-3.8**. The larger coupling constants in the *p*-OMe derivatives β-**3.1** or β-**3.5** can be readily rationalized in terms of a greater exo-anomeric effect, which leads to greater C-H bond strengths (see above). The origins of the increases in $J_{C1\text{-}H1}$ values for the *p*-CF_3 and *p*-NO_2 β-compounds are not as readily obvious. In these cases, the exo-anomeric effect is reduced.[16] We propose that these substituents cause field effects and inductive effects that cause a contraction of the C-H bond. The effect can be viewed as an electrostatic effect, as proposed by Pross and Radom[21] for the case of acetals. The invariance of the $J_{C1\text{-}H1}$ values in the α-isomers may be attributed to the counterbalance of the endo- and exo-anomeric effects. For example, the greater endo-anomeric effect in **3.8**

relative to that in **3.6** is offset by the decreased exo-anomeric effect.[14] The two effects thus partially cancel in **3.8**, giving a C-H bond strength that is similar to that in **3.6**.

3.5 Conclusions

There is no evidence to support the existence of a generalized reverse anomeric effect in neutral or protonated *N*-aryl-5-thioglucopyranosylamines and *N*-aryl-glucopyranosylamines. For the neutral compounds, the anomeric effect ranges from 0.85 kcal mol^{-1} in **3.2** to 1.54 kcal mol^{-1} in **3.10**. The compounds **3.1-3.4** and **3.9-3.11** show an enhanced endo-anomeric effect upon protonation. The anomeric effect in the protonated derivatives ranges from 1.73 kcal mol^{-1} in **3.2** to 2.57 kcal mol^{-1} in **3.10**. We estimate the increase in the anomeric effect upon protonation of **3.10** to be approximately 1.0 kcal mol^{-1}. However, this effect is offset by steric effects due to the associated counterion which we estimate to be approximately 1.2 kcal mol^{-1}. The values of K_{eq} in protonated **3.1-3.4** increase in the order $OMe<H<CF_3<NO_2$, in agreement with the dominance of steric effects (due to the counterion) over the endo anomeric effect. The values of K_{eq} in protonated **3.9-3.11** show the trend $OMe>H<NO_2$ that is explained by the balance of the endo-anomeric effect and steric effects in the individual compounds.

3.6 Experimental

3.6.1 Synthesis

The synthesis of compounds **3.1-3.8** are described elsewhere.[13b] Compounds **3.9-3.11** were synthesized by the method of Honeyman.[22] The α- and β-isomers of **3.10** and **3.11** have been characterized previously.[23] The synthesis of α- and β-**3.9** is described below. Compound **3.15** was synthesized as described.[24]

^{1}H NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 MHz. Chemical shifts are given in ppm downfield from TMS. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. Chemical shifts of the neutral and protonated species **3.1-3.4** and **3.9-3.11** are listed as supporting information in Tables 3.7-3.10.

***N*-(4-Methoxyphenyl) 2,3,4,6-tetra-*O*-acetyl-α- and β-D-glucopyranosylamine (3.9α and 3.9β).** A mixture of D-glucose (9.01 g, 50.0 mmol), *p*-anisidine (6.16 g, 50.0 mmol) in methanol (100 mL) and glacial acetic acid (0.2 g) was refluxed for 45 min. The reaction mixture was concentrated to give a red oil which was shown by ^{1}H NMR spectroscopy to contain a mixture of α- and β-isomers. The oil was dissolved in pyridine (80 mL) and acetic anhydride (60 mL) containing a catalytic amount of DMAP and the mixture was stirred for 1h at room temperature and then for 45 min at 45°C. The reaction mixture was poured into ice/water (800 mL) and stirred to give a reddish-brown precipitate that was recrystallized from ether to give a 2:1 α:β mixture of **3.9** (13.5 g, 64%). Selective recrystallization from ethanol gave pure **3.9α** (M.p. 131-132°C) and

pure **3.9β** (M.p. 140-141°C); Anal. Calcd for $C_{21}H_{27}NO_{10}$: C, 55.63; H, 6.00; N, 3.09%. Found (α: β mixture): C, 55.66; H, 6.02; N, 3.08%.

3.6.2 Equilibrations

The equilibrations of the neutral compounds **3.1-3.4** were carried out at 294 K in CD_3NO_2, CD_3OD, and $(CD_3)_2CO$. To a solution of either the α- or β-isomer (1.5 mg) in 0.6 mL of the appropriate deuterated solvent was added 10% $HgCl_2$ from a 100 mM stock solution in deuterated solvent. The equilibrations of the protonated compounds **3.1-3.4** were carried out at 294 K in $CDCl_3$, CD_2Cl_2, CD_3NO_2, CD_3OD, and $(CD_3)_2CO$. To a solution of either the α- or β-isomer (1.5 mg) in 0.6 mL of the appropriate deuterated solvent was added 1.5 eq of triflic acid from a 0.565 M stock solution in deuterated solvent. The ^1H NMR spectra were recorded periodically until equilibrium had been reached. Equilibration usually took place in less than a few hours. Because both isomers were used in the equilibrations, it was deemed that equilbrium had been reached when the spectra starting from both the α- and the β-isomers were identical.

The equilibrations of the neutral and protonated compounds **3.9-3.11** were carried out at 230 K in CD_2Cl_2 and CD_3OD. Attempts to extend these studies to other solvents, e.g. CD_3NO_2 were frustrated by line broadening effects that did not permit unambiguous assignment of the signals or their accurate integration. To a solution of either the α- or β-isomer (10 mg) in 0.6 mL of the appropriate deuterated solvent at 230 K was added 5% triflic acid for the equilibrations of the neutral species or 2.5 eq. triflic acid for the equilibriations of the protonated species. The equilibrations were immediate and the proton spectra did not change over time.

The equilibrium constants were derived from the integration of different pairs of peaks of both isomers. Isolated signals were integrated to ensure accuracy. The average values of several integrations were taken into account for the final calculation of K. The errors in K are the standard deviations of the measurements. The errors in ΔG were derived from errors in K and in temperature (± 2 K). Calculations of the magnitude of the anomeric effect were performed using the formula: $AE=\Delta G_2-\alpha(\Delta G_1)$, where $\alpha=\Delta G$(2-methyltetrahydropyran or 2-methyltetrahydrothiapyran)/ΔG(methylcyclohexane); ΔG_1=*N*-(phenyl)cyclohexylamine; ΔG_2=*N*-(aryl)glycopyranosylamine (X=S,O). The errors in the values of ΔG in Tables 3.4 and 3.5 were derived by standard treatment for the propagation of errors.

3.7 Acknowledgments

We are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support and to J.-C. Brodovitch for helpful discussions regarding the error analysis.

Supporting Information Available: Tables of ^{1}H NMR chemical shifts for neutral and protonated **3.1-3.4** and **3.9-3.11** in different solvents (6 pages). Ordering information is given on any current masthead page.

3.8 References

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3.9 Supporting Information: Is There a Generalized Reverse Anomeric Effect? Substituent and Solvent Effects on the Configurational Equilibria of Neutral and Protonated *N*-Aryl-Glucopyranosylamines and *N*-Aryl-5-Thioglucopyranosylamines

Karla D. Randell, Blair D. Johnston, David F. Green, and B. Mario Pinto

Table 3.7 ^{1}H NMR Chemical Shifts[a] and Coupling Constants[b] for the Neutral Compounds in which X=S

Solvent	Y	H1 ($J_{1,2}$)	H2 ($J_{2,3}$)	H3 ($J_{3,4}$)	H4 ($J_{4,5}$)	H5 ($J_{5,6a}$)	H6a ($J_{6a,6b}$)	H6b($J_{5,6b}$)	NH ($J_{1,NH}$)	Ar
$CDCl_3$[e,f]	3.1OMeα	4.79 (4.1)	5.40 (10.2)	5.47 (9.0)	5.34 (10.8)	3.47 (4.1)	4.44 (12.1)	3.98 (3.0)	4.10 (4.9)	6.80
CD_2Cl_2[e,f]		4.82 (4.2)	5.38 (10.4)	5.47 (9.4)	5.30 (10.5)	3.49 (4.4)	4.38 (12.0)	3.99 (3.4)	4.14 (4.2)	6.75
CD_3NO_2[e,f]		4.90 (4.3)	5.38 (10.5)	5.48 (9.2)	5.30 (10.8)	3.50 (4.7)	c (12.1)	4.03 (3.7)	d	6.80
C_3D_6O[e,f]		5.09 (4.6)	5.28 (10.8)	5.55 (9.5)	5.22 (11.0)	3.43 (4.6)	4.38 (12.0)	3.96 (3.5)	5.18 (9.5)	6.80
CD_3OD[e,f]		5.09 (4.7)	5.28 (10.5)	5.68 (9.3)	5.23 (10.8)	3.48 (4.5)	4.44 (12.1)	3.95 (3.1)	d	6.75
$CDCl_3$[e,f]	3.1OMeβ	4.69 (10.0)	5.31 (9.6)	5.18 (9.5)	5.30 (10.7)	3.37 (5.0)	4.32 (11.9)	4.08 (3.4)	3.69 (10.9)	6.70
CD_2Cl_2[e,f]		4.74 (10.2)	5.28 (9.7)	5.18 (9.5)	5.27 (10.6)	3.28 (5.3)	4.26 (11.9)	4.08 (3.5)	3.78 (10.9)	6.65/6.80
CD_3NO_2[e,f]		4.96 (9.8)	5.29 (8.9)	5.20 (8.6)	5.25 (9.8)	3.43 (5.3)	4.28 (12.0)	4.10 (3.6)	d	6.80
C_3D_6O[e,f]		5.22 (m)	5.22 (m)	5.22 (m)	5.22 (10.2)	3.59 (5.4)	4.28 (11.9)	4.04 (3.5)	4.73 (10.8)	6.80
CD_3OD[e,f]		4.90 (9.3)	5.24 (m)	5.24 (m)	5.24 (10.3)	3.48 (5.0)	4.33 (12.0)	4.03 (3.3)	d	6.75
$CDCl_3$[f]	3.2 H α	4.88 (4.3)	5.41 (10.2)	5.47 (8.8)	5.34 (10.9)	3.44 (4.2)	4.43 (12.1)	3.98 (3.1)	4.28(3.8)	6.80/6.85/7.20
CD_2Cl_2[f]		4.92 (4.4)	5.39 (10.4)	5.47 (9.1)	5.31 (10.8)	3.46 (4.4)	4.39 (12.0)	3.98 (3.4)	4.40 (3.5)	6.80/7.20
CD_3NO_2[f]		4.78 (4.4)	5.39 (10.5)	5.49 (9.2)	5.30 (10.8)	3.52 (4.6)	C (12.1)	4.03 (3.6)	d	6.85/7.20
C_3D_6O[f]		5.18 (4.7)	5.29 (10.6)	5.58 (9.4)	5.24 (10.9)	3.53 (4.6)	4.38 (12.1)	3.97 (3.4)	6.18 (9.3)	6.70/6.85/7.20
CD_3OD[f]		5.09 (4.7)	5.29 (10.4)	5.70 (9.3)	5.25 (11.0)	3.46 (4.6)	4.45 (12.1)	3.95 (3.1)	d	6.70/6.75/7.20
$CDCl_3$[f]	3.2 H β	4.78 (9.9)	5.32 (9.4)	5.19 (9.4)	5.31 (10.6)	3.28 (5.0)	4.33 (11.9)	4.10 (3.5)	4.03(10.0)	6.70/6.80/7.10
CD_2Cl_2[f]		4.83 (9.6)	5.31 (9.5)	5.19 (9.5)	5.29 (10.5)	3.32 (5.4)	4.28 (12.0)	4.10 (3.6)	4.09 (9.9)	6.70/6.80/7.10
CD_3NO_2[f]		5.01 (9.8)	5.31 (9.6)	5.22 (9.0)	5.27 (10.2)	3.48 (5.4)	4.29 (12.1)	4.11 (3.6)	d	6.85/7.20
C_3D_6O[f]		5.25 (9.6)	5.25 (m)	5.25 (m)	5.25 (10.2)	3.63 (5.4)	4.29 (11.9)	4.07 (3.5)	5.10 (10.2)	6.70/6.85/7.20
CD_3OD[f]		5.19 (9.5)	5.25 (m)	5.25 (m)	5.25 (10.3)	3.51 (5.1)	4.37 (12.0)	4.06 (3.3)	d	6.70/6.75/7.20
$CDCl_3$[f]	3.3 CF_3 α	4.90 (4.2)	5.41 (10.2)	5.45 (8.7)	5.33 (10.8)	3.39 (4.4)	4.43 (12.2)	3.99 (3.1)	4.68 (4.3)	6.80/7.50
CD_2Cl_2[f]		4.95 (4.2)	5.40 (10.4)	5.47 (9.0)	5.32 (10.8)	3.42 (4.5)	4.40 (12.1)	4.00 (3.3)	4.72 (4.7)	6.90/7.50
CD_3NO_2[f]		5.10 (4.3)	5.40 (10.5)	5.49 (9.1)	5.30 (10.6)	3.51 (4.9)	c (12.1)	4.02 (3.5)	d	6.91/7.50
C_3D_6O[f]		5.26 (4.7)	5.32 (10.4)	5.59 (9.3)	5.27 (10.9)	3.53 (4.8)	4.39 (12.1)	3.99 (3.4)	6.78 (8.9)	7.00/7.50
CD_3OD[f]		5.15 (4.6)	5.32 (10.4)	5.71 (9.3)	5.27 (10.9)	3.43 (4.5)	4.44 (12.1)	3.95 (3.1)	d	6.90/7.40

Table 3.7 Cont'd

Solvent	Y	H1 ($J_{1,2}$)	H2 ($J_{2,3}$)	H3 ($J_{3,4}$)	H4 ($J_{4,5}$)	H5 ($J_{5,6a}$)	H6a ($J_{6a,6b}$)	H6b($J_{5,6b}$)	NH ($J_{1,NH}$)	Ar
$CDCl_3$[f]	3.3 CF_3 β	4.78 (9.7)	5.32 (9.5)	5.20 (9.5)	5.31 (10.5)	3.30 (5.2)	4.33 (11.9)	4.12 (3.4)	4.45 (9.5)	6.70/7.50
CD_2Cl_2[f]		4.84 (9.5)	5.31 (9.5)	5.20 (9.5)	5.29 (10.3)	3.35 (5.4)	4.30 (11.9)	4.11 (3.5)	4.58 (9.7)	6.80/7.50
CD_3NO_2[f]		5.01 (9.3)	5.35 (9.6)	5.23 (9.4)	5.28 (10.2)	3.51 (5.1)	4.29 (12.0)	4.13 (3.5)	d	6.91/7.50
C_3D_6O[f]		5.38 (10.1)	5.30 (m)	5.30 (10.0)	5.20 (10.3)	3.68 (5.5)	4.29 (12.0)	4.09 (3.5)	5.81 (10.4)	7.00/7.50
CD_3OD[f]		5.19 (9.5)	5.25 (m)	5.25 (m)	5.25 (10.3)	3.51 (5.2)	4.38 (12.0)	4.07 (3.3)	d	6.90/7.40
$CDCl_3$[f]	3.4 $NO_2\alpha$	4.96 (m)	5.40 (10.3)	5.45 (9.2)	5.35 (10.8)	3.38 (4.6)	4.41 (12.2)	4.00 (3.1)	5.21 (4.4)	6.80/8.10
CD_2Cl_2[f]		5.00 (4.2)	5.40 (10.2)	5.47 (8.9)	c (10.7)	3.40 (4.6)	4.38 (12.2)	4.00 (3.3)	5.10 (4.6)	6.80/8.10
CD_3NO_2[f]		5.18 (4.5)	5.41 (10.4)	5.49 (9.0)	5.32 (10.6)	3.52 (4.7)	c (12.1)	4.04 (3.4)	5.18 (6.4)	6.95/8.12
C_3D_6O[f]		5.35 (m)	5.35 (10.0)	5.58 (9.3)	5.28 (10.9)	3.57 (4.8)	4.49 (12.1)	4.00 (3.3)	7.32 (9.8)	6.95/8.10
CD_3OD[f]		5.22 (4.9)	5.33 (10.5)	5.74 (9.3)	5.28 (10.9)	3.43 (4.5)	4.44 (12.1)	3.98 (3.0)	d	6.90/8.10
$CDCl_3$[f]	3.4 $NO_2\beta$	4.78 (9.6)	5.315 (9.6)	5.21 (9.6)	5.31 (10.6)	3.34 (5.3)	4.34 (12.0)	4.13 (3.4)	4.94 (9.3)	6.70/8.10
CD_2Cl_2[f]		4.86 (m)	5.32 (m)	5.21 (9.5)	5.29 (10.0)	3.38 (5.4)	4.30 (12.0)	4.13 (3.5)	5.02 (9.2)	6.75/8.10
CD_3NO_2[f]		5.13 (8.0)	5.35 (8.5)	5.24 (9.5)	5.29 (10.4)	3.57 (5.3)	4.30 (12.0)	4.15 (3.5)	5.40 (9.7)	6.95/8.12
C_3D_6O[f]		5.28 (m)	5.49 (m)	5.28 (c)	5.28 (10.3)	3.72 (5.6)	4.30 (12.0)	4.10 (3.5)	6.48 (10.1)	6.98/8.08
CD_3OD[f]		5.27 (m)	5.27 (m)	5.27 (c)	5.27 (9.8)	3.60 (5.2)	4.33 (12.0)	4.09 (3.1)	d	6.85/8.10

[a]294 K; ppm downfield from TMS
[b]Hz
[c]overlapping with other peaks
[d]not observed
[e]OMe=3.70
[f]OAc=2.0

Table 3.8 ^{1}H NMR Chemical Shifts[a] and Coupling Constants[b] for the Neutral Compounds in which X=O

Solvent	Y	H1 ($J_{1,2}$)	H2 ($J_{2,3}$)	H3 ($J_{3,4}$)	H4 ($J_{4,5}$)	H5 ($J_{5,6a}$)	H6a ($J_{6a,6b}$)	H6b ($J_{5,6b}$)	NH ($J_{1,NH}$)	Ar
$CDCl_3$[c]	3.9OMeα	5.27 (5.1)	5.19 (10.3)	5.47 (9.3)	5.05(10.2)	4.18 (4.4)	4.31 (12.3)	4.02 (2.1)	d	6.63/6.80
CD_2Cl_2		5.24	5.13	5.46	5.05	4.16	4.28	3.92	4.35	6.74/6.81
CD_3OD		5.39	c	5.67	c	3.98	4.30	3.98	d	6.75
$CDCl_3$[c]	3.9OMeβ	4.68 (9.1)	5.08 (e)	5.36 (e)	5.08 (e)	3.8 (5.3)	4.28 (12.3)	4.09 (2.4)	d	6.63/6.80
CD_2Cl_2		4.76	5.05	5.35	5.05	3.81	4.24	4.02	4.60	6.63/6.74
CD_3OD		5.00	5.04	5.39	5.04	3.98	4.30	3.98	d	6.78
$CDCl_3$[c]	3.10 H α	5.35 (4.2)	5.21 (10.3)	5.48 (9.4)	5.08(10.3)	4.15 (4.5)	4.32 (12.4)	4.03 (2.2)	4.40 (bs)	6.85/6.90/7.20
CD_2Cl_2		5.32 (3.1)	5.16 (10.1)	5.48 (9.4)	5.06(10.1)	4.13(5.0)	4.29(12.1)	3.92(2.2)	4.53 (m)	6.85/6.90/7.20
CD_3OD		5.40 (5.0)	5.08 (m)	5.72 (m)	5.02 (m)	4.02 (4.6)	4.32 (12.2)	3.97 (m)	d	6.84/7.14
$CDCl_3$[c]	3.10 H β	4.76 (9.1)	5.08 (m)	5.38 (m)	5.08(10.0)	3.84 (5.3)	4.29 (12.2)	4.09 (2.3)	4.40 (bs)	6.60/6.90/7.20
CD_2Cl_2		4.81 (m)	5.06 (m)	5.38 (m)	5.06(10.1)	3.84(5.3)	4.24 (12.1)	4.03(2.4)	4.78 (m)	6.69/6.80/7.20
CD_3OD		5.05 (m)	5.05 (m)	5.40 (m)	5.05 (e)	3.97 (4.6)	4.32 (12.2)	3.97	d	6.73/7.14
$CDCl_3$[c]	3.11NO_2α	D	d	d	d	d	d	d	d	d
CD_2Cl_2		5.45	5.20	5.49	5.06	4.00	4.27	3.93	d	6.91/8.09
CD_3OD		D	d	d	d	d	d	d	d	d
$CDCl_3$[c]	3.11 NO_2β	4.80 (e)	5.09 (e)	5.40 (e)	5.05 (e)	3.87 (5.4)	4.30 (12.3)	4.10(2.3)	5.56 (8.3)	6.65/8.10
CD_2Cl_2		4.85	5.06	5.40	5.06	3.90	4.28	4.04	5.80	6.69/8.08
CD_3OD		C	c	5.40	c	4.04	4.30	4.04	D	6.84/8.09

[a]ppm downfield from TMS
[b]Hz
[c]overlapping with other peaks
[d]not observed
[e]OMe=3.70
[f]OAc=2.0

Table 3.9 ^{1}H NMR Chemical Shifts for Protonated Compounds in which X=S[a]

Solvent	Comp'd	H1	H2	H3	H4	H5	H6a	H6b	OAc	Ar	OMe
$CDCl_3$	**3.1**OMeα	4.80	5.45[b]	5.45[b]	5.30	3.61	4.30	4.00	2.00	6.80-7.30	3.80
CD_2Cl_2		4.80	5.30[b]	5.50	5.30[b]	3.60	4.28	4.05	2.00	6.80-7.50	3.80
CD_3NO_2		5.18	5.50	5.80	5.38	3.78	4.30	4.20	2.00	7.15/7.60	3.90
$(CD_3)_2CO$		5.43	5.40	5.60	5.30	3.65	4.38	4.08	2.00	6.98-7.73	3.80
CD_3OD		4.90	5.30[b]	5.60	5.30[b]	c	4.42	4.00	2.00	6.80-7.30	3.75
$CDCl_3$	**3.1**OMeβ	4.58	5.20[b]	5.20[b]	5.20[b]	3.23	4.20	4.09	2.00	6.80-7.30	3.80
CD_2Cl_2		4.80	5.20[b]	5.20[b]	5.20[b]	3.30	4.28	4.07	2.00	6.80-7.50	3.80
CD_3NO_2		5.20	5.23[b]	5.23[b]	5.23[b]	3.57	4.30	4.20	2.00	7.15/7.60	3.90
$(CD_3)_2CO$		5.40	5.30[b]	5.30[b]	5.30[b]	3.65	4.30	4.08	2.00	6.98-7.73	3.80
CD_3OD		4.90	5.30[b]	5.30[b]	5.30[b]	c	4.38	4.08	2.00	6.80-7.30	3.75
$CDCl_3$	**3.2** H α	4.85	5.44	5.48	5.38	3.6	4.38	4.00	2.00	7.25	d
CD_2Cl_2		4.81	5.50	5.58	5.35	3.83	4.28	4.10	2.00	7.25-7.70	d
CD_3NO_2		5.20	5.58	5.80	5.40	3.80	4.40	4.20	2.00	7.67	d
$(CD_3)_2CO$		5.25[c]	5.40	5.60	5.25[c]	3.65	4.38	4.02	2.00	6.80-7.30	d
CD_3OD		5.10	5.30[c]	5.70	5.30[c]	3.50	4.50	4.00	2.00	6.72/6.80/7.12	d
$CDCl_3$	**3.2** H β	4.80	5.33[c]	5.20	5.33[c]	3.30	4.32	4.10	2.00	7.25	d
CD_2Cl_2		4.87	5.21[c]	5.21[c]	5.21[c]	3.30	4.28	4.10	2.00	7.25-7.70	d
CD_3NO_2		5.24[c]	5.24[c]	5.24[c]	5.24[c]	3.56	4.30	4.20	2.00	7.67	d
$(CD_3)_2CO$		5.25[c]	5.25[c]	5.25[c]	5.25[c]	3.65	4.30	4.05	2.00	6.80-7.30	d
CD_3OD		5.12	5.30[c]	5.30[c]	5.30[c]	3.55	4.40	4.10	2.00	6.72/6.80/7.15	d
$CDCl_3$	**3.3** CF_3 α	4.90	5.33[c]	5.41	5.33[c]	3.40	4.20	4.00	2.00		d
CD_2Cl_2		4.92	5.43	5.52	5.33	3.50	4.38	4.01	2.00		d
CD_3NO_2		5.19	5.59	5.55	5.38	3.70	4.30	4.10	2.00	7.02-7.75	d
$(CD_3)_2CO$		5.30[c]	5.30[c]	5.59	5.30[c]	3.56	4.38	4.10	2.00		d
CD_3OD		5.19	5.37	5.75	5.30	3.48	4.48	4.00	2.00	6.90/7.44	d

Table 3.9 Cont'd

Solvent	Comp'd	H1	H2	H3	H4	H5	H6a	H6b	OAc	Ar	OMe
$CDCl_3$	**3.3** CF_3 β	4.78	5.33[c]	5.20	5.33[c]	3.30	4.32	4.12	2.00		d
CD_2Cl_2		4.82	5.30[c]	5.20	5.30[c]	3.38	4.30	4.12	2.00		d
CD_3NO_2		5.10	5.25[c]	5.25[c]	5.25[c]	3.50	4.30	4.15	2.00	7.02-7.75	d
$(CD_3)_2CO$		5.30[c]	5.30[c]	5.30[c]	5.30[c]	3.62	4.29	3.98	2.00		d
CD_3OD		5.20	5.30[c]	5.30[c]	5.30[c]	3.60	4.39	4.10	2.00	6.9/7.44	d
$CDCl_3$	**3.4** NO_2α	4.97	5.30[c]	5.40	5.30[c]	3.38	4.40	4.00	2.00	6.70/6.80/8.15	d
CD_2Cl_2		5.00	5.42[c]	5.42[c]	5.30	3.42	4.40	4.00	2.00	6.80/8.10	d
CD_3NO_2		5.18	5.42	5.48	5.38	3.53	4.30	4.03	2.00	6.93/8.10	d
$(CD_3)_2CO$		5.60	5.30[c]	5.30[c]	5.23	3.58	4.38	4.00	2.00	7.00/8.00	d
CD_3OD		5.24	5.38	5.75	5.30	3.50	4.48	4.00	2.00	6.90/8.10	d
$CDCl_3$	**3.4** NO_2β	4.78	5.30[c]	5.20	5.30[c]	3.36	4.38	4.15	2.00	6.70/6.80/8.15	d
CD_2Cl_2		4.84	5.25	5.20	5.30	3.39	4.30	4.13	2.00	6.80/8.10	d
CD_3NO_2		5.12	5.25[c]	5.25[c]	5.25[c]	3.53	4.30	4.16	2.00	6.93/8.10	d
$(CD_3)_2CO$		5.43	5.25[c]	5.25[c]	5.25[c]	3.70	4.29	4.10	2.00	7.00/8.00	d
CD_3OD		5.30[c]	5.30[c]	5.30[c]	5.30[c]	3.62	4.40	4.10	2.00	6.90/8.10	d

[a] 294 K, ppm downfield from TMS
[b] overlapping with other peaks
[c] center of a complex multiplet
[d] not relevant

Table 3.10 ^{1}H NMR Chemical Shifts[a] for Protonated Compounds in which X=O

Solvent	Comp'd	H1	H2	H3	H4	H5	H6a	H6b	OAc	Ar	OMe
CD_2Cl_2	**3.9**OMeα	5.25	5.17	5.44	5.05	4.20	4.25	3.95	2.00	6.79	3.71
CD_3OD		5.41	b	5.67	b	4.00	4.30	4.00	2.00	6.78	3.74
CD_2Cl_2	**3.9**OMeβ	4.92	5.05[c]	5.31	5.05[c]	3.84	4.19	4.11	2.00	6.87	3.77
CD_3OD		5.07[c]	5.07[c]	5.41	5.07[c]	4.00	4.33	4.00	2.00	6.89	3.74
CD_2Cl_2	**3.10** H α	5.32	5.18	5.47	5.08	4.14	4.27	3.92	2.00	6.91/7.22	d
CD_3OD		5.39	5.05[c]	5.71	5.05[c]	3.98	4.30	3.98	2.00	6.85/7.14	d
CD_2Cl_2	**3.10** H β	4.96	5.08	5.35	5.08	3.87	4.20	4.10	2.00	6.91/7.33	d
CD_3OD		5.05[c]	5.05[c]	5.39	5.05[c]	3.98	4.30	3.98	2.00	6.75/7.14	d
CD_2Cl_2	**3.11** NO_2α	5.45[c]	5.22[c]	5.45[c]	5.22[c]	4.10	4.25	3.97	2.00	8.12	d
CD_3OD		b	b	5.75	b	4.04	4.30	4.04	2.00	6.98/8.09	d
CD_2Cl_2	**3.11** NO_2β	5.00	5.10[c]	5.37	5.10[c]	3.89	4.20	4.11	2.00	8.21	d
CD_3OD		b	b	5.40	b	4.04	4.30	4.04	2.00	6.84/8.09	d

[a]230 K; ppm downfield from TMS
[b]overlapping with other peaks
[c]center of a complex multiplet
[d]not relevant

CHAPTER 4: SYNTHESIS OF GALACTOFURANOSYL-CONTAINING OLIGOSACCHARIDES CORRESPONDING TO THE GLYCOSYLINOSITOLPHOSPHOLIPID OF *TRYPANOSOMA CRUZI*

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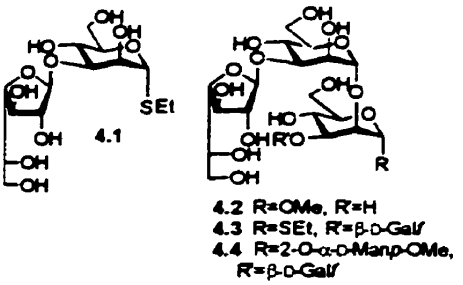
4.1 Graphical Abstract

Synthesis of Galactofuranosyl-Containing Oligosaccharides Corresponding to the Glycosylinositolphospholipid of *Trypanosoma cruzi*

Karla D. Randell, Blair D. Johnston,
Paula N. Brown, and B. Mario Pinto

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6



4.2 Abstract

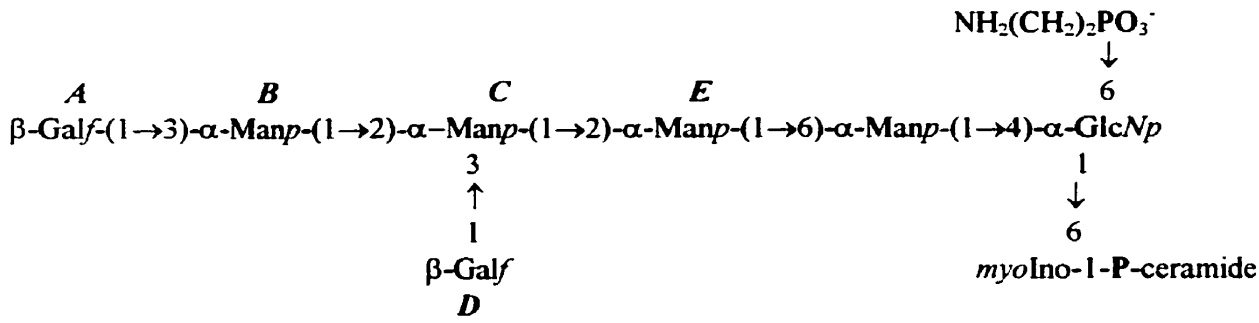
The oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-(β-D-Gal*f*-(1→3))-α-D-Man*p*-(1→2)-α-D-Man*p* corresponds to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease. Syntheses of methyl or ethylthio glycosides of the terminal disaccharide (**4.1**), trisaccharide (**4.2**), tetrasaccharide (**4.3**), and pentasaccharide (**4.4**) corresponding to this structure are described. These syntheses employ the selective activation of a phenyl 1-selenogalactofuranoside or a phenyl 1-selenomannopyranoside donor over ethyl 1-thioglycoside acceptors with NIS/TfOH.

Keywords: glycosylation; glycosides; galactofuranose; *Trypanosoma cruzi*

4.3 Introduction

Galactofuranose (Gal*f*) is present as a constituent of external cellular structures in protozoa,[1] bacteria,[2,3] and fungi.[4] These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection.[5] Many glycoconjugates are anchored to the plasma membrane *via* glycosylphosphatidylinositol (GPI) anchors. The GPI anchors of plasma membrane proteins have been detected in organisms ranging from yeast to man, but occur with a much higher frequency in lower eukaryotes such as protozoa.[6,7] All of the GPI anchors which have been characterized to date contain an identical ethanolamine-phosphate-α-Man-(1→2)-α-Man-(1→6)-α-Man-(1→4)-α-Glc*N*-(1→6)-*myo*inositol backbone, suggesting that this sequence is likely to be conserved in all GPI anchors. In protozoa, GPI anchors have been widely studied in their role as anchors for cell surface proteins. Several protozoa also synthesize unique GPI derivatives which are not covalently linked to protein or modified by additional glycoconjugates. These low molecular weight structures, referred to as glycosylinositolphospholipids (GIPLs) are included as members of the GPI family by virtue of the core sequence α-Man-(1→4)-α-Glc*N*-(1→6)-*myo*inositol.[6] It is known that Gal*f* is part of the oligosaccharide core of the glycosylinositolphospholipid from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[8] The GIPL structure of *T. cruzi* contains the same tetrasaccharide core sequence as the protein-bound GPI anchors, but diverges from the protein anchors beyond this sequence. The GIPL

contains up to two additional β-Gal*f* residues and there is a 2-aminoethylphosphonic acid group located at the C-6 position of the glucosamine residue. The lipid moiety is a ceramide containing sphinganine and *N*-linked lignoceric ($C_{24:0}$) acid instead of the alkylglycerol found in the protein anchors: [6, 9, 10]



This structure is the most abundant cell surface glycoconjugate present in the insect dwelling epimastigote stage of the *T. cruzi* life cycle.[1] The glycoconjugates on the cell surface during the infectious stage of *T. cruzi* are not modified with galactofuranose; however, it has been shown that the β-D-Gal*f* moiety is recognized by antibodies that inhibit *T. cruzi* internalization into mammalian cells.[5] Recently, it was demonstrated that *T. cruzi* GIPLs are able to block T-lymphocyte activation.[11] Thus, interaction between host cellular defense mechanisms and the GIPLs of *T. cruzi* may play a role in establishment and maintenance of chronic infection.[12]

Galactofuranose has also been isolated from another class of GIPLs found in *Leishmania*.[13] This GIPL differs from *T. cruzi* by having a glycerolipid instead of a ceramide and the β-D-Gal*f*-(1→3)-α-Man*p* moiety as an internal unit in the oligosaccharide core. Glycoconjugates containing galactofuranose have also been found

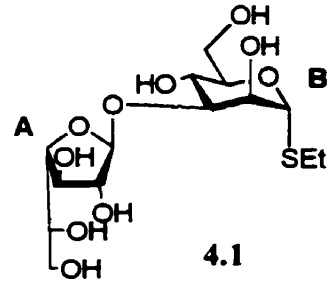
in the GIPLs of *Leptomonas samueli*[14] and *Endotrypanum schaudinni*,[15] both of which contain a ceramide lipid. The Gal*f* moiety is the terminal non-reducing sugar in *L. samueli* and it is internal in *E. schaudinni*. In all of the above examples, the β-D-galactofuranose is linked (1→3) to an α-Man*p*. This specificity suggests that a β-(1→3)-galactofuranosyltransferase might be involved in the biosynthesis, although a sugar donor has not been identified.[10]

The specific capsular polysaccharide produced by *Streptococcus pneumoniae* type 20 contains both an internal Gal*f* and a branched Gal*f* moiety.[16] The acidic glycolipid, purified from *Paracoccidioides brasiliensis*, containing a terminal Gal*f*, has been shown to be reactive with sera of patients infected with paracoccidioidomycosis.[17] The synthesis of oligosaccharides containing Gal*f* may therefore be useful for understanding the role Gal*f* plays in microorganisms and for studying the biosynthesis of furanosyl-containing glycoconjugates. Compounds containing Gal*f* may also be used as inhibitors to probe the development of infections, to develop diagnostic methods, or as vaccines.

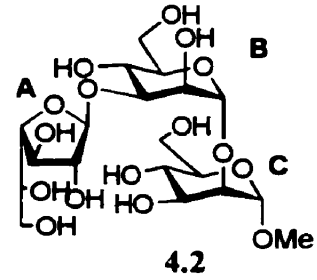
Oligosaccharide synthesis involving furanosyl glycosyl donors has not been studied to the same extent as with pyranosyl donors, but methods have been developed that employ thioglycosides,[18] *n*-pentenyl glycosides,[19,20] anomeric benzoates,[21] anomeric xanthates,[22] trichloroacetimidates,[23,24] and selenoglycosides.[25, 26] Anomeric benzoates[21] and *n*-pentenyl glycosides[19] give stereoselective syntheses of the β-anomers, while activation of anomeric xanthates leads to α:β mixtures. In addition, an indirect approach to galactofuranosyl-containing disaccharides involving acyclic glycosyl donors has

recently been reported.[27] The syntheses of α-D-galactofuranose-containing oligosaccharides have not been as widely investigated as those of the β–anomers, but have been achieved in high yields, using ethyl 2,3,5,6-tetra-*O*-benzyl-α-D-thiogalactofuranoside and *N*-bromosuccinimide.[18]

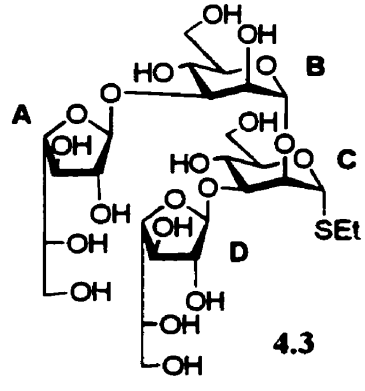
A previous report from our laboratory described the viability of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside **4.16** as a glycosyl donor.[26] As an extension of this and other studies of the selective activation of selenoglycoside donors in the presence of thioglycoside acceptors,[28, 29] we now report the syntheses of the terminal disaccharide **4.1**, trisaccharide **4.2**, tetrasaccharide **4.3**, and pentasaccharide **4.4** corresponding to the terminus of the glycosylinositolphospholipid oligosaccharide of *T. cruzi*, that employ **4.16** as a glycosyl donor.



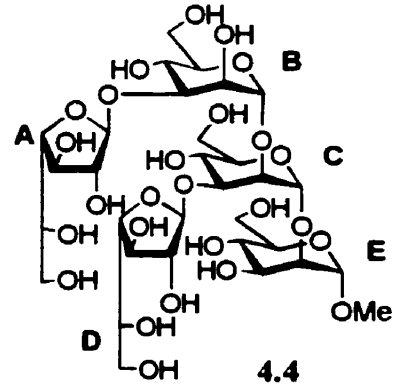
4.1



4.2



4.3



4.4

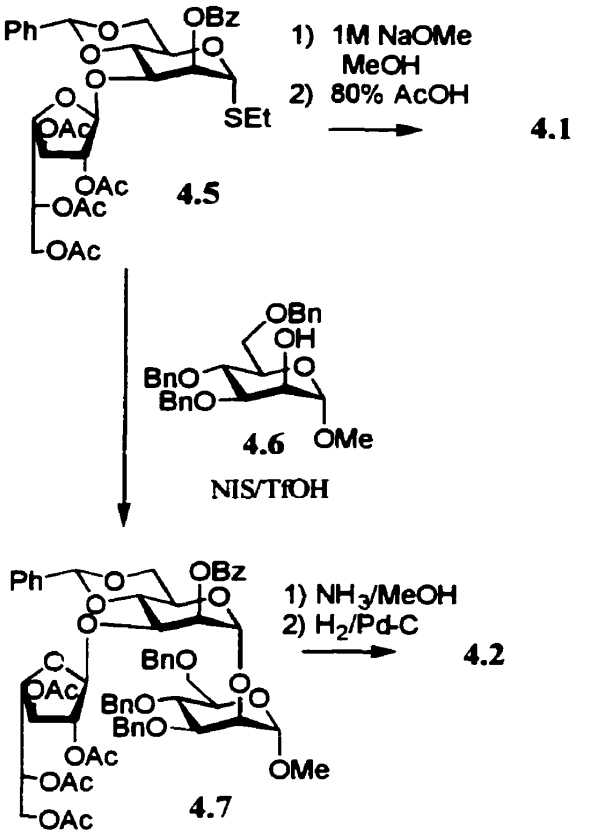
4.4 Results and Discussion

The required disaccharide, ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-α-D-thiomannopyranoside **4.5**[26] and the monosaccharides methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **4.6**[30] and phenyl 2,3,5,6-tetra-O-acetyl-β-D-selenogalactofuranoside **4.16**[26] were synthesized following literature methods. The disaccharide **4.5** was deprotected by methanolysis of the esters followed by hydrolysis of the benzylidene acetal to give compound **4.1** in 70% yield.

The reaction of the glycosyl donor, disaccharide **4.5**, with an excess of the acceptor **4.6** mediated by NIS/TfOH resulted in the protected trisaccharide **4.7** as a colorless foam in 68% yield (see Scheme 4.1). The trisaccharide was deprotected by aminolysis of the esters followed by hydrolysis of the benzylidene acetal and hydrogenolysis of the benzyl ethers to give compound **4.2** in 85% yield.

The stereochemical integrity of the oligosaccharides **4.1** and **4.2** was confirmed by examination of the J_{C1H1} values of the mannopyranosyl residues and NOE contacts for the galactofuranosyl residues. The J_{C1H1} values cannot be used as a reliable indicator of the α- or β-configuration for the galactofuranosyl residues.[31] For the trisaccharide **4.2**, the 2D NOESY spectrum showed an NOE between H-1A and H-3A (of the Gal*f* ring A) and no NOE between H-1A and H-4A, indicating the presence of a β-linkage between the Gal*f* (A) and Man*p* (B) rings. The J_{C1H1} values are 171 Hz for the Man*p* (B) ring and 172 Hz for the Man*p* (C) ring, indicating the presence of α–configurations about C-1 for both mannopyranosyl residues.[32]

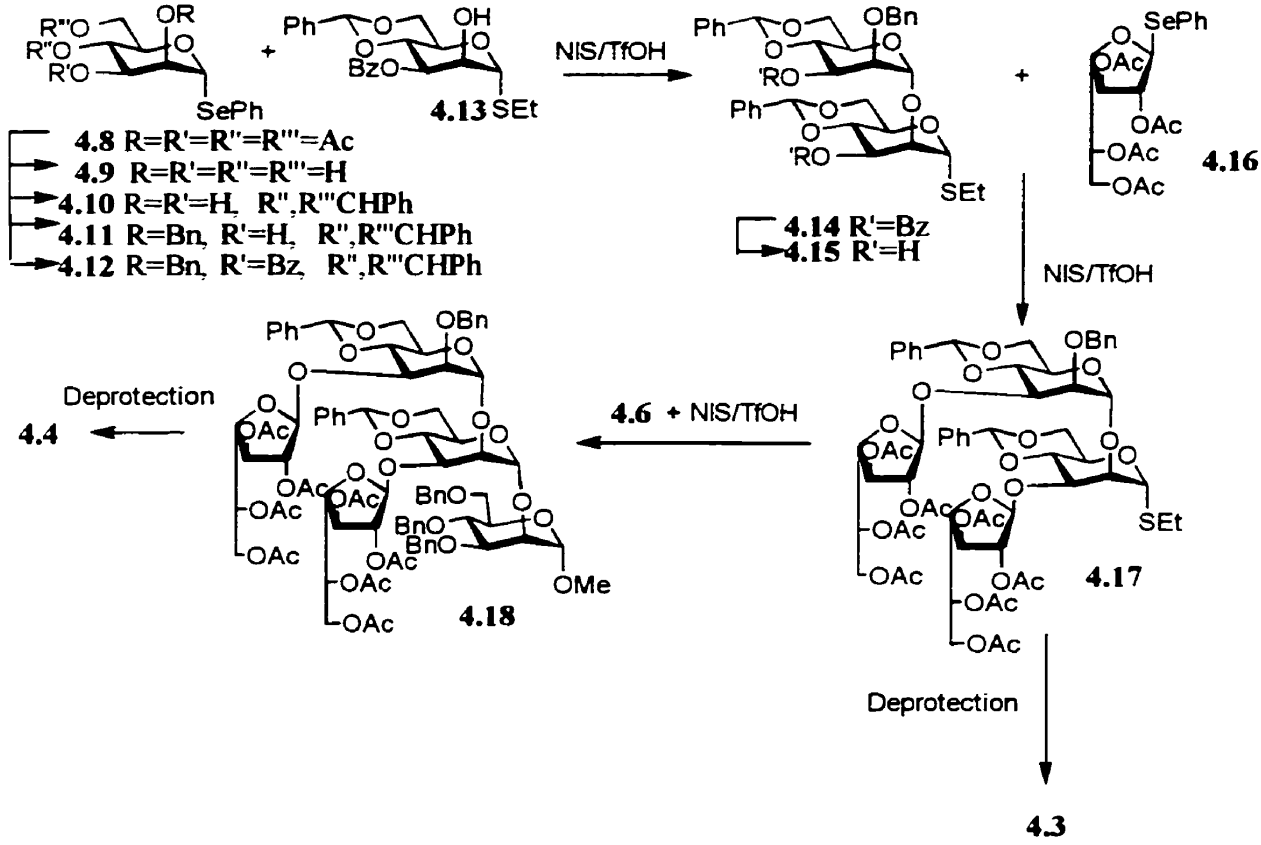
Scheme 4.1



Synthesis of the monosaccharide acceptor phenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-selenomannopyranoside **4.12** was carried out from phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-selenomannopyranoside **4.8**. Compound **4.8** was deacetylated using NaOMe/MeOH and the crude product **4.9** was selectively protected as a 4,6-*O*-benzylidene acetal, in a manner analogous to that reported by Franzyk *et al.*,[33] to give **4.10** (see Scheme 4.2). Phase transfer-catalyzed benzylation gave the 2-*O*-benzyl compound **4.11** that was benzoylated to give **4.12**.[34] Ethyl 3-*O*-benzoyl-4,6-*O*-

benzylidene-α-D-thiomannopyranoside **4.13** was synthesized in an analogous manner to that reported by Seymour[35] for the methyl glycoside.

Scheme 4.2



Glycosylation of the acceptor **4.13** with the donor **4.12** was again performed using NIS/TfOH, with selective activation of the phenyl selenomannoside over the ethyl thiomannoside; the protected disaccharide **4.14** was obtained as a white foam in 60% yield (see Scheme 4.2). The benzoate esters at the 3- positions of both mannose residues were removed using NaOMe/MeOH to give the disaccharide **4.15**, which was

subsequently used as an acceptor. The tetrasaccharide **4.17** was synthesized in one pot using the disaccharide **4.15** and 2.4 equiv. of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside **4.16**. Again, selective activation of the phenyl selenogalactofuranoside over the ethyl thiomannopyranoside yielded the desired tetrasaccharide **4.17** as white crystals in 85% yield. Compound **4.17** was then deprotected by aminolysis of the esters followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ether to give compound **4.3** in 71% yield.

Assignment of the NMR signals of the Man*p* (C) ring of the tetrasaccharide **4.3** was based on the fact that C-1 for this residue has a characteristic upfield chemical shift due to the ethyl thioglycoside. A C-H correlation spectrum, together with a COSY and a TOCSY spectrum then permitted complete assignment of the ^{1}H and ^{13}C NMR signals. The assignment of signals for the Man*p* (B) ring was based on the presence of a NOE contact across the glycosidic linkage between H-1B and H-2C, and COSY and TOCSY transfer between ^{1}H NMR signals of the B ring. Assignment of signals of the Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H-1A and H-3B, and H-1D and H-3C, respectively. The 2D NOESY spectrum also showed an NOE between H-1A and H-3A (of the Gal*f* ring A), and another NOE between H-1D and H-3D (of the Gal*f* ring D), indicating the presence of a β-linkage between the Gal*f* (A) and Man*p* (B) rings, and also between the Gal*f* (D) and Man*p* (C) rings. The J_{C1H1} values are 170 Hz for the Man*p* (B) ring and 167 Hz for the Man*p* (C) ring, indicating the presence of α-configurations about C-1 for both mannopyranosyl residues.[32]

The tetrasaccharide **4.17** contains the ethyl thioglycoside at C-1 of the Man*p* (C) ring, and without any manipulation, could be used as a donor to make the pentasaccharide. Thus, glycosylation of the acceptor **4.6** with the donor tetrasaccharide **4.17** gave the desired compound **4.18** as a clear syrup. Both the donor **4.17** and the product of the reaction **4.18** had similar *Rf* values; a ^{1}H NMR spectrum showed the presence of **4.18**:**4.17** in a ratio of 3:1, with an estimated 35% yield of the desired pentasaccharide **4.18**. Extensive chromatography gave an analytically pure sample of **4.18**. In subsequent experiments, the mixture of **4.17** and **4.18** was subjected to methanolysis, hydrolysis, and hydrogenolysis; the deprotected pentasaccharide **4.4** could be isolated as a pure compound (see below).

In a recent paper from the group of C.-H. Wong, the synthesis of oligosaccharides in one pot by sequential addition of acceptors and promoters was reported.[36] Using this approach, a second attempt at the synthesis of **4.18** was carried out in one pot starting with disaccharide **4.15**. The tetrasaccharide **4.17** was first synthesized by reacting the disaccharide **4.15** with 2.1 equiv. of the galactofuranosyl donor **4.16** at 0°C; then, without any workup, the acceptor **4.6** was added together with an additional 1.2 equiv. of NIS and activated 4Å molecular sieves. Again, a mixture of the tetrasaccharide **4.17** and the product **4.18** was obtained, but **4.18** was formed in a slightly better yield (28% for 2 steps). The pentasaccharide **4.18** was deprotected by methanolysis of the esters followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ethers to give the target pentasaccharide **4.4** in 60% yield.

The assignment of the NMR signals of the Man*p* (E) ring of the pentasaccharide **4.5** was based on the ^{1}H NMR spectrum in which this H-1 signal was the most upfield. The 2D NOESY spectrum also showed a contact between H-1E and the methyl aglycon. The assignment of the remaining signals of the Man*p* (E) ring then followed from a COSY experiment. The signals of the Man*p* rings B and C were assigned based on NOE contacts across the glycosidic linkages between H-1C and H-2E, and H-1B and H-2C, respectively. Assignment of signals of the Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H-1A and H-3B, and H-1D and H-3C, respectively. The 2D NOESY spectrum also showed an NOE between H-1A and H-3A (of the Gal*f* ring A), and another NOE between H-1D and H-3D (of the Gal*f* ring D), indicating the presence of β-linkages between the Gal*f* (A) and Man*p* (B) rings, and between the Gal*f* (D) and Man*p* (C) rings. The J_{C1H1} values were 171 Hz for the Man*p* (B) ring, 172 Hz for the Man*p* (C) ring, and 173 Hz for the Man*p* (E) ring, indicating the presence of α-configurations about C-1 for all three mannopyranosyl residues.[32]

In summary, di- up to penta-saccharides **4.1-4.4** corresponding to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, have been synthesized by selective activation of selenoglycoside donors in the presence of thioglycoside acceptors. The selenoglycoside **4.16** is a versatile furanosyl donor, that gives oligosaccharides with β selectivity. The four target compounds **4.1-4.4** will be tested as inhibitors against *T. cruzi* proliferation and also in the inhibition of proliferation of B lymphocytes.

4.5 Experimental

4.5.1 General Methods.

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured at 21°C with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. Chemical shifts are given in ppm downfield from TMS for those measured in $CDCl_3$ or CD_2Cl_2 and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional $^{1}H/^{1}H$ (COSYDFTP), $^{1}H/^{13}C$ (INVBTP), ^{1}H (NOESYTP), and ^{1}H (MLEVTP) experiments using standard Bruker pulse programs and an inverse detection, $^{1}H/X$ double-resonance probe. Sugar rings are denoted A, B, C, D, and E, respectively, as shown in the diagrams for compounds **4.1-4.4**; intermediates are labeled correspondingly. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated. Compounds were purified by flash column chromatography on Kieselgel 60 (230-400

mesh). Solvents were distilled before use and were dried as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40°C.

4.5.2 General Procedure for Glycosylation Reactions.

A mixture of the glycosyl donor, the acceptor, and activated 4Å molecular sieves was stirred in dry CH_2Cl_2 at room temperature under an N_2 atmosphere. The reaction mixture was cooled in an ice bath and NIS (1.2-1.3 eq. relative to the donor) was added, followed by addition of TfOH (0.05 eq). The reaction mixture was stirred at 0°C, under an N_2 atmosphere, until TLC showed that the reaction was complete. The mixture was quenched by addition of Et_3N, diluted with CH_2Cl_2 and filtered through a pad of Celite. The mixture was washed with 10% $Na_2S_2O_3$, followed by satd. $NaHCO_{3(aq)}$. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography.

4.5.3 General Procedure for Deprotection.

The protected oligosaccharide was dissolved in freshly distilled MeOH and $NH_3(g)$ was bubbled through the solution, while stirring under an N_2 atmosphere, until TLC indicated that no further change was occurring. The reaction mixture was concentrated by rotary-evaporation, then placed under high vacuum (~ 0.05 Torr), at 50°C overnight to remove NH_4OAc. The residue was purified by column chromatography to give the desired partially deprotected oligosaccharide. The ^1H NMR spectrum confirmed that the acyl groups had been removed. The partially deprotected

oligosaccharide was dissolved in 4:1 HOAc:H_2O (10 mL) and stirred with Pd-C (100 mg) under H_2 (52 psi). After 20 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with water. The combined filtrates were evaporated to dryness and the residue co-evaporated several times with distilled H_2O to remove any traces of HOAc. The target oligosaccharide was purified by column chromatography.

4.5.4 Synthesis

Ethyl 3-*O*-(β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (4.1). To a solution of the disaccharide **4.5** (45 mg, 0.06 mmol) in freshly distilled MeOH (5 mL) was added 1 M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred at room temperature, overnight, under an N_2 atmosphere. The reaction mixture was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The crude product was then dissolved in 80% AcOH (5 mL) and the solution was stirred at room temperature overnight. The solvent was removed and the crude product was purified by column chromatography using EtOAc:MeOH:H_2O (7:2:1) as the eluant to yield the desired disaccharide **4.1** as a clear glass, (13 mg, 70%). $[\alpha]_D^{22}$ 35 ° (*c* 0.028, H_2O); ^{1}H NMR (D_2O): δ 5.31 (d, 1 H, $J_{1,2}$ 1.6 Hz , H-1B), 5.09 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1A), 4.21 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2B), 4.11 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2A), 4.04 (dd, 1 H, $J_{3,4}$ 6.6 Hz, H-3A), 4.01 (m, 1 H, H-5A), 4.00 (dd, 1 H, $J_{4,5}$ 3.8 Hz, H-4A), 3.85 (dd, 1 H, $J_{5,6}$ 2.3, $J_{6,6'}$ 12.4 Hz, H-6A), 3.81 (m, 2 H, H-3B, H-5B), 3.74 (dd, 1 H, $J_{5,6}$ 6.0 Hz, H-6A'), 3.70 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4B), 3.67 (dd, 1 H, $J_{5,6}$ 4.5, $J_{6,6'}$ 11.7 Hz, H-6B), 3.61 (dd, 1 H, $J_{5,6}$ 7.4 Hz, H-6B'), 2.64 (m, 2 H, SCH_2CH$_3$), 1.24 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH_3), ^{13}C NMR (D_2O): δ 106.90 (C-1A), 86.60 (C-1B), 85.51 (C-4A), 83.97 (C-

2A), 79.62 (C-3A), 78.31 (C-3B), 75.65 (C-5A), 73.28 (C-5B), 71.06 (C-2B), 67.99 (C-4B), 65.41 (C-6B), 63.47 (C-6A), 27.45 (SCH_2CH_3), 16.67 (SCH_2CH_3). Anal.Calcd for $C_{14}H_{26}O_{10}S$: C, 43.52; H, 6.78. Found: C, 43.85; H, 6.78.

Methyl 2-*O*-(3-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (4.7). The methyl mannopyranoside acceptor **4.6** (308 mg, 0.66 mmol) was glycosylated with the thioglycoside donor **4.5** (379 mg, 0.51 mmol) following the general procedure. An immediate reaction to produce a dark purple-brown color ensued; reaction time was 3.5 h at room temperature. The crude product was purified by column chromatography using toluene-EtOAc, (3:1) as the eluant. The desired trisaccharide **4.7** was obtained as a colorless foam, (393 mg, 68 %). $[\alpha]_D^{22}$ –36 ° (*c* 0.25, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.12-7.09 (m, 25 H, aromatic), 5.70 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.6 Hz, H-2B), 5.64 (s, 1 H, PhC*H*), 5.29 (bs, 1 H, H-1A), 5.25 (ddd, 1 H, $J_{4,5}$ 3.4, $J_{5,6}$ 7.5, $J_{5,6'}$ 4.1 Hz, H-5A), 5.24 (d, 1 H, H-1B), 4.91 (d, 1 H, $J_{2,3}$ 1.5 Hz, H-2A), 4.85, 4.57 (2 d, 2 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.82 (dd, 1 H, $J_{3,4}$ 5.6 Hz, H-3A), 4.80 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.70, 4.66 (2 d, 2 H, J_{gem} 11.8 Hz, OCH_2Ph), 4.64, 4.58 (2 d, 2 H, J_{gem} 12.2 Hz, OCH_2Ph), 4.41 (m, 1 H, H-3B), 4.32 (dd, 1 H, $J_{5,6}$ 4.1, $J_{6,6'}$ 10.2 Hz, H-6B), 4.24 (dd, 1 H, H-4A), 4.11-4.01 (m, 4 H, H-2C, H-4B, H-5B, H-6A), 3.96 (dd, 1 H, $J_{6,6'}$ 11.8 Hz, H-6A'), 3.92-3.83 (m, 3 H, H-3C, H-6B', H-4C), 3.77-3.72 (m, 3 H, H-5C, H-6C, H-6C'), 3.37 (s, 3 H, -OC*H*$_3$), 2.11, 2.08, 1.89, 1.82 (4 s, 3 H each, -C(O)C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 170.55, 170.28, 170.20, 169.45 (*C*OCH_3), 165.62 (*C*OPh), 139.13-126.46 (30 C, aromatic), 103.14 (C-1A), 102.21 (CHPh), 100.64 (C-1B), 100.24 (C-1C), 81.18 (C-2A), 81.05 (C-4A), 80.41

(C-3C), 77.69 (C-4B), 77.25 (C-3A), 75.37 (2C, C-4C CH_2Ph), 75.05 (C-2C), 73.60 (CH_2Ph), 72.76 (CH_2Ph), 72.25 (C-5C), 70.47 (C-3B), 69.98 (C-6C), 69.64 (2C, C-5A and C-2B), 69.17 (C-6B), 64.76 (C-5B), 62.96 (C-6A), 55.01 (-OCH_3), 20.97, 20.94, 20.78, 20.54 ($COCH_3$). Anal. Calcd for $C_{62}H_{68}O_{21}$: C, 64.80; H, 5.96. Found: C, 64.95; H, 5.91.

Methyl 2-*O*-(3-*O*-(β-D-galactofuranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (4.2). The trisaccharide **4.7** (129 mg, 0.11 mmol) was deprotected following the general procedure. After deacylation, the partially deprotected trisaccharide was purified by column chromatography using CH_2Cl_2:MeOH (5:1) as the eluant. This product was further deprotected to yield the target trisaccharide **4.2** as a colorless foam, (43 mg, 96 %). $[\alpha]_D^{22}$ -54.5 ° (*c* 0.16, H_2O); ^1H NMR (D_2O): δ 5.13 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1A), 5.03 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 4.96 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.24 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2B), 4.20 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2A), 4.04 (dd, 1 H, $J_{3,4}$ 6.5 Hz, H-3A), 4.01 (dd, 1 H, $J_{4,5}$ 3.8 Hz, H-4A), 3.93 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2C), 3.89 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3B), 3.83 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3C), 3.83-3.36 (m, 11 H, H-4C, H-4B, H-5C, H-5B, H-5A, H-6C, H-6C', H-6B, H-6B', H-6A, H-6A'), 3.37 (s, 3 H, -OCH_3). ^{13}C NMR (D_2O): δ 107.02 (C-1A), 104.73 (C-1B), 101.93 (C-1C), 85.50 (C-4A), 83.98 (C-2A), 81.27 (C-2C), 79.63 (C-3A), 77.87 (C-3B), 75.82, 75.20, 75.35, 72.81, 69.60 (C-4B, C-4C, C-5A, C-5B, C-5C), 69.31 (C-2B), 67.80, 65.48, 63.58 (C-6A, C-6B, C-6C), 63.76 (C-3C), 57.46 (-OCH_3). Anal. Calcd for $C_{19}H_{34}O_{16}$: C, 44.02; H, 6.61. Found: C, 43.68; H, 6.30.

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-D-mannopyranoside (4.8). To a solution of 50% H_3PO_2 (90 mL) was added diphenyldiselenide (9.1g, 29 mmol) and the mixture was rapidly stirred at reflux, under an N_2 atmosphere until the yellow color disappeared. The reaction mixture was cooled to 0°C and extracted with CH_2Cl_2 and washed with cold H_2O. The combined extracts were washed with half-saturated NaCl and dried over $MgSO_4$. The solution was filtered into a flask containing peracetylated mannose (15g, 39 mmol) and $BF_3.Et_2O$ (9.9 mL, 78 mmol) was added. The reaction was stirred overnight under an N_2 atmosphere. The reaction mixture was cooled in an ice bath and quenched with Et_3N (4 mL) and satd. $NaHCO_{3(aq)}$ (50 mL), and extracted with CH_2Cl_2. The extracts were washed with satd. $NaHCO_{3(aq)}$, followed by cold H_2O and dried over Na_2SO_4. The crude product was purified by column chromatography using hexanes:EtOAc (2:1) as the eluant. The white solid was recrystallized from hexanes:EtOAc to yield white crystals of compound **4.8** (9.2 g, 49%). Mp 89-92 °C; $[\alpha]_D^{22}$ 138° (*c* 0.33, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.60-7.20 (m, 5 H, aromatic), 5.75 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.56 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 5.34 (dd, 1 H, H-4), 5.28 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 4.46 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 5.8, $J_{5,6'}$ 2.3 Hz, H-5), 4.31 (dd, 1 H, $J_{6,6'}$ 12.3 Hz, H-6), 4.09 (dd, 1 H, H-6'), 2.13, 2.07, 2.06, 2.01 (4 s, 3 H each, -C(O)C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.40, 169.76, 169.71, 169.61 (*C*OCH$_3$), 134.37-128.30 (6C, aromatic), 82.51 (C-1), 71.57 (C-2), 71.31 (C-5), 69.82 (C-3), 66.41 (C-4), 62.41 (C-6), 20.76, 20.60, 20.52 (4C, CO*C*H$_3$). Anal. Calcd for $C_{20}H_{24}O_9Se$: C, 49.17; H, 4.96. Found: C, 49.38; H, 5.03.

Phenyl 4,6-*O*-benzylidene-1-seleno-α-D-mannopyranoside (4.10). To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-D-mannopyranoside **4.8** (2.0 g, 4.0 mmol) in freshly distilled MeOH (60 mL) was added 1M NaOMe/MeOH (2 mL). The reaction mixture was stirred overnight, under an N_2 atmosphere. The solution was neutralized with Rexyn 101 (H^+). The resin was filtered, the filtrate was concentrated in vacuo and placed under high vacuum overnight. To a solution of the crude product **4.9** in DMF (6 mL) was added *p*-toluenesulfonic acid (7.6 mg, 0.04 mmol) and benzaldehyde dimethyl acetal (0.72 mL, 4.8 mmol). The reaction mixture was heated at 45-50°C for 1.5 h. An excess of K_2CO_3 was added and the solvent was removed by rotary-evaporation under a high vacuum. The white solid was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4 and the solvent removed in vacuo. The white solid was recrystallized from hexanes:EtOAc to yield white powdery crystals of compound **4.10**, (1.20 g, 74%). This compound was very insoluble in both EtOAc and CH_2Cl_2, and in subsequent repetitions of this reaction it was not purified. M.p. softens at ~70°C, melts 214-216°C; $[\alpha]_D^{22}$ 237° (*c* 0.54, DMSO); ^{1}H NMR (DMSO-d_6): δ 7.60-7.30 (m, 10 H, aromatic), 5.71 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.62 (s, 1 H, C*H*Ph), 5.54 (d, 1 H, $J_{2,OH}$ 4.0 Hz, OH-2), 5.20 (d, 1 H, $J_{3,OH}$ 6.2 Hz, OH-3), 4.07 (m, 2 H, H-2, H-6), 3.95 (m, 2 H, H-4, H-5), 3.79 (m, 1 H, H-6'), 3.73 (m, 1 H, H-3). ^{13}C NMR (DMSO-d_6): δ 137.75-126.34 (12C, aromatic), 101.13 (*C*HPh), 87.79 (C-1), 78.32 (C-4), 73.02 (C-2), 68.32 (C-3), 67.36 (C-6), 66.93 (C-5). Anal. Calcd for $C_{19}H_{20}O_5Se$: C, 56.03; H, 4.95. Found: C, 56.18; H, 4.91.

Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-seleno-α-D-mannopyranoside (4.11). To a solution of **4.10** (1.63 g, 4.0 mmol) in CH_2Cl_2 (75 mL) was added $(Bu)_4NHSO_4$ (272 mg, 0.8 mmol), benzyl bromide (0.83 mL, 7.0 mmol) and 5% $NaOH_{aq}$ (8 mL). The reaction mixture was stirred at reflux, under an N_2 atmosphere, for 40 h. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. The solvent was removed in vacuo and the white solid was purified by column chromatography using hexanes:EtOAc (4:1) as the eluant. The product **4.11** was recrystallized from hexanes:EtOAc to give white needle-like crystals, (1.35 g, 68%). M.p. 149-150 °C; $[\alpha]_D^{22}$ 142° (*c* 0.76, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.56-7.25 (m, 15 H, aromatic), 5.85 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.59 (s, 1 H, C*H*Ph), 4.73, 4.59 (2 d, 2 H, J_{gem} 11.6 Hz, OCH_2Ph) 4.25-4.15 (m, 2 H, H-5, H-6), 4.17 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 4.11, (m, 1 H, H-3), 3.99 (bt, 1 H, $J_{3,4}$, $J_{4,5}$ 9.3 Hz, H-4), 3.84 (m, 1 H, H-6'), 2.42 (bd, 1 H, O*H*). ^{13}C NMR ($CDCl_3$): δ 137.33-126.34 (18C, aromatic), 102.19 (*C*HPh), 83.96 (C-1), 80.72 (C-2), 79.56 (C-4), 73.04 (CH_2Ph), 69.34 (C-3), 68.34 (C-6), 66.59 (C-5). Anal. Calcd for $C_{26}H_{26}O_5Se$: C, 62.78; H, 5.27. Found: C, 63.01; H, 5.26.

Phenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-1-seleno-α-D-mannopyranoside (4.12). To a solution of **4.11** (620 mg, 1.25 mmol) in pyridine (10 mL) at 0°C; was added benzoyl chloride (0.26 mL, 2.3 mmol) and DMAP (catalytic). The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction was quenched with MeOH and the pyridine was removed by rotary-evaporation under a high vacuum. The crude product was purified by column chromatography using hexanes:EtOAc (4:1) as the eluant. The desired product **4.12** was

obtained as a white foam, (650 mg, 86%). $[\alpha]_D^{22}$ 67° (*c* 0.60, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 8.20-7.10 (m, 20 H, aromatic), 5.83 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.64 (s, 1 H, C*H*Ph), 5.54 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.3 Hz, H-3), 4.63, 4.50 (2 d, 2 H, J_{gem} 12.0 Hz, OCH_2Ph), 4.43 (dd, 1 H, H-4), 4.41, (dd, 1 H, H-2), 4.36 (m, 1 H, $J_{5,6}$ 4.9, $J_{4,5}$, $J_{5,6'}$ 10.0 Hz, H-5), 4.28 (dd, 1 H, $J_{6,6'}$ 10.2 Hz, H-6), 3.93 (dd, 1 H, H-6'). ^{13}C NMR ($CDCl_3$): δ 165.82 (*C*OPh), 137.28-126.20 (24 C, aromatic), 101.81 (*C*HPh), 83.91 (C-1), 78.44 (C-2), 76.34 (C-4), 73.04 (CH_2Ph), 71.44 (C-3), 68.42 (C-6), 67.22 (C-5). Anal. Calcd for $C_{33}H_{30}O_6Se$: C, 65.77; H, 5.02. Found: C, 65.75; H, 5.01.

Ethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4.13). To a solution of ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[34] (770 mg, 2.47 mmol) in pyridine (15 mL) at -25°C; was added benzoyl chloride (0.34 mL, 2.9 mmol). The reaction mixture was stirred at -25°C for 4 h. The pyridine was removed by rotary-evaporation under a high vacuum. The residue was dissolved in $CHCl_3$, washed with 0.1M HCl, followed by satd. $NaHCO_{3(aq)}$ and then H_2O. The solution was dried over Na_2SO_4 and the solvent removed in vacuo to give a white solid. The crude product was purified by column chromatography using hexanes:EtOAc (3:1) as the eluant. The desired product **4.13** was obtained as a white foam, (711 mg, 69%). $[\alpha]_D^{22}$ 80° (*c* 0.28, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 8.10-7.30 (m, 10 H, aromatic), 5.60 (s, 1 H, C*H*Ph), 5.48 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.1 Hz, H-3), 5.38 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.42 (ddd, 1 H, $J_{5,6}$ 4.8, $J_{5,6'}$ $J_{4,5}$ 9.9 Hz, H-5), 4.36 (m, 1 H, H-2), 4.31, (dd, 1 H, H-4), 4.28 (dd, 1 H, $J_{6,6'}$ 10.3 Hz, H-6), 3.91 (dd, 1 H, H-6'), 2.70 (m, 2 H, SCH_2CH_3), 2.49 (d, 1 H, $J_{OH,2}$ 4.0 Hz, OH), 1.32 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3). ^{13}C NMR (CD_2Cl_2): δ 165.78 (*C*OPh), 137.93-

126.56 (12C, aromatic), 102.27 (*C*HPh), 85.55 (C-1), 76.86 (C-4), 72.03 (C-3), 71.84 (C-2), 69.04 (C-6), 64.91 (C-5), 25.61 (S*C*H_2CH_3), 15.1 (SCH$_2$*C*H_3). Anal. Calcd for $C_{22}H_{24}O_6S$: C, 63.45; H, 5.81. Found: C, 63.19; H, 5.91.

Ethyl 2-*O*-(3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4.14). The thioglycoside acceptor **4.13** (375 mg, 0.9 mmol) was glycosylated with the selenoglycoside donor **4.12** (650 mg, 1.08 mmol) following the general procedure. The reaction time was 1.5 h at 0°C. The disaccharide was purified by column chromatography using hexanes:EtOAc (4:1) as the eluant. The desired product **4.14** was obtained as a white foam, (464 mg, 60 %). %). $[\alpha]_D^{22}$ -49° (*c* 0.60, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.12-7.00 (m, 25 H, aromatic), 5.70 (s, 1 H, C*H*Ph), 5.67 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$10.4 Hz, H-3B), 5.61 (s, 1 H, C*H*Ph), 5.51 (dd, 1 H, $J_{2,3}$ 3.6, $J_{3,4}$ 9.9 Hz, H-3C), 5.50 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1C), 4.96 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 4.46-4.38 (m, 3 H, H-2C, H-4C, H-5C), 4.34, 4.21 (2 d, 2 H, J_{gem} 11.6 Hz, OCH$_2$Ph), 4.31-4.23 (m, 3 H, H-6B, H-6C, H-4B), 4.18-4.10 (m, 2 H, H-2B, H-5B), 4.01 (dd, 1 H, $J_{5,6}$, $J_{6,6'}$ 10.0 Hz, H-6C'), 3.86 (dd, 1 H, $J_{5,6}$, $J_{6,6'}$ 10.3 Hz, H-6B'), 2.72 (m, 2 H, SC*H*$_2$CH$_3$), 1.38 (t, 3 H, *J* 7.5 Hz, SCH$_2$C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 166.18, 166.04 (*C*OPh), 137.97-126.52 (30 C aromatic), 102.21 (*C*HPh), 102.12 (*C*HPh), 101.85 (C-1B), 84.94 (C-1C), 79.68 (C-2C), 77.56 (C-2B), 76.88 (C-4C), 76.69 (C-4B), 74.28 (*C*H$_2$Ph), 71.45 (C-3C), 71.26 (C-3B), 68.42 (2C, C-6B, C-6C), 65.36 (C-5B), 65.04 (C-5C), 26.01 (S*C*H_2CH_3), 15.19 (SCH$_2$*C*H_3). Anal. Calcd for $C_{49}H_{48}O_{12}S$: C, 68.35; H, 5.62. Found: C, 68.35; H, 5.66.

Ethyl 2-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4.15). To a solution of the disaccharide **4.14** (110 mg, 0.128 mmol) in freshly distilled MeOH (10 mL) was added 1M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred at room temperature, overnight, under an N_2 atmosphere. The solution was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The partially deprotected disaccharide was purified by column chromatography using hexanes: EtOAc (2:1) as the eluant. The desired product **4.15** was obtained as a white foam, (81 mg, 96%). $[\alpha]_D^{22}$ 69° (*c* 0.67, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.60-7.30 (m, 15 H, aromatic), 5.60 (s, 1 H, C*H*Ph), 5.59 (s, 1 H, C*H*Ph), 5.33 (bs, 1 H, H-1C), 5.21 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1B), 4.76, 4.66 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.25-4.15 (m, 4 H, H-6B, H-6C, H-5C, H-3B), 4.11 (m, 2 H, H-2C, H-3C), 3.98 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2B), 3.96-3.87 (m, 5 H, H-6B', H-6C', H-4B, H-4C, H-5B), 2.65 (m, 2 H, SCH_2CH_3), 1.35 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 137.61-126.17 (18 C aromatic), 102.29 (*C*HPh), 102.07 (*C*HPh), 100.71 (C-1B), 84.73 (C-1C), 79.93 (C-2C), 79.48 (2C, C-4B, C-4C), 78.39 (C-2B), 73.55 (CH_2Ph), 69.55 (C-3C), 68.74 (C-6C), 68.64, 68.60 (C-3B, C-6B), 64.34 (C-5B), 64.04 (C-5C), 25.62 (SCH_2CH_3), 14.94 (SCH_2CH_3). Anal. Calcd for $C_{35}H_{40}O_{10}S$: C, 64.39; H, 6.18. Found: C, 64.60; H, 6.16.

Ethyl 2-*O*-(3-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4.17). The thioglycoside acceptor **4.15** (156 mg, 0.239 mmol) was glycosylated with the

selenoglycoside donor **4.16** (280 mg, 0.574 mmol) following the general procedure. The reaction time was 40 min at 0°C. Pure **4.17** (265 mg, 85%) was crystallized from the crude product mixture using hexanes:EtOAc. An analytical sample was obtained as colorless needles by column chromatography using hexanes:EtOAc (2:1) as the eluant and recrystallization from hexanes:EtOAc. M.p. 137-140 °C; $[\alpha]_D^{22}$ -5° (*c* 0.60, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.50-7.25 (m, 15 H, aromatic), 5.62 (s, 1 H, C*H*Ph), 5.60 (s, 1 H, C*H*Ph), 5.40 (bs, 1 H, H-1C), 5.34 (m, 1 H, H5A), 5.29 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1B), 5.25 (m, 1 H, $J_{4,5}$ 4.0, $J_{5,6}$ 7.6 Hz, H-5D), 5.21 (s, 1 H, H-1A), 5.13 (s, 1 H, H-1D), 5.06 (d, 1 H, $J_{2,3}$ 1.1 Hz, H-2A), 4.96 (dd, 1 H, $J_{3,4}$ 5.8 Hz, H-3D), 4.93 (d, 1 H, $J_{2,3}$ 1.6 Hz, H-2D), 4.91 (bdd, 1 H, $J_{3,4}$ 5.4 Hz, H-3A), 4.79, 4.75 (2 d, 2 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.37 (dd, 1 H, $J_{4,5}$ 3.2 Hz, H-4A), 4.30 (dd, 1 H, H-4D), 4.28 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 10.1 Hz, H-3B), 4.25-4.18 (m, 3 H, H-5C, H-6C, H-6B), 4.18 (m, 1 H, H-2C), 4.17 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3C), 4.09 (dd, 1 H, $J_{5,6}$ 7.4 Hz, H-6A), 4.10-4.05 (m, 4 H, H-6D, H-4B, H-4C, H-2B), 3.95 (dd, 1 H, $J_{5,6'}$ 4.6, $J_{6,6'}$ 11.7 Hz, H-6A'), 3.93 (m, 1 H, H-5B), 3.92 (dd, 1 H, $J_{5,6'}$, $J_{6,6'}$ 11.6 Hz, H-6C'), 3.85 (dd, 1 H, $J_{5,6'}$ 4.1, $J_{6,6'}$ 12.0 Hz, H-6D'), 3.84 (dd, 1 H, $J_{5,6'}$, $J_{6,6'}$ 10.0 Hz, H-6B'), 2.65 (m, 2 H, SCH_2CH_3), 2.10, 2.08 (2 s, 3 H each, -C(O)CH_3), 2.06 (s, 6 H, -C(O)CH_3), 1.93, 1.92, 1.91, 1.90 (4 s, 3 H each, C(O)CH_3), 1.30 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.62, 170.34 (8C, $COCH_3$), 138.9-126.5 (18 C, aromatic), 103.22 (C-1D), 103.04 (C-1A), 102.13 (3C, C-1B, 2 x *C*HPh), 85.33 (C-1C), 82.58 (C-2D), 82.04 (C-2A), 81.16 (2C, C-4A, C-4D), 77.81 (C-2C), 77.47 (2C, C-4B and C-4C), 77.35 (C-3A), 76.98 (C-3D), 76.35 (C-2B), 74.53 (CH_2Ph), 72.42 (2C, C-3B, C-3C), 69.85 (C-5A), 69.75 (C-5D), 69.14 (C-6C), 68.99 (C-6B), 65.58 (C-5B), 65.44 (C-5C), 63.08 (C-6D), 62.84 (C-6A), 26.14 (SCH_2CH_3), 21.0, 20.84, 20.74 (8C, $COCH_3$),

15.29 (SCH_2CH_3). Anal. Calcd for $C_{63}H_{76}O_{28}S$: C, 57.60; H, 5.84. Found: C, 57.80; H, 5.99.

Ethyl 2-*O*-(3-*O*-(β-D-galactofuranosyl)-α-D-mannopyranosyl)-3-*O*-β-D-galactofuranosyl-1-thio-α-D-mannopyranoside (4.3). The tetrasaccharide **4.17** (50 mg, 0.038 mmol) was deprotected as described in the general procedure. The intermediate deacylated product was purified by column chromatography using CH_2Cl_2:MeOH (12:1) as the eluant to give a clear glass. The compound was then subjected to hydrogenolysis and purified by column chromatography using EtOAc:MeOH:H_2O (5:2:1) as the eluant. The tetrasaccharide **4.3** was obtained as a syrup, (19 mg, 71%). $[\alpha]_D^{22}$ -10° (*c* 0.079, H_2O); ^{1}H NMR (D_2O): δ 5.55 (d, 1 H, $J_{1,2}$ 1.4 Hz, H 1C), 5.13 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1A), 5.11 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1D), 5.08 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.29 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2C), 4.21 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2B), 4.12 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2A), 4.08 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2D) 4.07-3.96 (m, 5 H, H-3A, H-3D, H-4A, H-4D, H-5C), 3.92-3.74 (m, 9 H, H-3B, H-3C, H-5A, H-5B, H-5D, H-6B, H-6C, H-6C', H-4C), 3.71 (dd, 1 H, $J_{5,6'}$ 4.2, $J_{6,6'}$ 10.9 Hz, H-6B'), 3.69 (m, 2 H, H-6A, H-6D), 3.65 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.9 Hz, H-4B), 3.62 (dd, 1 H, $J_{5,6'}$ 7.4, $J_{6,6'}$ 11.6 Hz, H-6A'), 3.61 (dd, 1 H, $J_{5,6'}$ 7.4, $J_{6,6'}$ 11.6 Hz, H-6D'), 2.65 (m, 2 H, SCH_2CH_3), 1.30 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3). ^{13}C NMR (D_2O): δ 107.30 (C-1D), 107.18 (C-1A), 104.47 (C-1B), 85.53 (2C, C-4A, C-4D), 85.46 (C-1C), 83.98 (2C, C-2A, C-2D), 79.68 (C-2C), 79.60 (C-3A), 79.45 (C-3D), 78.51 (C-3B), 78.05 (C-3C), 76.06 (C-5D), 78.85 (C-5C), 73.35 (C-5A), 73.26 (C-5B), 69.56 (C-2B), 68.29 (C-4C), 67.92 (C-4B), 65.50, 65.45 (2C, C-

6A, C-6D), 63.78 (C-6B), 63.54 (C-6C), 27.84 (SCH_2CH_3), 16.91 (SCH_2CH_3). Anal. Calcd for $C_{26}H_{46}O_{20}S$: C, 43.93; H, 6.53. Found: C, 43.71; H, 6.39.

Methyl 2-*O*-(2-*O*-((3-*O*-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (4.18). The methyl mannopyranoside acceptor **4.6** (30 mg, 0.064 mmol) was glycosylated with the thioglycoside donor **4.17** (100 mg, 0.076 mmol) following the general procedure. The reaction time was 4 h at room temperature. Purification of the pentasaccharide **4.18** was attempted by column chromatography, but the donor and product eluted together. The ^{1}H NMR spectrum showed a 3:1 ratio of product:donor (57 mg, 35%, corrected for presence of donor). Extensive column chromatography using toluene:EtOAc (1.5:1) as the eluant gave a small amount of pure material for characterization. $[\alpha]_D^{22}$ -6.4° (*c* 2.35, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.60-7.15 (m, 30 H, aromatic), 5.64 (s, 1 H, C*H*PhB), 5.59 (s, 1 H, C*H*PhC), 5.33 (d, 1 H, H-1C), 5.32 (d, 1 H, H-1B), 5.30 (m, 1 H, H-5A), 5.26 (m, 1 H, H-5D), 5.23 (s, 1 H, H-1A), 5.17 (s, 1 H, H-1D), 5.06 (d, 1 H, $J_{2,3}$ 1.2 Hz, H-2A), 4.96 (m, 2 H, H-2D, H-3D), 4.93 (m, 1 H, H-3A), 4.83, 4.54 (2 d, 2 H, J_{gem} 10.9 Hz, OCH_2PhE), 4.80, 4.75 (2 d, 2 H, J_{gem} 12.5 Hz, OCH_2PhB), 4.77 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1E), 4.73, 4.68 (2 d, 2 H, J_{gem} 12.3 Hz, OCH_2PhE), 4.63, 4.57 (2 d, 2 H, J_{gem} 12.2 Hz, OCH_2PhE), 4.36 (dd, 1 H, $J_{3,4}$ 5.3, $J_{4,5}$ 3.2 Hz, H-4A), 4.30 (dd, 1 H, $J_{3,4}$ 6.0, $J_{4,5}$ 3.2 Hz, H-4D), 4.26 (dd, 1 H, $J_{5,6}$ 3.3, $J_{6,6'}$ 9.9 Hz, H-6B), 4.26-4.22 (m, 3 H, H-2C, H-3C, H-3B), 4.17 (dd, 1 H, $J_{5,6}$ 4.6, $J_{6,6'}$ 10.0 Hz, H-6C), 4.10 (dd, 1 H, $J_{5,6}$ 7.6, $J_{6,6'}$ 11.7 Hz, H-6A), 4.08 (dd, 1 H, $J_{1,2}$, $J_{2,3}$ 1.7 Hz, H-2B),

4.07 (dd, 1 H, $J_{3,4}$ 8.1, $J_{4,5}$ 9.1 Hz, H-4B), 4.06 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4C), 4.05 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 11.9 Hz, H-6D), 4.01 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2E), 3.99-3.85 (m, 2 H, H-5B, H-5C), 3.91 (dd, 1 H, $J_{5,6}$ 4.4 Hz, H-6A'), 3.89 (dd, 1 H, $J_{5,6'}$ 9.9 Hz, H-6B'), 3.88 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3E), 3.81 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4E), 3.80 (dd, 1 H, $J_{5,6'}$ 3.8 Hz, H-6D'), 3.79 (dd, 1 H, $J_{5,6}$ 10.3 Hz, H-6C'), 3.77-3.68 (m, 3 H, H-5E, H-6E, H-6E'), 3.35 (s, 3 H, OCH_3), 2.10, 2.08, 2.06, 2.04, 1.951, 1.950, 1.89, 1.86 (8 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.57, 170.47, 170.40, 170.31, 170.27, 170.08 (8C, $COCH_3$), 136.21-126.41 (36 C, aromatic), 103.26 (C-1D), 102.94 (C-1A), 102.06 (CHPhB), 101.96 (CHPhC), 101.68 (2C, C-1B, C-1C), 100.24 (C-1E), 82.45 (C-2D), 81.90 (C-2A), 81.05 (C-4A), 81.00 (C-4D), 79.59 (C-3E), 77.26 (C-3A), 77.16 (2C, C-4B, C-4C), 76.96 (C-3D), 76.22 (C-2B), 75.51 (C-4E), 75.40 (C-2C), 75.32 (CH_2PhE), 75.19 (C-2E), 74.42 (CH_2PhB), 75.57, 72.42 (2C, CH_2PhE), 72.32 (C-5E), 72.08 (C-3C), 69.92 (C-3B), 69.73 (C-5A), 69.65 (2C, C-5D, C-6E), 68.97 (2C, C-6B, C-6C), 65.41 (C-5C), 65.08 (C-5B), 63.02 (C-6D), 62.77 (C-6A), 55.02 (OCH_3), 21.00, 20.75 (8C, $COCH_3$). Anal. Calcd for $C_{89}H_{102}O_{34}$: C, 62.30; H, 5.99. Found: C, 62.62; H, 5.88.

One pot synthesis of 4.18. The thioglycoside acceptor **4.15** (58 mg, 0.088 mmol) was glycosylated with the selenoglycoside donor **4.16** (94 mg, 0.19 mmol) following the general procedure. After 40 min at 0°C, a solution of the methyl glycoside acceptor **4.6** (74 mg, 0.16 mmol) in dry CH_2Cl_2 (2 mL), activated 4Å molecular sieves (200 mg), and NIS (52 mg, 0.23 mmol) were added. The reaction mixture was stirred for 1 h at 0°C and then warmed to room temperature and stirred for an additional 2 h. The color of the solution changed from a deep purple to dark rose. The reaction was worked up as

described in the general procedure. The ^{1}H NMR spectrum showed a 3:1 ratio of product:donor, (42 mg, 28%, corrected for presence of donor).

Methyl 2-*O*-((2-*O*-(3-*O*-β-D-galactofuranosyl)-α-D-mannopyranosyl)-3-*O*-β-D-galactofuranosyl-α-D-mannopyranosyl)-α-D-mannopyranoside (4.4). To a solution of the impure pentasaccharide **4.18** (55 mg, 0.032 mmol) in freshly distilled MeOH (5 mL) was added 1 M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred at room temperature, overnight, under an N_2 atmosphere. The reaction mixture was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The crude product was purified by column chromatography using EtOAc:MeOH:H_2O (10:1:1) as the eluant to give a clear glass. This compound was then subjected to hydrogenolysis as described in the general procedure for deprotection. The solvent was removed in vacuo and the crude product purified by column chromatography using EtOAc:MeOH:H_2O (5:2:1) as the eluant. The pentasaccharide **4.4** was obtained as a clear syrup, (12 mg, 60%). $[\alpha]_D^{22}$ -30 ° (*c* 0.029, H_2O); ^{1}H NMR (D_2O): δ 5.24 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1C), 5.18 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1D), 5.14 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1A), 5.09 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.97 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1E), 4.31 (dd, 1 H, H-2C), 4.21 (dd, 1 H, $J_{2,3}$ 2.9 Hz H-2B), 4.12 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2A), 4.09 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2D), 4.08-4.01 (m, 4 H, H-3A, H-3D, H-4A, H-4D), 4.00 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.2 Hz, H-3C), 3.93 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2E) 3.91-3.55 (m, 20 H, H-3B, H-3E, H-4B, H-4C, H-4E, H-5A, H-5B, H-5C, H-5D, H-5E, H-6A, H-6B, H-6C, H-6D, H-6E, H-6A', H-6B', H-6C', H-6D', H-6E'), 3.39 (s, 3 H, OCH_3). ^{13}C NMR (D_2O): δ 107.47 (C-1D), 107.20 (C-1A), 104.23 (C-1B), 103.40 (C-1C), 101.94 (C-1E), 85.51, 85.43 (C-4A, C-

4D), 83.99 (2C, C-2A, C-2D), 81.40 (C-2E), 79.61, 79.46 (C-3A, C-3D), 78.05 (2C, C-3B, C-3C), 77.18 (C-2C), 75.96, 75.89 (C-4B, C-4C), 75.25 (C-4E), 73.37, 73.32 (C-5A, C-5D), 72.83, 68.10, 67.75 (4C, C-3E, C-5B, C-5C, C-5E), 65.51 (2C, C-6A, C-6D), 63.61 (3C, C-6B, C-6C, C-6E), 57.52 (OCH_3). Anal. Calcd for $C_{31}H_{54}O_{26}$: C, 44.18; H, 6.46. Found: C, 43.81; H, 6.22.

4.6 Acknowledgements

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CHAPTER 5: SYNTHESIS OF OLIGOSACCHARIDE FRAGMENTS OF THE GLYCOSYLINOSITOLPHOSPHOLIPID OF *TRYPANOSOMA CRUZI*: A NEW SELENOGLYCOSIDE GLYCOSYL DONOR FOR THE PREPARATION OF 4-THIOGALACTOFURANOSYL ANALOGUES*

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* This work is dedicated to P. Sinay in celebration of his 62nd birthday.

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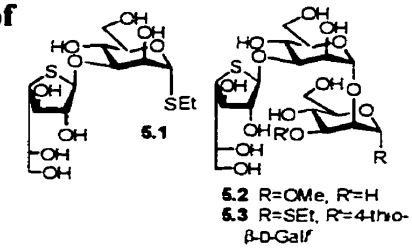
5.1 Graphical Abstract

Synthesis of Oligosaccharide Fragments of the Glycosylinositol-phospholipid of *Trypanosoma cruzi*: A New Selenoglycoside Glycosyl Donor for the Preparation of 4-Thiogalactofuranosyl Analogues



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5.2 Abstract

A new selenoglycoside, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-1-selenogalactofuranose has been synthesized. This 4-thiogalactofuranosyl donor was used in the syntheses of heteroatom analogues of the di-, tri-, and tetra-saccharides corresponding to the oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-(β-D-Gal*f*-(1→3))-α-D-Man*p*. These compounds represent fragments of the terminal end of the glycosylinositolphospholipid oligosaccharide found in the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, and are intended for use as inhibitors of the enzymes that construct the native oligosaccharides. The syntheses employed the selective activation of a phenyl 4-thio-1-selenogalactofuranoside glycosyl donor over ethyl 1-thioglycoside glycosyl acceptors with NIS/TfOH.

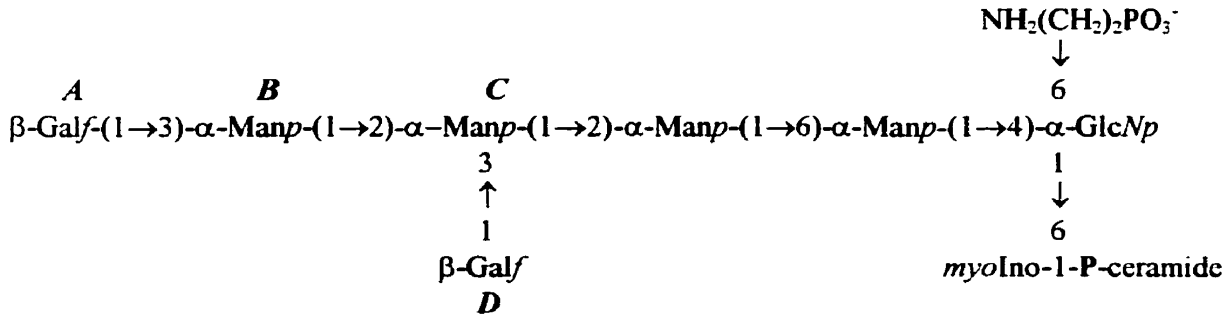
Keywords: carbohydrates, glycosidation, thiosugars, glycosides

5.3 Introduction

Carbohydrates are highly functionalized molecules that are used by Nature for diverse tasks. They play an important role in biochemical recognition pathways. They are involved in growth and development, immune responses, infection by viruses and bacteria, host-pathogen interactions, cell adhesion, tumor metastasis, and signal transduction.[1] For example, inhibition of tumor metastasis is an important area of research and this goal may be achieved through the inhibition of glycosidases used in the oligosaccharide processing pathways.[2,3,4] The synthesis of heteroanalogues of sugars as potential glycosidase inhibitors has been a focus of our laboratory. The synthesis of novel glycosidase inhibitors as analogues of methyl maltoside and alkyl kojibiosides in which the ring and/or the interglycosidic oxygen atoms have been replaced with sulfur or selenium have been reported.[5,6,7,8] More recently, the syntheses of disaccharide analogues containing sulfur in the ring and nitrogen in the interglycosidic linkage have been achieved.[9,10,11]

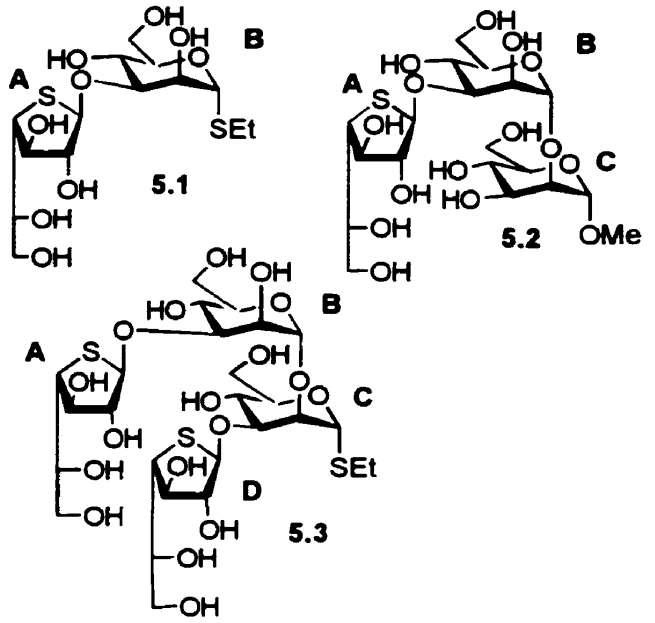
Previous reports from our laboratory demonstrated the viability of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside as a glycosyl donor.[12,13] The synthesis of oligosaccharides containing galactofuranose is important because it has been determined that galactofuranose (Gal*f*) is present as a constituent of external cellular structures in protozoa,[14] bacteria,[15,16] and fungi.[17] These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection.[18] It is known that Gal*f* is part of the oligosaccharide core of the glycosylinositolphospholipid (GIPL)

from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[19] The GIPL structure is:[20,21]



This structure is the most abundant cell surface glycoconjugate present in the insect dwelling epimastigote stage of the *T. cruzi* life cycle.[21] The glycoconjugates on the cell surface during the infectious stage of *T. cruzi* are not modified with galactofuranose; however, it has been shown that the β-D-Gal*f* moiety is recognized by antibodies that inhibit *T. cruzi* internalization into mammalian cells.[18] Oligosaccharides containing 4-thio-Gal*f* may therefore be useful for understanding the role Gal*f* plays in microorganisms, for studying the biosynthesis of furanosyl-containing glycoconjugates, and may also be used as inhibitors to probe the development of infections. In this study, we report the first synthesis of oligosaccharides containing 4-thio-Gal*f* using a new glycosyl donor, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-D-selenogalactofuranoside, **5.5**. This work is an extension of previous studies on the selective activation of selenoglycoside glycosyl donors in the presence of glycosyl acceptors containing a latent thioglycoside.[12, 13, 22, 23, 24, 25] In the present application, these methods led to the ready preparation of heteroanalogues containing sulfur in the non-reducing (and branched) galactofuranose ring(s) of the disaccharide **5.1**, trisaccharide **5.2** and tetrasaccharide **5.3**

corresponding to the terminus of the glycosylinositolphospholipid oligosaccharides of *T. cruzi*.



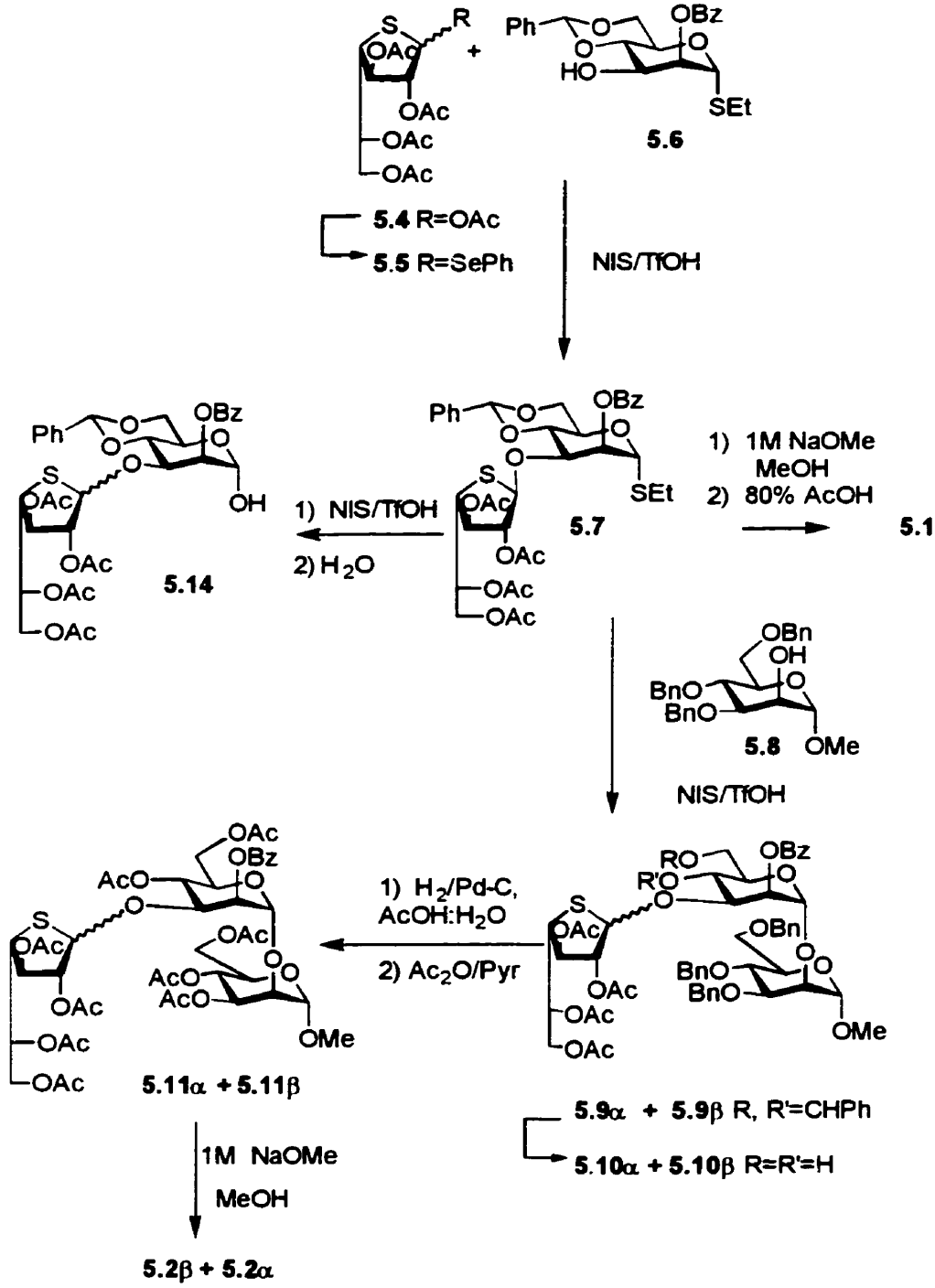
5.4 Results and Discussion

The required monosaccharides 1,2,3,5,6-penta-*O*-acetyl-4-thio-D-galactofuranose **5.4**,[26] ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **5.6**,[27] and methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **5.8**,[28] and the disaccharide ethyl 2-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **5.12**[13] were synthesized following literature methods.

Phenyl 2,3,5,6-tetra-*O*-acetyl-1-seleno-4-thio-D-galactofuranoside **5.5** was prepared by reaction of peracetylated 4-thiogalactofuranose **5.4** with phenylselenol and

BF_3:Et_2O in 94% yield. A ^1H NMR spectrum showed the presence of an α:β mixture in a ratio of 1:3 (see Scheme 5.1). The 2D NOESY spectrum showed an NOE between H-1

Scheme 5.1



and H-3 for the major isomer, indicating the presence of a β- linkage, the minor isomer showed no NOE between H-1 and H-3, indicating the presence of an α- linkage. The J_{C1H1} values are very similar for both the isomers **5.5α** (163 Hz) and **5.5β** (160 Hz). As with furanose sugars in general,[29] J_{C1H1} values do not seem to be reliable indicators of α- or β- linkage in the 4-thio-furanosyl residues.

Glycosylation of the acceptor **5.6** with the new glycosyl donor **5.5** was performed using NIS/TfOH. Activation of the phenyl 1-selenogalactofuranoside over the ethyl 1-thiomannopyranoside was achieved with good selectivity, and the protected (1→3)-linked disaccharide **5.7** was obtained as a white foam in 80% yield (see Scheme 5.1). The stereochemical integrity of the disaccharide **5.7** was confirmed by examination of the NOE contacts for the galactofuranosyl residue and the J_{C1H1} values of the mannopyranosyl residue. The 2D NOESY spectrum showed the presence of an NOE between H-1A and H-3A (of the 4-thio-Gal*f* ring A) and the absence of an NOE between H-1A and H-4A, indicating the presence of a β–linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. The β-isomer was the only compound isolated even though an α:β mixture of the donor was used. The J_{C1H1} value is 168 Hz for the Man*p* (B) ring, indicating the presence of an α-configuration about C-1 for the mannopyranosyl residue.[30] The disaccharide **5.7** was deprotected by methanolysis of the esters followed by hydrolysis of the benzylidene acetal to give compound **5.1** in 75% yield. Again, the 2D NOESY spectrum showed an NOE between H-1A and H-3A (of the 4-thio-Gal*f* ring A), indicating the preservation of the β–linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. The J_{C1H1} value is 167 Hz for the Man*p* (B) ring, indicating the expected α-configuration about C-1 for the mannopyranosyl residue.[30]

The protected disaccharide **5.7** was used as a donor in the next glycosylation reaction without any further manipulation. Glycosylation of the acceptor **5.8** with the donor thioglycoside **5.7** was considerably slower using NIS/TfOH than the previous reaction with the selenoglycoside. The reaction gave a mixture of products that included compounds **5.9α** and **5.9β** (see Scheme 5.1) as the major components, which could not be separated by column chromatography. The mixture was analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy. We initially considered the possibility that one of the major compounds was an orthoester because a high-field, three-proton singlet was observed at δ1.48 ppm in the ^{1}H NMR spectrum. However, a ^{13}C DEPT experiment indicated that there was no quaternary carbon in the region around 120 ppm as would be expected for an orthoester. In a subsequent glycosylation reaction, the reaction mixture was warmed to room temperature before quenching, but the ^{1}H NMR spectrum of the crude product mixture showed that the peak at δ 1.48 ppm was present with the same intensity. Since these conditions (which should promote rearrangement of any orthoester product) made no difference, the possibility of an orthoester was discounted. The high-field singlet was dismissed as resulting from an unusually shielded acetate group. A COSY spectrum, together with a C-H correlation spectrum, permitted assignment of the other ^{1}H and ^{13}C NMR signals, as expected for the structures **5.9α** and **5.9β**. Next, the benzylidene acetal was removed by hydrolysis to give **5.10α** and **5.10β**. The ^{1}H NMR spectrum indicated that there was no longer a singlet at 1.48 ppm, and all the acetate peaks were found between 2.01-1.85 ppm. A COSY spectrum, together with a C-H correlation spectrum, permitted assignment of the ^{1}H and ^{13}C NMR signals that was consistent with the presence of **5.10α** and **5.10β** (see Scheme 5.1). Finally, the benzyl ethers were removed

by hydrogenolysis and the mixture was acetylated for purification purposes (see Scheme 5.1). In subsequent experiments, the deprotection sequence was simplified by first removing both the benzylidene and the benzyl ethers by hydrogenolysis, followed by acetylation to give a mixture of **5.11α** and **5.11β**. The ^{1}H NMR spectrum showed the presence of two compounds in a ratio of 2:3. A NOESY spectrum showed an NOE between H-1A and H-3A (of the 4-thio-Gal*f* ring A) for the major compound, indicating the presence of a β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. This confirmed that the major isomer was indeed the desired compound **5.11β**. No NOE is found between H-1A and H-3A for the minor compound, indicating the presence of an α-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. A COSY and a TOCSY spectrum, together with a C-H correlation spectrum, then permitted complete assignment of the ^{1}H and ^{13}C NMR signals for **5.11α** as the minor product and **5.11β** as the major product. The J_{C1H1} values are 172 Hz for both the Man*p* (B) ring and the Man*p* (C) ring, indicating the presence of α–configurations about C-1 for both mannose residues, in both the major and minor compounds.[30]

In the ^{13}C{^{1}H} NMR spectra for all three sets of compounds, **5.9-5.11**, the Gal*f* C-4 resonances are found between 40-50 ppm due to shielding by the sulfur atom. The C-1β and C-4β signals are always downfield from the C-1α and C-4α signals. This can be explained by the *syn* vs. *anti* orientation of the ring substituents in the molecules.[31] In Gal*f*, the C-1α and C-4α are shielded because of a *syn* arrangement between the aglycon and O-2 and between the aglycon and the substituent at C-4, respectively. Also, a *syn* arrangement between the aglycon and O-3 of the β-Gal*f* causes C-4β to be deshielded.

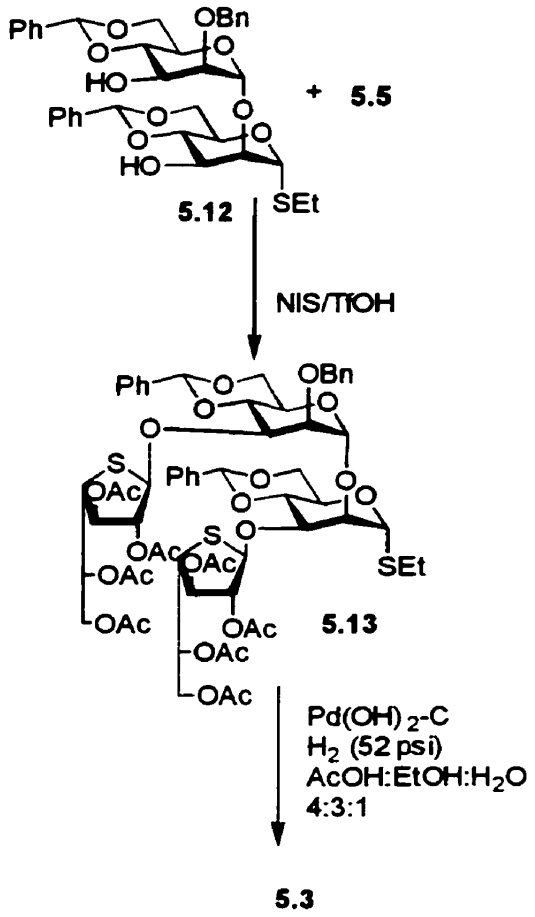
The latter effect has been observed with C-4 of pento- or hexofuranosides, containing *gluco*, *manno*, and *allo* configurations.[31]

The synthesis of the trisaccharide **5.9** was also attempted using either methyl triflate[32] or iodonium di-*sym*-collidine perchlorate[33] as the promoter, but both reactions were unsuccessful. It was hoped that these promoters would be more selective for the ethylthio aglycon of **5.7**.

The trisaccharides **5.11α/β** were deprotected by methanolysis of the esters to give the target trisaccharide **5.2β**, plus the α-anomer in 94% yield, with an α:β ratio of 1:1.6. Again, COSY, TOCSY, and C-H correlation spectra enabled the assignment of the signals of rings A, B, and C for both the α- and the β- isomer. The 2D NOESY spectrum showed an NOE between H-1A and H-3A (of the 4-thio-Gal*f* ring A) for the major compound, indicating the preservation of the β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. No NOE is found between H-1A and H-3A for the minor compound, indicating the preservation of the α-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings.

The tetrasaccharide **5.13** was synthesized by double glycosylation of the disaccharide **5.12** with 2.2 equivalents of phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-D-selenogalactofuranoside **5.5** (see Scheme 5.2). This reaction was immediate and resulted in tetrasaccharide products in 93% yield. The desired compound **5.13** containing β-linkages between both Gal*f* rings and the mannosyl residues comprised >80% of the tetrasaccharide. An isomeric tetrasaccharide containing an α-linkage between one of the Gal*f* rings and a mannosyl residue could not be separated from the desired compound.

Scheme 5.2

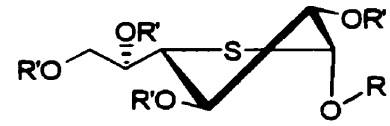


This reaction was repeated at room temperature and much less α/β selectivity was observed. The tetrasaccharide was deprotected by methanolysis of the esters, followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ether to give the target tetrasaccharide **5.3**, containing a minor amount of the α anomeric isomer, in 25% yield. The low overall yield for deprotection steps resulted from poisoning of the palladium catalyst by the sulfur atoms during the hydrogenolysis step. It was therefore necessary to use large amounts of Pd/C and to replace the catalyst three times in order to completely remove the benzyl group, thus leading to a reduction in overall yield because some of the compound was adsorbed on the Pd/C catalyst.

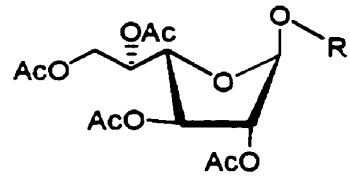
Assignment of the NMR signals of the Man*p* (C) ring of the tetrasaccharide **5.3** was based on the fact that C-1 for this residue has a characteristic upfield chemical shift due to the ethyl thioglycoside. A C-H correlation spectrum, together with a COSY and TOCSY spectrum then permitted complete assignment of the ^{1}H and ^{13}C NMR signals of this ring. The assignment of signals for the Man*p* (B) ring was based on the presence of an NOE contact across the glycosidic linkage between H-1B and H-2C, and COSY and TOCSY transfer between ^{1}H NMR signals of the B ring. Assignment of signals of the 4-thio-Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H-1A and H-3B, and H-1D and H-3C, respectively. The 2D NOESY spectrum showed an NOE between H-1A and H-3A (of the 4-thio-Gal*f* ring A), and another NOE between H-1D and H-3D (of the 4-thio-Gal*f* ring D), indicating the presence of a β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings, and also between the 4-thio-Gal*f* (D) and Man*p* (C) rings. The J_{C1H1} values are ~172 Hz for the Man*p* (B) ring and 167 Hz for the Man*p* (C) ring, indicating the presence of α-configurations about C-1 for both mannopyranosyl residues.[30]

The coupling constants observed in the ^{1}H NMR spectrum for the 4-thio-galactofuranosyl residues are of interest. The *J* values for the 4-thio-Gal*f* residue in **5.11α** and **5.2α** suggest that the ring is in a $^{2}T_{3}$ (D) conformation (see Scheme 5.3).[34] In this conformation, the anomeric linkage from 4-thio-Gal*f* (A) to Man*p* (B) is quasi-axially oriented and the other substituents are quasi-equatorial. The large values of $J_{1,2}$ (4.2 Hz), $J_{2,3}$ (9.8 Hz) and $J_{3,4}$ (8.2 Hz) indicate that H-2 and H-3 and also H-3 and H-4 must have dihedral angles near 180°. This was also observed by Valera *et al.*[26] for 1,2,3,5,6-penta-*O*-acetyl-4-thio-α-D-galactofuranose **5.4α**.

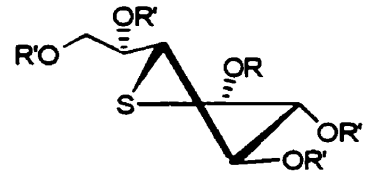
Scheme 5.3



α-S-series $^{2}T_{3}$ (D)
5.11α / 5.2α



β-O-series E_{O} (D)



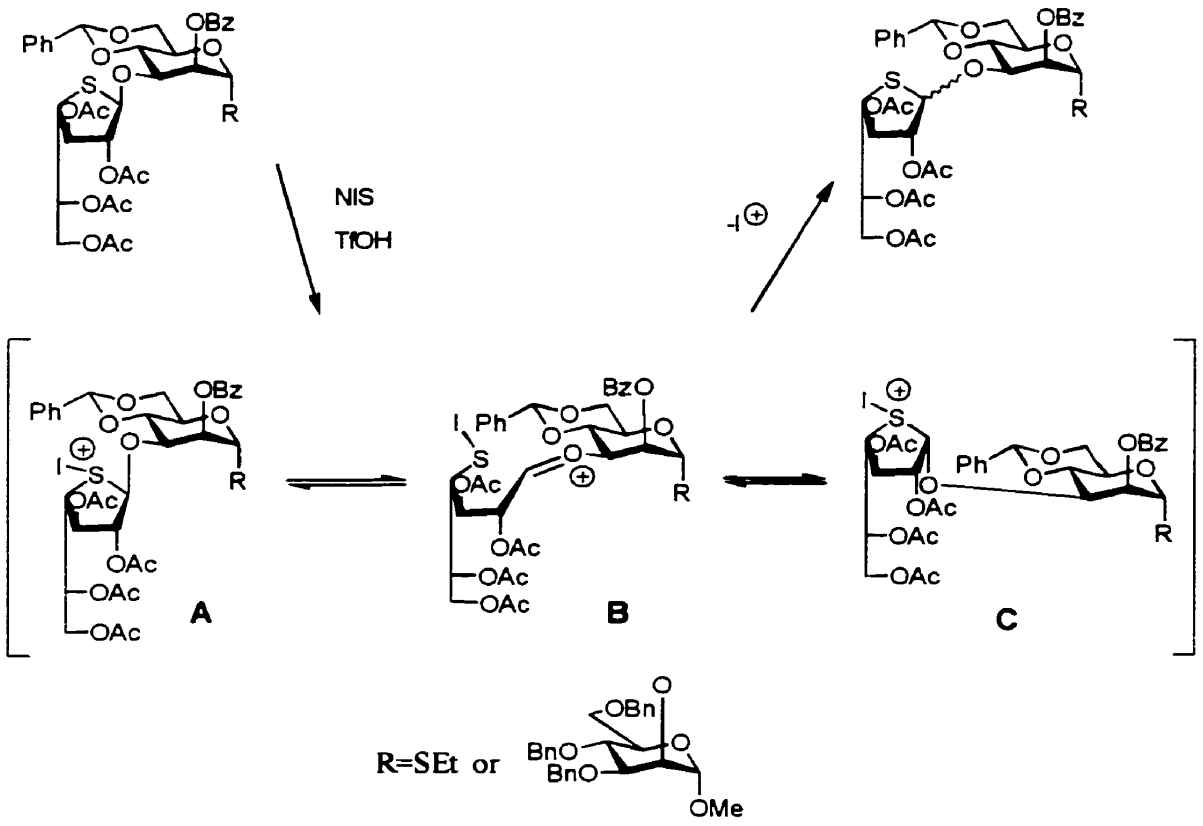
β-S-series $^{4}T_{3}$ (D)
5.1, 5.2β, 5.3, 5.7, 5.11β, 5.13

The coupling constants $J_{1,2}$ (2.4-2.6 Hz), $J_{2,3}$ (4.0-4.6 Hz) and $J_{3,4}$ (6.6-7.6 Hz) for **5.7**, **5.11β**, and **5.13** indicate that the 4-thio-galactofuranosyl residue in these compounds exists as mixture of several conformations. This is very different from the E_{O} (D) conformation observed for the β-D-galactofuranosides (i.e. the *O*-series) [$J_{1,2}$ (<1 Hz), $J_{2,3}$ (1.5 Hz) and $J_{3,4}$ (5.6 Hz)], as seen in Scheme 5.3.[13, 35] The coupling constants that have been reported for **5.4β** [$J_{1,2}$ (3.1 Hz), $J_{2,3}$ (5.4 Hz), $J_{3,4}$ (6.4 Hz)], methyl 2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranoside [$J_{1,2}$ (2.5 Hz), $J_{2,3}$ (5.0 Hz), $J_{3,4}$ (5.3 Hz)] and 6-deoxy-2,3,5-tri-*O*-acetyl-4-thio-β-D-galactofuranose [$J_{1,2}$ (3.3 Hz), $J_{2,3}$ (6.0 Hz), $J_{3,4}$ (7.4 Hz)] were attributed to result from an equilibrium mixture of several conformations,

including $^4T_3$ (D) (see Scheme 5.3).[34, 36] In a study of 4-thiofuranoside derivatives of D-galactosamine, it was observed that all the β-isomers had coupling constants, $J_{1,2}$ (3.3-5.7 Hz), $J_{2,3}$ (6.1-7.6 Hz) and $J_{3,4}$ (6.7-7.7 Hz), that indicated the presence of a mixture of conformations.[37] Upon deprotection, the β-linked Gal*f* residue(s) in compounds **5.1**, **5.2** and **5.3** also adopt a conformation similar to the $^4T_3$ (D) conformation (see Scheme 5.3), with $J_{1,2}$ (5.5-5.9 Hz), $J_{2,3}$ (8.3 Hz) and $J_{3,4}$ (8.8 Hz). In an attempt to determine whether hydrogen-bonding networks were responsible for the conformational changes, the ^{1}H NMR spectrum of **5.1** in DMSO-d_6 was obtained, but addition of D_2O showed no significant changes in the resonances of the OH groups.[38]

We propose that the isomerization of the Gal*f* unit during the glycosylation to give the trisaccharide **5.9** may occur either in the disaccharide donor **5.7** or in the trisaccharide product by way of open-chain intermediates (Scheme 5.4). If there is a competition between the sulfur atom of the aglycon and the sulfur atom of the Gal*f* ring in the donor **5.7** for the electrophilic iodine generated from the NIS/TFOH reagent, then isomerization may occur before glycosylation. In a control experiment, the disaccharide **5.7** was reacted with NIS/TfOH in the absence of the acceptor. The ^{1}H NMR spectrum indicated that isomerization occurred at the glycosidic linkage between the Gal*f* ring and the mannosyl residue to give compound **5.14α/β** (Scheme 5.1). If the ring sulfur atom of the Gal*f* moiety reacts preferentially, the β sulfonium iodide intermediate **A** (Scheme 5.4) may be in equilibrium with an open-chain oxonium ion **B** which can reclose to regenerate **A**. Alternatively, **B** may, by rotation about single bonds, attain a conformation in which attack of the sulfenyl iodide occurs from the opposite face of the oxonium ion to give the

Scheme 5.4



α sulfonium ion intermediate **C**. Loss of I^- from either **A** or **C** would then lead to a mixture of α and β isomers in the donor **5.7** and hence, to the observed anomeric mixture in trisaccharide **5.9** (Scheme 5.1). On the other hand, there still remains the possibility that the Gal*f* in the trisaccharide **5.9** may isomerize by a similar open-chain mechanism catalyzed by excess NIS. A glycosylation reaction performed with only 1 equiv. of NIS, showed that a noticeable amount of isomerization still occurred, albeit to a lesser extent. We conclude that oligosaccharide thioglycoside donors that also contain 4-thio-Gal*f* units are likely to be of only limited use in NIS/TfOH-promoted glycosylation reactions.

The absence of this isomerization in the glycosylation reaction with the selenoglycoside donor **5.5** to give disaccharide **5.7**, and the much smaller extent of isomerization observed during the formation of the tetrasaccharide **5.13,** is a reflection of the almost instantaneous glycosylation reactions with the selenoglycoside donor. This results in exposure of the reactants and products to the isomerizing NIS/TfOH reagent for much less time. The selectivity for reaction of NIS/TfOH at the selenium atom of **5.5** over reaction with the sulfur atom of the Gal*f* ring or with the sulfur atom of ethylthioglycoside acceptors such as **5.12** appears to be excellent. Acceptable α/β ratios in the products, even for such challenging reactions as the double-glycosylation of **5.12** to give tetrasaccharide **5.13**, are therefore attainable.

In summary, di-, tri- and tetra-saccharide heteroanalogues **5.1-5.3** corresponding to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi,* the causative agent of Chagas disease, have been synthesized by selective activation of selenoglycoside donors in the presence of thioglycoside acceptors. The selenoglycoside **5.5** is a versatile furanosyl donor that gives oligosaccharides with β-selectivity. The three target compounds **5.1-5.3** will be tested as inhibitors against *T. cruzi* proliferation and also in the inhibition of proliferation of B-lymphocytes.

5.5 Experimental

5.5.1 General Methods

Optical rotations were measured at 21°C with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. Chemical shifts are given in ppm downfield from TMS for those measured in $CDCl_3$ or CD_2Cl_2 and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional $^{1}H/^{1}H$ (COSYDFTP), $^{1}H/^{13}C$ (INVBTP), ^{1}H (NOESYTP), and ^{1}H (MLEVTP) experiments using standard Bruker pulse programs and an inverse detection, $^{1}H/X$ double-resonance probe. Sugar rings are denoted A, B, C, and D, respectively, as shown in the diagrams for compounds **5.1-5.3.** High resolution liquid secondary ion mass spectra (FAB) were recorded on a Kratos Concept H instrument using glycerine-thioglycerine as the matrix. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated. Compounds were purified by flash column chromatography on Kieselgel 60 (230-400

mesh). Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40°C.

5.5.2 Synthesis

Phenyl 2,3,5,6-tetra-*O*-acetyl-1-seleno-4-thio-α/β-D-galactofuranoside (5.5). To a solution of 50% H_3PO_2 (50 mL) was added diphenyldiselenide (1.0 g, 3.2 mmol) and the mixture was rapidly stirred at reflux, under an N_2 atmosphere until the yellow color disappeared. The reaction mixture was cooled to 0°C and extracted with CH_2Cl_2 (2 x 30 mL). The combined extracts were washed with ice cold water (20 mL) and dried over $MgSO_4$. The solution was filtered into a round bottom flask and cooled to 0°C. Peracetylated 4-thiogalactofuranose **5.4** (1.29 g, 3.17 mmol) and $Et_2O{:}BF_3$ (0.60 mL, 4.7 mmol) were added. The reaction mixture was stirred at 0°C for 40 min. The mixture was quenched with cold satd. $NaHCO_{3(aq)}$. and extracted with CH_2Cl_2 (2 x 30 mL). The combined extracts were washed with additional satd. $NaHCO_{3(aq)}$ and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by column chromatography using hexanes:EtOAc (2:1) as the eluant. The desired monosaccharide donor **5.5** was obtained as a colorless syrup, (1.5 g, 94 %, α:β=1:3).

5.5α. ^{1}H NMR ($CDCl_3$): δ 7.65-7.30 (m, 5 H, aromatic), 5.64 (dd, 1 H, $J_{2,3}$ 7.3, $J_{3,4}$ 6.3 Hz, H-3), 5.38 (dd, 1 H, $J_{1,2}$ 5.2 Hz, H-2), 5.24 (m, 1 H, H-5), 4.96 (d, 1 H, H-1), 4.36 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 12.1 Hz, H-6), 4.06 (dd, 1 H, $J_{5,6'}$ 5.7 Hz, H-6'), 3.52 (dd, 1 H, $J_{4,5}$ 6.4 Hz, H-4), 2.16, 2.06, 2.05, 1.85 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.33-169.54 (4 C, 4 x *C*(O)CH_3), 135.74-127.69 (6 C, aromatic), 78.15 (C-2), 74.94

(C-3), 70.41 (C-5), 63.43 (C-6), 47.56 (C-4), 45.56 (C-1, J_{C1H1} 163 Hz), 20.86-20.32 (4 C, 4 x C(O)CH_3).

5.5β. ^{1}H NMR ($CDCl_3$): δ 7.65-7.30 (m, 5 H, aromatic), 5.46 (dd, 1 H, d$J_{3,4}$ 6.4 Hz, H-3), 5.26 (dd, 1 H, $J_{2,3}$ 6.6 Hz, H-2), 5.23 (m, 1 H, H-5), 4.59 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.27 (dd, 1 H, $J_{5,6}$ 3.9, $J_{6,6'}$ 12.1 Hz, H-6), 4.04 (dd, 1 H, $J_{5,6'}$ 5.9 Hz, H-6'), 3.66 (dd, 1 H, $J_{4,5}$ 6.7 Hz, H-4), 2.10, 2.047, 2.045, 1.99 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.33-169.54 (4 C, 4 x C(O)CH_3), 135.74-127.69 (6 C, aromatic), 81.31 (C-3), 76.45 (C-2), 69.80 (C-5), 63.43 (C-6), 49.01 (C-4), 45.14 (C-1, J_{C1H1} 160 Hz), 20.86-20.32 (4 C, 4 x C(O)CH_3). Anal. Calcd. for $C_{20}H_{24}O_8SeS$: C, 47.72; H, 4.81. Found: C, 47.99; H, 4.81 (for the α/β mixture).

General Procedure for Glycosylation Reactions. A mixture of the glycosyl donor, the acceptor, and activated 4Å molecular sieves was stirred in dry CH_2Cl_2 (25 mM-30 mM of acceptor) at room temperature under an N_2 atmosphere. The reaction mixture was cooled in an ice bath and NIS (1.2-1.3 eq. relative to the donor) was added, followed by addition of TfOH (0.05 eq). The reaction mixture was stirred at 0°C, under an N_2 atmosphere, until TLC showed that the reaction was complete. The mixture was quenched with Et_3N, diluted with CH_2Cl_2 and filtered through a pad of Celite. The mixture was washed with 10% $Na_2S_2O_3$, followed by satd. $NaHCO_{3(aq)}$. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography.

Ethyl 3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (5.7). The thioglycoside acceptor **5.6** (99 mg, 0.24 mmol) was glycosylated with the selenoglycoside donor **5.5** (143 mg, 0.28 mmol) following the general procedure. The reaction time was 5 min. at 0°C. The disaccharide was purified by column chromatography using hexanes:EtOAc (2:1) as the eluant. The desired disaccharide **5.7** was obtained as a white foam, (143 mg, 80 %). $[\alpha]_D^{22}$ -69° (*c* 0.18, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 8.13-7.32 (m, 10 H, aromatic), 5.66 (s, 1 H, C*H*Ph), 5.58 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.4 Hz, H-2B), 5.41 (d, 1 H, H-1B), 5.22 (d, 1 H, $J_{1,2}$ 2.6 Hz, H-1A), 5.19 (dd, 1 H, $J_{2,3}$ 4.6 Hz, H-2A), 5.17 (ddd, 1 H, $J_{5,6}$ 3.8 Hz, H-5A), 5.11 (dd, 1 H, $J_{3,4}$ 7.4 Hz, H-3A), 4.33 (ddd, 1 H, H-5B), 4.28 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3B), 4.27 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 10.0 Hz, H-6B), 4.17 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4B), 4.05 (dd, 1 H, $J_{6,6'}$ 12.0 Hz, H-6A), 3.91 (dd, 1 H, $J_{5,6'}$ 9.8 Hz, H-6B'), 3.89 (dd, 1 H, $J_{5,6'}$ 6.5 Hz, H-6A'), 3.75 (dd, 1 H, $J_{4,5}$ 5.4 Hz, H-4A), 2.76-2.60 (m, 2 H, SCH_2CH$_3$), 2.04, 1.95, 1.94, 1.84 (4 s, 3 H each, C(O)CH_3), 1.31 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.54, 170.17, 170.11, 169.62 (*C*(O)CH$_3$), 165.77 (*C*(O)Ph), 137.99-126.60 (12 C, aromatic), 102.13 (*C*HPh), 87.58 (C-1A, J_{C1H1} 167 Hz), 83.80 (C-1B, J_{C1H1} 168 Hz), 82.90 (C-2A), 78.33 (C-4B), 77.50 (C-3A), 74.14 (C-3B), 71.75 (C-2B), 69.49 (C-5A), 68.99 (C-6B), 64.96 (C-5B), 64.07 (C-6A), 50.0 (C-4A), 26.01 (S*C*H$_2$CH$_3$), 20.89, 20.84, 20.77, 20.67 (C(O)*C*H$_3$), 15.11 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{36}H_{42}O_{14}S_2$: C, 56.68; H, 5.55. Found: C, 56.44; H, 5.55.

Ethyl 3-*O*-(4-thio-β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (5.1). To a solution of the disaccharide **5.7** (40 mg, 0.052 mmol) in freshly distilled MeOH (5

mL) was added 1M NaOMe/MeOH (1 mL). The reaction mixture was stirred under an N_2 atmosphere for 2.5 h and then neutralized by the addition of Rexyn 101 (H^+) ion exchange resin. The resin was removed by filtration and the filtrate was concentrated in vacuo. The crude product was dissolved in 80% AcOH(aq) and stirred overnight at room temperature. The reaction mixture was concentrated and co-concentrated with distilled water to remove traces of AcOH. The crude product was purified by column chromatography using $CHCl_3$:MeOH (3:1) as the eluant. The disaccharide **5.1** was obtained as a clear syrup, (15 mg, 71 %). $[\alpha]_D^{22}$ -34° (*c* 0.029, H_2O); ^{1}H NMR (D_2O): δ 5.31 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1B), 5.13 (d, 1 H, $J_{1,2}$ 5.5 Hz, H-1A), 4.20 (dd, 1 H, $J_{2,3}$ ~3.0 Hz, H-2B), 4.06 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2A), 3.99 (m, 1 H, H-5B), 3.92 (m, 1 H, H-5A), 3.90 (dd, 1 H, H-3A), 3.85 (dd, 1 H, $J_{5,6}$ 2.2, $J_{6,6'}$ 12.4 Hz, H-6B), 3.74 (dd, 1 H, $J_{5,6'}$ 6.1 Hz, H-6B'), 3.70 (dd, 1 H, $J_{3,4}$ $J_{4,5}$ 9.4 Hz, H-4B), 3.67 (dd, 1 H, H-3B), 3.55 (dd, 1 H, $J_{5,6}$ 4.7, $J_{6,6'}$ 11.7 Hz, H-6A), 3.50 (dd, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 3.9 Hz, H-4A), 3.48 (dd, 1 H, $J_{5,6'}$ 7.0 Hz, H-6A'), 2.72-2.55 (m, 2 H, SCH_2CH_3), 1.23 (t, 3 H, *J* 7.3 Hz, SCH_2CH_3). ^{13}C NMR (D_2O): δ 88.46 (C-1A, J_{C1H1} 165 Hz), 86.66 (C-1B, J_{C1H1} 167 Hz), 84.29 (C-2A), 81.87 (C-3B), 77.77 (C-3A), 75.78 (C-5B), 72.53 (C-5A), 71.34 (C-2B), 68.17 (C-4B), 67.02 (C-6A), 63.46 (C-6B), 52.89 (C-4A), 27.50 (SCH_2CH_3), 16.00 (SCH_2CH_3). Anal. Calcd for $C_{14}H_{26}O_9S_2$: C, 41.78; H, 6.51. Found: C, 41.65; H, 6.63.

Methyl 2-*O*-(3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-α/β-D-galactofuranosyl)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (5.11). The methyl glycoside acceptor **5.8** (121 mg, 0.26 mmol) was glycosylated with the thioglycoside donor **5.7** (238 mg, 0.31 mmol) following the general

procedure. The reaction time was 2.5 h at 0°C. The trisaccharide **5.9** was obtained as a mixture of compounds (α:β 1.3:1). The mixture could not be purified by column chromatography.

5.9α. ^{1}H NMR (CD_2Cl_2): δ 8.20-7.10 (m, 25 H, aromatic), 5.67 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2B), 5.66-5.62 (m, 3 H, H-3A, H-1A, CHPh), 5.25 (ddd, 1 H, H-5A), 5.21 (d, 1 H, H-1B), 5.00 (dd, 1 H, $J_{1,2}$ 4.1, $J_{2,3}$ 9.5 Hz, H-2A), 4.89-4.53 (m, 6 H, 3 x CH_2Ph), 4.79 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1C), 4.34 (dd, 1 H, $J_{2,3}$ 3.7, $J_{3,4}$ 9.3 Hz, H-3B), 4.31 (dd, 1 H, $J_{5,6}$ 4.7, $J_{6,6'}$ 10.1 Hz, H-6B), 4.25 (dd, 1 H, $J_{5,6}$ 3.9, $J_{6,6'}$ 12.1 Hz, H-6A), 4.12-4.01 (m, 4 H, H-6A', H-2C, H-5B, H-4B), 3.93-3.84 (m, 3 H, H-4C, H-3C, H-6B'), 3.78-3.71 (m, 3 H, H-5C, H-6C, H-6C'), 3.50 (dd, 1 H, $J_{3,4}$ 7.4, $J_{4,5}$ 6.1 Hz, H-4A), 3.37 (s, 3 H, OCH_3), 2.0-1.46 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 172.52-169.74 (4 C, *C*(O)CH_3), 165.53 (*C*(O)Ph), 139.06-125.61 (30 C, aromatic), 102.00 (*C*HPh), 100.60 (C-1B), 100.48 (C-1C), 82.35 (C-1A), 80.24 (C-3C), 78.40 (C-2A), 78.00 (C-4B), 75.58 (C-2C), 75.38-75.18 (2 C, C-4C, *C*H_2Ph), 74.36 (C-3A), 73.84 (C-3B), 73.59 (*C*H_2Ph), 72.80 (*C*H_2Ph), 72.23 (C-5C), 71.14 (C-5A), 69.92 (C-6C), 69.47 (C-2B), 69.07 (C-6B), 64.07 (C-5B), 63.95 (C-6A), 55.01 (-O*C*H_3), 44.58 (C-4A), 21.51-20.05 (4 C, C(O)*C*H_3).

5.9β. ^{1}H NMR (CD_2Cl_2): δ 8.20-7.10 (m, 25 H, aromatic), 5.76 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.7 Hz, H-2B), 5.66-5.62 (s, 1 H, CHPh), 5.27 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1A), 5.22 (dd, 1 H, $J_{2,3}$ 4.8 Hz, H-2A), 5.20 (d, 1 H, H-1B), 5.17 (ddd, 1 H, $J_{5,6}$ 4.0, $J_{5,6'}$ 9.3, $J_{4,5}$ 5.2 Hz, H-5A), 5.12 (dd, 1 H, $J_{3,4}$ 7.5 Hz, H-3A), 4.89-4.53 (6 H, CH_2Ph), 4.79 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1C), 4.47 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3B), 4.31 (dd, 1 H, $J_{5,6}$ 4.7, $J_{6,6'}$ 10.1 Hz, H-6B), 4.15 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4B), 4.12-4.01 (m, 3 H, H-6A, H-2C, H-5B), 3.93-3.84 (m, 4 H, H-3C, H-4C, H-6A', H-6B'), 3.78-3.71 (m, 4 H, H-4A, H-6C, H-6C', H-5C), 3.37 (s,

3 H, OCH_3), 2.00-1.84 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.52-169.74 (4 C, *C*(O)CH_3), 165.46 (*C*(O)Ph), 139.06-125.61 (30 C, aromatic), 102.09 (*C*HPh), 100.60 (C-1B), 100.18 (C-1C), 87.64 (C-1A), 82.76 (C-2A), 80.41 (C-4B), 80.10 (C-3C), 77.43 (C-3A), 75.43 (C-2C), 75.38-75.18 (2 C, C4C, *C*H_2Ph), 73.56 (*C*H_2Ph), 72.71 (*C*H_2Ph), 72.16 (C-5C), 71.77, 71.73 (C-2B, C-3B), 69.69 (C-6C), 69.47 (C-5A), 69.03 (C-6B), 64.66 (C-5B), 63.32 (C-6A), 55.01 (-O*C*H_3), 49.70 (C-4A), 21.51-20.05 (4 C, C(O)*C*H_3).

In another experiment, the methyl glycoside acceptor **5.8** (121 mg, 0.26 mmol) was glycosylated with the thioglycoside donor **5.7** (238 mg, 0.31 mmol) following the general procedure, except that 1 equiv. of NIS was used. The reaction time was 2.5 h at 0°C-RT. The trisaccharide **5.9** was obtained as a mixture of compounds (α:β 0.47:1). The mixture could not be purified by column chromatography.

The mixture of trisaccharides **5.9α** and **5.9β** was dissolved in 80% AcOH(aq) and stirred overnight at room temperature. The reaction mixture was concentrated and co-concentrated with distilled toluene to remove traces of AcOH. Compounds **5.10α** and **5.10β** were obtained as a clear glass (α:β 1:1.3).

5.10α. ^{1}H NMR (CD_2Cl_2): δ 8.10-7.10 (m, 20 H, aromatic), 5.62 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 7.8 Hz, H-3A), 5.57 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2B), 5.49 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1A), 5.18 (m, 1 H, H1B), 5.11 (ddd, 1 H, H-5A), 5.07 (dd, 1 H, H-2A), 4.90-4.54 (6 H, CH_2Ph), 4.79 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.18 (dd, 1 H, $J_{5,6}$ 3.9, $J_{6,6'}$ 12.0 Hz, H-6A), 4.10-3.93 (m, 4 H, H-6A', H-2C, H-3B, H-4B*), 3.92-3.79 (m, 6 H, H-6C, H-6C', H-6B, H-6B', H-3C, H-4C*), 3.78-3.67 (m, 2 H, H5C*, H5B*), 3.52 (dd, 1 H, $J_{4,5}$ 5.9 Hz, H-4A), 3.34 (s, 3 H, OCH_3), 2.01-1.85 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2):

δ 171.18-170.13 (4 C, *C*(O)CH_3), 165.54 (*C*(O)Ph), 139.04-127.88 (24 C, aromatic), 100.04 (C-1C), 99.50 (C-1B), 82.34 (C-1A), 80.16 (C-3C), 78.40 (C-2A), 77.29 (C-2C), 75.44-75.21 (2 C, C-3B, *C*H_2Ph), 75.11-66.75 (4 C, C-4C, C-4B, C-5B, C-5C), 74.25 (C-3A), 73.52 (*C*H_2Ph), 72.87 (*C*H_2Ph), 72.03 (C-5A), 71.99 (C-2B) 63.09 (C-6A), 62.72, 62.68 (C-6B, C-6C), 55.05 (-O*C*H_3), 44.59 (C-4A), 21.15-20.75 (4 C, C(O)*C*H_3). *assignments may be interchanged

5.10β. ^{1}H NMR (CD_2Cl_2): δ 8.10-7.10 (m, 20 H, aromatic), 5.55 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.1 Hz, H-2B), 5.33 (d, 1 H, H-1A), 5.28 (dd, 1 H, $J_{1,2}$ 2.7, $J_{2,3}$ 4.8 Hz, H-2A), 5.25-5.19 (m, 2 H, H-3A, H-5A), 5.18 (m, 1 H, H-1B), 4.90-4.54 (m, 6 H, 3 x CH_2Ph), 4.81 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.24 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 12.1 Hz, H-6A), 4.10-3.93 (m, 4 H, H-6A', H-3B, H-2C, H-4B*), 3.92-3.79 (m, 7 H, H-3C, H-4C*, H-4A, H-6B, H-6B', H-6C, H-6C'), 3.78-3.67 (m, 2 H, H-5C*, H-5B*), 3.35 (s, 3 H, OC*H*$_3$), 2.01-1.85 (4 s, 3 H each, C(O)C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 171.18-170.13 (4 C, *C*(O)CH_3), 165.54 (*C*(O)Ph), 139.04-127.88 (24 C, aromatic), 100.04 (C-1C), 99.80 (C-1B), 87.33 (C-1A), 82.18 (C-2A), 80.16 (C-3C), 78.54 (C-2C), 76.95 (C-3A), 75.44-75.21 (2 C, C-3B, *C*H_2Ph), 75.11-66.75 (4 C, C-4C, C-4B, C-5B, C-5C), 73.52 (*C*H_2Ph), 72.64 (*C*H_2Ph), 70.83 (C-5A), 69.77 (C-2B), 63.92 (C-6A), 62.72, 62.68 (C-6B, C-6C), 55.05 (-O*C*H_3), 50.45 (C-4A), 21.15-20.75 (4 C, C(O)*C*H_3). *assignments may be interchanged

In subsequent experiments the mixture of compounds was dissolved in 4:1 HOAc:H_2O (10 mL) and stirred with Pd-C (100 mg) under H_2 (52 psi). After 20 h the reaction mixture was filtered through a pad of Celite, which was then washed with water. The combined filtrates were evaporated to dryness and the residue was co-evaporated several times with distilled H_2O to remove any traces of AcOH. The mixture was

acetylated using acetic anhydride (5 mL) and pyridine (10 mL). After 15 h the solvent was removed by rotary evaporation under high vacuum. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with H_2O, dried over Na_2SO_4 and the solvent was removed in vacuo. The resulting crude product was purified by column chromatography using hexanes:EtOAc (1:1.5) as the eluant. The ^{1}H NMR spectrum showed a 2:3 ratio of an α:β mixture of the desired trisaccharide **5.11β** and its isomer **5.11α**. The products (**5.11α/β**) were obtained as a white foam, (160 mg, 60 % α:β 2:3).

5.11α. ^{1}H NMR ($CDCl_3$): δ 8.10-7.42 (m, 5 H, aromatic), 5.69 (dd, 1 H, dd, $J_{2,3}$ 9.8, $J_{3,4}$ 8.2 Hz, H-3A), 5.55 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.0 Hz, H-2B), 5.41 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.8 Hz, H-4B), 5.36 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1A), 5.33-5.27 (m, 3 H, H-3C, H-4C, H-5A), 5.07 (d, 1 H, H-1B), 4.92 (dd, 1 H, H-2A), 4.87 (d, 1 H, H-1C), 4.28-4.04 (m, 10 H, H-2C, H-3B, H-5B, H-5C, H-6A, H-6A', H-6B, H-6B', H-6C, H-6C'), 3.58 (dd, 1 H, $J_{4,5}$ 6.6 Hz, H-4A), 3.40 (s, 3 H, OCH_3), 2.14, 2.12, 2.10, 2.08, 2.03, 2.02, 2.01, 2.00, 1.88 (9 s, 3 H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.59-169.39 (9 C, *C*(O)CH_3), 165.33 (*C*(O)Ph), 133.53-128.41 (6 C, aromatic), 99.56, 99.32 (C-1B, C-1C, both J_{C1H1} ~172 Hz), 81.59 (C-1A, J_{C1H1} 165 Hz), 77.72 (C-2A), 77.24 (C-2C), 73.08 (2 C, C-3A, C-3B), 71.51 (C-2B), 70.49, 70.12 (C-3C, C-5A), 69.39, (C-5B), 68.41 (C-5C), 67.92 (C-4B), 66.21 (C-4C), 62.72, 62.57 (3 C, C-6A, C-6B, C-6C), 55.21 (-O*C*H_3), 43.69 (C-4A), 20.96-20.27 (9 C, C(O)*C*H_3).

5.11β. ^{1}H NMR ($CDCl_3$): δ 8.10-7.42 (m, 5 H, aromatic), 5.50 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.2 Hz, H-2B), 5.36 (dd, 1 H, *J* 8.4, *J* 9.8 Hz, H-4B), 5.33-5.27 (m, 2 H, H-3C, H-4C), 5.24 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1A), 5.22-5.19 (m, 2 H, H-2A, H-5A), 5.18 (dd, 1 H, $J_{2,3}$ 4.6, $J_{3,4}$ 6.6 Hz, H-3A), 5.03 (d, 1 H, H-1B), 4.87 (dd, 1 H, H-1C), 4.30 (dd, 1 H, $J_{5,6}$ 3.8,

$J_{6,6'}$ 12.1 Hz, H-6A), 4.28-4.04 (m, 6 H, H-2C, H-3B, H-6B, H-6B', H-6C, H-6C'), 4.02 (dd, 1 H, $J_{5,6}$ 7.2 Hz, H-6A'), 3.96-3.90 (m, 2 H, H-5B, H-5C), 3.74 (dd, 1 H, $J_{4,5}$ 6.4 Hz, H-4A), 3.41 (s, 3H, OCH_3), 2.15, 2.13, 2.11, 2.10, 2.09, 2.04, 2.03, 1.99, 1.91 (9 s, 3 H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.59-169.39 (9 C, *C*(O)CH_3), 165.33 (*C*(O)Ph), 133.53-128.41 (6 C, aromatic), 99.56, 99.32 (C-1B, C-1C, both J_{C1H1} ~172 Hz), 87.51 (C-1A, J_{C1H1} 167 Hz), 82.45 (C-2A), 77.24 (C-2C), 76.89 (C-3A), 74.48 (C-3B), 70.20 (C-3C), 69.59, 69.30 (C-5A, C-5C), 68.41 (C-5B), 68.20 (C-2B), 66.61, 66.28, (C-4B, C-4C), 63.55 (C-6A), 62.72, 62.57 (C-6B, C-6C), 55.21 (-O*C*H_3), 49.92 (C-4A), 20.96-20.27 (9 C, C(O)*C*H_3). Anal. Calcd for $C_{44}H_{56}O_{25}S$: C, 51.95; H, 5.55. Found: C, 51.82; H, 5.50.

Methyl 2-*O*-(3-*O*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (5.2). To a solution of the trisaccharides **5.11α/β** (29 mg, 0.028 mmol) in freshly distilled MeOH (3 mL) was added 1M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred overnight, under an N_2 atmosphere. The solution was neutralized with Rexyn 101 (H^+), the resin was filtered and the solvent was removed in vacuo. The crude product was purified by column chromatography using $CHCl_3$:MeOH (1:1) as the eluant. The desired oligosaccharide **5.2β** and its α anomer were obtained as a clear syrup, (14 mg, 94 % α:β 1:1.6).

5.2α. ^{1}H NMR (D_2O): δ 5.20 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1A), 5.02 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.96 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1C), 4.39 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2B), 4.21 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 8.2 Hz, H-3A), 4.08 (dd, 1 H, H-2A), 3.97-3.81 (m, 6H, H-2C, H-5A, H-6C, H-6C', H-3B, H-3C), 3.80-3.61 (m, 5 H, H-4B, H-6B, H-6B', H-4C, H-5C), 3.60-

3.46 (m, 3 H, H-5B, H-6A, H-6A'), 3.37 (s, 3 H, -OCH_3), 3.23 (dd, 1 H, $J_{4,5}$ 3.8 Hz, H-4A). ^{13}C NMR (D_2O): δ 104.50 (C-1B), 101.95 (C-1C), 86.81 (C-1A), 81.67 (C-3B), 81.07 (2 C, C-2A, C-2C), 77.81 (C-3A), 75.73 (C-4B), 75.22 (C-5B), 73.44 (C-5A), 72.82 (C-3C), 71.29 (C-2B), 69.57 (C-4C), 68.81 (C-5C), 66.85 (C-6A), 63.69, 63.55 (C-6B, C-6C), 57.49 (-O*C*H_3), 51.05 (C-4A).

5.2β. ^1H NMR (D_2O): δ 5.18 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1A), 5.03 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.95 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1C), 4.24 (dd, 1 H, $J_{2,3}$ 2.4 Hz, H-2B), 4.08 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2A), 3.97-3.81 (m, 6 H, H-2C, H-5A, H-3A, H-6C, H-6C', H-3C) 3.80-3.61 (m, 6 H, H-3B, H-4B, H-6B, H-6B', H-4C, H-5C), 3.60-3.46 (m, 4 H, H-5B, H-6A, H-4A, H-6A'), 3.37 (s, 3 H, -OC*H*$_3$). ^{13}C NMR (D_2O): δ 104.64 (C-1B), 101.95 (C-1C), 88.55 (C-1A), 84.23 (C-2A), 81.49 (C-3B), 81.07 (C-2C), 77.58 (C-3A), 75.95 (C-4B), 75.22 (C-5B), 72.82 (C-3C), 72.50 (C-5A), 69.57 (C-4C) 69.53 (C-2B), 67.94 (C-5C), 67.01 (C-6A), 63.69, 63.55 (C-6B, C-6C), 57.49 (O*C*H_3), 52.67 (C-4A). FAB HRMS: Calcd for $C_{19}H_{34}O_{15}S$ + Na: 557.1516. Found: M + Na: 557.1521.

Ethyl 2-*O*-(3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranosyl)-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (5.13). The thioglycoside acceptor **5.12** (76 mg, 0.116 mmol) was glycosylated with the selenoglycoside donor **5.5** (140 mg, 0.279 mmol) following the general procedure. The reaction time was 10 min at 0°C. The crude product was purified by column chromatography using hexanes:EtOAc (1:1) as the eluant. The desired tetrasaccharide **5.13**, accompanied by 20% of an isomeric tetrasaccharide, was obtained as a white foam,

(145 mg, 74% based on **5.13**). ^{1}H NMR (CD_2Cl_2): δ 7.58-7.29 (m, 15 H, aromatic), 5.63 (s, 1 H, C*H*Ph), 5.62 (s, 1 H, C*H*Ph), 5.39 (dd, 1 H, $J_{1,2}$ 2.4, $J_{2,3}$ 4.0 Hz, H-2A), 5.36 (bs, 1 H, H-1C), 5.30 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 5.29 (d, 1 H, 1A), 5.25 (dd, 1 H, $J_{1,2}$ 2.6, $J_{2,3}$ 4.3 Hz, H-2D), 5.25-5.16 (m, 5 H, H-3A, H-3D, H-5A, H-5D, H-1D), 4.85, 4.74 (2 d, 2 H, J_{gem} 11.2 Hz, OCH_2Ph), 4.26-4.19 (m, 4 H, H-3B, H-5B or H-5C, H-6B, H-6C), 4.18-4.10 (m, 4 H, H-2C, H-3C, H-4C, H-4B), 4.10-4.01 (m, 3 H, H-2B, H-6A, H-6D), 3.96-3.80 (m, 6 H, H-5B or H-5C, H-6A', H-6B', H-6C', H-6D', H-4A), 3.77 (dd, 1 H, $J_{3,4}$ 6.8, $J_{4,5}$ 5.9 Hz, H-4D), 2.67 (m, 2 H, SCH_2CH$_3$), 2.07, 2.06 (2 s, 3 H each, C(O)CH_3), 2.03 (s, 6 H, C(O)CH_3), 1.95 (s, 6 H, C(O)CH_3), 1.94, 1.93 (2 s, 3 H each, C(O)CH_3), 1.32 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.55-169.95 (8 C, *C*(O)CH$_3$), 138.8-126.4 (18 C, aromatic), 101.96 (*C*HPhB), 101.88 (*C*HPhC), 101.58 (C-1B, J_{C1H1} 175 Hz), 88.67, 88.49 (C1A, J_{C1H1}=167 Hz, C1D, J_{C1H1}=167 Hz), 85.15 (C1C, J_{C1H1} ~166Hz), 83.77 (C-2A), 83.60 (C-2D), 78.37, 78.12, 78.02, 77.87, 77.41 (C-2C, C-3A, C-3D, C-4B, C-4C), 76.82 (C-2B), 76.12 (C-3C), 75.92 (C-3B), 74.33 (*C*H$_2$Ph), 69.91, 69.84 (C-5A, C-5D), 68.95, 68.86 (C-6B, C-6C), 65.25, 64.98 (C-5B, C-5C), 64.11, 63.94 (C-6A, C-6D), 50.49 (C-4A), 50.45 (C-4D), 25.98 (S*C*H$_2$CH$_3$), 21.16, 21.07, 20.99, 20.88, 20.84, 20.76, 20.75, (8 C, C(O)*C*H$_3$), 15.20 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{63}H_{76}O_{26}S_3$: C, 56.23; H, 5.70. Found: C, 56.12; H, 5.76.

Ethyl 2-*O*-(3-*O*-(4-thio-β-D-galactofuranosyl)-α-D-mannopyranosyl)-3-*O*-(4-thio-β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (5.3). To a solution of the tetrasaccharide **5.13** (140 mg, 0.10 mmol) in freshly distilled MeOH (10 mL) was added 1M NaOMe/MeOH (1 mL). The reaction mixture was stirred at room temperature,

overnight, under an N_2 atmosphere. The reaction mixture was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The partially deprotected tetrasaccharide was dissolved in 80% AcOH (8 mL) and stirred overnight at room temperature. The solvent was removed in vacuo and the residue was co-concentrated with H_2O several times to remove the AcOH. The compound was then dissolved in 4:2:1 H_2O:EtOH:AcOH (10 mL) and stirred with $Pd(OH)_2$-C (200 mg) under H_2 (52 psi). After 24 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with EtOH. The combined filtrates were evaporated to dryness and the hydrogenolysis reaction was repeated twice more, using the same quantities of solvent and Pd. The residue was co-evaporated several times with H_2O to remove any traces of AcOH. The crude product was purified by column chromatography using EtOAc:MeOH:H_2O (6:2:1) as the eluant. The tetrasaccharide **5.3** (80% pure) was obtained as a clear glass, (20 mg, 25%). ^{1}H NMR (D_2O): δ 5.52 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1C), 5.18 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1A), 5.13 (d, 1 H, $J_{1,2}$ 5.9 Hz, H-1D), 5.08 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 4.22 (m, 1 H, H2C), 4.20 (dd, 1 H, $J_{2,3}$ 2.6 Hz, H-2B), 4.05 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2A), 3.99 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2D), 3.96-3.65 (m, 13 H, H-5C, H-5A, H-5D, H-3A, H-3D, H-6B, H-6C, H-5B, H-6C', H-4C, H-3C, H-3B, H-6B'), 3.62 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz H-4B), 3.52 (dd, 2 H, $J_{5,6A}$, $J_{5,6D}$, 4.6, $J_{6,6'A}$, $J_{6,6'D}$, 11.8 Hz, H-6A, H-6D), 3.49 (dd, 2 H, $J_{3,4A}$, $J_{3,4D}$, 8.9, $J_{4,5A}$, $J_{4,5D}$ 3.4 Hz, H-4A, H-4D), 3.47 (dd, 2 H, $J_{5,6'A}$, $J_{5,6'D}$ 7.0 Hz, H-6A', H-6D'), 2.63 (m, 2 H, SCH_2CH_3), 1.22 (t, 3 H, J 7.0 Hz, SCH_2CH_3). ^{13}C NMR (D_2O): δ 104.39 (C-1B, J_{C1H1} ~174 Hz), 88.43 (C-1A, J_{C1H1} 164 Hz), 88.26 (C-1D, J_{C1H1} 164 Hz), 85.46 (C-1C, J_{C1H1} 167 Hz), 84.31 (C-2D), 84.21 (C-2A), 81.89, 81.35 (C-3B, C-3C), 79.88 (C-2C), 77.63, 77.50 (C-3A, C-3D), 76.12 (C-5B), 75.84 (C-5C), 72.48

(2 C, C-5A, C-5D), 69.50 (C-2B), 68.42 (C-4C), 67.99 (C-4B), 66.98 (2 C, C-6A, C-6D), 63.69 (C-6B), 63.42 (C-6C), 52.69, 52.69 (C-4A, C-4D), 27.79 (SCH_2CH_3), 16.89 (SCH_2CH_3). FAB HRMS: Calcd for $C_{26}H_{46}O_{18}S_3$ + Na: 765.1744. Found: M + Na: 765.1746.

3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-α/β-D-galactofuranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranose (5.14). The thioglycoside donor **5.7** (60 mg, 0.079 mmol) was reacted with NIS (21.2 mg, 0.094 mmol) and TfOH (0.4 µl, 0.039 mmol). The reaction time was 45 min. at 0°C. The disaccharide was purified by column chromatography using toluene:EtOAc (1.5:1) as the eluant. The desired disaccharide **5.14α/5.14β** was obtained as a clear glass, (32 mg, 58 %)

5.14α: ^{1}H NMR (CD_2Cl_2): δ 8.18-7.09 (m, 10 H, aromatic), 5.70 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.6 Hz, H-2B), 5.65 (dd, 1 H, H-3A), 5.63 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1A), 5.61 (s, 1 H, C*H*Ph), 5.32 (dd, 1 H, H-1B), 5.29 (ddd, 1 H, H-5A), 5.02 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2A), 4.59 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3B), 4.32 (dd, 1 H, $J_{5,6}$ 3.4 Hz, H-6B), 4.29 (dd, 1 H, d H-6A), 4.22-4.14 (m, 2 H, H-5B, H-6A'), 4.13 (dd, 1 H, $J_{4,5}$ 8.7 Hz, H-4B), 3.87 (dd, 1 H, $J_{5,6'}$, $J_{6,6'}$ 10.0 Hz, H-6B'), 3.51 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 7.4 Hz, H-4A), 3.48 (d, 1 H, $J_{1,OH}$ 3.6 Hz, OH), 2.08, 2.03, 2.00, 1.45 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.66-171.17 (4 C, *C*(O)CH_3), 139.33-127.96 (12 C, aromatic), 103.37 (*C*HPh), 95.40 (C-1B), 84.64 (C-1A), 82.03 (C-4B), 80.73 (C-2A), 76.40 (C-2B), 74.08 (C-5A), 73.73 (C-3A), 72.59 (C-3B), 70.70 (C-6B), 65.82-65.19 (C-5B), 64.64 (C-6A), 46.43 (C-4A), 22.51-21.55 (4 C, C(O)*C*H_3).

5.14β: ^{1}H NMR (CD_2Cl_2): δ 8.13-7.32 (m, 10 H, aromatic), 5.64 (s, 1 H, C*H*Ph), 5.54 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.5 Hz, H-2B), 5.32 (dd, 1 H, H-1B), 5.26 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1A), 5.22 (dd, 1 H, $J_{2,3}$ 4.8 Hz, H-2A), 5.18 (ddd, 1 H, H-5A), 5.12 (dd, 1 H, $J_{3,4}$ 7.6 Hz, H-3A), 4.37 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3B), 4.28 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 10.0 Hz, H-6B), 4.22-4.13 (m, 1 H, H-5B), 4.12 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4B), 4.05 (dd, 1 H, $J_{5,6}$ 3.8, $J_{6,6'}$ 12.0 Hz, H-6A), 3.90 (dd, 1 H, $J_{5,6'}$ 6.5 Hz, H-6A'), 3.87 (dd, 1 H, $J_{5,6'}$ 9.8 Hz, H-6B'), 3.77 (dd, 1 H, $J_{4,5}$ 5.4 Hz, H-4A), 3.13 (d, 1 H, $J_{1,OH}$ 3.9 Hz, OH), 2.04, 1.95, 1.93, 1.85 (4 s, 3 H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.66-171.17 (4 C, *C*(O)CH_3), 139.33-127.96 (12 C, aromatic), 103.56 (*C*HPh), 95.40 (C-1B), 88.95 (C-1A), 84.31 (C-2A), 79.54 (C-4B), 78.95 (C-3A), 74.93 (C-3B), 71.53 (C-2B), 70.90 (C-5A), 70.75 (C-6B), 65.82-65.19 (C-5B, C-6A), 51.30 (C-4A), 22.51-21.55 (4 C, C(O)*C*H_3).

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CHAPTER 6: NOVEL 4-THIOGALACTOFURANOSYL-CONTAINING DISACCHARIDES WITH NITROGEN IN THE INTERGLYCOSIDIC LINKAGE

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6.1 Graphical Abstract

Novel 4-Thiogalactofuranosyl-Containing Disaccharides with Nitrogen in the Interglycosidic Linkage

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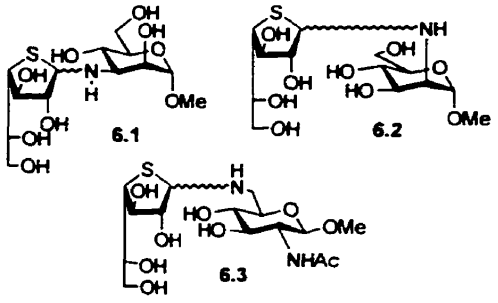


6.2 Abstract

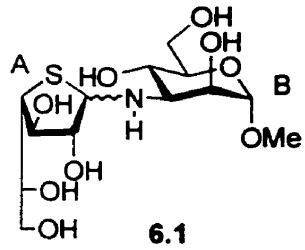
The syntheses of three novel disaccharides containing a 4-thiogalactofuranosyl residue as the non-reducing unit and a nitrogen in the interglycosidic linkage are described. Acid-catalyzed condensation reactions of 4-thio-α/β-D-galactofuranose with either methyl 3-amino-3-deoxy-α-D-mannopyranoside, methyl 2-amino-2-deoxy-α-D-mannopyranoside, or methyl 2-acetamido-6-amino-2,6-dideoxy-β-D-glucopyranoside gave methyl 3-amino-3-deoxy-3-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (**6.1**), methyl 2-amino-2-deoxy-2-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (**6.2**), or methyl 2-acetamido-6-amino-2,6-dideoxy-6-*N*-(4-thio-α/β-D-galactofuranosyl)-β-D-glucopyranoside (**6.3**).

Keywords: disaccharide; heteroanalogues; 4-thiogalactofuranose; *S, N*-acetals

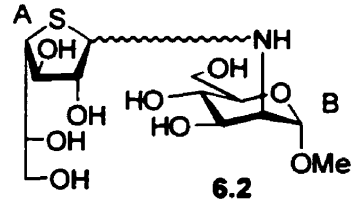
6.3 Introduction

The synthesis, conformational analysis, and enzyme inhibitory activity of disaccharide analogues containing sulfur in the ring and nitrogen in the interglycosidic linkage has been a focus of our laboratory. [1,2,3,4] Thus far, our efforts have targeted disaccharides containing 5-thiohexopyranosides as the non-reducing sugar. We now report the synthesis of the first examples of a new class of disaccharide (**6.1**, **6.2**, and **6.3**) containing 4-thiogalactofuranose (4-thio-Gal*f*) as the non-reducing sugar and nitrogen in the interglycosidic linkage.

The synthesis of oligosaccharides containing galactofuranose (Gal*f*) is of interest because Gal*f* is present as a constituent of the external cellular structures of protozoa,[5] bacteria,[6,7] and fungi.[8] For example, Gal*f* forms part of the oligosaccharide core of the glycosylinositolphospholipid (GIPL) from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[9] Gal*f* is found β-(1→3)-linked to a mannopyranosyl unit at the terminal end of the GIPL structure and as a branched unit, also β-(1→3)-linked to an internal mannopyranosyl residue.[10] Previous reports from our laboratory have described the synthesis of oligosaccharides containing β-D-Gal*f* and 4-thio-β-D-Gal*f*, with oxygen atoms in the interglycosidic linkages.[11,12,13] It is also of interest to synthesize heteroanalogues that might function as substrate mimics to inhibit the processing of native substrates by the enzymes, and we now report the synthesis of **6.1-6.3** as representatives of a potential new class of inhibitor.



6.1



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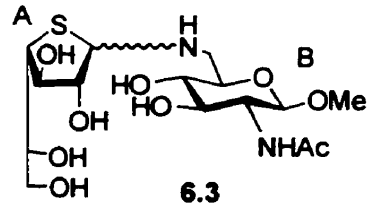
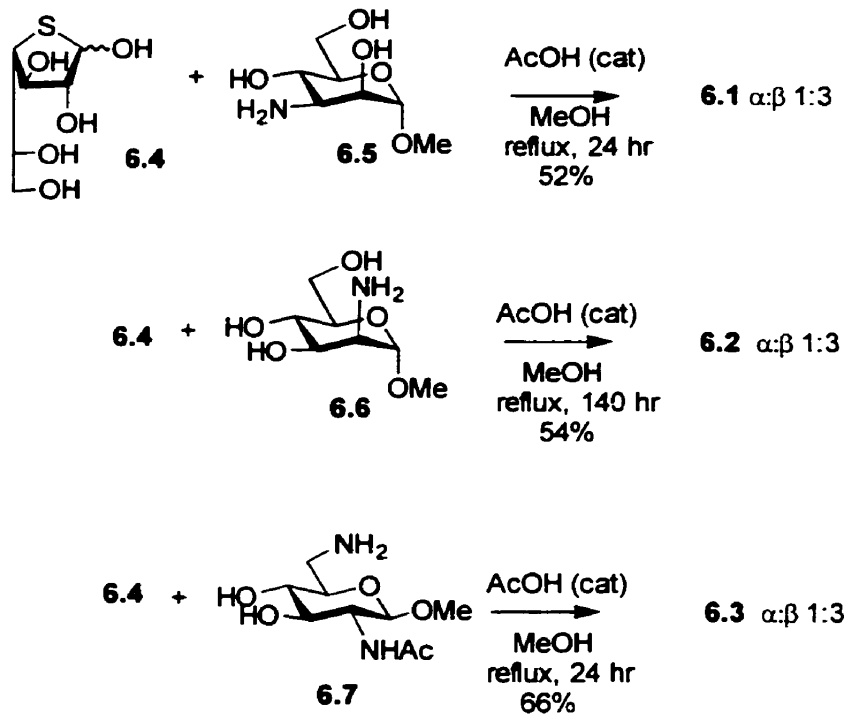


6.3

6.4 Results and Discussion

The required monosaccharide derivatives 4-thio-D-Gal*f* (**6.4**),[14] methyl 3-amino-3-deoxy-α-D-mannopyranoside (**6.5**),[15] methyl 2-amino-2-deoxy-α-D-mannopyranoside (**6.6**),[16] and methyl 2-acetamido-6-amino-2,6-dideoxy-β-D-glucopyranoside (**6.7**)[17] were synthesized by literature methods. Syntheses of the glycosylamines were performed using acetic acid as a catalyst in refluxing methanol, without the use of protecting groups on the monosaccharide units. Reactions of **6.4** with **6.5**, **6.6**, or **6.7** gave α:β mixtures of products **6.1**, **6.2**, and **6.3**, respectively (see Scheme 6.1). The isomers were not separable by column chromatography. The α:β mixtures were characterized by NMR spectroscopy using a COSY spectrum, together with a C-H correlation spectrum to fully assign the ^{1}H and ^{13}C NMR signals of the 4-thio-Gal*f* ring (A) and the mannopyranosyl ring (B).

Scheme 6.1



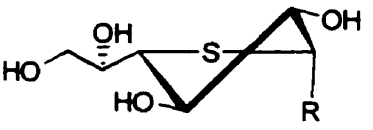
In an attempt to assign the anomeric configuration of the major and minor isomers in **6.1-6.3**, 2D NOESY spectra were obtained in D_2O. There was substantial overlapping of the ^1H NMR signals of the 4-thio-Gal*f* (A) rings in the spectra of **6.1-6.3** obtained in D_2O at 400 MHz, including two multiplets containing H-2A and H-3A of the major and minor isomers, respectively. The cross-peak between H-1A and H-3A, which is indicative of a β-linkage, was overlapped with the cross-peak between H-1A and H-2A. This latter cross-peak would be present in both the α- and β-isomers.

In the case of **6.1**, the 1D and 2D NOESY spectra were obtained in D_2O at 600 MHz in order to increase spectral dispersion. The ^1H NMR spectrum of **6.1** showed less overlap of the signals in the minor isomer, but unfortunately, the signals attributable to

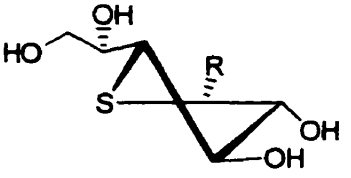
the major isomer were still not fully resolved. There did not appear to be a cross-peak between H-1A and H-3A in the spectrum of the minor isomer, but to be certain, another set of spectra for **6.1** was obtained using methanol-d_4 as a solvent. In this case, the multiplet containing H-2A and H-3A of the major isomer separated. The 2D NOESY spectra of **6.1** in methanol-d_4 showed the presence of only one H-1A and H-3A cross-peak, which corresponded to the signals of the major isomer. This enabled definitive assignment of the major isomer as the β-isomer. The absence of the H-1A-H-3A cross-peak in the spectra of the minor isomer in both D_2O and methanol-d_4 was consistent with the presence of an α-linkage between the 4-thio-Gal*f* ring (A) and the mannopyranosyl ring (B).

The coupling constants observed in the ^1H NMR spectra for the 4-thio-Gal*f* residues are of interest. The *J* values in D_2O [$J_{1,2}$ (5.3 Hz), $J_{2,3}$ (8.7 Hz) and $J_{3,4}$ (8.7 Hz)] for the 4-thio-Gal*f* residue in **6.1α** suggest that the ring is in a $^2T_3$ (D) conformation (see Scheme 6.2).[18] In this conformation, the anomeric linkage from 4-thio-Gal*f* (A) to Man*p* (B) is quasi-axially oriented and the other substituents are quasi-equatorial. The large *J* values indicate that H-2 and H-3 and also H-3 and H-4 must have dihedral angles near 180°. This was also observed by Varela *et al.*[14] for the case of 1,2,3,5,6-penta-*O*-acetyl-4-thio-α-D-galactofuranose.

Scheme 6.2

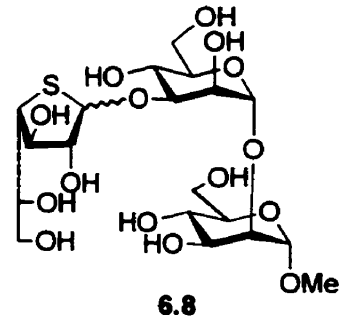


α-S-series $^{2}T_{3}$ (D)
6.1α



β-S-series $^{4}T_{3}$ (D)
6.1β, 6.8

The J values in methanol-d_4 [$J_{1,2}$ (7.2 Hz), $J_{2,3}$ (8.1 Hz) and $J_{3,4}$ (7.2 Hz)] for the 4-thio-Gal*f* residue in **6.1β** suggest that it exists as a mixture of conformations, including $^{4}T_{3}$ (D) (see Scheme 6.2).[13,18,19,20] In the case of methyl (4-thio-α/β-D-galactofuranosyl)-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside **6.8**,[13] the 4-thio-Gal*f* ring adopts a twist conformation causing $J_{1,2}$ for the β-isomer (5.8 Hz) to be larger than $J_{1,2}$ for the α-isomer (4.2 Hz). In furanosides, the $J_{1,2}$ values are usually indicative of α or β configuration,[21] where a *syn* orientation between H-1 and H-2 leads to a larger J value than an *anti* orientation between H-1 and H-2, but this was not found to be true for the 4-thio-Gal*f* compounds. The conformation of the 4-thio-Gal*f* ring in **6.1** of the minor isomer was judged to be very similar in methanol-d_4 to that observed in D_2O, so it was assumed that the conformation of the major isomer would be similar in D_2O or methanol-d_4. In support of this contention, the NOE contacts were similar in both solvents.



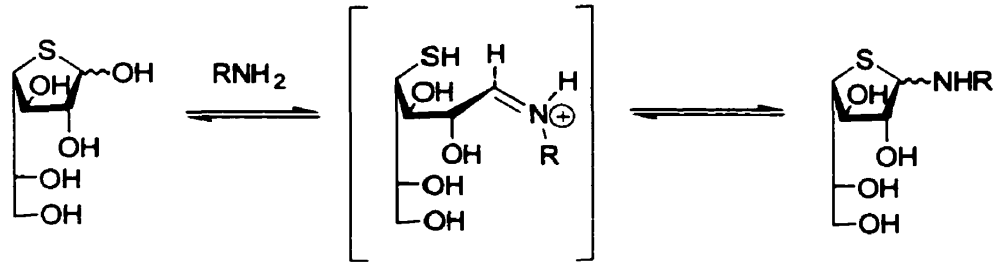
6.8

The α:β ratios (1:3) of **6.1-6.3** were found to be the same, so the major isomer was tentatively assigned as the β-isomer in **6.2** and **6.3**. This assignment was confirmed by several methods. 1) The $J_{1,2}$ values were consistently larger for the major isomer in **6.2** and **6.3**, as was the case with **6.1** and **6.8**. 2) ^{1}H NMR resonances observed for the 4-thio-Gal*f* H-4 protons in the minor isomers consistently showed an upfield chemical shift as compared to those of the major isomers. In the case of **6.1** and **6.8**,[13] we have shown that an α-linked 4-thio-Gal*f* moiety has a similar upfield chemical shift for H-4 compared to a β-linked 4-thio-Gal*f* moiety. Therefore, the major and minor isomers of **6.2** and **6.3** were assigned as β- and α-, respectively. The 2D NOESY spectra of **6.1, 6.2,** and **6.3** also showed the presence of cross-peaks between H-1A and H-3B, H-1A and H-2B, and H-1A and H-6B, respectively, across the interglycosidic linkages.

The occurrence of these compounds **6.1-6.3** as anomeric mixtures is not a serious concern since we expect that processing enzymes will bind one of the anomers preferentially, as was the case with glucoamylase binding of the α-anomer in an α:β equilibrium, mixture of a disaccharide analogue containing a 5-thiohexopyranosylamine.[1,4]

Since compounds **6.1-6.3** do not hydrolyze appreciably in aqueous solution, we propose that anomerization proceeds by endocyclic C-S bond cleavage of the sulfur-containing ring to give the intermediate iminium ions. Subsequent ring closure by nucleophilic attack of the thiol/thiolate on the opposite face of the iminium ion then occurs in preference to nucleophilic attack by water (see Scheme 3). This mechanism is in agreement with results from a recent study of the lifetime of an acyclic aliphatic iminium ion, $CF_3CH_2N^+$-(CH_3)=CH_2, in aqueous solution, formed during the solvolysis of the corresponding thiol, $CF_3CH_2N(CH_3)CH_2SC_6H_4$-2-$COO^-$.[22] The lifetime of the iminium ion was determined to be ~5.5 x 10^{-8} s, and the relative rates of the diffusion-controlled reaction of the nucleophilic leaving group RS^- versus the reaction with solvent (H_2O) were determined to be k_{RS^-}/k_{H_2O} = 280.[22] An attempt to increase the efficiency of the synthesis of **1** using mercuric chloride ($HgCl_2$) catalysis, as was described in our earlier work with 5-thiohexopyranosylamines,[2,3] was not successful in this case.

Scheme 6.3



In summary, the synthesis of a new class of disaccharides (**6.1**, **6.2**, and **6.3**) containing sulfur in the non-reducing galactofuranose ring and nitrogen in the interglycosidic linkage has been achieved.

6.5 Experimental

6.5.1 General Methods

Optical rotations were measured at 21°C with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. The ^{1}H NMR spectra of **6.1** were also recorded on a Bruker AMX-600 NMR spectrometer at 600.13 MHz. Chemical shifts are given in ppm downfield from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional $^{1}H/^{1}H$ (COSYDFTP), $^{1}H/^{13}C$ (INVBTP), and ^{1}H (NOESYTP) experiments using standard Bruker pulse programs and an inverse detection, $^{1}H/X$ double-resonance probe. High resolution liquid secondary ion mass spectra (FAB) were recorded on a Kratos Concept H instrument using *m*-nitrobenzyl alcohol as the matrix. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated. Compounds were purified by flash column chromatography on Kieselgel 60 (230-400 mesh). Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40°C.

6.5.2 General Procedure for Glycosylation Reactions

A mixture of 4-thio-D-galactofuranose **6.4**, the deoxyamino sugar **6.5, 6.6** or **6.7** (1.5 eq.), and AcOH (0.05 eq. relative to the amine) was heated to 85°C in dry MeOH (15-20 mL/mmol of **6.4**) in a sealed tube for 24 h-140 h. The reaction mixture was cooled to room temperature and the solvent removed in vacuo. The residue was purified by column chromatography using EtOAc:MeOH:H_2O (6:2:1) (**6.1** and **6.2**) or EtOAc:MeOH:H_2O (4:2:1) (**6.3**) as the eluant.

6.5.3 Characterization

Methyl 3-amino-3-deoxy-3-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (6.1). R_f 0.68; EtOAc:MeOH:H_2O (4:2:1), (α:β 1:3, 52%).

6.1β. ^{1}H NMR (D_2O): δ 4.72 (d, 1 H, H-1B), 4.40 (m, 1 H, H-1A), 3.95-3.89 (m, 4 H, H-2B, H-2A, H-3A, H-5A), 3.86 (dd, 1 H, $J_{5,6}$ 2.1, $J_{6,6'}$ 12.1 Hz, H-6B), 3.71 (dd, 1 H, $J_{5,6'}$ 6.2 Hz, H-6B'), 3.62 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5B), 3.55-3.50 (m, 2 H, H-4B, H-6A), 3.47 (dd, 1H, $J_{5,6'}$ 7.0, $J_{6,6'}$ 11.8 Hz, H-6A'), 3.42 (m, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 3.5 Hz, H-4A), 3.39 (s, 3 H, OCH_3), 2.87 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.9 Hz, H-3B). ^{13}C NMR (D_2O): δ 102.78 (C-1B), 83.08, 77.85 (C-2A, C-3A), 75.31 (C-5B), 72.69 (C-5A), 68.86 (C-2B), 67.77, 67.05 (C-4B, C-6A), 66.80 (C-1A), 63.71 (C-6B), 60.32 (C-3B), 57.42 (O*C*H_3), 51.36 (C-4A). ^{1}H NMR (methanol-d_4): δ 4.64 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1B), 4.35 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1A), 3.92 (dd, 1 H, $J_{3,4}$ 7.9 Hz, H-3A), 3.90-3.81 (m, 1 H, H-5A), 3.82 (dd, 1 H, $J_{2,3}$ 8.1 Hz, H-2A), 3.79 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2B), 3.76-3.64 (m, 2 H, H-6B, H-6B'), 3.58-3.36 (m, 5 H, H-5B, H-4B, H-6A, H-6A', H-4A), 3.38 (s, 3 H, OCH_3), 2.89 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3B).

6.1α. ^{1}H NMR (D_2O): δ 4.72 (d, 1 H, H-1B), 4.47 (d, 1 H, $J_{1,2}$ 4.6 Hz, H-1A), 4.12 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 2.9 Hz, H-2B), 4.08-4.05 (m, 2 H, H-2A, H-3A), 3.95-3.89 (m, 1 H, H-5A), 3.85 (dd, 1 H, $J_{5,6}$ 2.1, $J_{6,6'}$ 11.91 Hz, H-6B), 3.70 (dd, 1 H, $J_{5,6'}$ 6.0 Hz, H-6B'), 3.62 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5B), 3.55-3.50 (m, 2 H, H-4B, H-6A), 3.47 (dd, 1 H, $J_{5,6'}$ 7.2, $J_{6,6'}$ 11.4 Hz, H-6A'), 3.39 (s, 3 H, OCH_3), 3.24 (dd, 1 H, $J_{4,5}$ 3.7, $J_{3,4}$ 6.7 Hz, H-4A), 2.86 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3B). ^{13}C NMR (D_2O): δ 103.04 (C-1B), 80.22, 79.21 (C-2A, C-3A), 75.44 (C-5B), 73.35 (C-5A), 70.74 (C-2B), 68.64 (C-1A), 67.77, 67.05 (C-4B, C-6A), 63.71 (C-6B), 62.76 (C-3B), 57.42 (OCH_3), 52.56 (C-4A). ^{1}H NMR (methanol-d_4): δ 4.65 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.31 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1A), 4.13 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2B), 4.06 (dd, 1 H, $J_{3,4}$ 6.4 Hz, H-3A), 3.97 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2A), 3.90-3.81 (m, 1 H, H-5A), 3.76-3.64 (m, 2 H, H-6B, H-6B'), 3.58-3.36 (m, 4 H, H-5B, H-4B, H-6A, H-6A'), 3.38 (s, 3 H, OCH_3), 3.19 (dd, 1 H, $J_{4,5}$ 2.6 Hz, H-4A), 2.74 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3B). FAB HRMS. Calcd for $C_{13}H_{25}NO_9S$ + H: 372.1328. Found: M + H: 372.1337.

Methyl 2-amino-2-deoxy-2-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (6.2). R_f 0.66; EtOAc:MeOH:H_2O (4:2:1), (α:β 1:3, 54%).

6.2β. ^{1}H NMR (D_2O): δ 4.74 (d, 1 H, H-1B), 4.38 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1A), 3.91 (ddd, 1 H, H-5A), 3.89-3.80 (m, 4 H, H-3B, H-2A, H-3A, H-6B), 3.72 (dd, 1 H, $J_{5,6}$ 5.2, $J_{6,6'}$ 12.2 Hz, H-6B'), 3.58 (m, 1 H, H-5B), 3.56-3.48 (m, 2 H, H–4B, H-6A), 3.46 (dd, 1 H, $J_{5,6'}$ 7.1, $J_{6,6'}$ 11.7 Hz, H-6A'), 3.39 (dd, 1 H, dd, $J_{3,4}$ 8.5, $J_{4,5}$ 3.5 Hz, H-4A), 3.36 (s, 3 H, OCH_3), 3.04 (dd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 4.6 Hz, H-2B). ^{13}C NMR (D_2O): δ 101.89 (C-1B), 82.70, 77.79 (C-2A, C-3A), 74.89 (C-5B), 72.71 (C-5A), 71.99 (C-3B),

69.37 (C-4B), 68.97 (C-1A), 67.09 (C-6A), 63.39 (C-6B), 61.31 (C-2B), 57.50 (OCH_3), 51.40 (C-4A).

6.2α. ^{1}H NMR (D_2O): δ 5.06 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1B), 4.38 (d, 1 H, H-1A), 4.04-4.00 (m, 2 H, H-2A, H-3A), 3.91 (ddd, 1 H, H-5A), 3.89-3.80 (m, 1 H, H-6B), 3.78 (dd, 1 H, $J_{2,3}$ 4.6, $J_{3,4}$ 9.1 Hz, H-3B), 3.73 (dd, 1 H, $J_{5,6}$ 5.0, $J_{6,6'}$ 12.4 Hz, H-6B'), 3.58 (m, 1 H, H-5B), 3.56-3.48 (m, 2 H, H-4B, H-6A), 3.46 (dd, 1 H, $J_{5,6'}$ 7.0, $J_{6,6'}$ 11.5 Hz, H-6A'), 3.37 (s, 3 H, OCH_3), 3.22 (dd, 1 H, $J_{3,4}$ 6.7, $J_{4,5}$ 3.7 Hz, H-4A), 3.04 (dd, 1 H, $J_{2,3}$ 4.6 Hz, H-2B). ^{13}C NMR (D_2O): δ 102.34 (C-1B), 80.34, 79.01 (C-2A, C-3A), 74.89 (C-5B), 73.48 (C-5A), 72.71 (C-3B), 69.93 (C-4B), 69.22 (C-1A), 66.98 (C-6A), 64.15 (C-2B), 63.39 (C-6B), 57.50 (OCH_3), 52.52 (C-4A). FAB HRMS. Calcd for $C_{13}H_{25}NO_9S$ + H: 372.1328. Found: M + H: 372.1325.

Methyl 2-acetamido-6-amino-2,6-dideoxy-2,6-*N*-(4-thio-α/β-D-galactofuranosyl)-β-D-glucopyranoside (6.3). R_f 0.49; EtOAc:MeOH:H_2O (4:2:1), (α:β 1:3, 66%).

6.3β. H NMR (D_2O): δ 4.39 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1B), 4.32 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1A), 3.94-3.87 (m, 1 H, H-5A), 3.87-3.78 (m, 2 H, H-2A, H-3A), 3.65 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.3 Hz, H-2B), 3.52-3.42 (m, 4 H, H-6A, H-6A', H-3B, H-5B), 3.47 (s, 3 H, OCH_3), 3.37 (dd, 1 H, $J_{3,4}$ 8.6, $J_{4,5}$ 3.5 Hz, H-4A), 3.31 (dd, 1 H, $J_{3,4}$ $J_{4,5}$ 9.9 Hz, H-4B), 3.08 (dd, 1 H, $J_{5,6}$ 2.4, $J_{6,6'}$ 13.5 Hz, H-6B), 2.82 (dd, 1 H, $J_{5,6'}$ 8.5 Hz, H-6B'), 1.99 (s, 3 H, NHC(O)CH_3). ^{13}C NMR (D_2O): δ 177.31 (NHC(O)CH_3), 104.49 (C-1B), 82.81, 77.65 (C-2A, C-3A), 77.19, 76.51 (C-3B, C-5B), 74.55 (C-4B), 72.66 (C-5A), 69.58 (C-

1A), 67.02 (C-6A), 60.07 (OCH_3), 58.13 (C-2B), 51.21 (C-4A), 50.47 (C-6B), 24.81 ($NHC(O)CH_3$).

6.3α. H NMR (D_2O): δ 4.42 (d, 1 H, $J_{1,2}$ 5.9 Hz, H-1A), 4.40 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1B), 4.12-4.07 (m, 2 H, H-2A, H-3A), 3.94-3.87 (m, 1 H, H-5A), 3.65 (d, 1 H, d, $J_{2,3}$ 10.2 Hz, H-2B), 3.52-3.42 (m, 4 H, H-6A, H-6A', H-3B, H-5B), 3.45 (s, 3 H, OCH_3), 3.31 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.1 Hz, H-4B), 3.29 (dd, 1 H, $J_{3,4}$ 6.3, $J_{4,5}$ 4.0 Hz, H-4A), 2.99 (dd, 1 H, $J_{5,6}$ 2.9, $J_{6,6'}$ 12.7 Hz, H-6B), 2.92 (dd, 1 H, $J_{5,6'}$ 8.6 Hz, H-6B'), 1.99 (s, 3 H, $NHC(O)CH_3$), ^{13}C NMR (D_2O): δ 177.31 ($NHC(O)CH_3$), 104.49 (C-1B), 79.80, 79.29 (C-2A, C-3A), 76.72, 76.44 (C-3B, C-5B), 74.71 (C-4B), 73.22 (C-5A), 69.00 (C-1A), 66.86 (C-6A), 59.91 (OCH_3), 57.86 (C-2B), 52.96 (C-4A), 51.21 (C-6B), 24.81 ($NHC(O)CH_3$). FAB HRMS. Calcd for $C_{15}H_{28}N_2O_9S$ + H: 413.1593. Found: M + H: 413.1591

6.6 Acknowledgement

We are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support.

6.7 References

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CHAPTER 7: CONFORMATIONAL ANALYSIS OF THREE DISACCHARIDES CONTAINING GALACTOFURANOSE OR 4-THIOGALACTOFURANOSE MOIETIES

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7.1 Graphical Abstract

Conformational Analysis of Three Disaccharides Containing Galactofuranose or 4-Thiogalactofuranose Moieties

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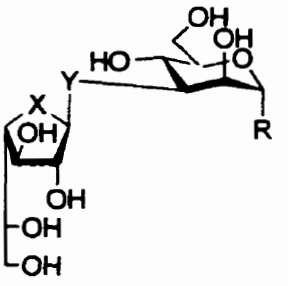


4.1 X=O, Y=O, R=SEt
5.1 X=S, Y=O, R=SEt
6.1 X=S, Y=NH, R=OMe

7.2 Abstract

The conformational analysis of three novel disaccharides containing galactofuranose (Gal*f*) or 4-thiogalactofuranose (4-thio-Gal*f*) moieties, namely, ethyl 3-*O*-(β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (**4.1**), ethyl 3-*O*-(4-thio-β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (**5.1**), and methyl 3-amino-3-deoxy-3-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (**6.1**), is performed using NOE data and coupling constants. Compound **4.1** is the ethylthio glycoside corresponding to the terminal end of the glycosylinositolphospholipid of *Trypanosoma cruzi*, the causative agent of Chagas disease, and **5.1** and **6.1** are the 4-thioGal*f* heteroanalogues, with the latter containing a nitrogen atom in the interglycosidic linkage. The $^{3}J_{H,H}$ values of the 4-thio-galactofuranosyl residues are used to determine the populations of the 4-thio-Gal*f* ring conformers for each disaccharide. The experimental NOE data indicate a similarity in the conformations about the glycosidic linkages for compounds **4.1**, **5.1**, and **6.1**.

Keywords: disaccharide; galactofuranose; heteroanalogues; conformational analysis; nuclear Overhauser enhancement; glycosidic linkage, coupling constants

7.3 Introduction

Galactofuranose (Gal*f*) is present as a constituent of external cellular structures in protozoa,[1] bacteria,[2,3] and fungi.[4] These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection.[5] It is known that Gal*f* is part of the oligosaccharide core of the glycosylinositolphospholipid from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[6] Gal*f* is found β-(1→3) linked to a mannopyranosyl unit at the terminal end of the GIPL structure and as a branched unit, also β-(1→3) linked to an internal mannopyranosyl residue.[7] The glycoconjugates on the cell surface during the infectious stage of *T. cruzi* are not modified with galactofuranose; however, it has been shown that the β-D-Gal*f* moiety is recognized by antibodies that inhibit *T. cruzi* internalization into mammalian cells.[5] Recently, it was demonstrated that *T. cruzi* GIPLs are able to block T-lymphocyte activation.[8] Thus, interaction between host cellular defense mechanisms and the GIPLs of *T. cruzi* may play a role in establishment and maintenance of chronic infection.[9]

Knowledge of the conformations of oligosaccharides is important in the understanding of the molecular mechanism of oligosaccharide-protein interactions. The pyranose forms of sugars normally exist in well-defined chair conformations in solution, but still have flexibility around the glycosidic linkages (Φ, Ψ) and the exocyclic C-C bond (torsion angle ω).[10] The furanose rings themselves are very flexible, so in addition to Φ, Ψ, and ω torsion angles, furanoses also possess a ring torsion angle χ (see Figure 7.1).[11] Furanose rings can adopt a wide-range of twist (T) and envelope (E) conformations of comparable energies as shown in Figure 7.2.[11] It is assumed that there

are at least two conformers existing in equilibrium in solution, typically, one from the northern hemisphere and one from the southern hemisphere.[12] The conformers interconvert through a series of pseudorotations around the ring as opposed to inversion *via* a planar ring conformation.[13] Conformational analysis of furanoses is not as well studied as their pyranose counterparts; however, there are several reports of computational studies on furanose ring conformation.[12,14] Some of the theoretical work is supported by solution NMR studies.[14c-g] Very recently the crystal structures of four pentofuranosyl methyl glycosides have been solved.[15] Thus, it is of interest to compare the furanose ring conformations and the conformation around the glycosidic linkage of ethyl 3-*O*-(β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (**4.1**), ethyl 3-*O*-(4-thio-β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside (**5.1**), and methyl 3-amino-3-deoxy-3-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (**6.1**). These compounds are currently being tested as inhibitors of *T. cruzi* proliferation. The conformational preferences may play a role in dictating inhibitory potency.

Figure 7.1 **Relevant Torsional Angles in Furanose Rings**

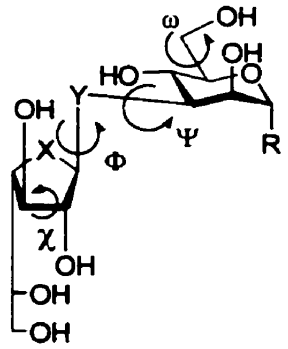


Figure 7.2 **Pseudorotational Wheel of a D-Aldofuranosyl Ring**

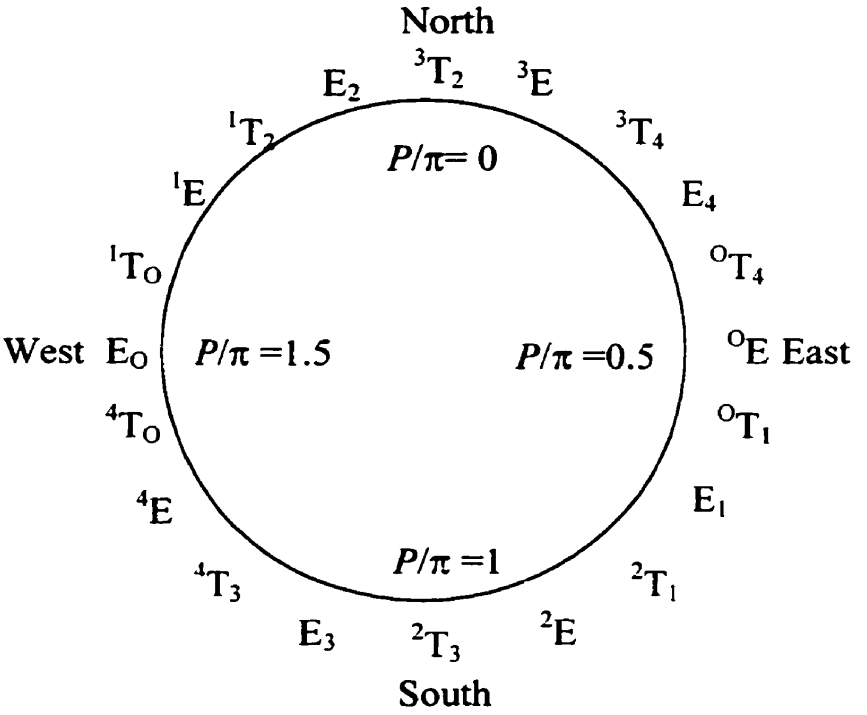
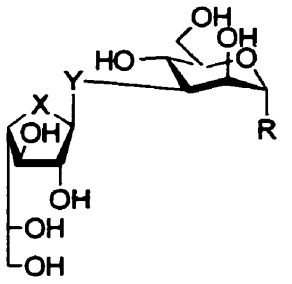


Chart 7.1



4.1 X=O, Y=O, R=SEt
5.1 X=S, Y=O, R=SEt
6.1 X=S, Y=NH, R=OMe

7.4 Results and Discussion

Previous reports from our laboratory described the synthesis of compounds **4.1**,[16] **5.1**,[17] and **6.1**.[18] First order analysis of the ^{1}H NMR spectra led to the determination of the coupling constants listed in Table 7.1. The coupling constants observed in the ^{1}H NMR spectrum for the 4-thiogalactofuranosyl (4-thio-Gal*f*) residues of **5.1** and **6.1** are of

Table 7.1 **Coupling Constants in Hz for Compounds 4.1-6.1**

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
4.1[a]	1.4	3.2	6.6	3.8
5.1[a]	5.5	8.3	8.8	3.9
6.1[b]	7.3	8.1	7.9	[c]

[a]in D_2O

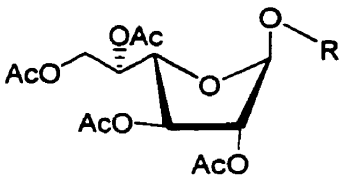
[b]in methanol-d_4

[c]not determined

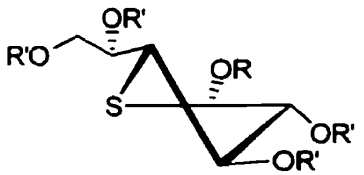
interest. They indicate that the 4-thio-Gal*f* residue in these compounds exists as a mixture of several conformations, including $^4T_3$ (D).[17] The dihedral angle between H-1A and H-2A is closer to 180° in **6.1** than in **5.1**. This is very different from the E_O (D) conformation observed for the β-D-galactofuranoside **4.1**, as seen in Figure 7.3.[16,19] The coupling constants that have been reported for 1,2,3,5,6-penta-*O*-acetyl-4-thio-β-D-galactofuranose [$J_{1,2}$ (3.1 Hz), $J_{2,3}$ (5.4 Hz), $J_{3,4}$ (6.4 Hz)], methyl 2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranoside [$J_{1,2}$ (2.5 Hz), $J_{2,3}$ (5.0 Hz), $J_{3,4}$ (5.3 Hz)] and 6-deoxy-

2,3,5-tri-*O*-acetyl-4-thio-β-D-galactofuranose [$J_{1,2}$ (3.3 Hz), $J_{2,3}$ (6.0 Hz), $J_{3,4}$ (7.4 Hz)] were attributed to result from an equilibrium mixture of several conformations, including $^4T_3$ (D) (see **Figure 7.3**).[20,21] In a study of 4-thiofuranoside derivatives of D-galactosamine, it was observed that all the β-isomers had coupling constants, $J_{1,2}$ (3.3-5.7 Hz), $J_{2,3}$ (6.1-7.6 Hz) and $J_{3,4}$ (6.7-7.7 Hz), that indicated the presence of a mixture of conformations.[22]

Figure 7.3 **Furanose Ring Conformations**



β-O-series E_O (D) **4.1**



β-S-series $^4T_3$ (D) **5.1, 6.1**

The 2D NOESY spectra for compounds **4.1**, **5.1**, and **6.1**, obtained in D_2O, are shown in Figures 7.4-7.6, respectively. Compound **6.1** was synthesized as an inseparable α:β mixture, so there was substantial overlapping of the ^1H NMR signals in the spectrum obtained in D_2O at 400 MHz. In an attempt to separate the multiplets found in the ^1H NMR spectrum of **6.1**, the 1D and 2D NOESY spectra were obtained in D_2O at 600 MHz. The proton spectrum of **6.1** showed less overlap of the signals in the minor isomer,

but unfortunately, the signals attributable to the major isomer were still not fully resolved. In order to determine the coupling constants and NOE contacts, a second set of spectra for **6.1** was obtained using methanol-d_4 as a solvent (see Figure 7.7). The multiplet containing H-2A, H-3A and H-2B separated, permitting determination of the coupling constants. The conformation of the 4-thio-Gal*f* ring of the minor isomer was judged to be very similar to that observed in D_2O, so it was assumed that the conformation of the major isomer would be similar in D_2O or methanol-d_4. In support of this contention, the NOE contacts were similar in both solvents, as seen in Figures 7.6 and 7.7. The 2D NOESY spectra of **4.1**, **5.1**, and **6.1** showed the presence of a cross-peak between H-1A and H-3A, indicating the presence of a β-linkage between the two rings and a cross-peak between H-1A and H-3B across the glycosidic linkage. The 2D NOESY spectra of **6.1** in methanol-d_4 showed the presence of only one H-1A and H-3A cross-peak, which corresponded to the proton signals of the major isomer. This enabled definitive assignment of the major isomer as the β-isomer. The absence of this contact in both D_2O and methanol-d_4 for the minor isomer is consistent with an α-linkage between the 4-thioGal*f* ring (A) and the mannopyranosyl ring (B). All three disaccharides showed the presence of an NOE cross-peak between H-1A and H-2B (see Figure 7.8). This NOE contact suggests that the two rings must be oriented in a conformation that brings H-1A close to H-2B, while still keeping H-1A and H-3B in close proximity. The absence of a contact between H-1A and H-4B indicates that the conformation about the glycosidic linkage must have H-1A and H-4B more than 4 Å apart.[23]

Figure 7.4 **The 2D NOESY Spectrum of 4.1 in D_2O**

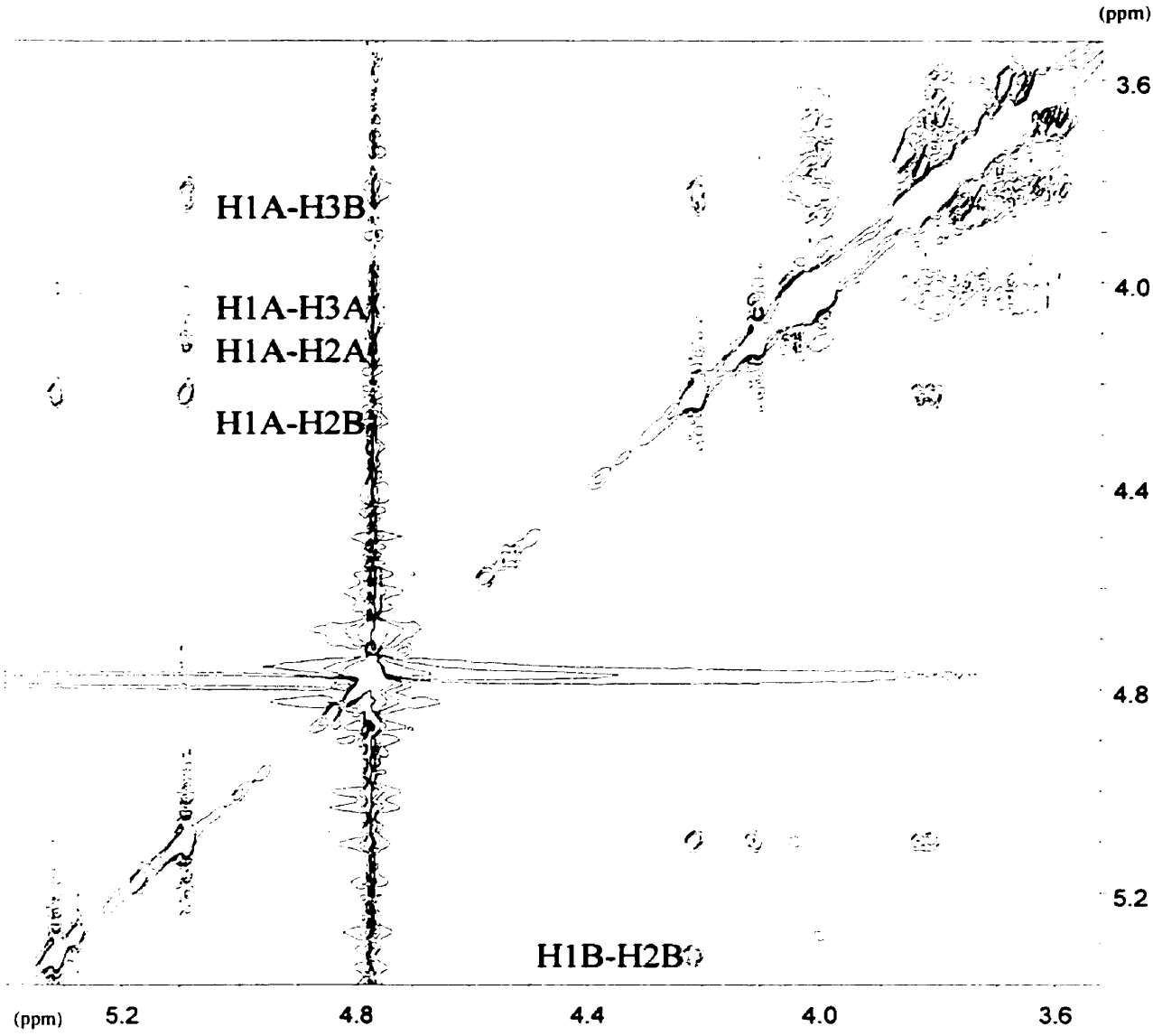


Figure 7.5 **The 2D NOESY Spectrum of 5.1 in D_2O**

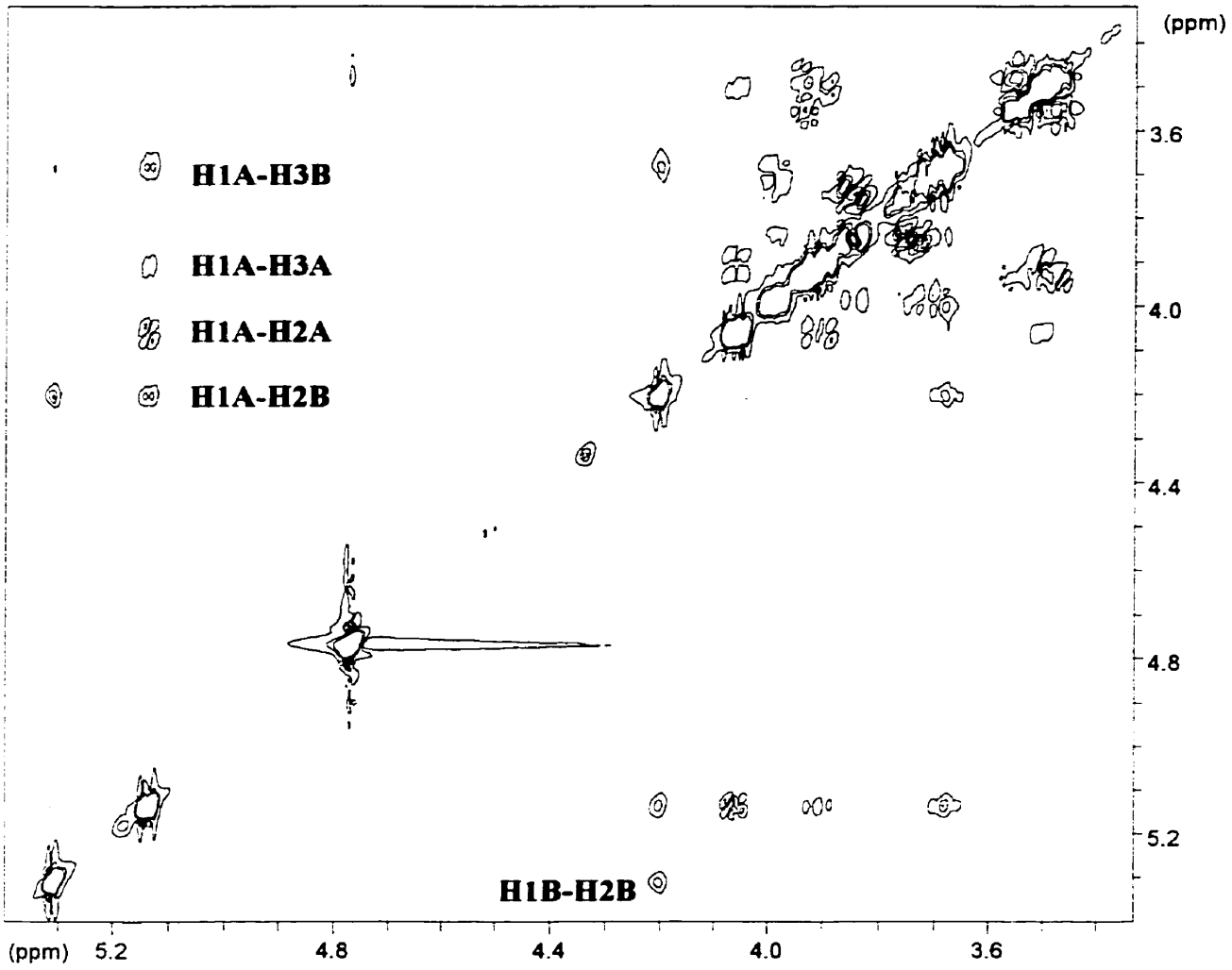


Figure 7.6 **The 2D NOESY Spectrum of 6.1 in D_2O**

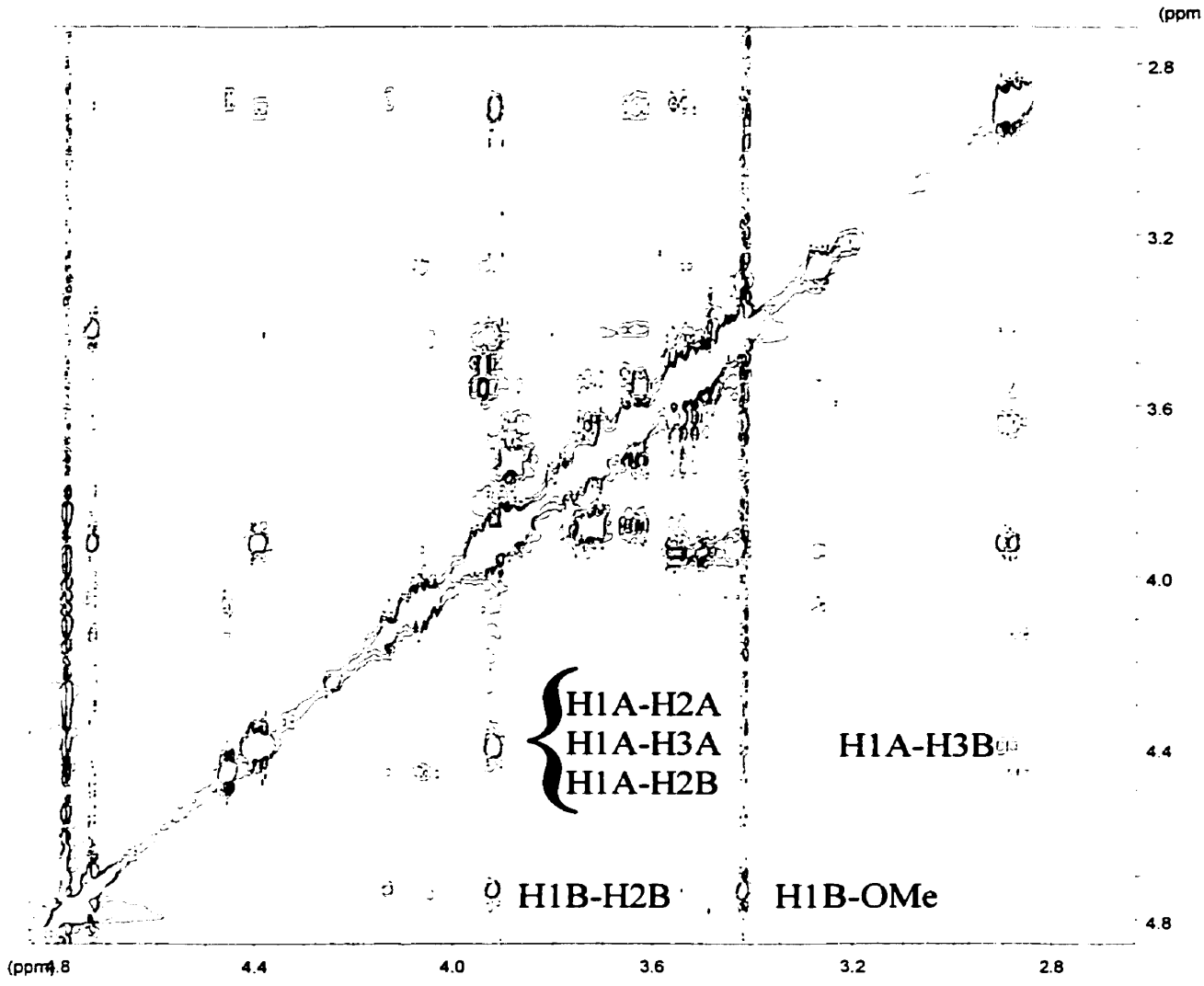


Figure 7.7 **The 2D NOESY Spectrum of 6.1 in Methanol-d_4**

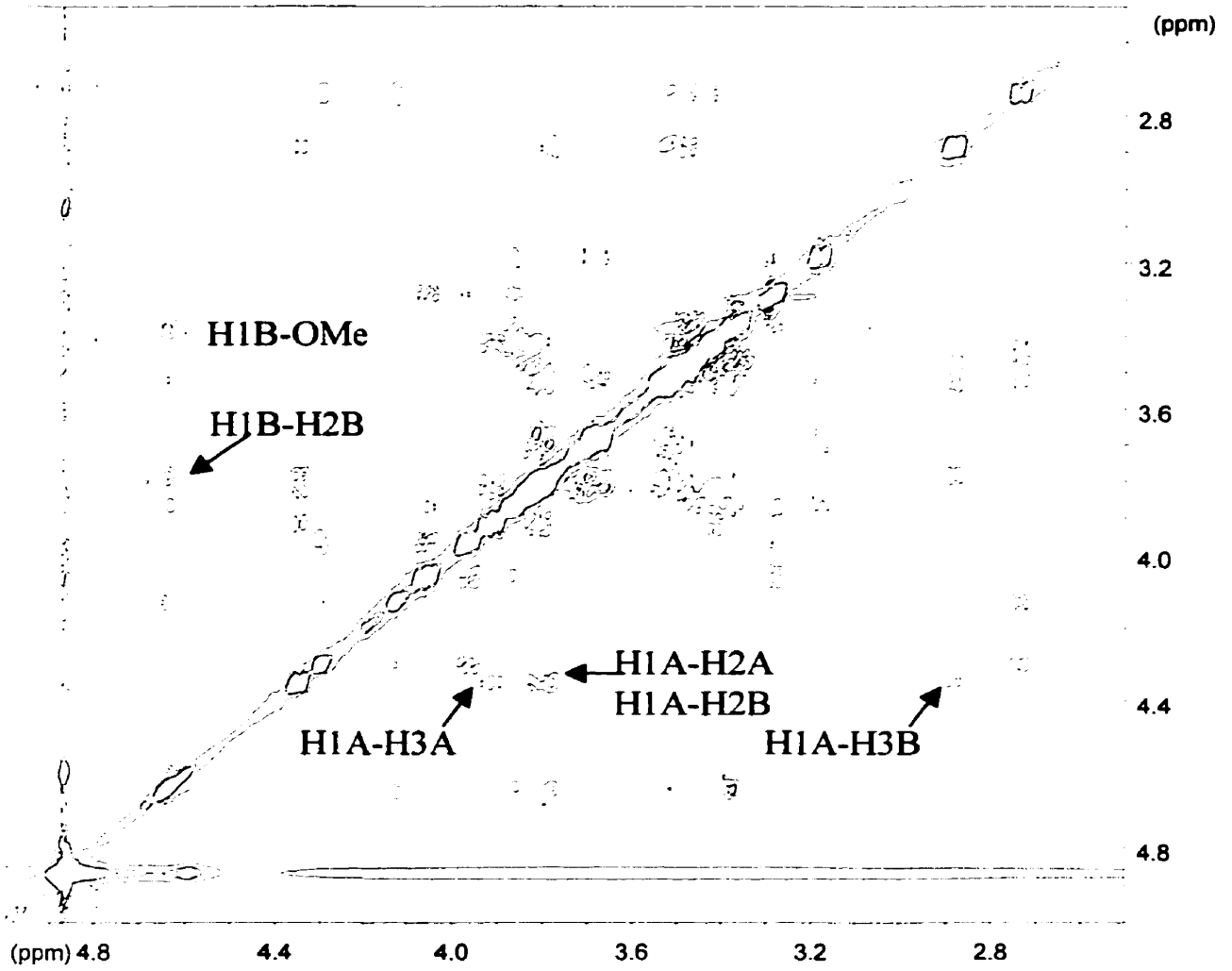
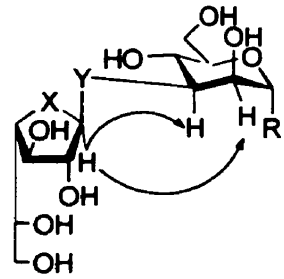


Figure 7.8 **NOE Contacts Across the Glycosidic Linkage**



The information obtained from the NOE contacts was used to construct initial models of conformation for **4.1**, **5.1**, and **6.1**. The data used as initial input and those obtained after minimization are shown in Table 7.2.

Table 7.2 **Distances and Dihedral Angles Before and After Minimization for 4.1-6.1**

Compound	H-1A/H-3B	H-1A/H-2B	H-1A/H-4B	Ψ	Φ
4.1[a]	2.97	1.78	4.19	180	-60
4.1[b]	3.04	2.58	4.56	153	-53
5.1[a]	2.95	1.76	4.19	180	-60
5.1[b]	2.61	2.49	4.56	147	-77
6.1[a]	2.96	1.76	4.19	180	-60
6.1[b]	2.84	2.46	4.66	151	-63

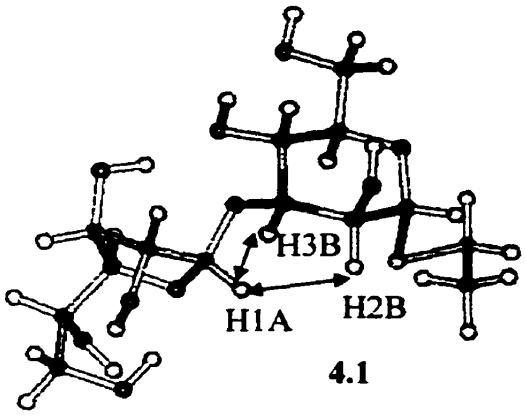
[a]before minimization

[b]after minimization

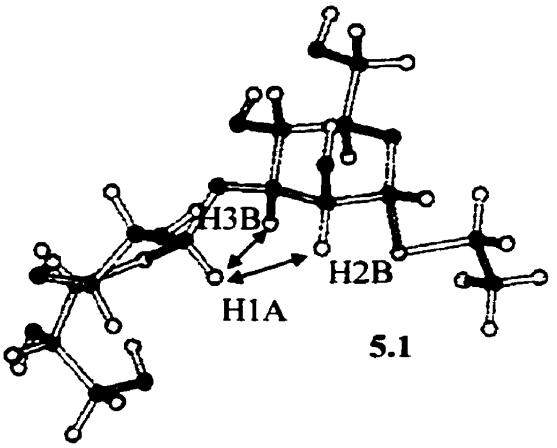
The minimized 3-D conformations of **4.1**, **5.1**, and **6.1** are presented in Figure 7.9. The glycosidic torsion angles in the minimized structures were in good agreement with those defined by the experimental data obtained from 2D NOESY spectra. A minimization was also performed using unrealistic dihedral angles and this structure minimized to a different conformation, but this conformation was predicted to give rise to strong NOE cross-peaks between H-1A and H-4B and a very weak, if any, NOE cross-peak between H-1A and H-2B, which is inconsistent with the data obtained experimentally. The Ψ angle was changed in increments of 10° and it was found that the experimental NOE cross-peaks were consistent with the population of conformations with Ψ angles of 90° to -30°. The conformations of the furanose rings obtained after minimization were not completely consistent with coupling constant data obtained from ^{1}H NMR spectra, most likely because the force field is not parameterized for furanose ring conformations. The furanose ring of **4.1** was in an E_2 (D) conformation, while the 4-thio-Gal*f* rings were in a $^{2}T_{3}$ (D) conformation. The dihedral angles Φ and Ψ of **4.1**, **5.1**, and **6.1** were very similar, as were the distances between the interglycosidic protons. It is interesting that the conformations across the glycosidic linkage are similar, even though the conformations of the furanosyl residues are different.

In summary, the conformations of the three disaccharides **4.1**, **5.1**, and **6.1** were analyzed using coupling constants and 2D NOESY data. This information led to a putative conformation for each disaccharide, that was similar about the glycosidic linkage.

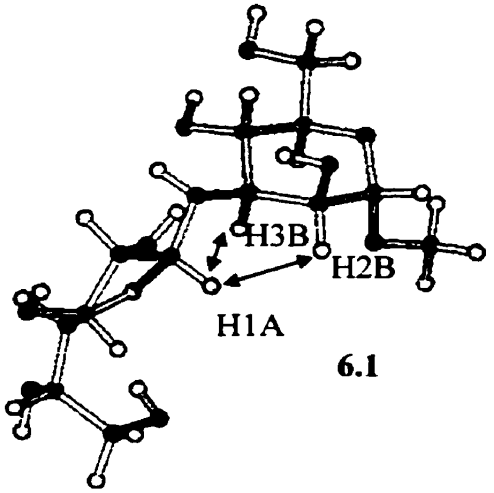
Figure 7.9 **The Minimized Structures of 4.1-6.1**



4.1



5.1



6.1

7.5 Experimental

7.5.1 NMR Experiments

Spectra for **4.1**, **5.1** and **6.1** were acquired on an AMX 400 Bruker spectrometer at 400.13 MHz (and spectra for compound **6.1** were also acquired on an AMX 600 Bruker spectrometer at 600.13 MHz), with a spectral width of 6 ppm. The compounds (9 mg) were lyophilized twice from 2 mL of D_2O (99.9%, CDN ISOTOPES) and then dissolved in 0.5 mL of D_2O. A second sample of **6.1** was prepared by dissolving the compound in 0.5 mL of methanol-d_4. The 2D NOESY experiments were acquired at 298K, non-spinning, in phase-sensitive mode using TPPI with 64 scans per increment, preceded by 4 dummy scans, a relaxation delay of 2 s, and a mixing time of 800 ms.

Processing of the spectra was performed with standard UXNMR (Bruker) and WINNMR software. Zero filling of the acquired data (512 t_1 values and 2 K data points in t_2) led to a final data matrix of 1 K x 1 K (F_1 x F_2) data points. Chemical shifts were referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

7.5.2 Energy Minimizations

Calculations were performed on a SGI O2 workstation. The calculations were achieved using INSIGHTII/Discover (Molecular Simulations Inc). The structures **4.1**, **5.1**, and **6.1** were assigned a Ψ angle of 180° and a Φ angle of –60°. These structures were in accordance with the NOE contacts present in the 2D NOESY spectra. These structures were then minimized using the Consistent-valence forcefield (CVFF).[24] A

conjugate gradient method was used; the minimizations occurred within 1000 iterations using a 0.001 derivative.

7.5.3 Charactreization

Methyl 3-amino-3-deoxy-3-*N*-(4-thio-α/β-D-galactofuranosyl)-α-D-mannopyranoside (6.1).

6.1β. ^{1}H NMR (methanol-d_4): δ 4.64 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1B), 4.35 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1A), 3.92 (dd, 1 H, $J_{3,4}$ 7.9 Hz, H-3A), 3.90-3.81 (m, 1 H, H-5A), 3.82 (dd, 1 H, $J_{2,3}$ 8.1 Hz, H-2A), 3.79 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2B), 3.76-3.64 (m, 2 H, H-6B, H-6B'), 3.58-3.36 (m, 5 H, H-5B, H-4B, H-6A, H-6A', H-4A), 3.38 (s, 3 H, OCH_3), 2.89 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3B).

6.1α. ^{1}H NMR (methanol-d_4): δ 4.65 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.31 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1A), 4.13 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2B), 4.06 (dd, 1 H, $J_{3,4}$ 6.4 Hz, H-3A), 3.97 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2A), 3.90-3.81 (m, 1 H, H-5A), 3.76-3.64 (m, 2 H, H-6B, H-6B'), 3.58-3.36 (m, 4 H, H-5B, H-4B, H-6A, H-6A'), 3.38 (s, 3 H, OCH_3), 3.19 (dd, 1 H, $J_{4,5}$ 2.6 Hz, H-4A), 2.74 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3B). ^{1}H NMR (D_2O): δ 4.73 (d, 1 H, H-1B), 4.46 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1A), 4.12 (dd, 1 H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.2 Hz, H-2B), 4.07 (dd, 1 H, $J_{3,4}$ 8.7 Hz, H-3A), 4.06 (dd, 1 H, $J_{2,3}$ 8.7 Hz, H-2A), 3.95-3.89 (m, 1 H, H-5A), 3.89-3.83 (m, 1 H, H-6B), 3.70 (dd, 1 H, $J_{5,6'}$ 6.0, $J_{6,6'}$ 12.1 Hz, H-6B'), 3.62 (m, 1 H, H-5B), 3.55-3.46 (m, 3 H, H-4B, H-6A, H-6A'), 3.39 (s, 3 H, OCH_3), 3.38 (dd, 1 H, $J_{4,5}$ 3.9 Hz, H-4A), 2.84 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3B).

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