SYNTHESIS, CONFORMATIONAL ANALYSIS AND BIOLOGICAL ACTIVITY OF DISACCHARIDE HETEROANALOGUES CONTAINING SULFUR AND/OR NITROGEN

by

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Abstract

The syntheses of novel heteroanalogues of propyl β-kojibioside (α-D-Glc*p*-(1-2)-β-D-Glc*p*-(1)-OPr) containing sulfur in the interglycosidic linkage and/or non-reducing ring are described. The trichloroacetimidate of tetra-O-benzyl-D-glucopyranoside was used as a glycosyl donor in glycosylation reactions, catalysed by triethylsilyl trifluoromethanesulfonate, with a selectively protected glucopyranosyl donor containing a 2-SH function, to give rise exclusively to the desired α-thio-linked disaccharide. Deprotection gave propyl 2-S-β-kojibioside. Kinetic inhibition studies indicated that the compound was a competitive inhibitor of glucosidase II, with a Ki value of 1.0 mM. The corresponding glycosylation reaction with the trichloroacetimidate of tetra-O-acetyl-D-glucopyranose was also investigated in order to determine the stereochemical requirements of glycosyl donors for α-thioglycoside formation.

Glycosylation reactions with the trichloroacetimidate of tetra-O-acetyl-5-thio-D-glucopyranose and a selectively protected 2-thioglucopyranoside unit gave rise to a 1.6:1 mixture of the α- and β-disaccharides, kojibioside and sophoroside analogues, respectively, containing sulfur in the non-reducing ring and interglycosidic linkage.

Using similar methodology, a methyl maltoside analogue containing sulfur in the non-reducing ring and interglycosidic linkage was synthesised and evaluated as an inhibitor of maltose binding by glucoamylase G2. The conformational preferences of this S,S-maltoside analogue were studied by comparison of experimental and theoretical NOE curves using a combined NMR spectroscopic/molecular mechanics protocol. Evidence

was obtained for both global and local minima. A molecular structure of the analogue derived from X-ray crystallographic studies indicated a conformation that is similar to that of the global minimum. Transferred NOE NMR studies of this compound when bound by the enzyme, glucoamylase G1, indicated a bound conformation that also resembles the global minimum conformation.

The synthesis of a novel class of disaccharides in which the non-reducing ring oxygen and interglycosidic oxygen are replaced by sulfur and nitrogen, respectively, is also presented. Acid catalysed condensation of 5-thio-D-glucose with either methyl 2-amino-2-deoxy-β-D-glucopyranoside or methyl 4-amino-4-deoxy-α-D-glucopyranoside gave rise to interconverting mixtures of methyl 5'-S-2-N-β-kojibioside/methyl 5'-S-2-N-β-sophoroside and methyl 5'-S-4-N-α-maltoside/methyl 5'-S-4-N-α-cellobioside, respectively. The maltoside analogue was evaluated as an inhibitor of glucoamylase G2 and the kojibioside analogue as an inhibitor of α-glucosidase I and II. A comparison of NOE effects in free methyl 5'-S-4-N-α-maltoside and transferred NOE effects in a mixture of methyl 5'-S-4-N-α-maltoside and glucoamylase G1 suggested that, although the compound populates both global and local minimum conformations, it is bound by the enzyme in a conformation in the area of the global minimum of the free disaccharide.

For Mum, Dad, Martyn, Nick, Donna and Carolyn

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I would like to express my sincere gratitude to my Senior Supervisor Professor Mario Pinto for his guidance and patience over the last six years.

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LIST OF ABBREVIATIONS

Ac	Acetyl
AIDS	Acquired Immunodeficiency Syndrome
All	Allyl
AMBER	Assisted Model Building and Energy Refinement
Asn	Asparagine
$BF_3 \cdot Et_2O$	Boron trifluoride etherate
Bz	Benzoyl
Bzl	Benzyl
COSY	Correlated spectroscopy
DMF	*N*,*N*-dimethylformamide
ELAM	Endothelial leukocyte adhesion molecule
ER	Endoplasmic reticulum
Et	Ethyl
Glc	Glucose
Glu	Glutamic acid
GlcNAc	*N*-acetylglucosamine
HIV	Human Immunodeficiency Virus
HMPA	Hexamethylphosphoramide
Man	Mannose
Me	Methyl
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Enhancement
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PIMM	Pi SCF Molecular Mechanics
PPDol	Dolichol pyrophosphate
Pr	Propyl
Py	Pyridine

TESOTf	Triethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Triflate (OTf)	Trifluoromethanesulfonate
TPPI	Time Proportional Phase Increments
TRNOE	Transferred Nuclear Overhauser Enhancement
TRNOESY	Transferred Nuclear Overhauser Enhancement Spectroscopy
UDP	Uridine diphosphate

"How sweet it is"

Jackie Gleason

CHAPTER 1

GENERAL INTRODUCTION

1.1. Biological Significance of Carbohydrates

Carbohydrates have traditionally taken a "back seat" role in terms of biological significance relative to other classes of biological macromolecules. Aminoacids and nucleotides have long been recognised as the biological building blocks in eucaryotes and their roles pertaining to information carriage and transfer have been extensively studied. The roles of carbohydrates in the storage and mobilisation of energy and in their contribution to the structural integrity of cells were well known, although their significance in information transfer was considered to be minimal. Carbohydrates were, in fact, often regarded as "impurities" in proteins and lipids when the biological importance of glycoproteins and glycolipids was being investigated.

As carriers of biological information, macromolecules require vast diversity in structure and surface topography in order to mediate countless recognition events. For example, the immune system requires the conference of explicit specificity towards the surface of any number of foreign antigens for an effective immune response to be mounted. In terms of structural diversity, carbohydrates are superior to aminoacids and nucleotides; the combination of three monosaccharide units can theoretically yield 1056 different trisaccharides, while the combination of three different aminoacids can yield a maximum of six different tripeptides. Further structural variation in carbohydrates is achieved *via* branching, and acetylation, phosphorylation or sulfation.

The recent improvements in analytical techniques for the study of complex organic molecules have allowed the determination of the structure and conformation of complex carbohydrates and, thus the extent of their structural diversity has become more fully recognised. The structurally diverse nature of carbohydrates, therefore, renders them ideal as carriers of biological information, and over the last two to three decades their implication in cell-recognition events has been recognised. In fact, cell surface carbohydrates are involved, almost without exception, whenever specific cell-interactions take place.

Oligosaccharides, covalently attached to proteins or lipids (glycoconjugates), have been shown to play a major role in cell-cell and cell-molecule recognition events.[1] The biological information in glycoconjugates is carried mainly by the carbohydrate portion[2,3] which are often large in comparison to the protein or lipid to which they are attached. Glycoconjugates are involved in many biological functions some of which include cell adhesion (fertilisation), blood clotting, hormonal regulation, immunological protection and structural support.[4] In addition, carbohydrates play a vital role as receptors for viruses, bacteria and toxins.[1,5] Carbohydrate-protein interactions are the basis for these and other biological processes and include the enzymatic synthesis and degradation of oligosaccharides during the maturation of glycoproteins. The processing of N-linked glycoproteins by specific enzymes and the study of these interactions by way of carbohydrate-based enzyme inhibitors will be discussed in the following sections, along with the study of the specificity and inhibition of hydrolase enzymes in lower organisms.

1.1.i The Role of Carbohydrates in Cell Adhesion and Inflammation

The recruitment of leukocytes to sites of inflammation to destroy pathogenic microorganisms is mediated in part by endothelial leukocyte adhesion molecule-1 (ELAM-1) expressed on the surface of endothelial cells on blood vessel walls.[6] ELAM-1 is a member of the selectin family of adhesion proteins containing a lectin motif which recognises carbohydrate epitopes expressed on the surface of leukocytes. It is now known that carbohydrate epitopes containing the tetrasaccharide sialyl Lewis x (Sle^x) (Figure 1.1) are recognised by ELAM-1 and are sufficient to cause ELAM-1 mediated cell adhesion.

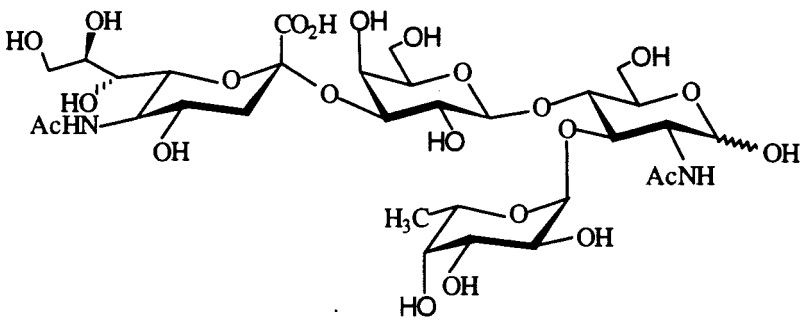


Figure 1.1 Structure of the sialyl Lewis x tetrasaccharide

Although the recruitment of leukocytes during an inflammatory response is crucial for tissue repair, the destruction of normal tissue by leukocytes may occur in chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. Since the discovery of the involvement of sialyl Lewis x in the inflammatory response much effort has focused on

the chemical[7] and enzymatic[8] syntheses of SLe^x and analogues as potential antiinflammatory agents.

1.1.ii Maturation of N-linked Glycoproteins

Asparagine-linked (N-linked) oligosaccharides are present in many secretary and membrane proteins. The maturation of glycoproteins[9] occurs as a sequence of enzyme controlled transformations within the endoplasmic reticulum (ER) and Golgi apparatus and results in the formation of active mature glycoproteins. The glycoprotein processing sequence and the structures of the resulting glycoproteins is remarkably conserved throughout eukaryotes, suggesting that the oligosaccharides are biologically important. The maturation process occurs in three distinct parts and is initiated from dolichol pyrophosphate within the ER. A series of glycosyl transfers mediated by specific transferase enzymes convert dolichol pyrophosphate (PPDol) to a lipid-linked glycan precursor $Glc_3Man_9GlcNAc_2PPDol$ in what is termed the dolichol pathway (Figure 1.2). Transfer of the precursor to specific sites on nascent protein is achieved by an oligosaccharyl transferase in the ER to give the high mannose type glycoprotein which is immediately processed by removal of the three glucose residues by specific trimming glucosidases. The distal α 1-2 linked glucose residue is removed (hydrolysed) by glucosidase I followed by the subsequent removal of the two innermost α 1-3 linked glucose residues by glucosidase II to give glycoprotein containing high mannose-type $Man_9GlcNAc_2$ oligosaccharides (Figure 1.2). Further processing by ER- and Golgi-located mannosidases permits subsequent processing by various Golgi-resident

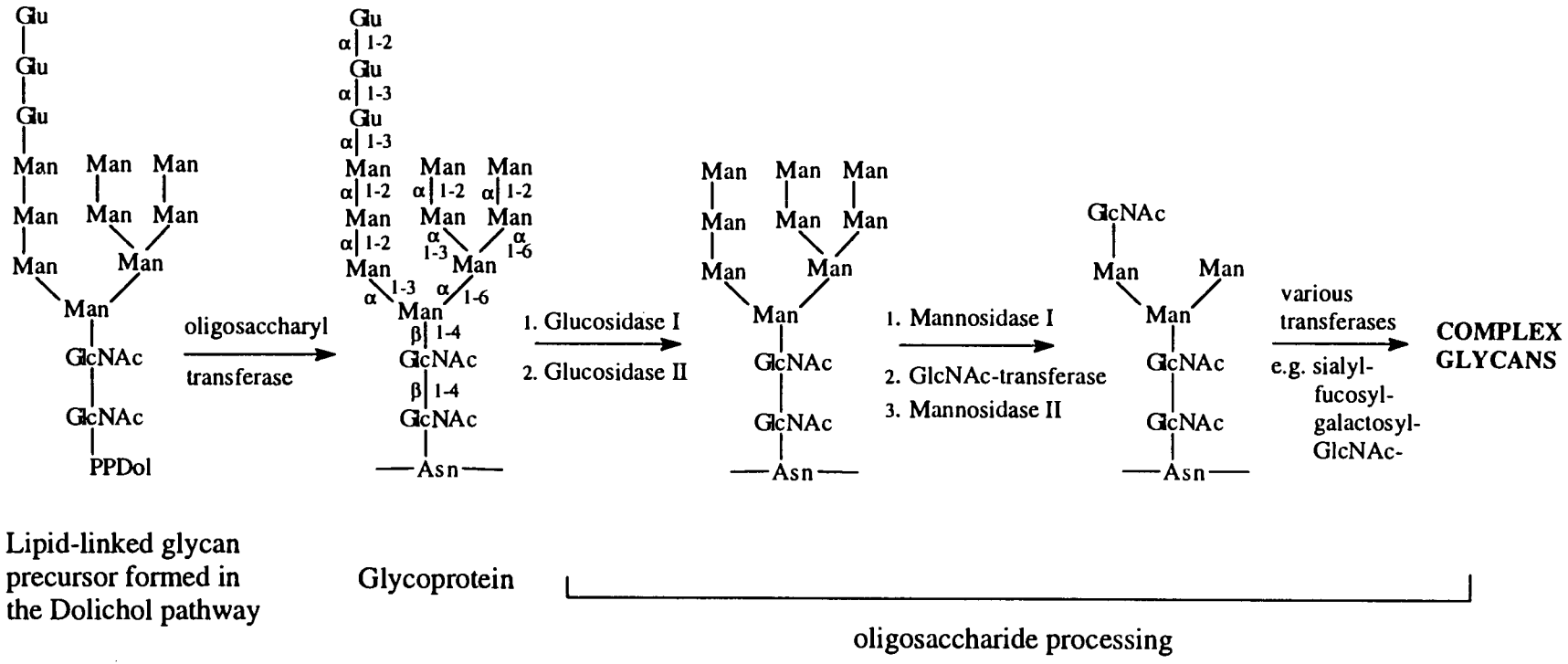


Figure 1.2 N-linked glycan biosynthesis

transferases such as sialyltransferase, fucosyltransferase, galactosyltransferases and N-acetylglucosaminyltransferase, to give mature complex and hybrid-type glycoproteins for secretion or cell-surface expression.

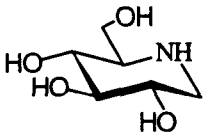
The specific structure of the oligosaccharides attached to surface glycoproteins is vital for their correct function. Alteration of the oligosaccharides by interference in the glycoprotein maturation by enzyme inhibitors has been employed for the study of these processes and the glycosidase inhibitors themselves have therapeutic potential as antiviral agents, for example, in HIV[10] and influenza[11] treatment, as anticancer[12] and antibacterial[13] agents, and in the treatment of metabolic disorders such as diabetes.[14]

1.1.iii Human Immunodeficiency Virus and Inhibitors of Glycoprotein Processing

Human immunodeficiency virus causes the destruction of T4 lymphocyte cells expressing the CD4 cell-surface antigen,[15] thereby interfering with the body's immune defenses and leaving it more susceptible to opportunistic infections, giving rise to the syndrome known as AIDS.[16] Infection of T4 lymphocytes by HIV is initiated by the interaction of the viral envelope glycoprotein GP120 and the cell-surface antigen CD4.[17] The envelope glycoproteins of HIV are heavily N-glycosylated, carbohydrates comprising approximately 50% of the total mass of GP120.[18] The viral infectivity is strongly dependent on the nature of the oligosaccharides on GP120. Altering the carbohydrate structure of GP120 has been shown to result in a lower affinity of GP120 for CD4, thereby interfering with viral infectivity.[19] Once the viral genome has been translated in to the cell, many of the normal glycoprotein maturation pathways of that cell are then

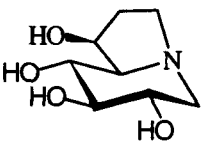
available to any viral protein expressing the correct potential glycosylation sites. By this method, viral membrane surface glycoproteins may be synthesised that are indistinguishable to those of the cell which it had invaded and camouflage the assembled viral particles towards immune scrutiny, and may also offer a means by which to reattach to cell surface receptors. Interference with the glycoprotein processing by glycosidase inhibitors may therefore result in aberrant viral glycoproteins and provide a potential anti-HIV therapy.[10]

Over the past five years a number of studies have demonstrated that 1-deoxynojirimycin **1.1** (Figure 1.3), a naturally occurring polyhydroxylated alkaloid, and derivatives, can interrupt the infection cycle of HIV, presumably *via* their action as



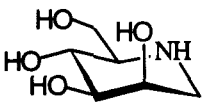
1.1

Deoxynojirimycin



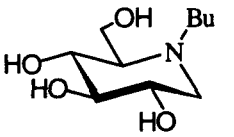
1.2

Castanospermine



1.3

Deoxymannonojirimycin



1.4

N-butyldeoxynojirimycin

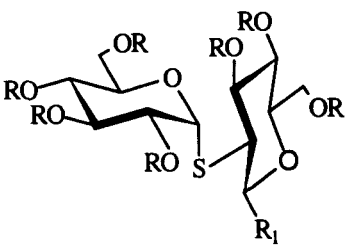
Figure 1.3 Azasugars tested for anti-HIV activity

glycosidase inhibitors.[20] Thus, the inhibition of syncytia formation (fusion of HIV infected T4 cells and healthy T4 cells) and interference with viral infectivity has been observed upon treatment of HIV-infected cells and CD4-expressing cells with known glucosidase inhibitors such as deoxynojirimycin **1.1**[21] and castanospermine **1.2**[21,22] (Figure 1.3). Deoxymannonojirimycin **1.3**, an inhibitor of mannosidases, was found to exhibit no effect, suggesting that drugs that act upon enzymes in the later stages of glycoprotein processing have no efficacy against HIV infections. Subsequently, it was reported that N-alkylation of deoxynojirimycin had a profound effect on α-glucosidase inhibition. In particular, the N-methyl and N-butyl **1.4** (Figure 1.3) derivatives were shown to be 10 times more potent inhibitors of calf liver α-glucosidase than the corresponding nonalkylated compounds.[23] Thereafter, similar results were found by Fleet *et al.*[24] who reported the screening of a large number of N-alkylated deoxynojirimycin analogues and demonstrated that N-butyldeoxynojirimycin **1.4** was a potent inhibitor of HIV infection with minimal cytotoxic effects. This compound was reported to inhibit purified glucosidase I with a Ki value of 0.22 μM.[19a]

Some of the reports mentioned above[24] suffer from the criticism[20b] that the anti-HIV activity exhibited by various deoxynojirimycin analogues was not shown unambiguously to be a result of modified GP120 glucans with retained glucose residues, derived from the inhibition of glucose trimming, as had been claimed. The most significant paper to date, therefore, is that by Karlsson *et al.*[19a] who report the effects of N-butyldeoxynojirimycin on the N-glycosylation of recombinant GP120. The characterisation of N-linked glycosylation of recombinant GP120 expressed in Chinese hamster ovary cells cultured in

the presence and absence of N-butyldeoxynojirimycin was performed, and using a variety of biochemical methods, they determined that glucosidase inhibition was, indeed, a possible mechanism for the anti-viral activity of this compound. A very recent paper by Asano *et al.*[25] has reported the conformational basis of inhibition of glycosidases and HIV replication by N-alkylated nitrogen-in-the-ring-sugars. The solution conformations of N-methyldeoxynojirimycin and N-butyldeoxynojirimycin **1.4**, and in particular the conformation of the C_6-OH bond, were studied primarily using NOE measurements and $^3J(H,H)$ coupling constants. The preferred axial conformation about the C_6-OH bond for these compounds, compared to the equatorial orientation in deoxynojirimycin **1.1**, was suggested as being responsible for the increased potency and specificity of these compounds towards processing α-glucosidase I.[25]

Very recently, various thiosugar analogues have been found to be effective inhibitors of HIV-induced cell killing and virus production in two different cell lines.[26]



1.5 R = Ac, R_1 = OAc

1.6 R = Ac, R_1 = SAc

Figure 1.4 Potent inhibitors of HIV-induced cell killing

Thus, peracetylated 2-thiokojibiose **1.5** and its 1,2-dithio analogue **1.6** (Figure 1.4) exhibited IC_{50} values (defined as the drug concentration that inhibits the viral cytopathic effect by 50%) in the μM range (8-50 μM) (see also Chapter 2). The mechanism of HIV inhibition was undetermined and neither of these compounds was evaluated as a glucosidase I or II inhibitor.

1.3 Glycosidase Inhibition: Mechanism and Structure-Function Relationships

An extensive amount of research has been performed over the last two decades on the synthesis of inhibitors of glycoprotein processing enzymes, namely, glycosidases and glycosyltransferases.[14,27] Inhibitors of these enzymes can not only lead to potential therapeutic agents, as mentioned previously, but also to an understanding of the structure-function relationships of enzyme specificity. With an understanding of these relationships it is envisaged that improved glycosidase inhibitors may be synthesised, leading to more effective drug therapy. To this end, a large number of sugar analogues, especially those of the aza sugars, have been synthesised and evaluated for their biological activity. It is beyond the scope of this Chapter to discuss in detail all of these studies; instead the Chapter will focus on the some of the more recent advancements in determining the structural/charge requirements of carbohydrate analogues for effective binding to enzyme active sites. This Chapter will focus primarily on aza sugars and derivatives but will refer to thio- and other sugar analogues in the description of transition-state *versus* ground state inhibitors. A more detailed description of biologically active thiosugar analogues is presented in Chapter 2.

1.3.i Mechanism of Enzymic Catalysis

In order to probe the structure-function relationships of carbohydrates towards enzyme-catalysed hydrolysis, an understanding of the catalytic mechanisms involved is necessary. The pioneering work by Emil Fischer in 1909 in which he formulated his "lock and key" hypothesis[28] initiated research on enzyme specificity and mechanism which still continues today.[29-31] Glycosidases can be divided into two classes; those hydrolysing the

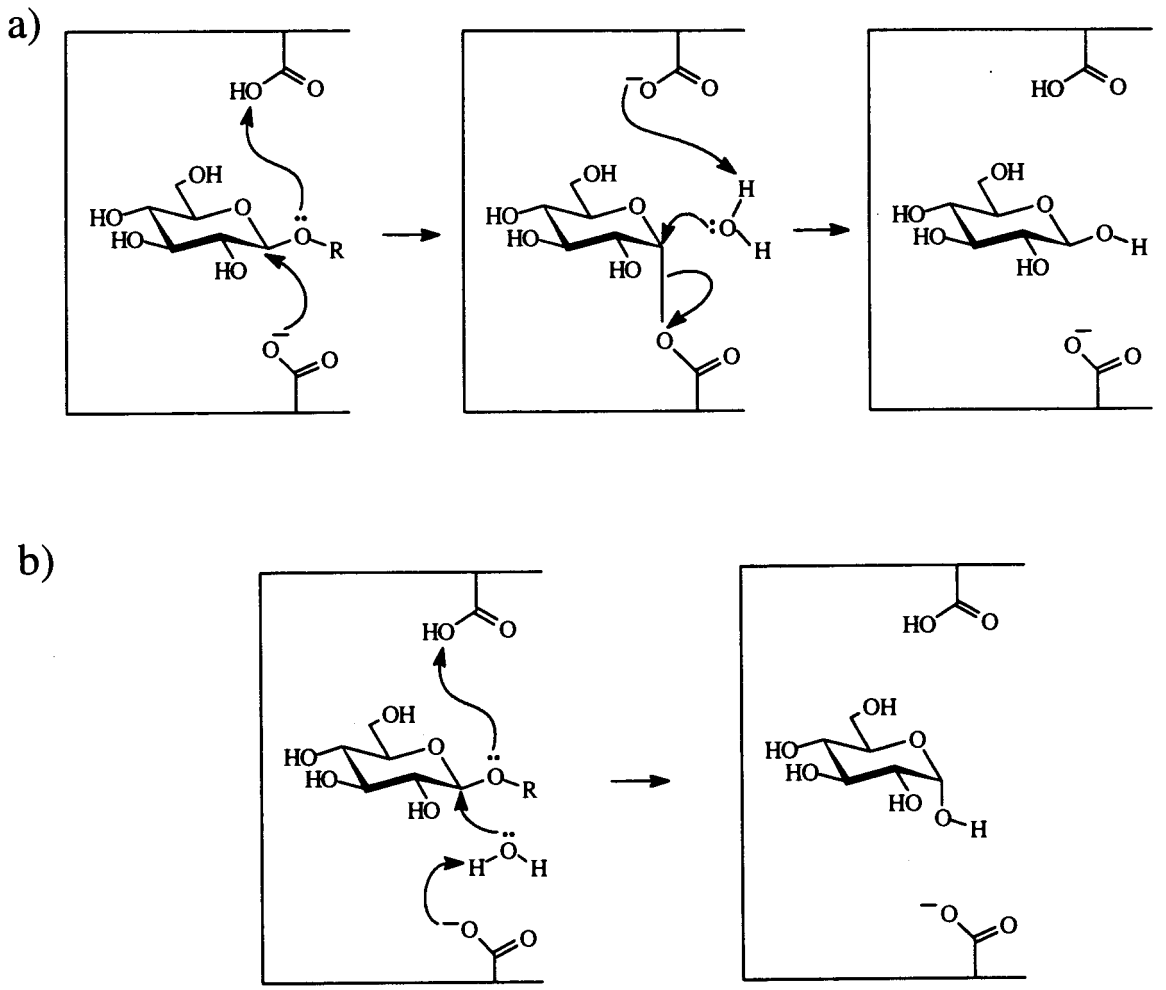


Figure 1.5 Presumed mechanism for a) retaining and b) inverting glycosidase enzymes

glycosidic bond with retention of anomeric configuration and those hydrolysing the glycosidic bond with inversion of anomeric configuration. It was first pointed out by Koshland[32] that retaining enzymes likely worked *via* a double displacement mechanism involving an enzyme nucleophile, while the inverting enzymes likely worked *via* a single step mechanism involving the displacement of the aglycon by a water molecule. This postulate is still widely accepted as the likely mechanism for enzymic hydrolysis (Figure 1.5). Although mechanistically distinct, the retaining and inverting enzymes both have two carboxyl groups within the active site that are essential for catalysis. Both mechanisms involve a transition-state with the following characteristics: i) significant positive charge, ii) a half-chair-like conformation, and iii) a trigonal anomeric centre (Figure 1.6).

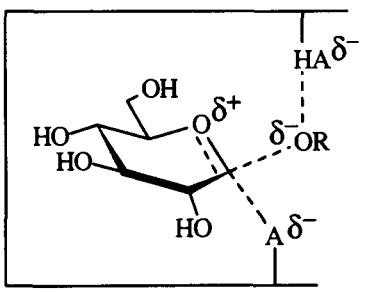


Figure 1.6 Putative transition-state structure

A breakthrough in the understanding of glycosidase mechanism came in 1966/1967 when the first crystal structure analysis of an enzyme-inhibitor complex was achieved. The three dimensional structure for a complex of lysozyme-chitotriose[33] revealed that the location of two carboxyl groups in the active site of the enzyme was essential to the

hydrolysis mechanism; one (Glu-35) donating a proton to the glycosidic oxygen atom, and the other (Asp-52) stabilising the positive charge resulting from the departure of the hydrolysed aglycon. Many protein-ligand crystal structures have been solved in the last five years, contributing greatly to the understanding of glycosidase mechanism. Retaining enzymes invariably have glutamate or aspartate residues present, acting as the catalytic nucleophile and proton donor/acceptor. In the retaining α-amylases on the other hand it has been shown that three conserved amino acids are present in the active site.[34] The presence of the third aminoacid has been suggested to likely provide further electrostatic stabilisation of the transition-state and may modulate active site pKa values.[31] A similar triad of active site amino acids has been observed in an inverting α-amylase by differential labelling experiments.[35] The three amino acids, Asp176, Glu179 and Glu180 were concluded to form an acidic cluster essential to the function of the enzyme.[35]

The mechanism of enzyme hydrolysis pertaining to the structure of the transition-state has, and continues to be, a contentious issue. The original proposal by Phillips[33] suggesting that the distortion of the substrate towards the oxocarbonium transition-state is important for catalysis has been further substantiated in two recent studies of lysozyme.[36,37] The crystal structure of a complex of lysozyme and a N-acetylmuramic acid- containing trisaccharide has shown that the N-acetylmuramic acid residue is distorted upon complexation from the usual $^4C_1$ ground-state chair conformation to one which possesses an approximately coplanar arrangement of atoms about the anomeric carbon atom.[36] Subsequently, T4 lysozyme with mutation of threonine for glutamic acid in the active site was shown to produce an enzyme with the carbohydrate substrate covalently

attached to the active site.[37] The crystal structure of this complex indicated that the covalently linked sugar ring was substantially distorted, suggesting partial attainment of the transition-state structure by the substrate.[37]

The hydrolysis mechanisms described thus far all involve exocyclic bond cleavage (i.e. glycosidic bond cleavage) as the crucial step. Alternative mechanisms involving endocyclic bond cleavage giving rise to enzyme-bound acyclic intermediates have been suggested[38] although all of these have been dismissed by Sinnott[39] as being unsubstantiated. The extent to which enzyme mechanisms are now understood is exemplified in a noteworthy paper describing the modification of a retaining glycosidase by site-directed mutagenesis to give an inverting enzyme.[40]

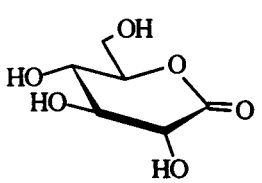
It has been suggested[41] that inhibitors capable of mimicking the transition-state structure during enzyme-catalysed hydrolysis would be more effective inhibitors of that enzyme. Much of the recent research, therefore, has concentrated upon the design and synthesis of transition-state analogues for the study of enzyme mechanism.

1.3.ii Structure-Function Characteristics of Glycosidase Inhibitors

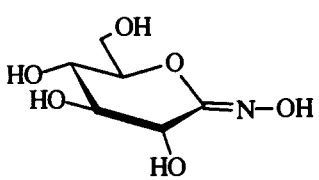
1.3.ii.a Nitrogen Analogues

Since the discovery that deoxynojirimycin **1.1** was a potent inhibitor of various glycosidases[14] many structural analogues have been synthesised with the expectation of improved inhibition. Direct evidence to support the suggested mechanism of aza sugar enzyme-catalysed hydrolysis[14] was realised in a study[23] in which the pH dependence of glucosidase I inhibition by various N-alkyl derivatives suggested that the cationic

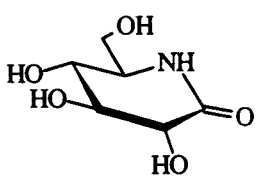
(protonated) form of the inhibitor was the active species. Thus, the protonated form of the inhibitor partially resembles the presumed transition-state structure by virtue of the positive charge.[42] Compounds that possess flattened pyranose rings have also been shown to inhibit glycosidases. Thus, D-gluconolactone[43] **1.7**, the corresponding oxime[44] **1.8** and 5-amino-5-deoxylactam[45] **1.9** (Figure 1.7) which all possess a distorted half-chair conformation, with only minor dipolar resonance structures contributing to a positive charge character, were shown to exhibit significant inhibition, suggesting that mimicry of the transition-state conformation is also an important factor in active site binding.[46] Inhibitors that have structural features resembling both the positive charge and flattened chair conformation of the transition-state would likely, therefore, be better inhibitors, and should also be more specific.[46] Clearly, a transition-state structure is impossible to mimic exactly; therefore, much of the recent progress in improved inhibitor design has focused on structures which are considered to mimic the transition-state as closely as possible.



1.7[43]



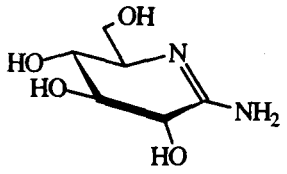
1.8[44]



1.9[45]

Figure 1.7 Analogues possessing a distorted chair conformation

Ganem *et al.*[47] reported in 1990 that the amidine **1.10** (Figure 1.8) was a potent, broad-spectrum inhibitor of glycosidases. Inhibition of β-glucosidase (sweet almond), α-mannosidase (jack bean) and β-galactosidase (bovine liver) were reported, with K_i values of ~10^{-5} M and **1.10** was categorised as a transition-state inhibitor. Subsequently, the amidrazone[48] **1.11** and the lactam oxime[49] **1.12** (Figure 1.8) were synthesised *via* a common intermediate that was used in the synthesis of **1.10** and tested for their inhibitory activity. Compounds **1.11** and **1.12** were tested with a variety of enzymes and were found to show similar behaviour to **1.10** (K_i = 8.4 μM and 13.8 μM, respectively, with β-glucosidase), although the inhibition of **1.12** was pH dependent. It was concluded from these studies, and based on the basicity of the analogues, that adoption of the flattened ring about the anomeric centre was more important than development of a full positive charge for transition-state binding by the enzyme and that the favourable electrostatic interactions resulting from this conformation override any minor effects of stereochemical discrimination between isomeric hexose units.[47] It has recently been noted[46,49] that a true tight-binding transition-state inhibitor should exhibit some enzymatic selectivity. The validity of categorising the "broad spectrum" inhibitors described by Ganem *et al.*[47,48] as being transition-state analogues has been called into question[46,49] since the factors determining an inhibitor as being strong did not seem to correlate with the lowered degree of observed inhibitor selectivity. It has been noted[46,49] that the amidine **1.10**, amidrazone **1.10** and lactam oxime **1.11**, by comparison with X-ray analyses in the Cambridge Data File and by quantum-chemical calculations,[49] have the structure resembling that of a hydroximolactam **1.13**, for example, and not of a hydroxylaminoimine **1.12** (Figure 1.8).



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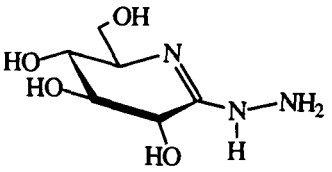

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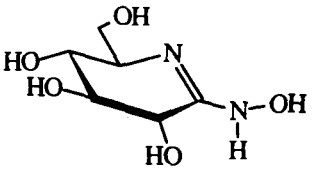

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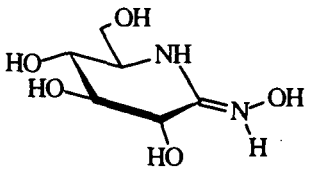


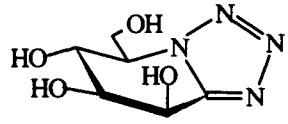
1.13

Figure 1.8 Aza analogues **1.10**-**1.12** with assumed[47,48] transition-state structure and the suggested[49] correct structure (**1.13**) of **1.12**

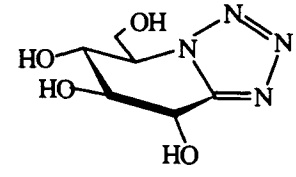
Based on this observation, it was suggested that the low discrimination of these inhibitors was, in fact, due to their basic nature and not to their assumed similarity to the transition-state structure.[46]

In order to assess the sole influence of conformation in the absence of basic influences on the inhibitory potential, the neutral species **1.14** ("mannonojiritetrazole") (Figure 1.9) was tested as an inhibitor.[46] Based on inhibition studies with **1.14** and the corresponding gluco analogue **1.15** ("nojiritetrazole),[50] it was shown[46] that the mannonojiritetrazole was considerably more effective as an inhibitor of β-mannosidase than the nojiritetrazole, and that the opposite was true for the inhibition of α-glucosidases. By comparison of various kinetic parameters for enzyme-catalysed and uncatalysed

reactions, it was suggested that inhibitors **1.14** and **1.15** can be truly categorised as transition-state analogues;[46] the work demonstrates a correlation between transition-state mimicry and inhibitor specificity.



1.14

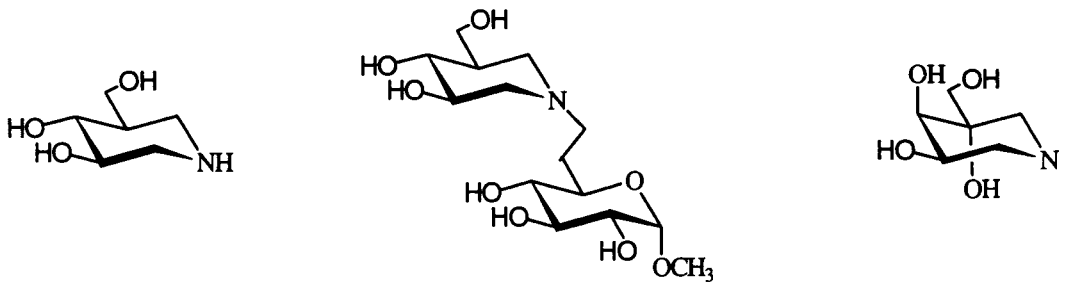


1.15

Figure 1.9 True transition-state inhibitors[46]

Wong *et al.*[27b,51] have reported the biological activity of a number of novel and known azasugars and thiosugars, and, in conjunction with molecular modelling studies, have concluded that good inhibitors should possess either a half-chair conformation and a positive charge character at the anomeric carbon and ring heteroatom, or a chair-like conformation with positive charge character and normal hydroxyl group stereochemistry. They also demonstrated that N-alkylation of deoxynojirimycin, for example, has positive and negative effects on the inhibitory activity, depending on the enzyme. Improved *in vivo* inhibition with some of these compounds was suggested to be as the result of enhanced transport of the N-alkylated inhibitors across the cell membrane.[27b] Also, N-oxidation of N-methylnojirimycin was shown to reduce the inhibition activity,[27b] presumably through incorporation of destabilising electrostatic interactions.

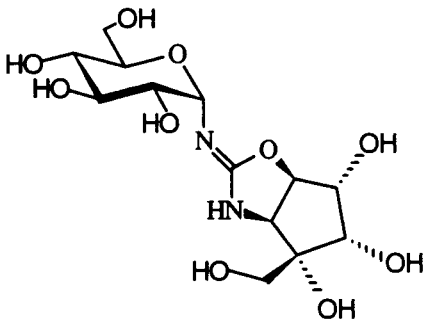
Inhibitors in which the anomeric carbon atom is replaced by nitrogen, which can accept a positive charge and thus mimic the transition-state, have recently been described.[52,53] The monosaccharide analogue, isofagomine, **1.17** and the disaccharide analogue **1.18** (Figure 1.10) have been synthesised[52] and were demonstrated to be more potent inhibitors of almond β-glucosidase (K_i values of 0.11 and 2.32 μM, respectively) than deoxynojirimycin **1.1** (K_i = 47.5 μM), but less, although still potent, inhibitors of α-glucosidase (K_i = 85.9, 58.8 and 25.4 μM, respectively). The corresponding inhibition of β-glucosidase by the iminosugar **1.19** (Figure 1.10) at the same pH (6.8) gave a K_i value



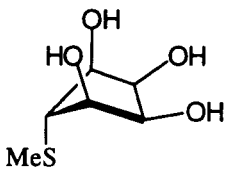
1.17 **1.18** **1.19**

Figure 1.10 Novel iminosugars

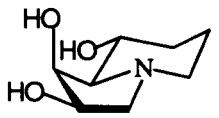
of 4.3 μM.[53a] Compound **1.19** was also recently found to be an extremely potent inhibitor of β-galactosidase from *Aspergillus orizae*, with a K_i value of 4.1 nM.[53b] These compounds represent, therefore, the most potent inhibitors of β-glucosidases to date. There are many other aza sugar analogues that have been synthesised and tested for their



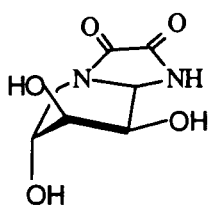
Trehalostatin
Trehalase inhibitor



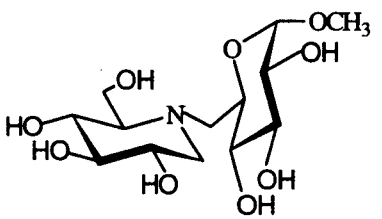
Mannostatin A
α-Mannosidase inhibitor



Swainsonine
α-Mannosidase II
inhibitor



Kifunesine
α-Mannosidase II inhibitor

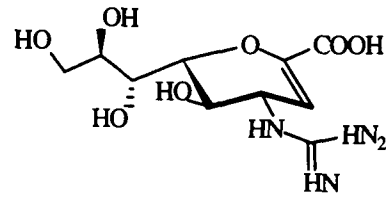


Synthetic sucrase inhibitor
Antidiabetic activity

Figure 1.11 Selected examples of azasugar glycosidase inhibitors

biological activity with a wide variety of enzymes, representative examples of which are shown in Figure 1.11.[27a]

Finally, in a remarkable testament to the "rational design" of potent carbohydrate-based enzyme inhibitors using a combination of X-ray crystal structure analysis and computer modelling[11], the unsaturated neuraminic (sialic) acid analogue 2,3-didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid **1.20** (Figure 1.12) has been



1.20

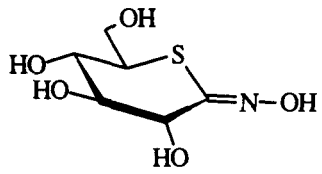
Figure 1.12 Anti-influenza drug candidate

synthesised[54] and demonstrated to be an extremely potent competitive influenza virus sialidase inhibitor, with a K_i value of 2×10^{-10} M.[11,55] Compound **1.20** was also found to be a potent inhibitor of influenza virus replication in cell culture and in animal models and is a potential anti-influenza drug. This result represents one of the strongest enzyme inhibitions so far observed for a carbohydrate-based inhibitor and exemplifies the potential for mechanism-based drug design. The imine-linked pseudotetrasaccharide acarbose also shows very potent enzyme inhibition,[14] with a K_i (K_d) value of $<6 \times 10^{-12}$ M^{-1} [56] and a binding constant of close to 10^{12} M [57] against glucoamylase from *Aspergillus niger*, and is by far the largest reported binding constant between a protein and a carbohydrate. The inhibitory activity of various pseudosugars (carba analogues) are discussed in detail in Chapter 3.

1.3.ii.b Sulfur Analogues

Thiosugar analogues with sulfur in the ring have been classified as substrate analogues rather than transition-state analogues[58] due to the inability of the ring sulfur to

be protonated. Thiosugar analogues, therefore, often exhibit less potent biological activity than the corresponding azasugar analogues.[58] For example, the thiolactone oxime



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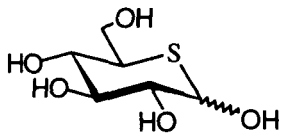

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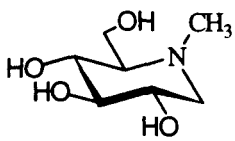
Figure 1.13 Examples of thioanalogues exhibiting potent glycosidase activity

1.21 (Figure 1.13) has been synthesised[58] and was found to be approximately 1000 times less potent against β-glucosidase than the corresponding aza analogue **1.13**. Although thioanalogues are poorer mimics of the transition-state structure than are azaanalogues, strong enzyme inhibition has been observed with a number of thiosugars. For example, 5-thioglucose **1.22** (Figure 1.13) has been shown to exhibit biological activity comparable to



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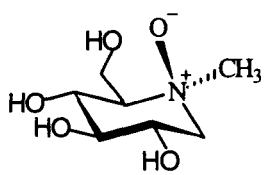


1.24

Figure 1.14 N-methylnojirimycin and the corresponding N-oxide

N-methyldeoxynojirimycin[27b] **1.23** with α-glucosidase from brewers yeast (K_i values, 7.50 x 10^{-4} M and 3.69 x 10^{-4} M, respectively), and stronger activity than the corresponding N-oxide **1.24** (Figure 1.14) (K_i >1.2 x 10^{-2} M).[27b] In addition, as will be described in greater detail in Chapter 2, 5-thio-α-L-fucose has been shown to be a very potent inhibitor of α-fucosidase.[59]

1.4 Thesis overview

As evident from the introduction in Chapter 1, thiosugars are important tools for understanding the structure-function characteristics of enzyme inhibitors. Although thiosugars tend to be generally less potent inhibitors than the corresponding nitrogen analogues, there are numerous examples of thiosugars with potent enzyme activity. In addition, based on the fact that 5-thioglucose is relatively non-toxic (LD_{50} = 14 g/Kg),[60] it is envisaged that thioanalogues will be less toxic than their nitrogen counterparts.

Chapter 2 describes the synthesis and biological activity of thioanalogues of alkyl kojibiosides and methyl maltoside with sulfur in the nonreducing ring and or interglycosidic linkage. The analogues are synthesised by glycosylation of the appropriate trichloroacetimidate donor and thiol acceptor, catalysed by triethylsilyl trifluoromethane-sulfonate. The biological activity of the analogues is compared and the effects of replacement of the oxygen atom in the interglycosidic linkage and/or the ring by sulfur are discussed.

Chapter 3 describes the synthesis and biological activity of methyl kojibioside and methyl maltoside analogues in which the oxygen atom of the nonreducing ring is replaced

by sulfur and the interglycosidic oxygen atom is replaced by nitrogen. Although such compounds are substrate analogues and not transition-state mimics, placement of a basic nitrogen on the anomeric carbon should give rise to an increase in biological activity. The nitrogen analogues described in Chapter 3 are a new class of disaccharide and it is expected that the aglycon moiety, being a sugar, should give rise to greater specificity for a particular glycosidase.

The conformational analysis of carbohydrates is an important tool for elucidating the three-dimensional structure in solution. Chapter 4 describes the results obtained for the conformational preferences of an S,S-maltoside analogue, studied by comparison of experimental and theoretical NOE curves using a combined NMR spectroscopic/molecular mechanics protocol. Also presented for this compound is a molecular structure of the analogue derived from X-ray crystallographic studies. Transferred NOE NMR studies of this compound in the presence of the enzyme, glucoamylase G1 are used to derive the conformation of the ligand when bound to the enzyme. Similar transferred NOE studies are also presented for the S,N-maltoside analogue bound to glucoamylase G1.

CHAPTER 2

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL GLYCOSIDASE INHIBITORS. 1: THIOANALOGUES OF DISACCHARIDES

2.1. Introduction

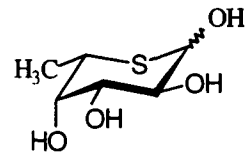
Carbohydrates containing sulfur in place of oxygen (thiosugars), unlike their amino counterparts, are rare in nature. The only naturally occurring 5-thiosugar, 5-thio-D-mannose, has been isolated fairly recently from the marine sponge *Clathria pyramida*.[61] Sulfur, being in the same group in the periodic table as oxygen makes the substitution of oxygen by sulfur ideal for the study of carbohydrates with slightly altered physiochemical characteristics. Thiosugars, by virtue of their physical resemblance to the naturally occurring sugar while at the same time possessing unique chemical characteristics, often show interesting biological properties.[62] The application of thiosugars as important synthetic intermediates will not be covered in this thesis.

Thiosugars may be divided into three basic classes: 1) those containing sulfur in place of one or more of the hydroxyl groups, 2) those in which the glycosyl or interglycosidic oxygen is replaced by sulfur, and lastly, 3) those in which the ring oxygen is replaced by sulfur. Of course, a thiosugar may also be a combination of two or more of the aforementioned classes. While carbohydrates in all three classes show interesting biological behaviour, this chapter will be concerned primarily with the synthesis and biological properties of thiosugars in which the ring and/or interglycosidic linkage is replaced by sulfur (classes 2 and 3).

2.2. Biological Properties of Thiosugars

2.2.i Enzyme Inhibitors

The synthesis of many 5-thio-sugars has been reported[63] and several have been shown to possess very interesting biological behaviour. For example, 5-thio-D-glucose **1.22** (Figure 1.13), first synthesised by Feather and Whistler in 1962,[63a] was found to be an inhibitor of the transport of D-glucose in many tissues,[64] and an inhibitor of the release of insulin.[65] Subsequently, low, non-toxic doses of 5-thio-D-glucose were found to induce temporary sterility in male mice and rats,[66] making this compound a prime candidate in the



5-thio-L-fucopyranose

2.1

Figure 2.1 A biologically active 5-thio-monosaccharide

search for a male contraceptive. 5-Thio-α-D-glucopyranose was reported to be a potent competitive inhibitor of α-glucosidase (Brewers yeast) with a K_i value of 7.5×10^{-4} M.[27b] 5-Thio-L-fucose **2.1** (Figure 2.1), synthesised from D-glucose,[63g] was found to show remarkable inhibitory effects on fucosidases from bovine epididymis and kidney ($K_i = 4.2 \times 10^{-5}$ M and 8.4×10^{-5} M, respectively).[63g]

Thioglycosides are hydrolysed significantly slower than their oxygen counterparts.[67] Thioglycoside glycomimetics of glycohydrolase substrates are often competitive inhibitors of the hydrolysis of the natural substrate by the enzyme due to their physical similarity but greater resistance towards enzymic hydrolysis. The analogy of the physical shape of thioglycosides to the natural substrate, and their relatively high K_i values, has resulted in the classification of such compounds as substrate,[58] or ground state analogues, as opposed to those that mimic the putative transition state of the substrate/active-site complex. Although thioglycoside analogues tend not to exhibit as potent inhibitory properties as many of the nitrogen-containing sugars, many of which are transition-state analogues, thioglycosides are still very important for assessing enzyme mechanisms and for mapping the active-site topography of many enzymes to assess structure-function relationships. For example, thio- and pseudothio-analogues of methyl α-maltotrioside **2.2** (Figure 2.2) have been synthesised by Blanc-Meusser *et al.*[68] as potential competitive inhibitors of pancreatic alpha-amylase. Methyl 4,4'-dithio-α-maltotrioside **2.3** was found to be resistant towards hydrolysis by alpha-amylase, although its activity (K_i = 9 mM) was lower than that for the corresponding trisaccharide **2.2** (K_i = 3 mM). The spacer-modified disaccharide **2.4** (Figure 2.2) with an acyclic, polar, flexible spacer, designed as a (thio)mimic of the trisaccharide **2.2**, was also found to be resistant to the enzyme with a similar affinity (K_i = 17 mM) to that of maltose (K_i = 20 mM). This latter result was suggested to be a demonstration of the so called "clustering effect",[69] in which the covalent linking of two or more potential ligands of a multisite substrate by a

flexible spacer of appropriate length results in an enhancement of the affinity towards the enzyme.

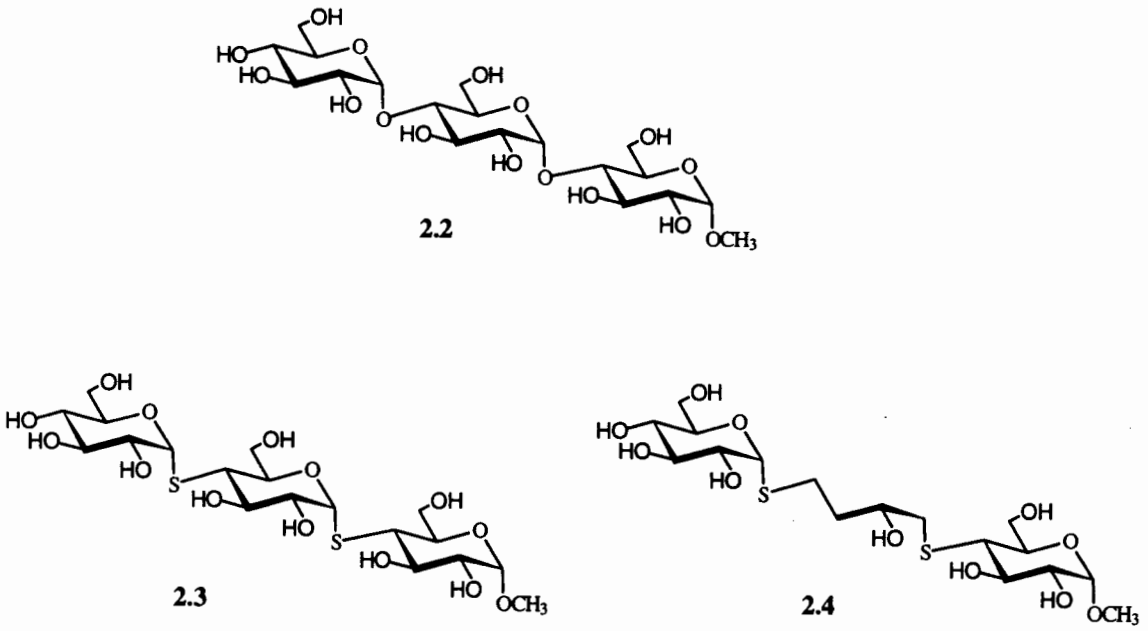
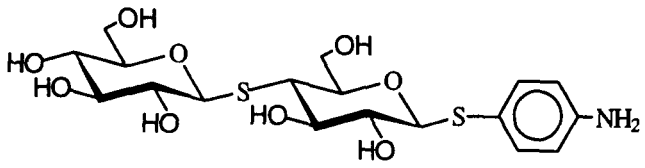


Figure 2.2 Thio- and pseudothio-analogues of **2.2** exhibiting inhibition of alpha-amylase

Cellulases, a family of enzymes that are responsible for the hydrolysis of cellulose into glucose and cellobiose, were found to be strongly inhibited by thio analogues of β-cellobioside and cellotrioside.[70,71] Thus, 4-aminophenyl 4-S-β-glucopyranosyl-1,4-dithio-β-D-glucopyranoside **2.5** and the analogous trisaccharide **2.6** (Figure 2.3) were found to be competitive inhibitors of the hydrolysis of a specific substrate, 4-methylumbelliferyl β-lactoside, by cellobiohydrolases (K_i = 24 and 6.5 μM, respectively);[70] the K_m for the substrate was 80 μM.



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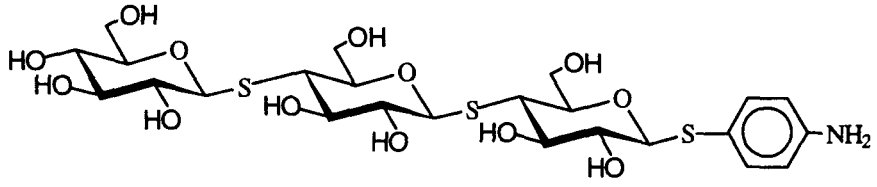

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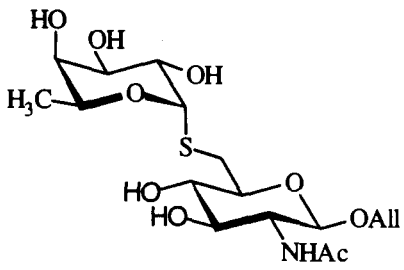
Figure 2.3 Cellobiohydrolase inhibitors[70] which have been coupled to Sepharose gel for use in affinity columns

In a study by Hashimoto *et al.*[72] a series of thio-linked α-L-fucopyranosyl disaccharides **2.7**, **2.8**, **2.9** and **2.10** (Figure 2.4) were synthesised for the purpose of characterising α-L-fucosidases from bovine epididymis and kidney. The most potent inhibition of bovine kidney α-L-fucosidase was observed with the α-1,3-linked disaccharide **2.9** (K_i = 0.65 mM, competitive inhibition). For **2.7**, **2.8** and **2.10** the inhibition (all mixed inhibition) K_i values were 4.5 mM, 4.9 mM and 0.88 mM, respectively. Comparable results were observed with α-L-fucosidase from bovine epididymis. Of interest to note is the comparison of the K_i values of **2.9** with its 5-thio-analogue (K_i = 30 μM against bovine kidney; see Section 2.2)



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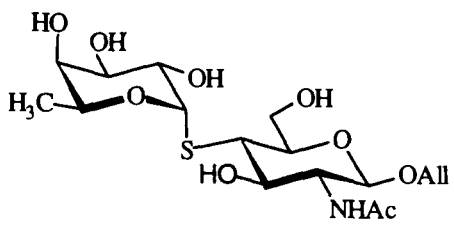

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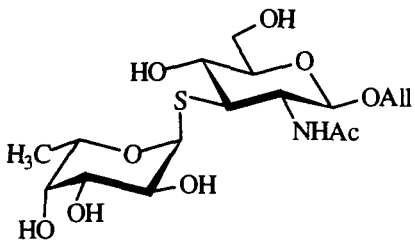
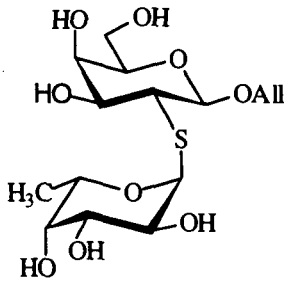


2.9



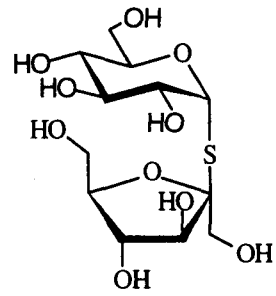
2.10

Figure 2.4 α-Fucosidase inhibitors[72]

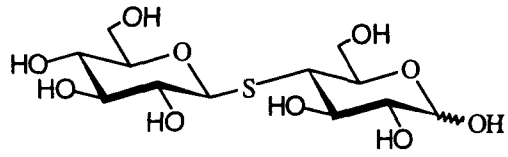
2.2.ii Enzyme Inducers

1-Thioglycosides[73] and various thio-linked oligosaccharides[74] have been shown to be excellent inducers of enzyme biosynthesis. The addition of enzymatically inert substrates (inhibitors) to enzyme cultures has been shown to induce the biosynthesis of enzymes specific to that substrate.[73] Inducers, therefore, are very important for the efficient production of enzymes from cultures. Since it has been shown that alkyl 1-thio-β-D-galactosides were able to induce the production of β-galactosidase in *E. coli*,[75] thio-linked oligosaccharides have been synthesised in the search for efficient inducers of a

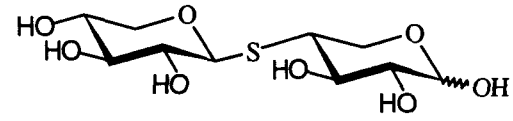
variety of enzymes. For example, 1-thiosucrose **2.11** (Figure 2.5) was found to be an inducer of levansucrase biosynthesis by *Bacillus subtilis* and a competitive inhibitor of the enzyme (K_i = 10 mM).[76] In related studies, thiocellobiose **2.12**[77] and 4-thioxylobiose **2.13**[78] (Figure 2.5) were found to be inducers of cellulase and xylanase, respectively.



2.11[76]



2.12[77]



2.13[78]

Figure 2.5 Enzyme inducers

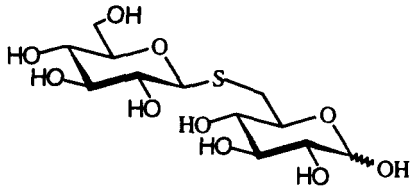
2.2.iii Affinity Chromatography

Thioglycosides which are reversible inhibitors of specific enzymes can be used as ligands for the purification of these enzymes by affinity chromatography,[79] thus simplifying the often tedious purification procedures. Inhibitors which are chemically attached to a

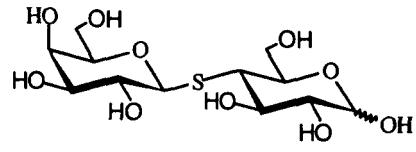
solid matrix can bind the associated enzyme from a crude enzyme preparation, after which they can be eluted in pure form from the affinity column. Thus, β-galactosidase from *E. coli* has been purified by affinity chromatography with β-thiolactose attached to γ-globulin[79a] and with p-aminophenyl β-D-thiolactopyranoside attached to agarose.[79b] Orgeret *et al.*[70] have used aminophenyl thio-oligosaccharides such as **2.5** and **2.6** (Figure 2.3), coupled to a Sepharose gel, as affinity columns for the purification of cellobio-hydrolases from crude commercial extracts.

2.3 Synthesis of Thiosugars

Several methods have been published for the synthesis of thio-linked oligosaccharides; these include an S_N2-type reaction involving the action of a thiolate anion on a glycosyl halide,[80] the displacement of a leaving group by a 1-thio-glycopyranose,[80] and the condensation of a 4-thio-glucopyranoside with a 1,6-anhydro glucopyranoside.[81] The first two of these methods have been used to synthesise thiogentiobiose[80] **2.14** (the first thioglycosidic analogue of a reducing disaccharide), thiolactose[82] **2.15** (Figure 2.6), and thiocellobiose **2.12**.[83] The second method has been used extensively by Defaye, Driguez and coworkers since 1982 for the synthesis of a thiomaltoside[83] **2.16**, various α-amylase inhibitors **2.3**, **2.4**[68] and **2.17**[84] (Figure 2.6) and 4-thiocellooligosaccharides[70] **2.4** and **2.5**. Hashimoto *et al.*[72] have used the displacement reaction by a 1-thio-α-L-unit of 6- and 4-sulfonates to give **2.7** and **2.8**, and the ring opening reactions of 2,3-aziridines and 2,3-oxiranes to give **2.9** and **2.10**, respectively.



2.14



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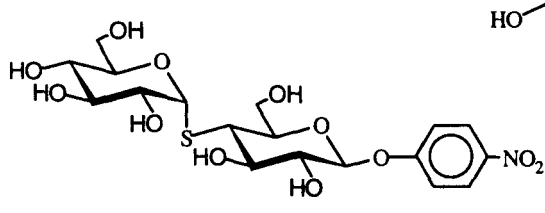

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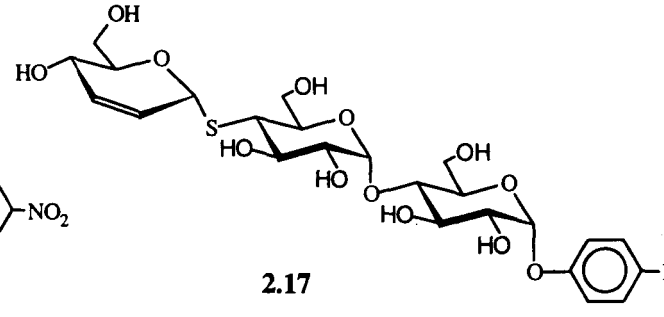


2.17

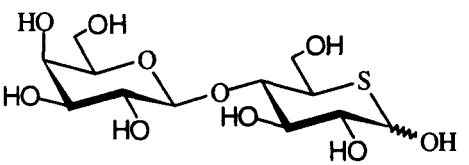
Figure 2.6 Thiosugars that have been synthesised using various methods

The efficiency of the displacement method relies heavily upon the propensity of a particular ring-carbon-sulfonate to displacement/inversion. Thus, all of the afore-mentioned examples utilise the displacement of either 4- or 6-sulfonate derivatives of D-galactopyranosides or D-glucopyranosides, respectively (with the exception of the ring opening of the 2,3-aziridine and 2,3-oxirane[72]). The corresponding S_N2 displacement reactions of 2-sulfonates of α-D-mannopyranosides (and glucopyranosides) do not proceed readily.[85,86] This has been attributed, in part, to stereoelectronic effects in the transition state.[85] However, the efficiency of the displacement reaction by thiols has

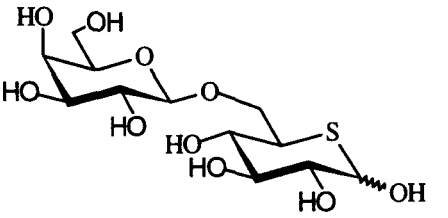
recently been improved *via* the *in situ* S-deacetylation and activation of 1-thiols,[84] although the method relies upon the use of the undesirable solvent, HMPA.

2.3.i Synthesis of Higher-Order 5-Thiosugars

This chapter has thus far dealt with the chemistry and biology of 5-thiosugars, thioglycosides and thio-linked di/oligosaccharides. Higher-order 5-thiosugars, for example, disaccharides, in which the ring oxygen of the reducing or non-reducing ring is replaced by sulfur are more elusive and their syntheses have only recently been reported. The first syntheses were those by Wong *et al.*[87,88] in which the disaccharides **2.18**[87] and **2.19**,[87,88] (Figure 2.7) both of which contain 5-thio-D-glucopyranose as the reducing sugar, were synthesised using an enzymatic approach.



2.18

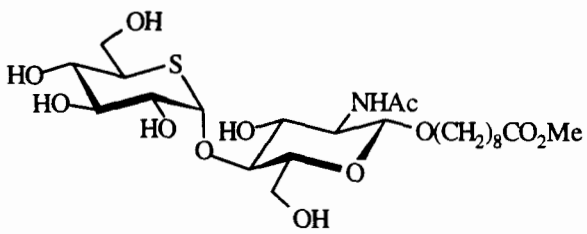


2.19

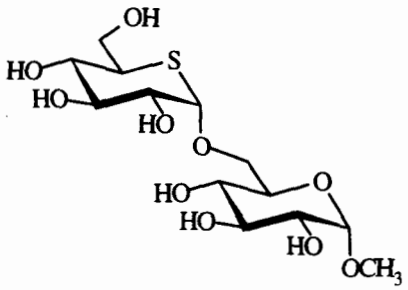
Figure 2.7 First disaccharide analogues containing sulfur in the reducing ring

The synthesis of a disaccharide with sulfur in the ring of the nonreducing sugar was first reported by Yuasa *et al.*[89] The disaccharide 8-(methoxycarbonyl)octyl 5'-thio-N-acetyllactosamine **2.20** (Figure 2.8) was synthesised by a chemo-enzymatic method which

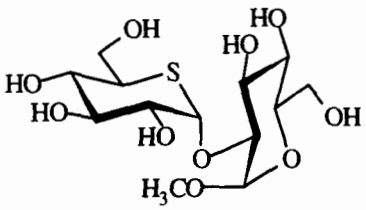
utilised the transfer of an unnatural UDP-5-thiogalactoside by a galactosyltransferase. This report was followed closely thereafter by Hashimoto *et al.*[90] who reported a new method for the synthesis of a sulfur-in-the-nonreducing-ring disaccharide, methyl 5'-thio-α-isomaltoside **2.21** (Figure 2.8), *via* an acyclic precursor.



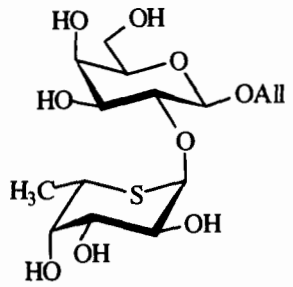
2.20[89]



2.21[90,91]



2.22[91]



2.23[92]

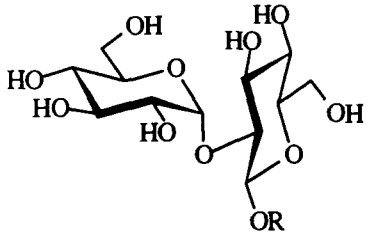
Figure 2.8 Existing thio-disaccharide analogues containing sulfur in the nonreducing ring

A direct chemical method for the synthesis of this type of disaccharide remained elusive, however, until Mehta *et al.*[91] reported the synthesis of the isomaltoside analogue **2.21** and

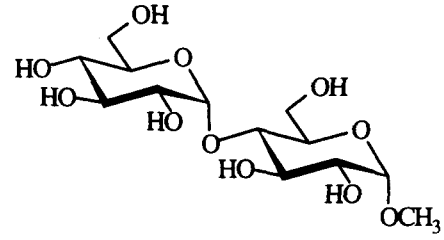
methyl 5'-thio-α-kojibioside **2.22** (Figure 2.8), which utilised the novel trichloroacetimidate of 5-thio-α-D-glucopyranose as a glycosyl donor. Subsequently, Hashimoto and Izumi[92] reported the synthesis of allyl 2-O-(5'-thio-α-L-fucopyranosyl)-β-D-galactopyranoside **2.23** (Figure 2.8), *via* chemical glycosylation; the compound showed potent inhibitory activity towards α-L-fucosidase (K_i = 30 μM).

2.4 Results and Discussion

Our group recently embarked upon a new programme of research to synthesise a series of novel disaccharide heteroanalogues in which the ring oxygen and/or interglycosidic oxygen atom are replaced by sulfur and/or selenium as potential glycosidase inhibitors, the foundation for which is presented in this chapter. The candidate disaccharides chosen were analogues of the kojibioside **2.24** and methyl maltoside **2.25** (Figure 2.9).



R = alkyl
2.24



2.25

Figure 2.9 Candidate disaccharides

The kojibioside heteroanalogues were synthesised as potential α-glucosidase inhibitors and the methyl maltoside heteroanalogues synthesised as potential glucoamylase inhibitors. The structures in Figure 2.10 represent some of the proposed target molecules at the commencement of the project.

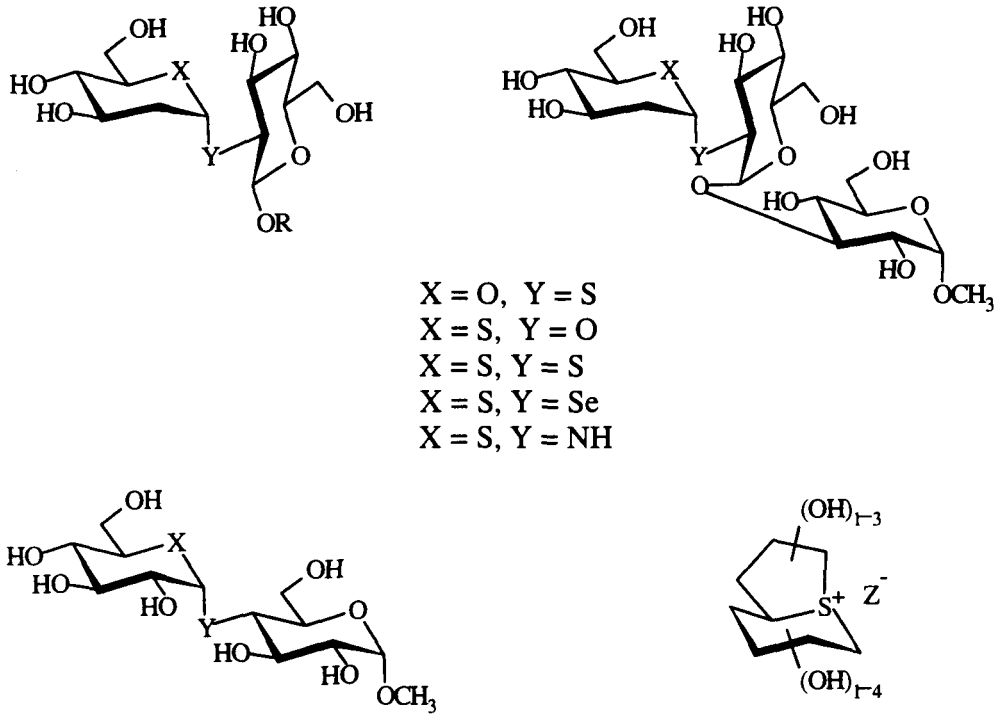
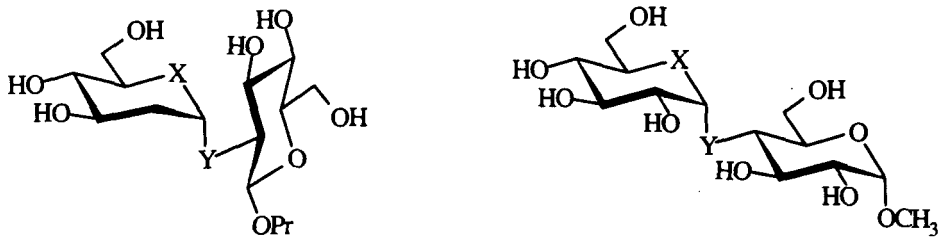


Figure 2.10 Proposed analogues

The trisaccharide analogues were also proposed since precedent existed that higher-order oligosaccharides exhibit greater inhibition.[93] The sulfonium ion was proposed as a transition-state analogue based on the premise that the presence of a permanent positive charge would lead to greater inhibition, since the protonated (cationic) form of the

inhibitor is assumed to be the active species.[23] Precedent for this assumption lies in the fact that the permanently cationic di-*N*-alkylated nojirimycin analogues are more potent inhibitors than their non-alkylated, neutral counterparts.[23] The target compounds that were synthesised in this study are indicated in Figure 2.11; the nitrogen heteroanalogues **3** and **5** are discussed in Chapter 3.



1 X = O, Y = S
2 X = S, Y = S
3 X = S, Y = NH

4 X = S, Y = S
5 X = S, Y = NH

Figure 2.11 Synthesised target compounds

2.4.i Synthesis of Sulfur Analogues of n-Propyl Kojibioside

The first target molecule was the kojibioside analogue **1**,[94] with the interglycosidic oxygen replaced by sulfur. Retrosynthetic analysis gave several possibilities. The most widely used method for the synthesis of similar compounds involves the S_N2 displacement of a sulfonate group by an anomeric thiolate. Thus, for **1** we would require the displacement of a 2-sulfonate of an α- or β-mannoside (Figure 2.12), or in the case of the

trisaccharide, the displacement of a 2'-sulfonate of an α-D-Man*p*-(1-3)-α-D-Glc*p*-OMe disaccharide. Earlier attempts at the displacement of the 2-sulfonate of the less stereoelectronically hindered β-D-mannopyranoside were found to proceed poorly with carbohydrate nucleophiles.[95] Our approach, based on more traditional glycosylation methodology, was to synthesise a 2-thio-glucopyranoside which, to the best of our knowledge, has not been reported as a glycosyl acceptor in glycosylation reactions with an appropriate glycosyl donor (Figure 2.12).

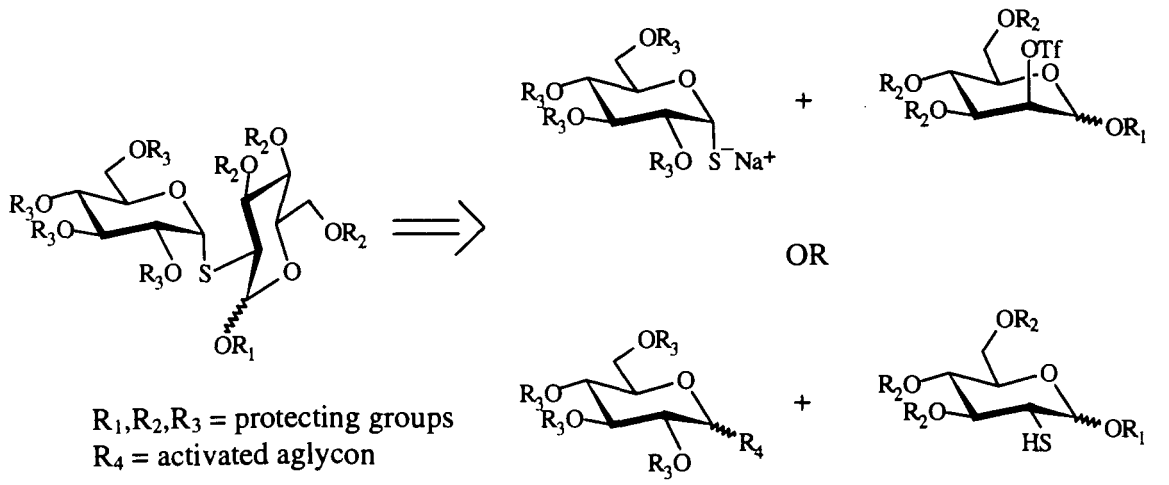


Figure 2.12 Retrosynthetic analyses for the thiodisaccharide **1**

Ideally, we would be able to synthesise a 2-thiol derivative of an α-D-glucopyranoside with the aglycon being either methyl or an α-D-glucopyranosyl unit. This would be quite feasible *via* manno-orthoesters[96] or the corresponding halide[97] to give

the α-glycosides. A problem arises in that the 2-triflate of α-D-mannopyranosides is difficult to displace. Attempted displacements using thioacetate or more powerful sulfur nucleophiles proceeded in low yields, giving predominantly the α/β unsaturated ketone, as Vos *et al.* have also found.[86] For example, treatment of methyl 3,4,6-tri-O-benzyl-2-O-trifluoromethanesulfonyl-α-D-mannopyranoside **6** with potassium thioacetate (2 equiv.) in DMF at room temperature for 48 h yielded the α/β unsaturated ketone **7** (47%, ^{1}H NMR agrees with Vos *et al.*[86]) and the desired methyl 3,4,6-tri-O-benzyl-2-thioacetyl-α-D-glucopyranoside **8** (13%) (Figure 2.13).

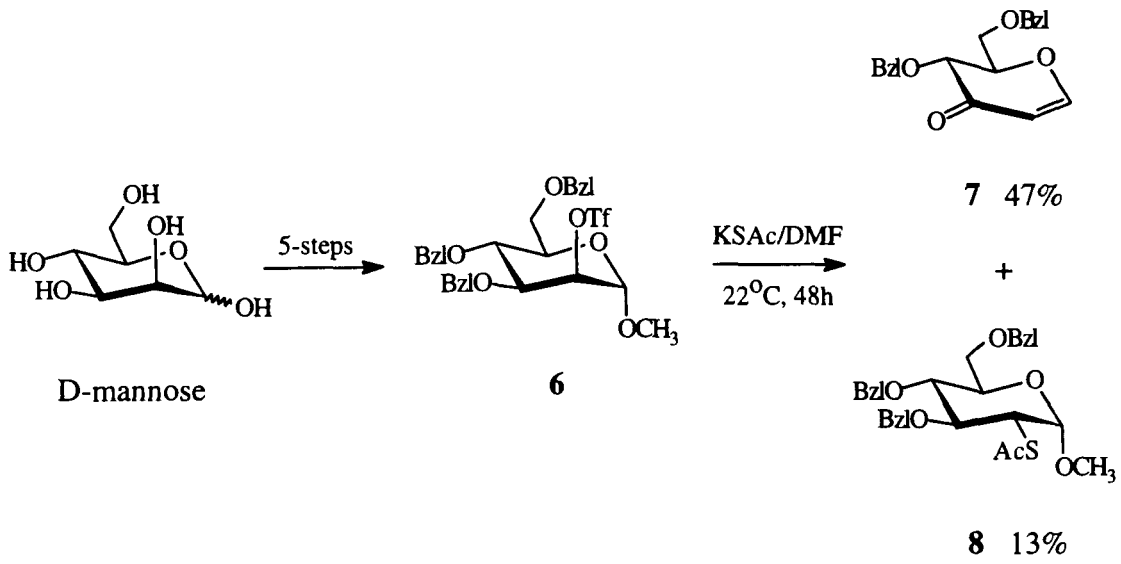


Figure 2.13 Attempted synthesis of the thiol precursor **8**

Further attempts using other sulfur nucleophiles such as potassium thiocyanate and sodium hydrogen sulfide did not lead to appreciable yields of the desired product.

Reaction of the triflate **6** with sodium hydrogen sulfide in DMF at room temperature gave the α/β unsaturated ketone **7** in quantitative yield, a plausible mechanism[86] for which is

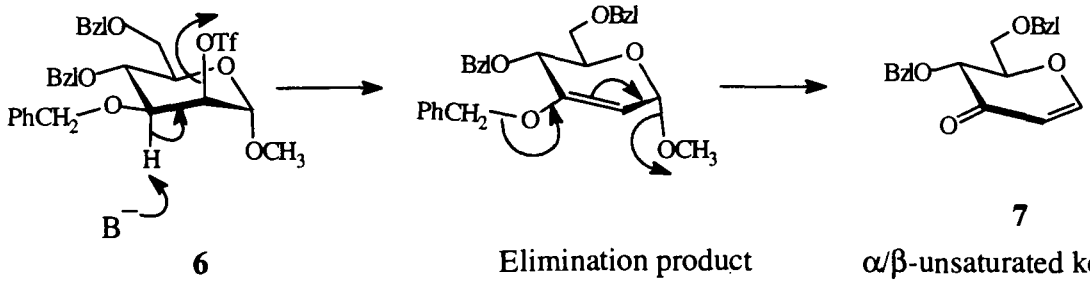


Figure 2.14 Possible mechanism[86] for the formation of the α/β-unsaturated ketone **7**

shown in Figure 2.14. We therefore concentrated our efforts on the synthesis of a 2-thio-β-D-glucopyranoside. We chose the β-allyl glycoside **12** as a suitable thiol glycosyl acceptor since the allyl group may be selectively deprotected for elaboration of higher order structures, for example, the trisaccharide. Thus, the triflate **10** was synthesised from the corresponding 2-hydroxy compound[98] **9** (obtained in 8 steps from D-mannopyranose) by the method of Pavliak *et al.*[99] using triflic anhydride in pyridine, in 82% yield (Figure 2.15). Of interest to note is the method by which the allyl group is introduced into the kinetically unfavourable β-position in mannose. The method used by Srivastava *et al.*[42] utilises the dibutyltin acetal of 3,4,6-tri-O-benzyl-D-mannopyranose, which can only form with *cis* oriented hydroxyl groups, to yield exclusively the corresponding β-allyl glycoside **9** when reacted with allyl

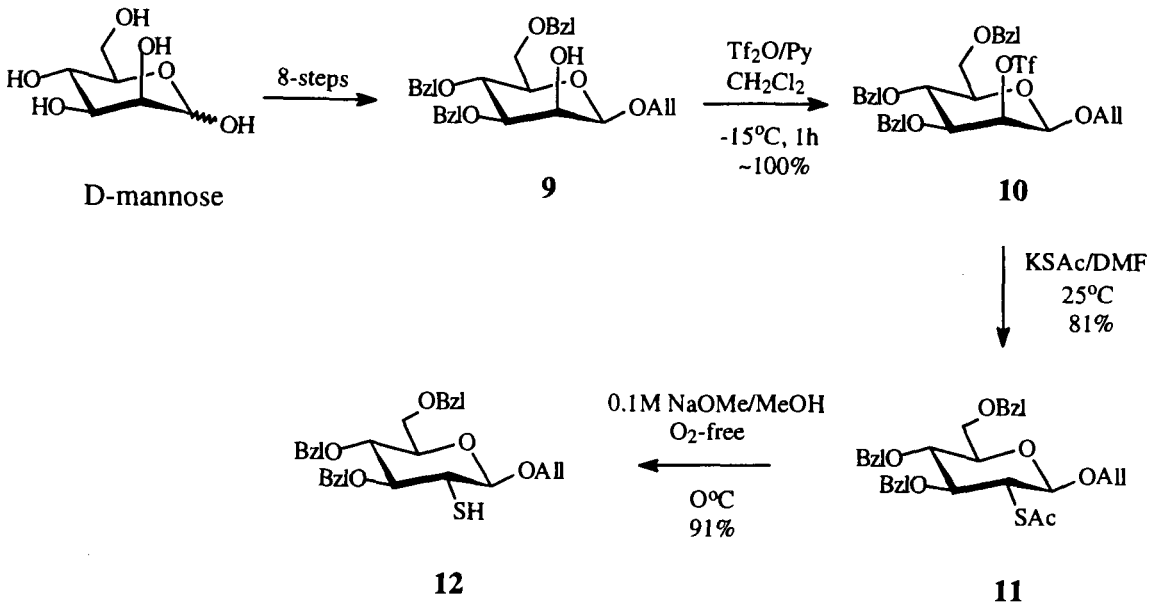


Figure 2.15 Synthesis of the thiol-glycosyl acceptor **12**

bromide. The inversion of the 2-triflate in **10** with sulfur nucleophiles to give a 2-thioglucopyranoside was best achieved with potassium thioacetate in DMF at room temperature to give the thioacetate **11** (81%) (Figure 2.15). None of the α/β-unsaturated ketone **7** was observed in this reaction. Selective deprotection of the S-acetate **11** was achieved in 91% yield with deoxygenated 0.1N NaOMe in methanol. In the presence of oxygen, the corresponding disulfide was formed in appreciable quantities. The thiol **12** was distinguishable from the disulfide by observation of the thiol resonance and coupling to H-2 in the ^{1}H NMR spectrum (2.05 [1H, d $J_{SH,H2}$ = 3.0 Hz, S-H]). Compound **12** is a convenient thio-glycosyl acceptor for the synthesis of oligosaccharides in which an inter-glycosidic sulfur atom is required; it can be stored indefinitely over P_2O_5 under vacuum at room temperature without appreciable oxidation to the disulfide. The thiol was also found to be stable towards oxidation during chromatography on silica gel, allowing purification if

necessary. Interestingly, the thiol is less polar on tlc plates relative to the thioacetate (R_f for **8** = 0.5, for **7** = 0.38 in hexane/ethyl acetate 5:1). The synthesis of the thiol **12** was also attempted *via* the corresponding 2-thiocyanate but this was found to be less efficient than the thioacetate route.

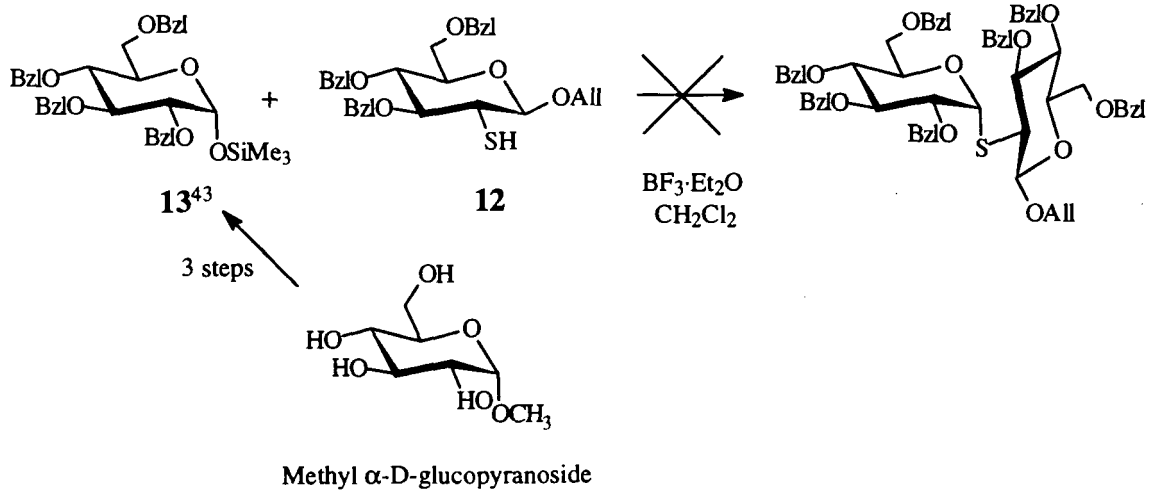


Figure 2.16 Attempted synthesis of a derivative of the thiodisaccharide **1**

We next attempted glycosylation reactions with the thiol **12** and other glycosyl donors. For example, attempted glycosylation reactions using the benzyl protected trimethylsilyl glycoside **13**[100] (Figure 2.16) as glycosyl donor using $BF_3.Et_2O$ as promoter were unsuccessful, giving rise to a multitude of products which were not isolated. Trichloroacetimidate donors developed by Schmidt *et al.* were next investigated. Glycosylation of the thiol **12** with either the α or β trichloroacetimidate of 2,3,4,6-tri-O-benzyl-D-glucopyranose **14**[101] using triethylsilyltriflate (TESOTf) as a catalyst gave rise

stereoselectively to the desired α-linked thiodisaccharide **15** as well as the α-amide **16**, resulting from the rearrangement of the trichloroacetimidates, a rearrangement also reported by Hoffmann and Schmidt.[102] Thus, the thiol **12** reacted with the β-trichloroacetimidate **14** in CH_2Cl_2 with 0.07 equivalents of TESOTf as catalyst to give the α-disaccharide **15** in 70% yield and the α-amide **16** (20%) (Figure 2.17). The use of an excess of the thiol in these reactions would be favourable except for the fact that the disulfide **17** (Figure 2.18), formed by oxidation of the thiol during the reaction or during neutralisation of the reaction mixture with base, is very difficult to separate from the disaccharide **15** by chromatography.

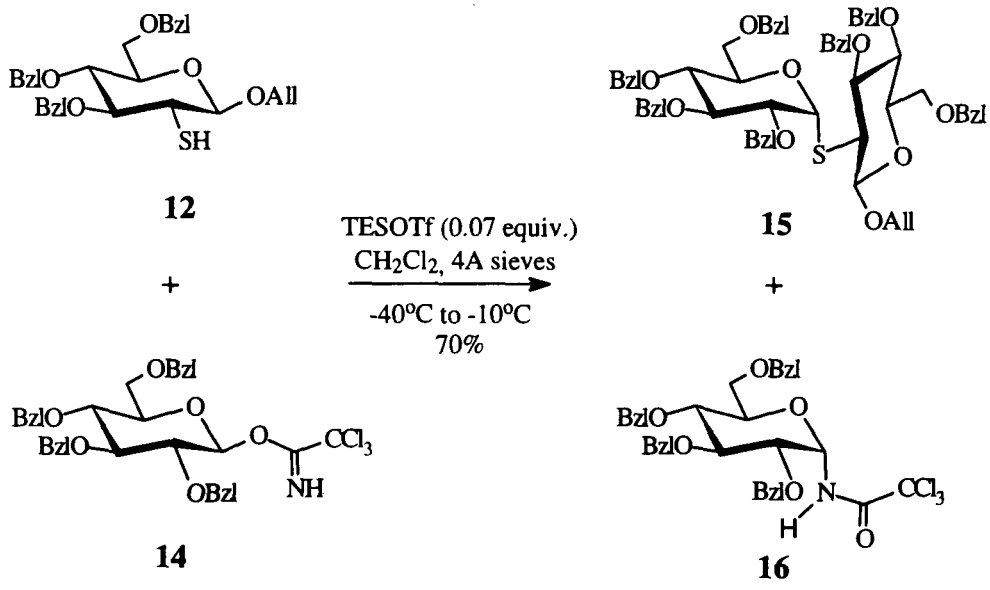
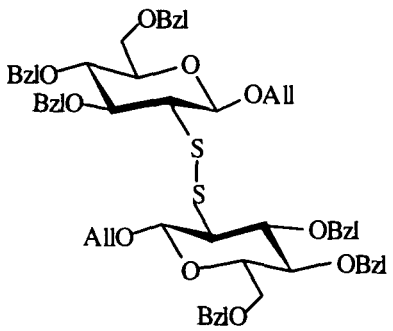


Figure 2.17 Synthesis of the thiodisaccharide **15**



17

Figure 2.18 Structure of the disulfide formed in glycosylation reactions

When solvents of a lower dielectric constant were used, the yield of the desired disaccharide was reduced with a concomitant increase in the yield of the amide. For example, glycosylation reactions using Et_2O and CCl_4 as solvents gave rise to the α-disaccharide **15** in 57% and 54% yield, respectively, compared with 70% in CH_2Cl_2. Addition of hexanes to CCl_4 resulted in a further increase in the formation of the amide. These results suggest a possible mechanism for the formation of the α-disaccharide **15** and the α amide **16**, that is shown (for the α-trichloroacetimidate only) in Figure 2.19. The β-trifluoromethanesulfonate glycoside intermediate, formed from the ion pair, resulting from the reaction of the α-trichloroacetimidate **14α** with triethylsilyl triflate, may be displaced by the thiol to give the α-disaccharide (mechanism a)). The polar solvent (CH_2Cl_2) stabilises the formation of the trifluoromethanesulfonate-silylated-amide ion pair, resulting in the formation of the β-trifluoromethanesulfonate glycoside, and hence, the preferential formation of the α-disaccharide. Conversely, in the presence of less polar solvents in

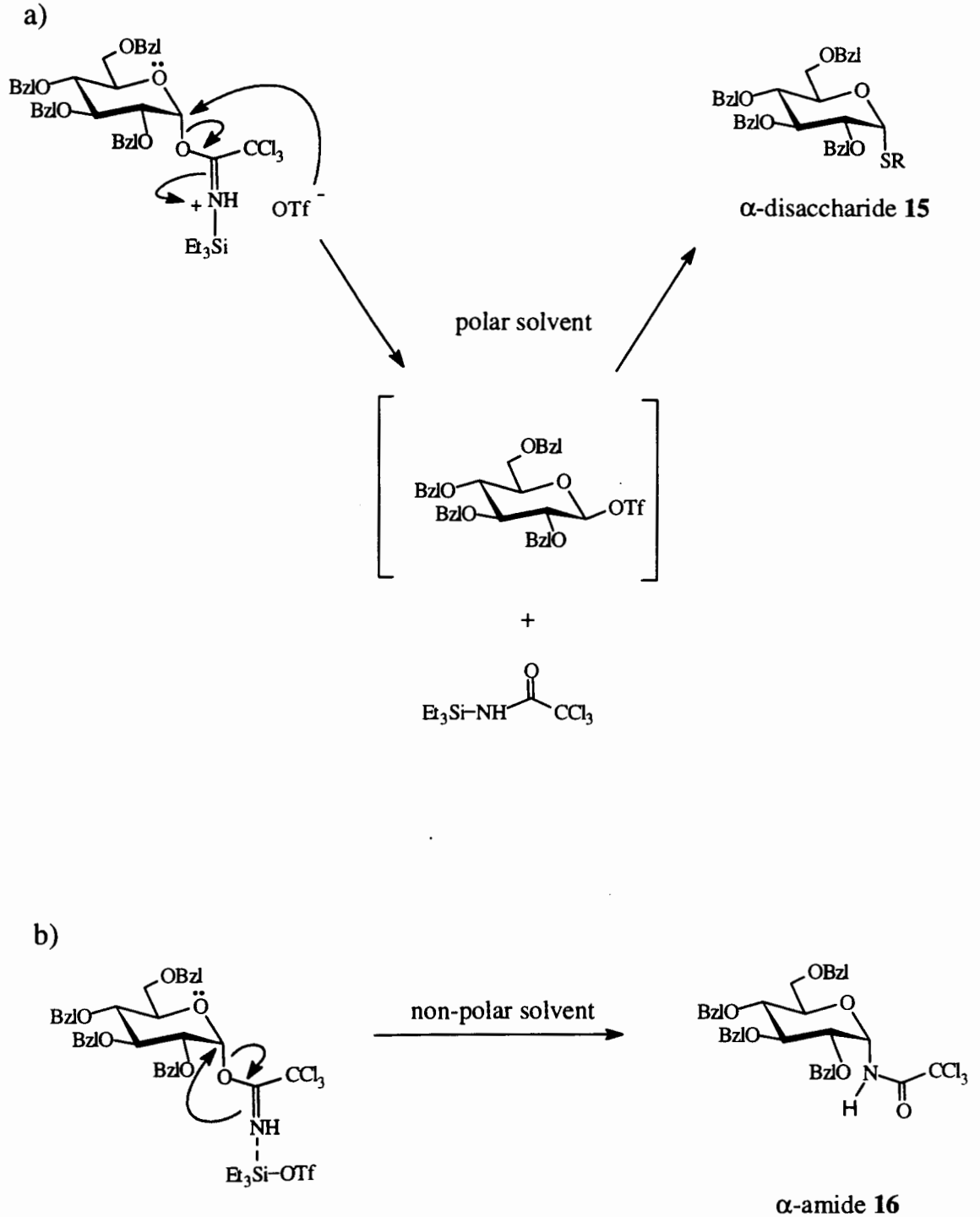
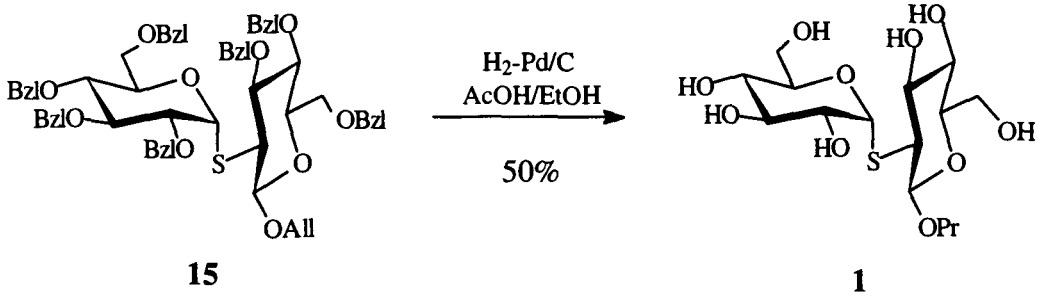


Figure 2.19 Suggested mechanisms for the preferential formation of a) the α-disaccharide **15** and b) the amide **16**

which the trifluoromethanesulfonate ion is less stable, a complex may be formed between the trichloroacetimidate **14α** and the triethylsilyl trifluoromethanesulfonate, followed by rearrangement and intramolecular delivery of the amide to the α-face of the ring, to give the α amide **16**.

The stereochemical integrity of the benzylated disaccharide **15** was confirmed by observation of the $^{1}J_{H1',H2'}$ and $^{1}J_{C1',H1'}$ coupling constants (5.6 Hz and 170 Hz, respectively) for the α-thioglycosidic linkage. Deprotection of **15** was achieved by hydrogenolysis in the presence of one equivalent of 10% palladium on activated carbon removing the benzyl ethers and simultaneously hydrogenating the allyl group to give the β-*n*-propyl glycoside **1** in 50% yield (Figure 2.20).



15 **1**

Figure 2.20 Deprotection to give the propyl 2-thio-β-kojibioside **1**

The large amount of palladium reagent required and the relatively low yield of the hydrogenolysis was presumably due to poisoning of the catalyst by the sulfur atom in the compound. Attempts to de-benzylate using Na/HMPA and Na/liq. ammonia were unsuccessful due to cleavage of the allyl glycoside to give the 1-O-acetates after

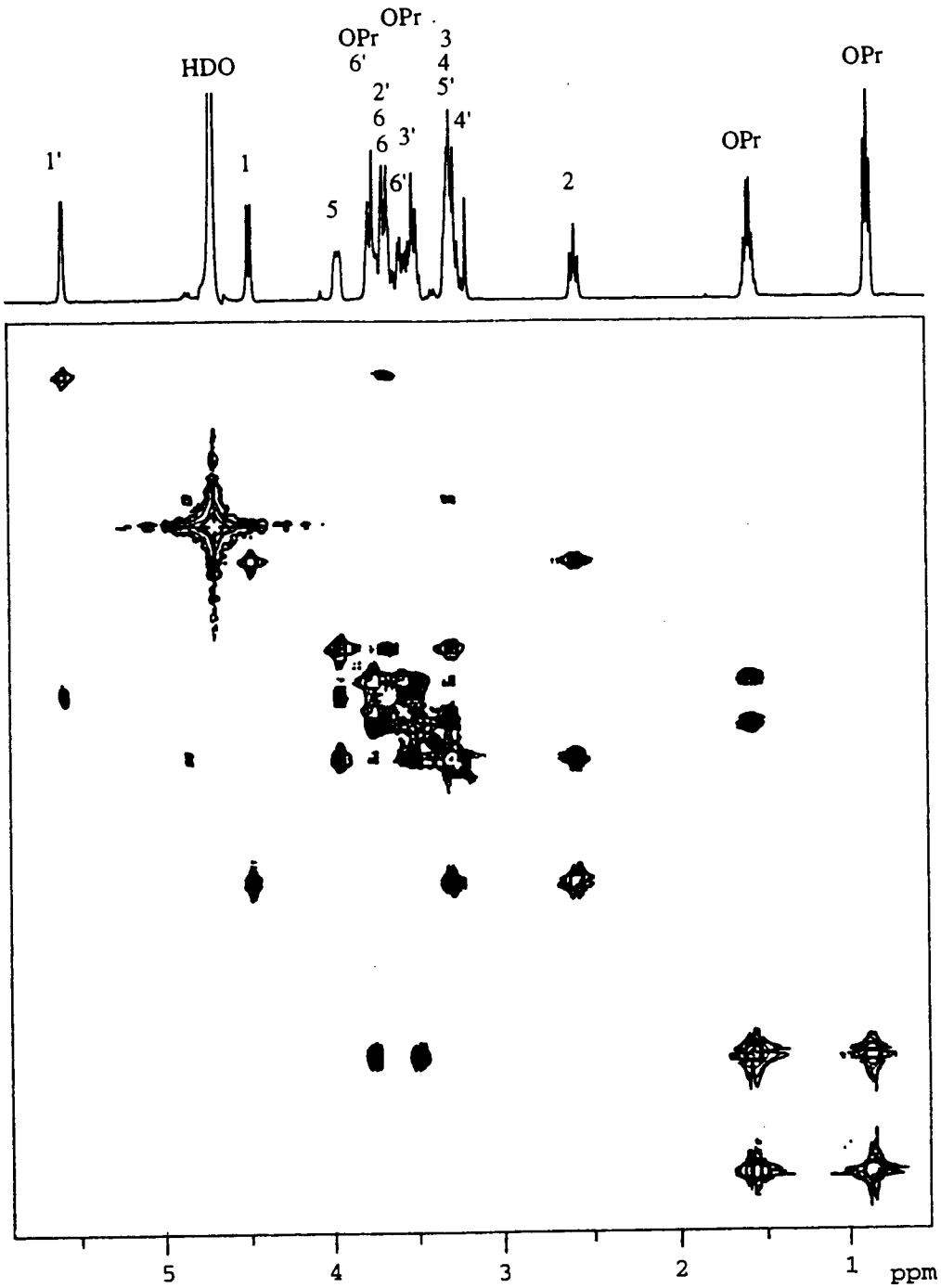


Figure 2.21 1D-^{1}H NMR and COSY spectrum of propyl 2-thio-β-kojibioside **1**

acetylation of the reaction mixture. Proof of the stereochemistry of the α-linkage of **1** was confirmed by observation of the $^{3}J_{H1',H2'}$ coupling constant (5.4 Hz) after assignment of all the proton resonances in the COSY spectrum (Figure 2.21). Further proof was obtained by observation of the $^{1}J_{C1',H1'}$ coupling constant in the $^{13}C\{^{1}H\}$ spectrum (171 Hz) after assignment of all ^{13}C resonances in the ^{13}C (decoupled) spectrum by correlation of all proton-carbon cross peaks in the ^{13}C-^{1}H chemical-shift correlated spectrum.

In order to ascertain the structural requirements of α-S-glycoside formation, in particular, the effect of a 2-acetate participating group on the control of stereochemistry of thioglycoside formation, the acetylated α-trichloroacetimidate **18**, prepared from the corresponding hemiacetals using potassium carbonate and trichloroacetonitrile,[101] was examined next as a glycosyl donor in reactions with the thiol acceptor **12**. When 0.14 equivalents of the catalyst, triethylsilyl triflate was used, both the α and β disaccharides **19** and **20** were formed in a ratio of 1:2.3 in 59% yield (Figure 2.22). However, when a lower amount of triethylsilyl triflate were used (0.07 eq.), the orthoester **21** was isolated as the only product (78%), even at room temperature (Figure 2.22). An isolated sample of the pure orthoester was rearranged in a separate experiment using 0.14 eq. of catalyst to give a 1:8 mixture of the α and β disaccharides. Schmidt *et al.*[103] have used the same trichloroacetimidates in glycosylation reactions with simple alkyl thiols, with $BF_3.Et_2O$ as catalyst, and have observed the exclusive formation of the β-glycoside.

The stereochemical integrity of **19** and **20** was confirmed by observation of the $^{1}J_{H1',H2'}$ coupling constants, 6.0 Hz for the α– and 10.0 Hz for the β-disaccharide, and by

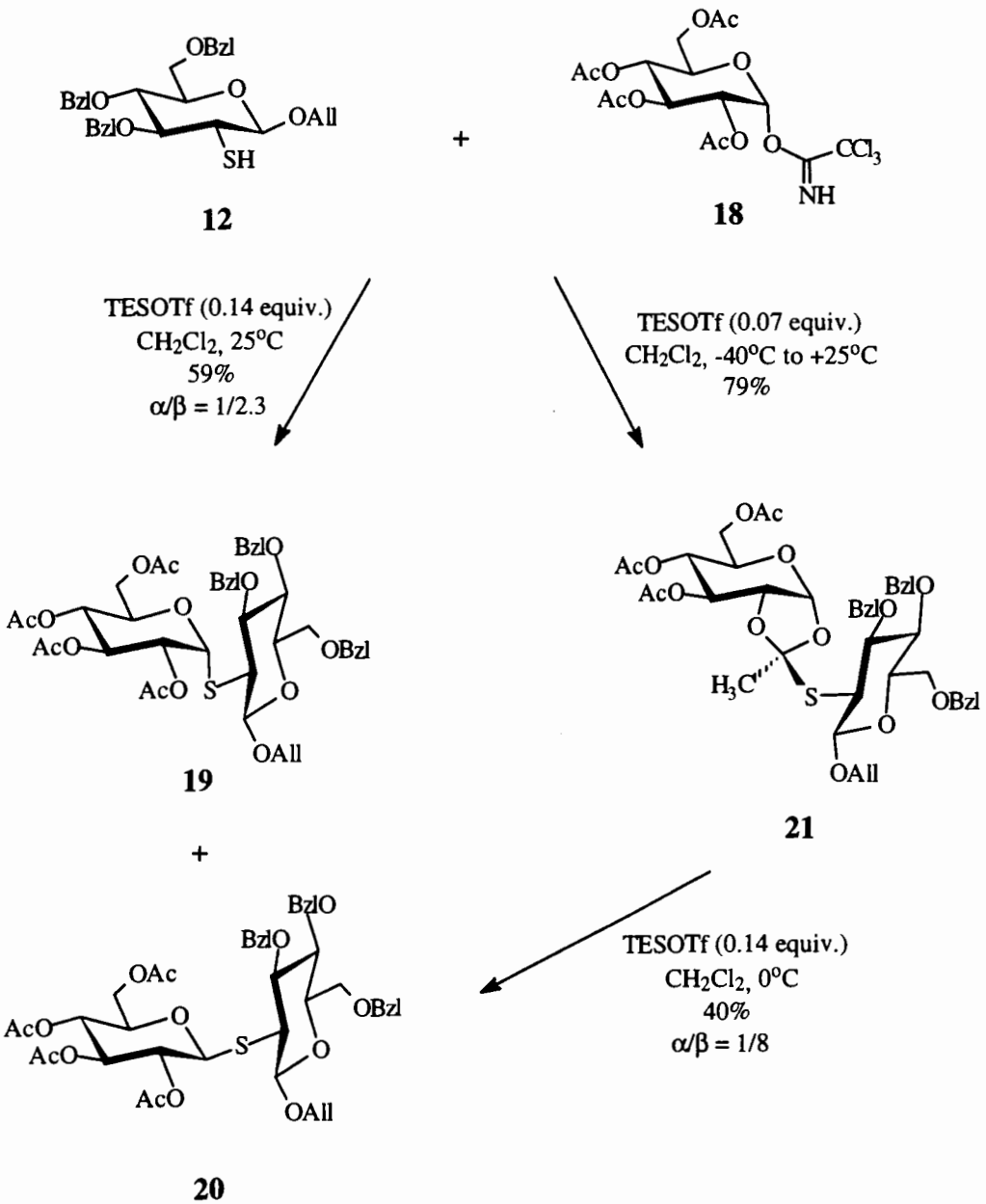


Figure 2.22 Synthesis of the α and β thiodisaccharides **19** and **20**, and the *exo* orthoester **21**

observation of the $^{1}J_{C1',H1'}$ coupling constants, 176 Hz for the α and 162 Hz for the β isomer. For the orthoester **21**, a NOESY experiment was performed in order to determine whether its configuration was either *exo* or *endo*. The NOESY contacts of interest were those between the orthoester-methyl and ring-protons of the two glucopyranose rings. The assignment of the orthoester-methyl protons and the acetate-methyl protons in the ^{1}H NMR (400 MHz) spectrum (2.08, 2.07, 1.96 and 1.95 ppm) was made on the basis of a ^{13}C-^{1}H chemical-shift correlated experiment. Thus, the ^{13}C orthoester-methyl resonance at 28.9 ppm (distinguishable from the acetate methyl carbon resonances at 20.7 ppm) correlated with the methyl proton resonance at 1.95 ppm in the ^{1}H NMR spectrum. NOESY contacts were observed between the orthoester-methyl protons and H-5' and H-2 ring protons,

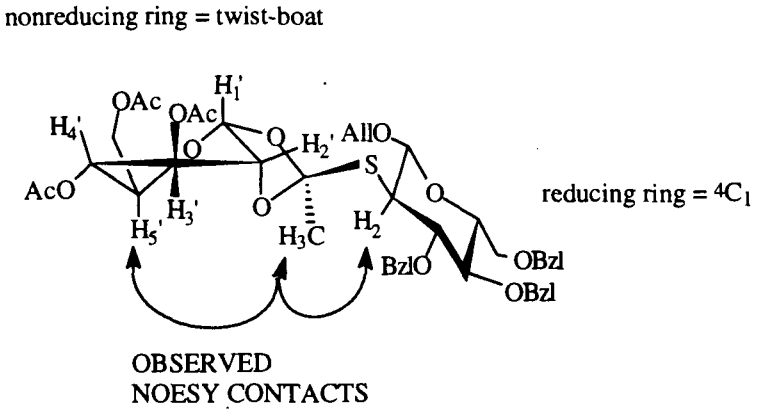


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Figure 2.23 Structure of the *exo*-orthoester **21**

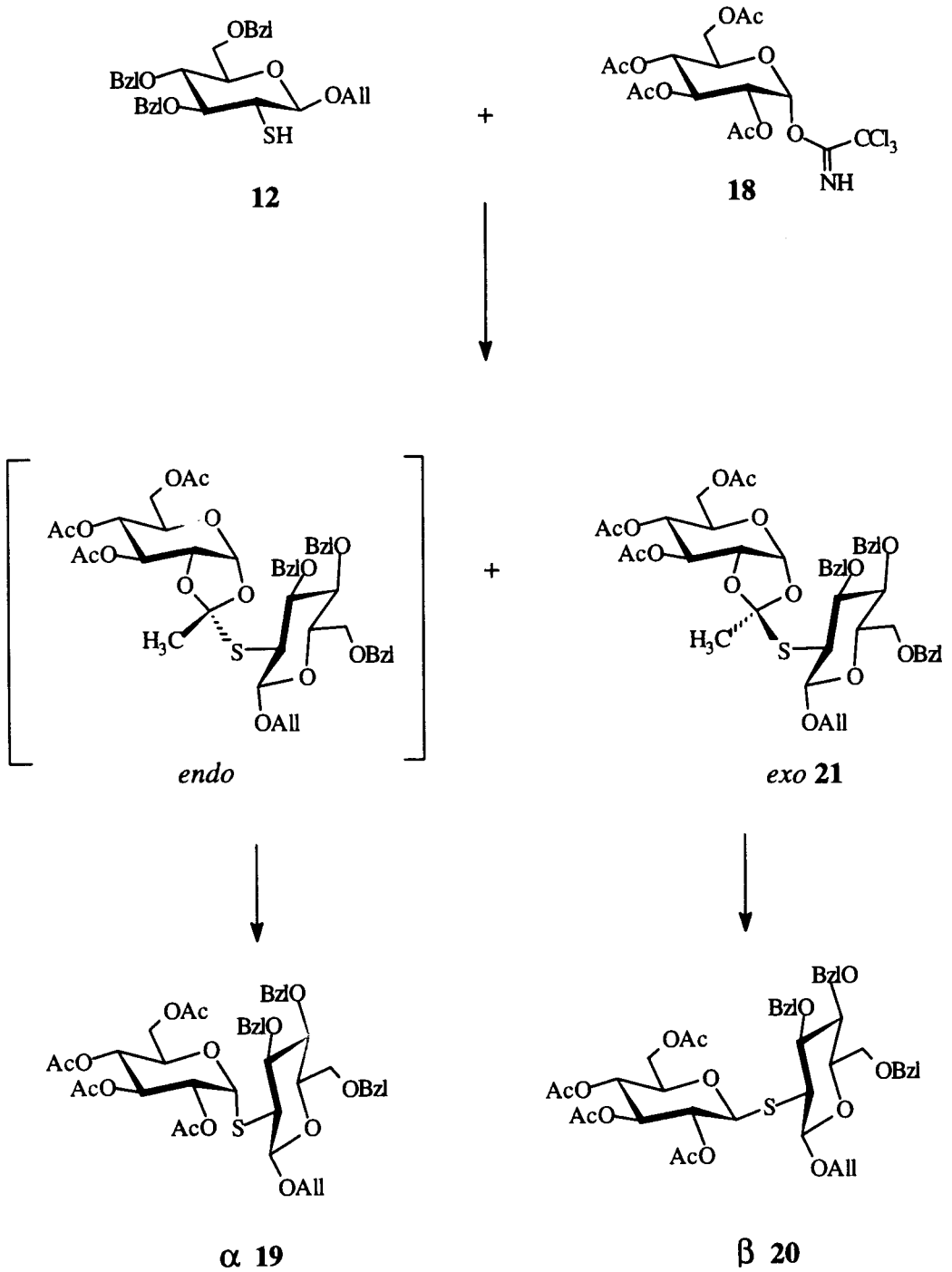


Figure 2.24 Possible route to α- and β-disaccharides *via endo*- and *exo*-orthoesters

suggesting the presence of the *exo* configuration. The expected contact with H-3' was not observed due to a changed conformation of the nonreducing ring of the orthoester. The $^{3}J_{H\text{-}H}$ coupling constants derived from the ^{1}H NMR spectrum indicated that the ring is in a twist-boat conformation (Figure 2.23). For example, $J_{2',3'} = 2.6$ Hz and $J_{3',4'} = 1.9$ Hz, and long-range coupling (1.0 Hz) was observed between H-2' and H-4'. The $^{1}J_{C\text{-}H}$ coupling constant for C-1' of the nonreducing ring was 182 Hz and agrees with typical values in the literature.[104] The reducing ring of the orthoester was determined to be in the expected $^{4}C_{1}$ conformation.

The experimental results for the formation of the orthoester, the *in situ* formation of α- and β-disaccharides and the rearrangement of isolated *exo*-orthoester to give predominantly the β-disaccharide suggests two possibilities. Firstly, a mixture of the *exo* and *endo* orthoesters is formed *in situ* which give the β- and α-disaccharides, respectively (Figure 2.24), and the *endo* orthoester is not stable enough to isolate (low temperature ^{1}H-NMR spectra of the reaction mixtures were too complex to be able to determine whether any *endo* orthoester was present). The second possibility (and considered the most likely), is that the α- and β-disaccharides are formed *via* different mechanisms; the β *via exo* orthoester rearrangement, and the α *via* the formation of a reactive β-trifluoromethanesulfonyl glycoside intermediate (Figure 2.24), as suggested in Figure 2.25.

Following the completion of this work, Defaye *et al.*[105] published the synthesis of 2-thiokojibiose and its β-anomer analogue, 2-thiosophorose. In their study Defaye *et al.*[105] synthesised the sodium salt of 2,3,4,6-tetra-O-acetyl-1-thio-α-D-glucopyranoside

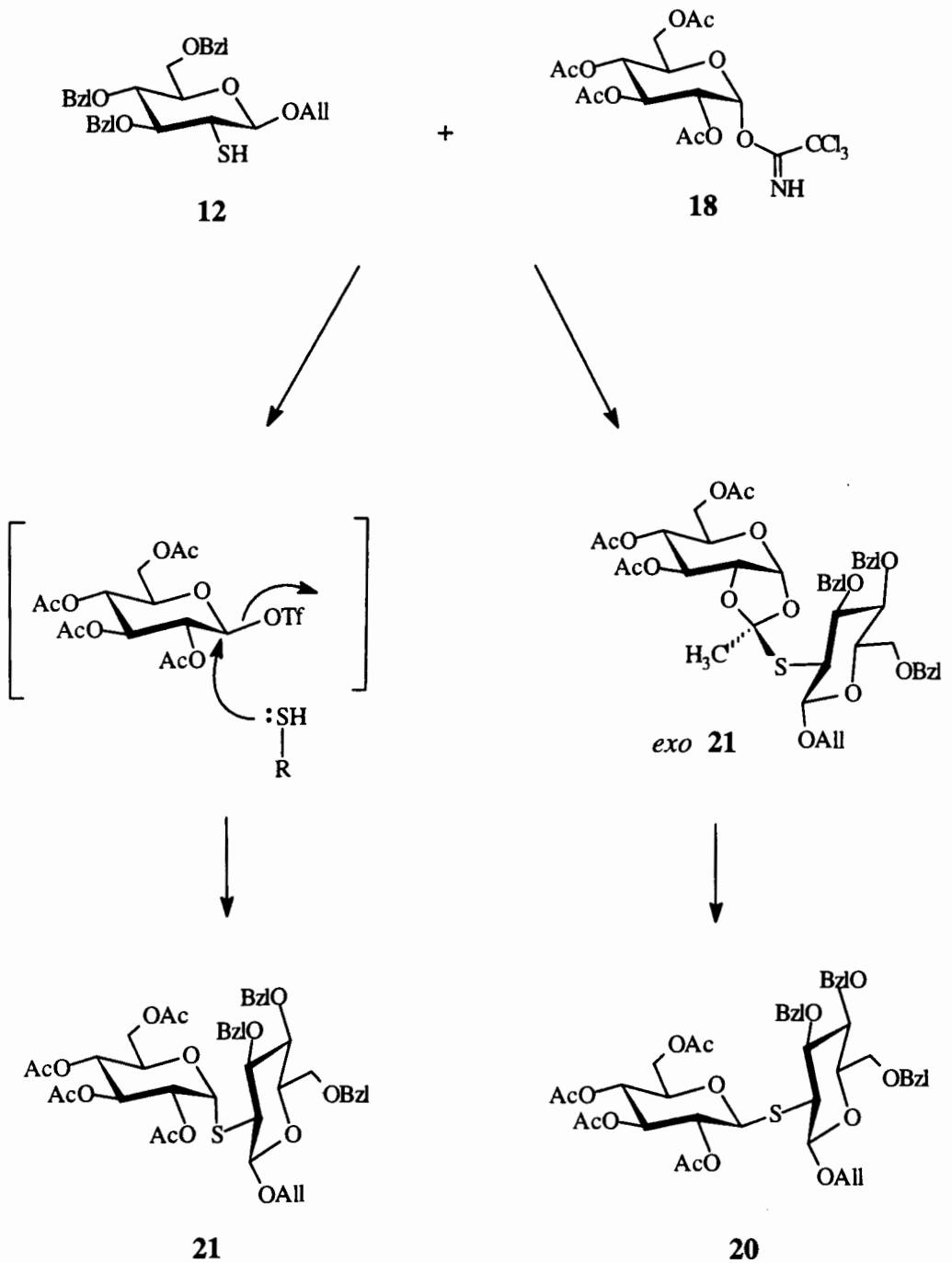


Figure 2.25 Possible route to α- and β-disaccharides *via* double displacement or *exo*-orthoester formation

was used to displace the triflate group in 1,3,4,6-tetra-O acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose in DMF to yield peracetylated 2-thiokojibiose in 87% yield. Deprotection in acidic or basic media was complicated due to the participating ability of the sulfur in reactions at the vicinal anomeric position,[105] as was first noticed during the attempted deprotection of similar compounds;[106] this contrasts with previous reports.[95] Deprotection was achieved instead *via* introduction of a β-allyl aglycon which was isomerised to the 1-propenyl glycoside after which Zemplen deacetylation and hydrolysis of the 1-propenyl group gave 2-thiokojibiose. This was followed shortly thereafter by a report by Comber *et al.*[26] describing the synthesis of various thio-linked disaccharides including several 2-thiokojibiose derivatives which were tested for their ability to inhibit HIV-induced cell killing and virus production in various cell lines. Of all the compounds tested only 2-thiokojibiose octaacetate and its 1-thioacetyl derivative were active, with IC_{50} values of 51 μg/mL and 48 μg/mL in CEM cells, respectively. Interestingly, with regard to the toxicity of thiosugars, all compounds were found to be nontoxic up to the highest dose tested (100 μg/mL).

Having established a readily accessible route to a suitable 2-thio-β-D-glucopyranoside and a method for synthesising disaccharide analogues in which the interglycosidic linkage is replaced by sulfur, we next turned our attention towards the synthesis of the 2,5'-dithiokojibioside **2** (Figure 2.11). Since a method had recently been developed in our laboratory for the chemical glycosylation of 5-thioglucose[91] to give disaccharides,[91,107] we decided to continue to use the same glycosylation procedures using 5-thioglucose trichloroacetimidates and the 2-thio-β-D-glucopyranosides developed for

the synthesis of the 2-thiokojibioside. Synthetic manipulations of 5-thioglucose are often fraught with complications due to the participatory nature of the ring sulfur with potential leaving groups.[108] The trichloroacetimidate of 5-thioglucose-tetraacetate[91] **22**, prepared in high yield in three steps from 5-thioglucose,[109] was therefore used as a glycosyl donor in glycosylation reactions.

Initial attempts at the synthesis of the dithiokojibioside **2** were made using allyl 3,4,6-tri-O-benzyl-2-thio-β-D-glucopyranoside **12**. Thus, in glycosylation reactions using triethylsilyl triflate as catalyst, a complex inseparable mixture was produced, whose ^{1}H NMR spectrum suggested a mixture of at least three different products of undetermined structure. It was therefore decided that a thiol glycosyl acceptor with ester protecting groups instead of benzyl ethers may simplify the reaction by firstly increasing the proportion of the α-disaccharide formed in the glycosylation reaction due to the expected lower reactivity of the ester protected thiol and secondly, by simplifying the deprotection which was envisaged, based on our previous experience,[94] to be complicated by the poisoning of the palladium catalyst by the two sulfur atoms in the compound.

The alcohol **23**[98] was acetylated using acetic anhydride in pyridine to give **24** followed by the simultaneous debenzylation and hydrogenation of the allyl to the propyl aglycon, with hydrogen and activated palladium on carbon, to give the triol **25**. Benzoylation of the triol using benzoyl chloride in pyridine then gave the propyl glycoside **26** in an 85% overall yield from **23** (Figure 2.26). Selective deprotection of the 2-acetate group was best achieved using anhydrous 1% HCl in methanol to give the desired 2-alcohol **27** in 89% yield. The selective deprotection of acetate esters in the presence of

benzoate esters can normally be achieved using 3% HCl in methanol.[110] However, at this concentration, substantial removal of the 6-benzoate was observed. Quantitative

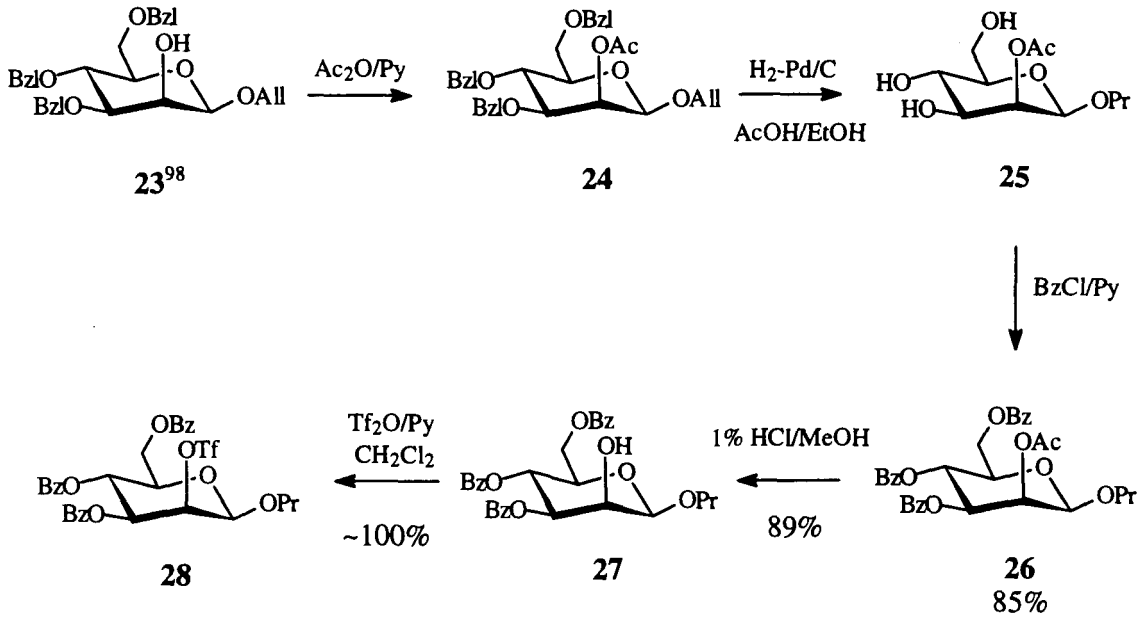


Figure 2.26 Synthesis of the triflate **28**

formation of the 2-triflate **28** was achieved according to a known procedure[99] using trifluoromethanesulfonic anhydride in CH_2Cl_2 containing pyridine (Figure 2.26).

The route to a suitable thiol precursor was examined next. The 2-thioacetyl glucopyranoside **29** was synthesised in 74% yield by stirring the crude triflate with potassium thioacetate in DMF for 1 h. The ^{1}H NMR spectra clearly indicated the change from a β-mannopyranoside derivative to a β-glucopyranoside derivative by observation of the change in $^3J_{H1,H2}$ coupling of the corresponding H-1 resonances, 1.0 Hz to 9.0 Hz. Selective deprotection of the 2-thioacetate **29** in the presence of the benzoate esters was

complicated due to the fact that S-acetates are more stable towards acid hydrolysis than are O-acetates. In order to hydrolyse the S-acetate, anhydrous, O_2-free, 2% HCl in $MeOH/CH_2Cl_2$ was required with heating to 40°C. However, under these conditions, the simultaneous hydrolysis of the 6-benzoate was observed if the hydrolysis of the S-acetate was allowed to reach completion. At room temperature, the reaction was slow (after 16 h the reaction was approximately 50% complete (by tlc)). Quenching the reaction with ice after heating the reaction mixture at 40°C for 3 h resulted in the isolation of the desired thiol **30** in 64% yield (74% based on unreacted thioacetate **29**).

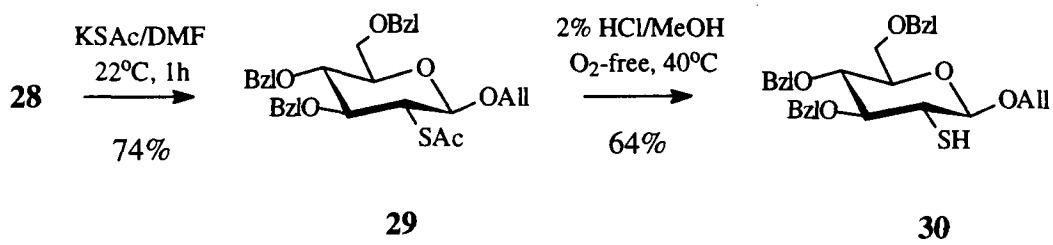


Figure 2.27 Synthesis of the thiol glycosyl acceptor **30**

As noticed with the thiol **12**, the thiol **30** was slightly less polar on tlc plates than the thioacetate. Degassed solvents were used in these reactions to avoid oxidation of the thiol to disulfide, although this occurs less readily in acidic rather than in basic solutions. Nevertheless, small amounts of the disulfide were detected by tlc although the thiol **30** was far more stable towards oxidation than the benzyl ether-protected thiol **12**, confirming our original expectation that the ester-protected thiol would be less reactive than the corresponding ether-protected one. Selective deprotection was also attempted using

cysteamine in acetonitrile[111] and diethylamine in DMF[112] but was unsuccessful for this compound. The corresponding thiocyanate was also synthesised in order to circumvent the problem of selective deprotection of the thioacetate **29**. This was achieved in approximately the same yield (77%) as the thioacetate by heating a crude mixture of the triflate with potassium thiocyanate in DMF at 70°C for 3 h. Attempted reduction of the thiocyanate using sodium borohydride in O_2-free THF/EtOH gave rise to a mixture of products including the desired thiol, as judged by comparison of tlc standards. This approach was discontinued in favour of the thioacetate route.

It should be noted that this synthetic route does not necessarily represent the most efficient method for the synthesis of the thiol **30** from a cheap commercially available starting material, but rather a route which utilised large stocks of compounds that were intermediates in the synthesis of the thiol. A more efficient route to this compound should

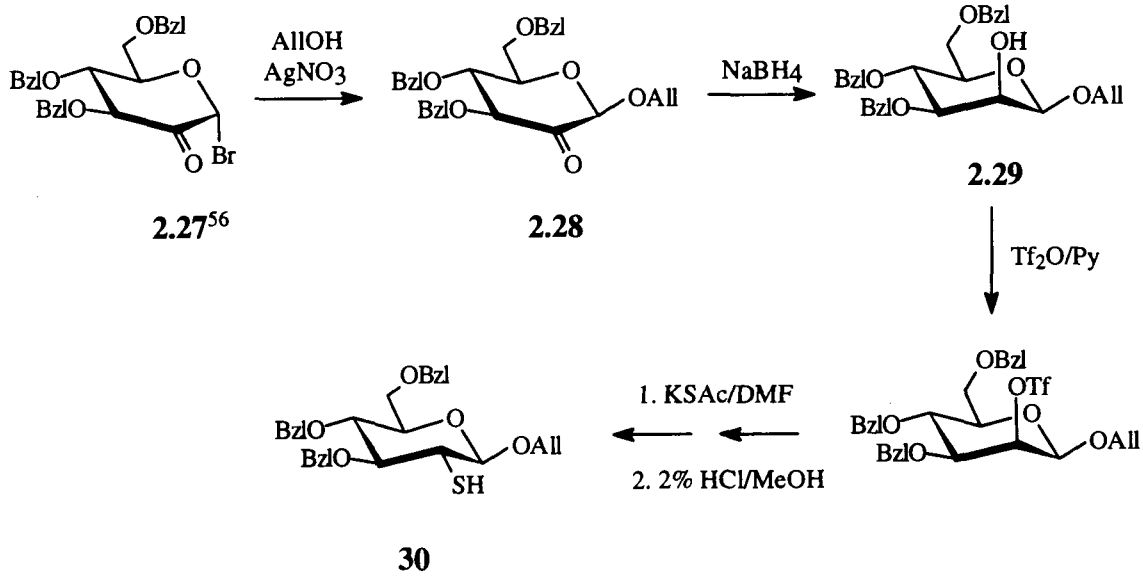


Figure 2.28 Proposed alternative scheme for the synthesis of the thiol **30** *via* ulosyl bromide chemistry

be feasible *via* ulosyl bromide chemistry developed by Lichenthaler *et al.*[113] Thus, starting from the ulosyl bromide **2.27**, formation of the β-allyl glycoside **2.28** followed by the stereoselective reduction of the ketone using sodium borohydride[113b] should yield the benzoate-protected alcohol **2.29**. Subsequent formation of the 2-triflate followed by displacement using sulfur nucleophiles would yield the thiol **30** (Figure 2.28). This synthetic route was initially investigated but was subsequently discontinued in favour of the less efficient but faster route described in Figures 2.26 and 2.27.

Initially, we attempted the glycosylation of the thiol **30** with the 2,3,4,6-tetra-O-acetyl-5-thio-α-D-glucopyranosyl trichloroacetimidate **22** using a 1:1 mixture of **30** and **22** and 0.25 equivalents of triethylsilyl triflate at -50 to +20°C. A 1:1 mixture of the α- and β-disacharides **31** and **32** was isolated as a single spot on tlc in 35% yield along with the 5-thio-D-glucal β-disaccharide **33** which accounted for 27% of the trichloroacetimidate **22**. Reduction of the amount of triethylsilyl triflate while retaining the same ratio of **22** and **30** resulted in the reduction of the glucal **33** formation (15%), although the isolated yield of the α-and β-disaccharides (~1:1) was 38%, or 78% based on recovered thiol. Based on observations during glycosylation reactions with similar systems, in which the use of an excess of the acceptor led to increased yields of the desired products,[107] the above reactions were repeated using a 50% excess of the thiol **30**. A significant reduction in the quantity of molecular sieves (4Å) was also made as it was assumed that an excess of sieves was slowing the glycosylation reaction down, resulting in incomplete reactions and elimination reactions with the disaccharides to give the β-glucal **33**. Indeed, when the glycosylation reaction was performed using 0.2 equivalents of

triethylsilyl triflate and a 50% excess of the thiol, an α/β mixture of the disaccharides (1.6:1 from ^{1}H NMR) was isolated in 72% yield (Figure 2.29). A reduction in the yield of the glucal **33** was also observed (11% yield). No corresponding orthoesters were observed or isolated due to a combination of the higher concentration of triethylsilyl triflate used in the reaction and the temperature to which the reaction mixture was warmed (22°C) before quenching with base.

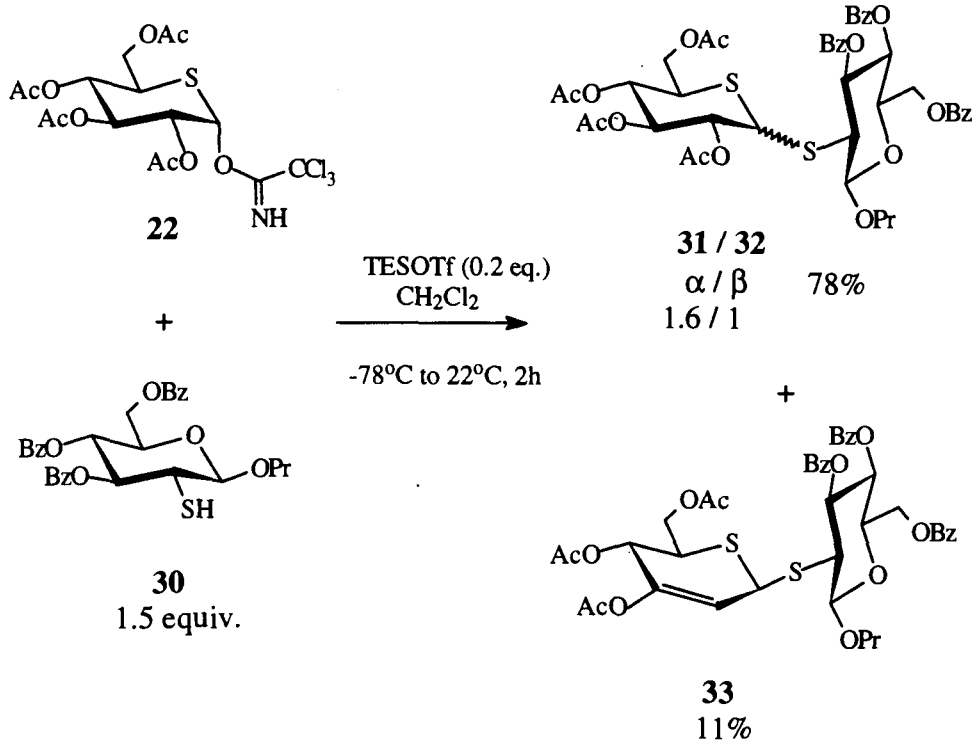


Figure 2.29 Synthesis of an anomeric mixture of **31/32**

The ^{1}H NMR spectrum of a mixture of the α- and β-disaccharides **31** and **32** was surprisingly clear with the resonances of H-1', H-2' and H-3' having distinct first-order coupling patterns, permitting the proof of anomeric configuration by observation of the

$^3J_{H1',H2'}$ coupling constants (4.5 Hz for the α- and 10.5 Hz for the β-thioglycosidic linkage). Pure samples of the β-anomer **32** were obtained by fractional crystallisation of the anomeric mixture of disaccharides from ether/dichloromethane/hexane but successive crystallisations did not yield the pure α-anomer due to preferential crystallisation of the β-anomer. Additional proof of structure of the pure β-anomer was obtained by observation of the $^1J_{C1',H1'}$ coupling constant, 156 Hz. The structural assignment of the 5-thioglucal disaccharide **33** was made on the basis of ^{1}H NMR and COSY spectra. After assignment of the three spin systems (two of which are in the unsaturated ring), observation of the $^3J_{H1',H2'}$ coupling constant (<1 Hz) and long range coupling between H-2' and H-4' led to the assignment of the structure of the unsaturated ring. Proof of anomeric configuration was made by observation of the $^1J_{C1',H1'}$ coupling constant, 156 Hz, the same value as that for **32**, suggesting the presence of the β-anomer.

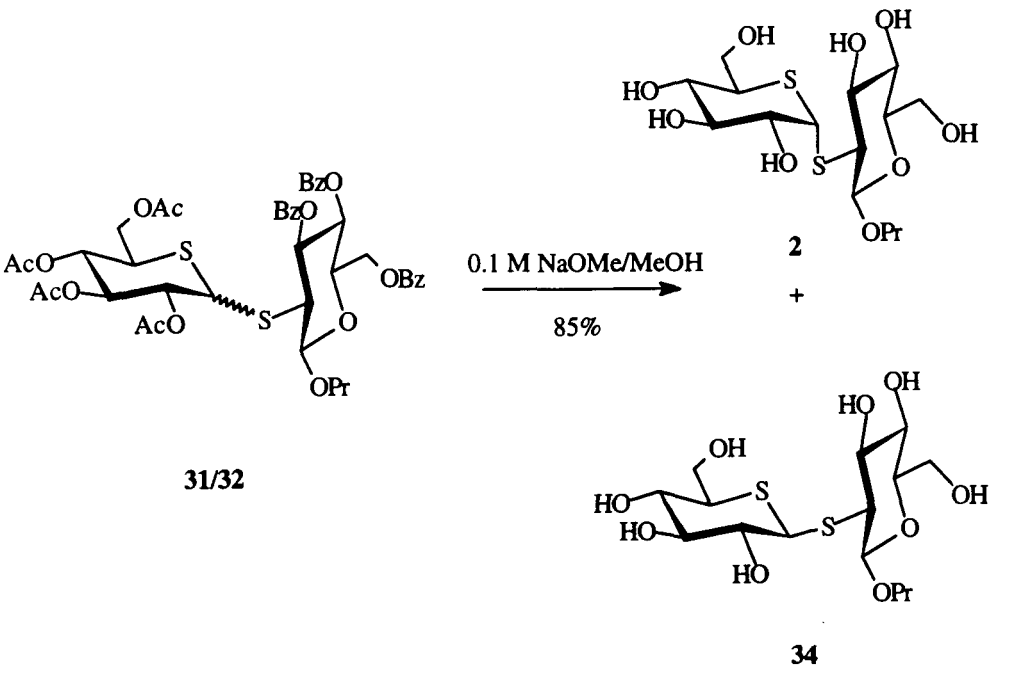


Figure 2.30 Deprotection of **31/32** to give **2** and **34**

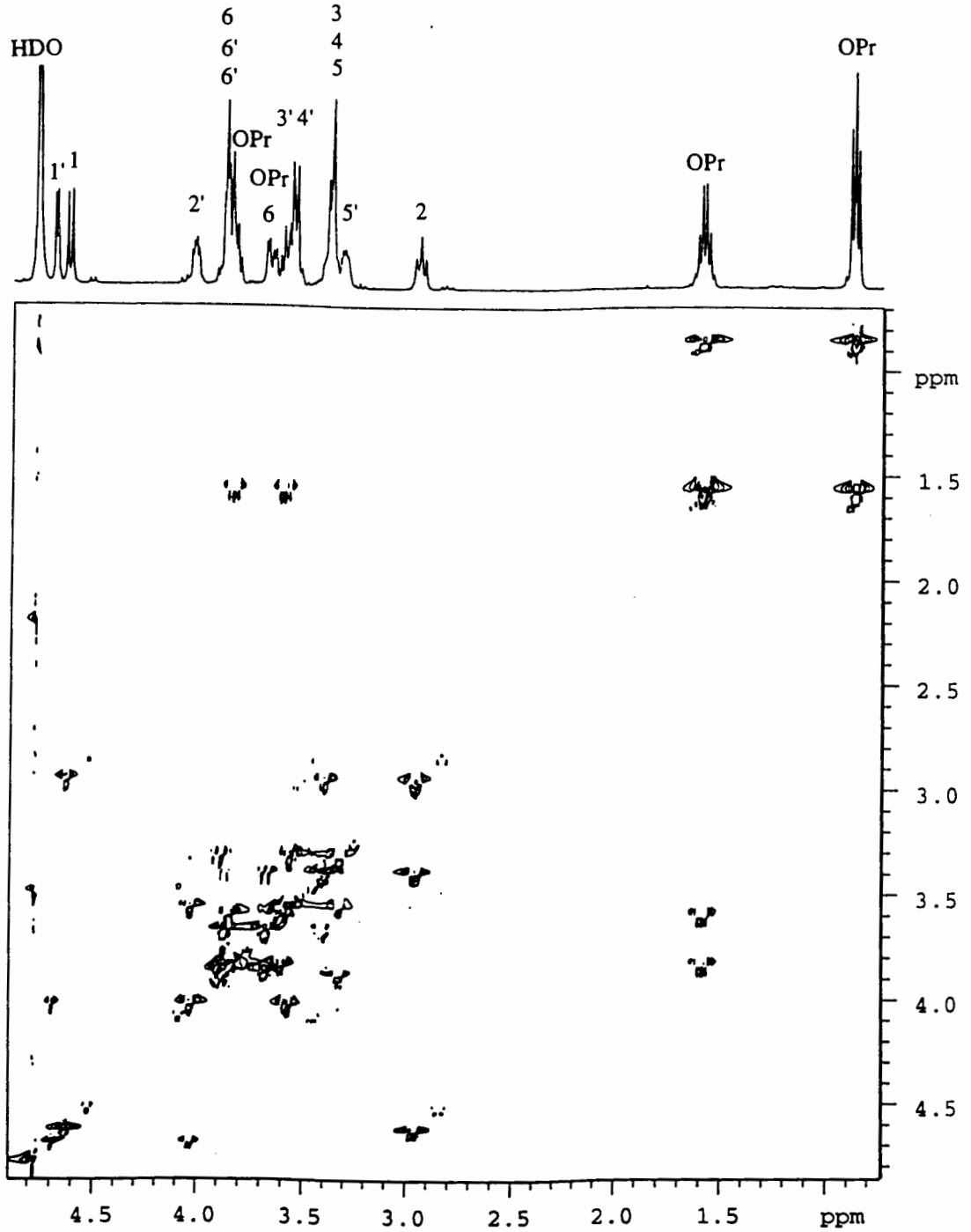


Figure 2.31 ^{1}H NMR and COSY spectrum of propyl 2,5'-dithio-β-kojibioside **2**

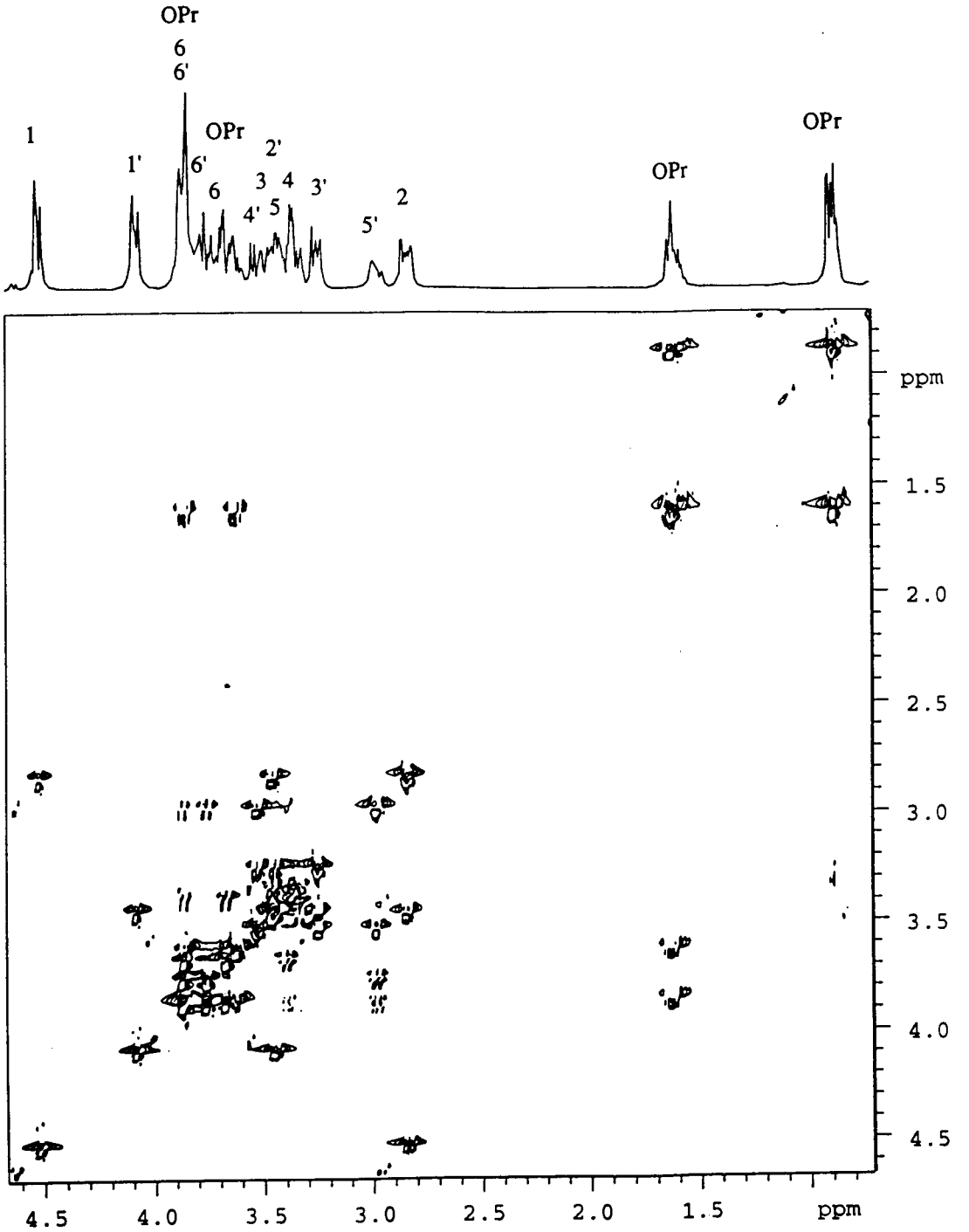


Figure 2.32 ^{1}H NMR and COSY spectrum of propyl 2,5'-dithio-β-sophoroside **34**

Deprotection of a mixture of the α- and β-disaccharides **31** and **32** was achieved in 85% yield using 0.1 M NaOMe in methanol (Figure 2.30). Fortunately, propyl 2,5'-dithio-β-kojibioside **2** and propyl 2,5'-dithio-β-sophoroside **34** were separable by careful chromatography using dichloromethane-methanol (2:1) as eluent (R_f 0.38-0.55). The stereochemical integrity of the two thiodisaccharides was confirmed by observation of the $^3J_{H1',H2'}$ and $^1J_{C1',H1'}$ coupling constants, 4.5 Hz and 159 Hz, respectively, for **2**, and 10.5 Hz and 156 Hz, respectively, for **34**. It should be noted that a reduction in the difference of the $^1J_{C,H}$ coupling constants is observed with these *S,S*-acetals compared to *O,O*-acetals and, therefore caution must be employed when using these values as proof of configuration. An explanation of thes effects is described in Chapter 3. The assignment of the ^1H and ^{13}C resonances was made on the basis of COSY (Figures 2.31 and 2.32) and ^{13}C-^1H chemical-shift correlated spectra.

Methyl maltoside analogues in which the interglycosidic linkage contains selenium in place of oxygen have been synthesised in our laboratory.[107] As a logical extension on our work on kojibioside analogues containing sulfur in the interglycosidic linkage, the corresponding selenium analogue was considered. Thus, the synthesis of the corresponding selenium analogue **35** was feasible *via* displacement of the triflate **28** with a suitable selenium nucleophile, the successful deprotection of which would give the selenol as a glycosyl acceptor for use in glycosylation reactions with the 5-thioglucose trichloroacetimidate **22**. The feasibility of this route initially appeared promising after the selenocyanate **36** was formed in 86% yield by the reaction of the triflate **28** with potassium selenocyanate in DMF. However, attempts at the reduction of the selenocyanate to the

selenol using sodium borohydride in the absence of oxygen completely failed, giving rise to the 2,3-glucal **37** as the sole product in the reaction (80%) (Figure 2.33).

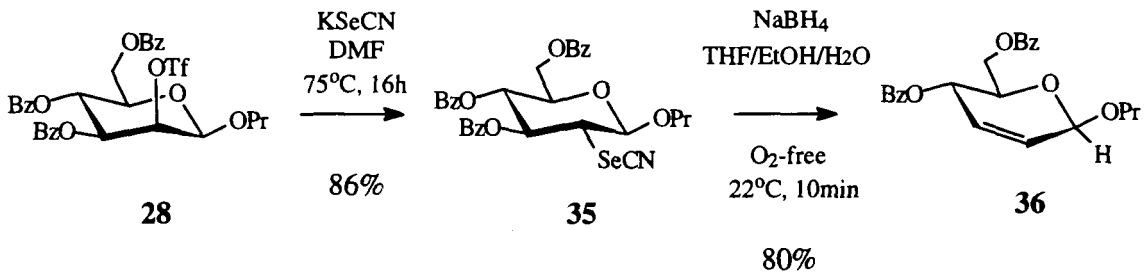


Figure 2.33 Synthesis of the 2,3-glucal **36** during the attempted reduction of the selenocyanate **35**

During the aqueous work-up of the reaction, a deep red colour formed which was indicative of the formation of selenium. It appears likely that the selenolate formed in the reaction displaces the neighbouring 3-O-benzoate giving rise to an unstable episelenide,[114] followed by elimination of selenium to give the glucal, as depicted in Figure 2.34.

The use of alternative protecting groups at the 3,4 and 6 positions that would not be displaced by the selenolate (e.g. benzyl ethers) would likely allow the isolation of the selenol, albeit, with some difficulty if the protecting groups activate the selenol towards oxidation. Although the selenium analogue of propyl kojibioside was desirable, it was decided at this stage, with knowledge that the selenium and corresponding sulfur analogues of methyl maltoside exhibited similar inhibition of glucoamylase,[115] that the

synthesis of the corresponding nitrogen analogues might be more beneficial. No further attempts of the synthesis of selenium analogues of kojibiosides were therefore made.

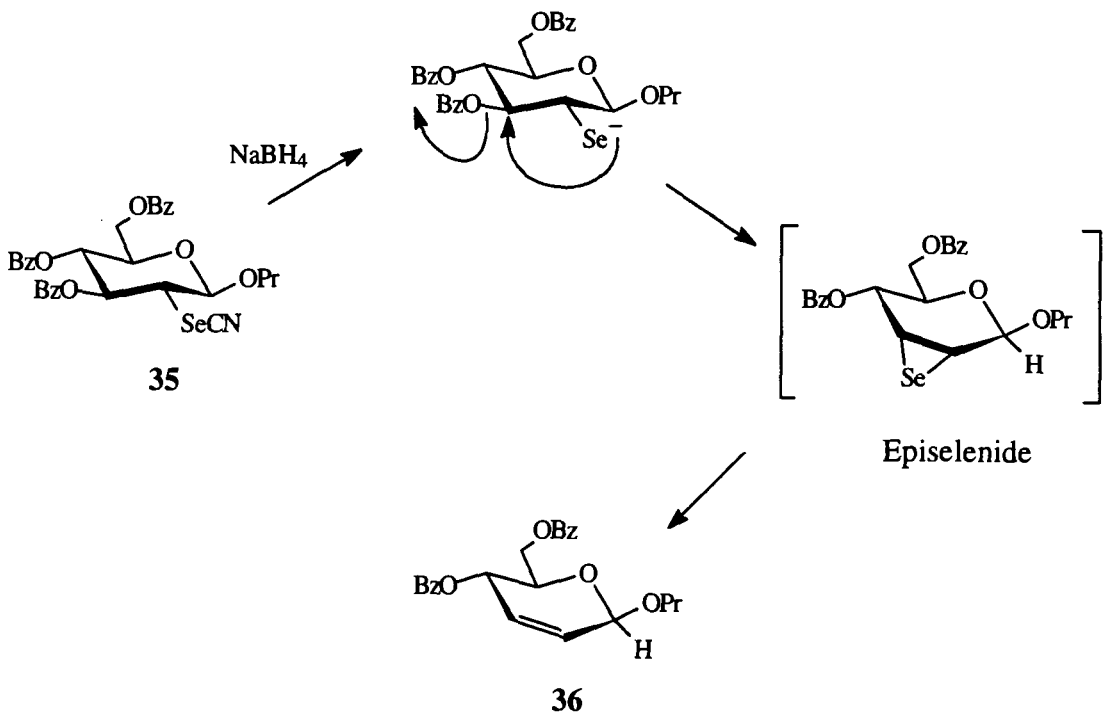


Figure 2.34 Possible mechanism for the formation of the glucal **36** *via* the episelenide

2.4.ii Synthesis of Sulfur Analogues of Methyl Maltoside

Our group has also been interested in a series of heteronuclear analogues of methyl maltoside containing sulfur in the nonreducing ring and either oxygen, sulfur or selenium in the interglycosidic linkage as potential α-amylase inhibitors. For the synthesis of the

4,5'-dithio analogue **4**, a similar strategy to that used for the synthesis of the 2,5'-dithiokojibioside **2** was employed, using the 5-thioglucose trichloroacetimidate **22** as a glycosyl donor. A suitably protected methyl 4-thio-4-deoxy-α-D-maltoside was therefore required as a glycosyl acceptor which would enable the straightforward deprotection, after successful glycosylation, without the need for palladium/carbon-mediated hydrogenolysis. The protecting groups chosen for the glycosyl acceptor were therefore ester groups which could be readily cleaved in high yield by acetolysis. Reed and Goodman[82] have reported

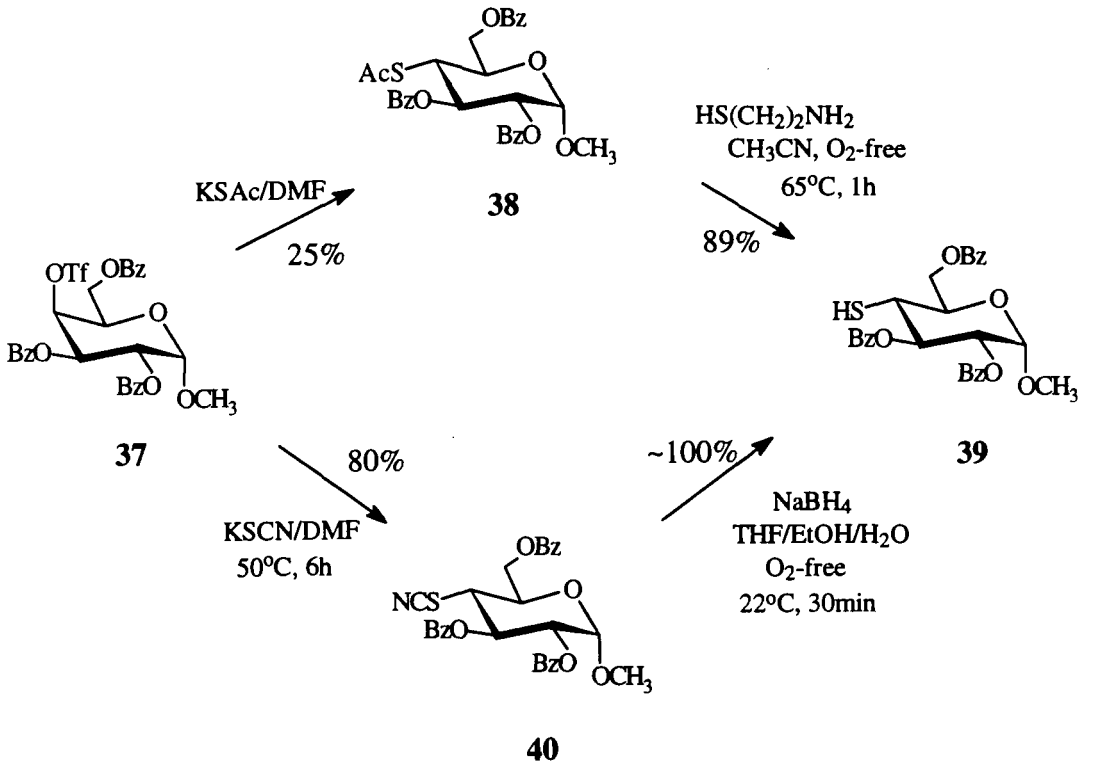


Figure 2.35 Alternative methods used for the synthesis of the thiol **39**

the synthesis of methyl 2,3,6-tri-O-benzoyl-4-thio-α-D-glucopyranoside **39** *via* selective benzoylation[116] of methyl α-D-galactopyranoside,[116,117] followed by triflation of the 4-OH, displacement of the crystalline 4-OTf by thiocyanate, and finally, reduction of the thiocyanate with zinc/acetic acid.

Initially, we attempted the synthesis of the 4-thiol **39** *via* the S-acetate **38** which was formed by displacement of the triflate in the galactoside derivative **37** using potassium thioacetate in DMF in only 25% yield, followed by selective de-S-acetylation using a deoxygenated solution of cysteamine in acetonitrile[111] (Figure 2.35). The de-S-acetylation to give the thiol **39** proceeded in high yield (89%; attempted acid-catalysed deacetylation in anhydrous HCl/MeOH was lower yielding [40%]). Due to the low yield of the formation of the thioacetate **38**, a slight modification of the method of Reed and Goodman[82] was therefore employed. Sodium borohydride (O_2-free, THF/95% EtOH, 0°C, 10 min) instead of zinc/acetic acid was used for the reduction of the thiocyanate **40** (synthesised from the triflate in 80% yield) to give the thiol **39** in essentially quantitative yield (the crude thiol was of sufficient purity to use without further purification) (Figure 2.35). Very little disulfide was observed in the isolated crude product, presumably a consequence of the short reaction time.

Glycosylation of the thiol **39** with the trichloroacetimidate **22** under triethylsilyl triflate catalysis and various conditions was examined next. Initial attempts utilising an excess ratio (1:1.3-1.8) of donor (trichloroacetimidate) to acceptor (thiol) and 0.4 equiv. (to donor) of triethylsilyltriflate resulted in the isolation of the α-linked disaccharide **41** (55%) as the sole glycosylated product together with large quantities of the glycal **43**[107]

(40%). When a two-fold excess of the thiol was used in glycosylation reactions the yield of the isolated α-disaccharide (53%) was the same as in the previous reaction but less glycal was formed (18%), simplifying the purification procedures (Figure 2.36). The larger scale of the latter reactions permitted the isolation of a minor component which was characterised as the corresponding β-disaccharide (2',3',4',6'-tetra-O-acetyl-2,3,6-tri-O-benzoyl-5',4'-α-sophoroside) **42** (1.5%; α/β = 36/1). The stereochemical outcome of this reaction is quite remarkable in that glycosylation reactions involving activated glycosyl donors of 5-O-sugars, in which the neighbouring acetate protecting group is able to participate, normally leads to the predominant formation of the β-linkage. Glycosylation reactions with the trichloroacetimidate of tetra-O-acetyl-5-thioglucose with the thiosugar acceptor (as well as oxo- and selenosugar acceptors[107]) give rise almost exclusively to the unexpected α-product. The preference for the stereoselective formation of the α-disaccharide has been shown to be affected by the reactivity of the nucleophilic glycosyl acceptor in the synthesis

of maltoside analogues.[107] In glycosylation reactions with the 5-thioglucose trichloroacetimidate **22**, as the reactivity of the glycosyl acceptor was increased from oxygen to sulfur to selenium, the observed stereoselectivity was shown to decrease. For example, glycosylation reactions with methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside afforded exclusively the α-disaccharide.[107] When the more reactive thiol glycosyl acceptor was used the α- and β-disaccharides were formed in a 36:1 ratio. A further reduction in the stereoselectivity was observed with the reaction of the selenol which yielded a ~4:1 ratio of the α- and β-disaccharides.[107]

Proof of the assigned stereochemistry of the α-and β-disaccharides **41** and **42** was confirmed by observation of the $^{3}J_{H1',H2'}$ coupling constants, 4.8 Hz and 11.0 Hz, respectively. COSY spectra were used to assign the spin systems for each ring which were correlated to their ^{13}C resonances by examination of the ^{13}C-^{1}H chemical-shift

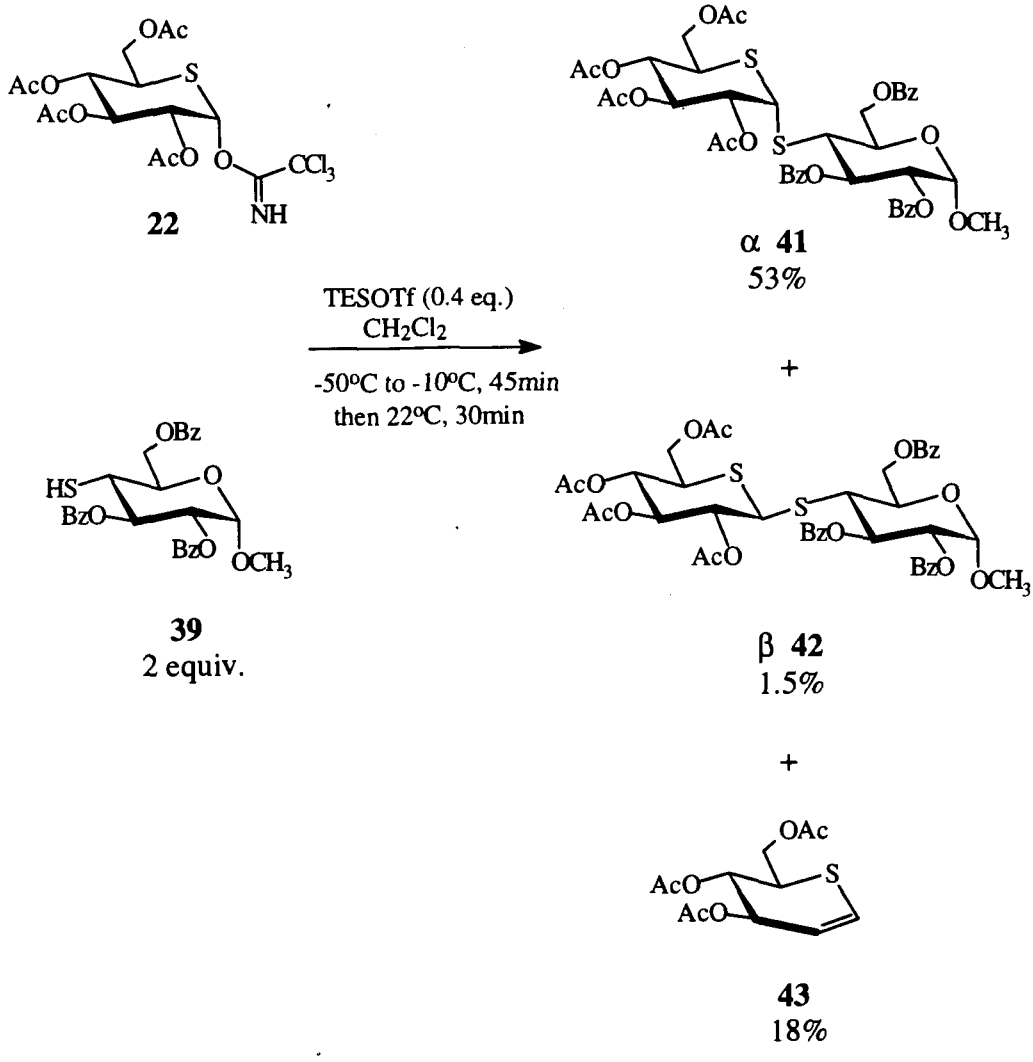
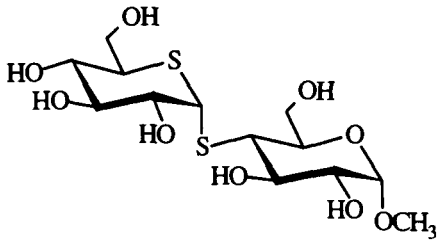


Figure 2.36 Synthesis of dithioanalogues of methyl maltoside and methyl cellobioside

correlated spectra. Consistent with the proposed stereochemistry was the observation of a larger $^{1}J_{C1',H1'}$ coupling constants, 158 Hz for the α compared to 155 Hz for the β isomer.



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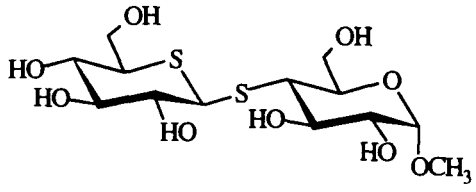


44

Figure 2.37 Methyl 4,5'-dithio-α-maltoside **4** and methyl 4,5'-dithio-α-cellobioside **44**

Deprotection of the α- and β-disaccharides **41** and **42** was readily achieved using 0.2 M NaOMe in methanol to give methyl 4,5'-dithio-α-maltoside **4** and methyl 4,5'-dithio-α-cellobioside **44** in 89% and 85% yield, respectively (Figure 2.37). The observed $^{3}J_{H1',H2'}$ and $^{1}J_{C1',H1'}$ coupling constants, 4.5 Hz and 155 Hz, respectively, for the α and 10.5 Hz and 153 Hz, respectively, for the β isomer, were in accord with the assigned stereochemistry. The assignment of the ^{1}H and ^{13}C resonances was made on the basis of COSY (Figures 2.38 (**4**) and 2.39 (**44**)) and ^{13}C-^{1}H chemical-shift correlated spectra.

Compound **4** was crystallised from hot methanol and its molecular structure was determined by X-ray crystallography by Drs. R. J. Batchelor and F. W. B. Einstein at Simon Fraser University ; the structure is presented in Chapter 4.

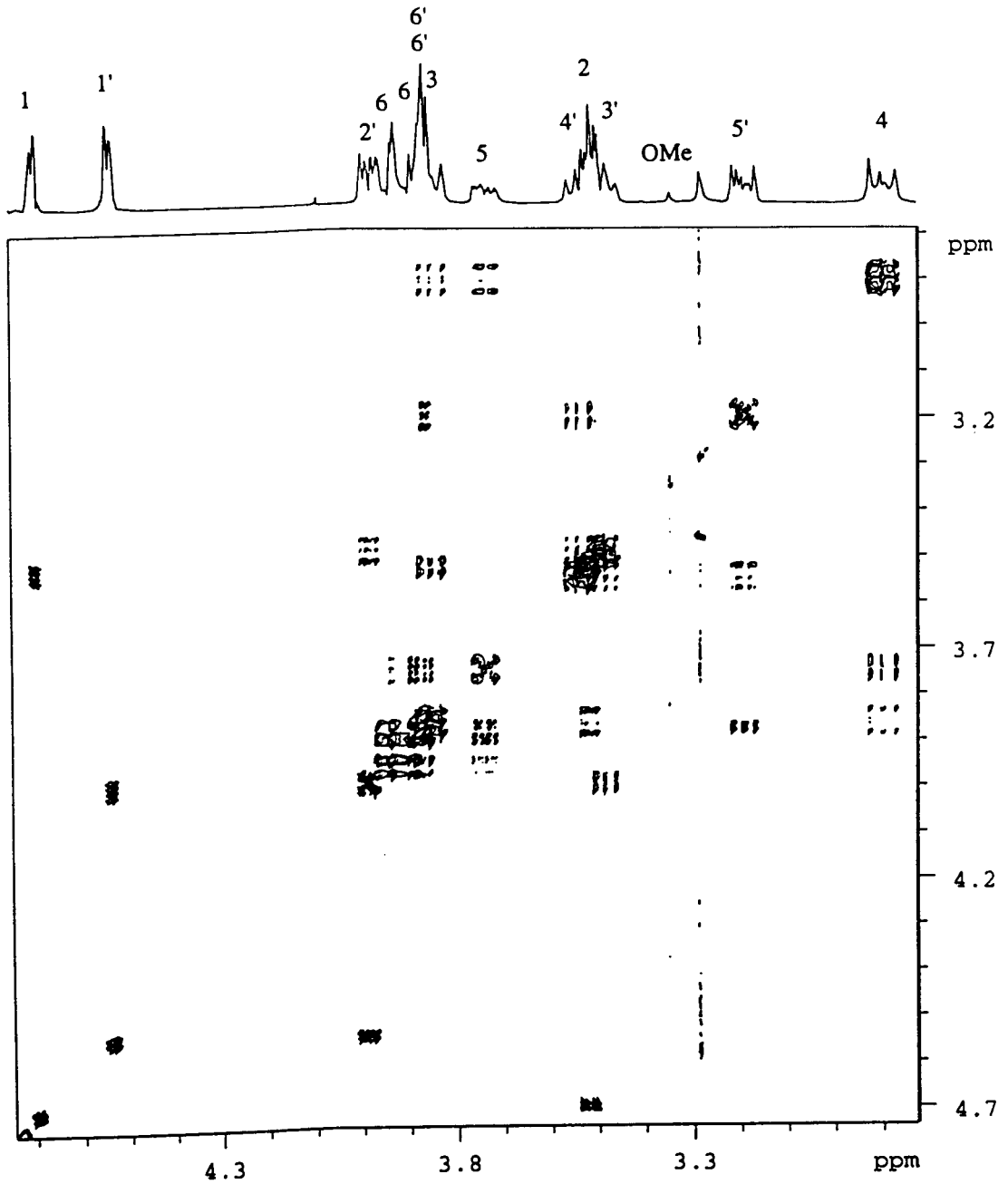


Figure 2.38 ^{1}H NMR and COSY spectrum of methyl 4,5'-dithio-α-maltoside **4**

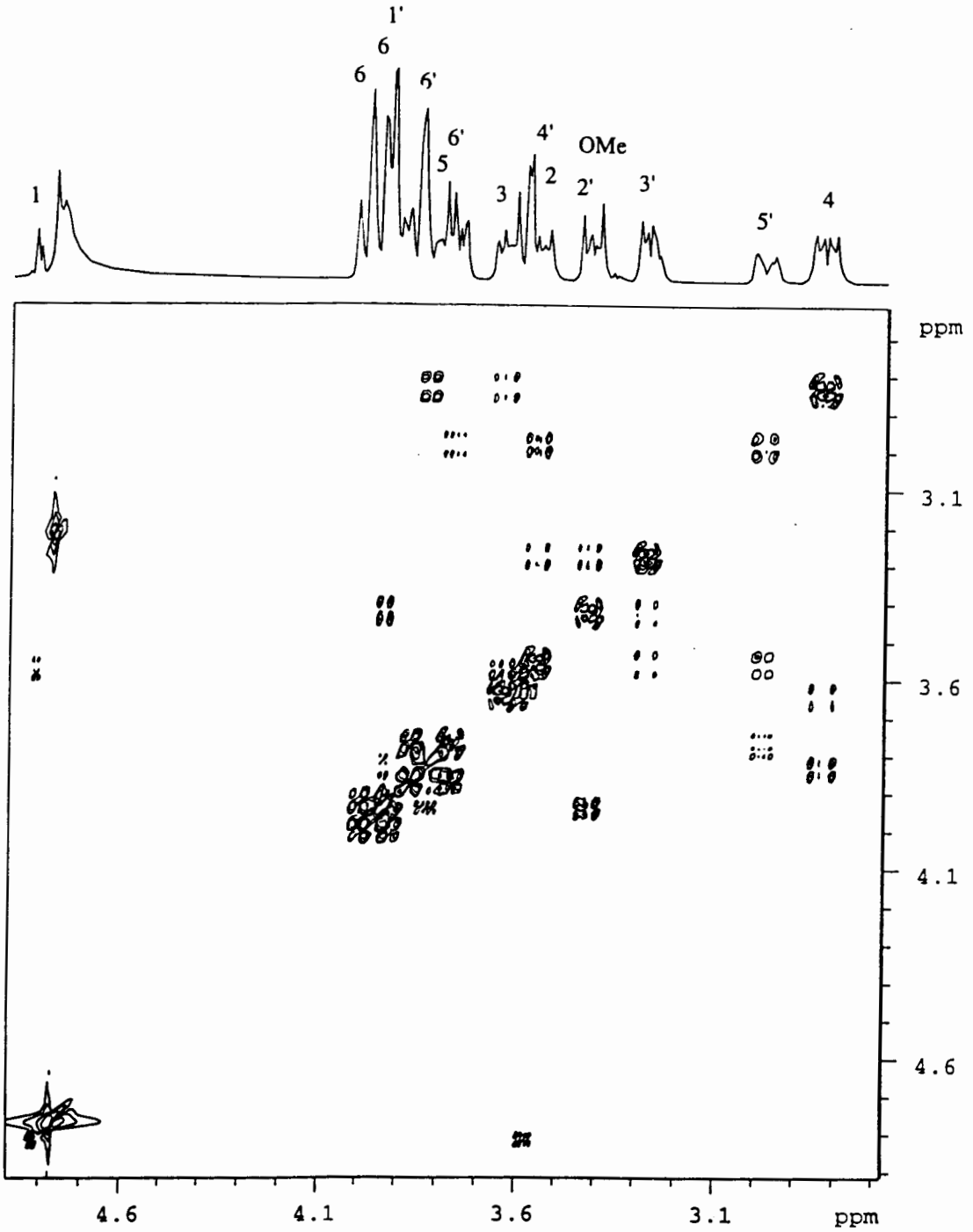


Figure 2.39 ^{1}H NMR and COSY spectrum of methyl 4,5'-dithio-α-cellobioside **44**

Following the publication of this work,[107] Lépine *et al.*[118] reported the chemical glycosylation of 2,3,4-tri-O-acetyl-5-thio-α-arabinopyranosyl bromide with 1,2,3,4-di-O-isopropylidene-α-D-glucopyranoside using silver triflate as promoter to give the corresponding α-disaccharide with sulfur in the nonreducing ring in 30% yield. However, no comments were made as to whether the corresponding β-disaccharide was formed during the reaction.

2.5 Biological Activity

2.5.i Inhibition of Glucosidase II by Propyl 2-S-β-Kojibioside 1

All inhibition studies of glucosidase I and II were carried out by Dr. M. Palcic and C. Scaman at the University of Alberta. The "trimming" enzymes glucosidase I and glucosidase II are responsible for the hydrolysis of the distal α-1-2 linked glucose residue and the two successive α-1-3 linked glucose residues, respectively, from the structure, Glc_3-Man_9-$GlcNAc_2$. Thus, propyl 2-S-β-kojibioside **1** was tested[119] 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 glucosidase II. Compound **1** was determined to be a poor inhibitor of glucosidase I, with an estimated K_i value >100 mM but a competitive inhibitor of glucosidase II, with a K_i value of 1.0 mM. The corresponding K_m value for α-D-Glc-(1-2)-α-D-Glc-(1-3)-α-D-Glc-$O(CH_2)_8$-CO_2CH_3 was 1.28 mM with glucosidase I. Interestingly, a 5'-thio analogue of **1**, allyl 5'-thio-β-kojibioside,[120] was found to be a poor inhibitor of glucosidase II but a competitive inhibitor of glucosidase I (K_i = 2.0 mM), and

phenyl α-D-selenoglucopyranoside[120] was found to be a competitive inhibitor of glucosidase I (K_i = 16 mM) and glucosidase II (K_i = 0.2 mM).

Propyl 2,5'-dithio-β-kojibioside **2** will also be tested as an inhibitor of glucosidase I and glucosidase II.

2.5.ii Inhibition of Glucoamylase G2 by Methyl 4,5'-dithio-α-maltoside 4

All inhibition studies on glucoamylase were carried out by Dr. B. Svensson and T. P. Frandsen at the Carlsberg Laboratory in Denmark. Glucoamylase G2 from *Aspergillus niger* catalyses the hydrolysis of maltose and related compounds with release of β-D-glucose.[121] The inhibitory action of the novel heteroanalogue of methyl α-maltoside **4** and of the corresponding 4-oxo and 4-seleno analogues[107], methyl 5'-thio-α-maltoside and methyl 5'-seleno-α-maltoside, has been tested.[122]

Methyl α- 5'-thiomaltoside was a very poor substrate with a $t_{½}$ of 28 h compared to 1.5 h for the substrate methyl β-D-maltoside under the same conditions. The lower rate of hydrolysis of the sulfur-in-the-ring compound is in accord with recent observations by Yuasa *et al.*[89] who reported that the stability of a glycoside of 5'-thio-N-acetyllactosamininide to hydrolysis by a β-galactosidase was 200 times greater than that of its counterpart with oxygen in the ring. The results are also consistent with the analysis of Jagannadham *et al.*[123] which shows that the thermodynamically more stable α-thia-stabilised carbocation (the presumptive intermediate in the glycoside hydrolysis reaction) is nevertheless formed more slowly than its oxygen counterpart because of a higher intrinsic barrier.

All of the heteroanalogues examined were competitive inhibitors with inhibition constants (K_i) of the same order of magnitude as the K_m values for maltose and 4-nitrophenyl α-D-glucopyranoside of 1.2 mM and 3.7 mM, respectively (Table 2.1).

Table 2.1 Inhibition of glucoamylase by heteroanalogues of methyl maltoside

Inhibitor	K_i (mM)	Ref.
methyl 5'-thio-α-maltoside	1.34 ± 0.06[a]	115
methyl 4,5'-dithio-α-maltoside **4**	2.04 ± 0.42	this work
methyl 4-seleno-5'-thio-α-maltoside	0.80 ± 0.03	115

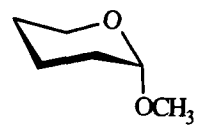
[a] Standard deviation

As a final point of interest, we comment on the thermodynamics of binding of the methyl 4,5'-dithio-α-maltoside **4**. Titration microcalorimetry[57] has given a K_a of 3.0 ± 1.6 × 10^3 M^{-1}; at 300 K this K_a corresponds to a free energy of interaction of -20.0 ± 1.4 kJ mol^{-1}. The enthalpy of interaction ΔH = -5.7 ± 1.7 kJ mol^{-1} and the $T\Delta S$ term was 14.3 ± 2.2 kJ mol^{-1}. The binding is thus dominated by the entropic contribution. The positive entropic term must result from the efficient release of water molecules to bulk solvent from the interacting complementary surfaces since a negative contribution would result from restrictions in translational, rotational, vibrational, and conformational degrees of freedom.[57]

2.6 Conclusions

This Chapter describes the synthesis of novel analogues of propyl kojibioside and methyl maltoside containing sulfur in the nonreducing ring and/or interglycosidic linkage for evaluation as α-glucosidase inhibitors. The preferential formation of the α-disaccharides resulting from the glycosylation of 5-thioglucose trichloroacetimidate is quite remarkable considering the presence of a neighbouring acetate group capable of participation. Similar results have also been noted in our laboratory with the synthesis of sulfur analogues of kojibiosides and isomaltosides[91] and of analogues of **4** containing oxygen or selenium in the interglycosidic linkage,[107] as well as with the synthesis of the corresponding nitrogen analogues (see Chapter 3). Hashimoto and Izumi[92] have also observed this unexpected preference for 1,2-*cis*-glycosylation during the synthesis of the disaccharide **2.24** derived from 5-thio-L-fucosyl trichloroacetimidates. This axial preference observed with 5-thiosugars can be accounted for by the enhanced thermodynamic stability of an axially oriented aglycon of 5-thiosugars relative to their oxygen counterparts. Pinto and Leung[124] have calculated the orbital interactions of the X-C-Z component of 2-methoxyoxacyclohexane **2.31** and 2-methoxythiacyclohexane **2.32** (Figure 2.39) pertaining to their equatorial and axial preferences, respectively. They concluded that the axial preference of the latter could be attributed to reduced steric effects as well as stabilisation from a combination of endo and exo anomeric orbital interactions.

Also noteworthy is the formation of the α-linkage during the glycosylation of the trichloroacetimidate **18** with the 2-thiol **12** (Figure 2.22). The corresponding reactions



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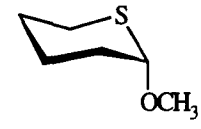


2.32

Figure 2.40 Compounds that have been used in orbital interaction analysis

with simple alkyl thiols catalysed with $BF_3.Et_2O$ have been shown to give rise exclusively to the β-linked compounds.[103] This result suggests that α-thioglycoside formation and β-thioglycoside formation proceed *via* different mechanisms, the α *via* a double displacement mechanism and the β *via exo*-orthoester formation (Figure 2.25).

Inhibition kinetics indicate that disaccharides in which either the nonreducing ring oxygen atom and/or the interglycosidic oxygen atom are replaced by sulfur are likely to be competitive inhibitors and therefore useful for examining the structure-function relationships of carbohydrate/protein interactions. However, the inhibition constants are of the same order of magnitude (mM) as the K_m values for the natural substrate. A conclusion as to the structural requirements of selective inhibitors of glucosidase I or glucosidase II is not clear with the results to date. The synthesis of higher-order structures containing more sugar residues may well be useful for determining the active site and aglycon site specificity.

CHAPTER 3

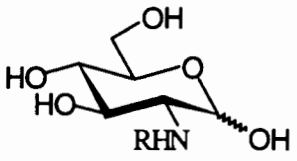
SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL GLYCOSIDASE INHIBITORS. 2: THIOANALOGUES OF *N*-LINKED DISACCHARIDES[125]

3.1. Introduction

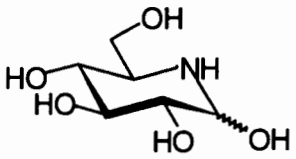
3.1.i Nitrogen Analogues

Carbohydrates in which one of the oxygen atoms in the molecule is replaced by nitrogen represent a very large and varied class of compounds within glycochemistry and glycobiology, which can be divided into several classes. Replacement of one (or more) of the hydroxyl groups by a free or *N*-substituted amino group gives rise to a class of compounds known as aminodeoxy (or amino) sugars. These compounds are very abundant in nature, the most common of these being 2-amino-2-deoxy-D-glucopyranose **3.1** and its *N*-acetylated derivative **3.2** (Figure 3.1). Replacement of the ring-oxygen by nitrogen gives rise to compounds such as the naturally occurring nojirimycin **3.3** (a 5-amino-5-deoxy-hexose) which commonly possess antibiotic behaviour and potent glycosidase inhibition (see Chapter 1).

This Chapter will be concerned primarily with a third class of compounds in which the glycosidic oxygen atom is replaced by nitrogen. These compounds can be further subdivided into several different types. Nucleosides **3.4**, in which a furanose sugar containing a β-glycosidic nitrogen attached to a heterocyclic base, such as adenine, occur in every living cell as components of nucleic acids. *N*-linked glycoproteins **3.5**, present in



3.1 R=H
3.2 R=OAc



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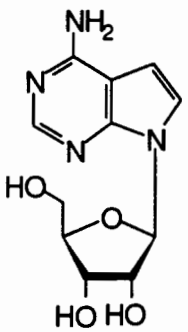

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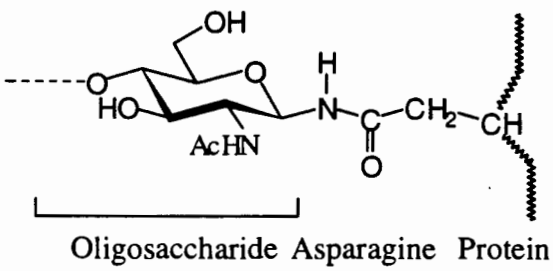

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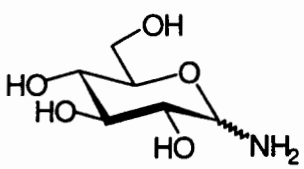

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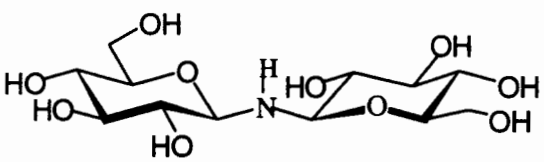


3.7

Figure 3.1 Examples of some common amino-sugars found in Nature

all eukaryotic organisms, contain oligosaccharides that are β-linked *via* an amide bond to an L-asparagine in the protein (Figure 3.1).

Glycosylamines containing a free or substituted amino group are well known. The free monosaccharide when treated with concentrated alcoholic solutions of ammonia produce the crystalline glycosylamine after a prolonged period;[126,127] for example, D-glucose gives the β-anomer of glucopyranosylamine **3.6**. This method of synthesis frequently gave rise to diglycosylamines[126] such as β-di-D-glucopyranosylamine **3.7** which is in equilibrium with the glycosylamine in alcoholic solutions.[126] The structure of diglycosylamines has been reported.[128] Improved methods for the synthesis of primary glycosylamines have been reported in which no diglycosylamine is produced.[129] Other methods have used the reaction between the free sugar and saturated aqueous solutions of ammonium hydrogen carbonate adjusted to pH 8.5 with ammonia to generate glycosylamines.[130] This method, however, often produces diglycosylamines, the pure glycosylamines being obtained by repeated ion-exchange chromatography.[130a]

N-alkyl glycosylamines are easily prepared from analogous reactions with primary or secondary aliphatic amines. For example, reaction of D-glucose with butylamine gives rise to *N*-butyl D-glucopyranosylamine.[131] Compounds of this type, however, are reactive and tend to hydrolyse rapidly (eg. *N*-alkyl galactosylamines[132] (half-life ~20 min at 25°C and pH 7). Far more resistant to hydrolysis are the *N*-aryl glycosylamines. These compounds have been known since 1870[133] and are commonly prepared by heating alcoholic[134] or aqueous[135] solutions of the free sugar and the appropriate aromatic amine.

3.1.ii Glycosylamines as Enzyme Inhibitors

Glycosylamines were first reported to exhibit specific enzyme inhibition in 1973 by Lai and Axelrod.[136] 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 Ki values for the inhibition ranged from between 0.002 mM for glucosylamine/β-glucosidase to 0.23 mM for galactosylamine/β-galactosidase. These values represent up to a 1000-fold increase in inhibition as compared to the free sugars, which are also known to be specific inhibitors of the corresponding glycosidase. For these studies, β-glucosylamine, which does not mutarotate in solution,[126] was used and showed inhibition towards both α- and β-glucosidases. Galactosylamine and mannosylamine, on the other hand, are known to mutarotate (ie. form an equilibrium mixture of the α- and β-anomers) rapidly in solution[125] and therefore, no conclusions as to the anomeric requirements for inhibition were made. A mechanistic rationale for the binding of a substrate/analogue to an enzyme active site had been suggested,[137] based upon the structure of lysozyme, the only glycosidase structure that had been elucidated at that time. Free carboxylic acid groups of the aspartic and glutamic residues located in the active site were suggested to participate in the formation and stabilisation of the intermediate oxocarbocation by electrostatic interactions. Lai and Axelrod[136] speculated, based on their results, that the strong affinity of the glycosylamines for glycosidases originated not only at the site that defines the glycon specificity, but also through the basic glycosidic amine at the acidic site in the catalytic centre.

This work was extended by Legler[138] in which he looked at the inhibition of β-glucosidases from bitter and sweet almonds by cationic and neutral β-glucosyl derivatives. It was observed that basic β-glucosyl derivatives (e.g. β-D-glucosylamine) inhibited the enzymes much more strongly than their neutral analogues (e.g. β-D-glucose). Furthermore, it was concluded that protonation of the inhibitor at the glycosyl nitrogen was a structural prerequisite for strong binding. Structurally similar compounds which could not be protonated at the glycosyl nitrogen were found to be very weak inhibitors. Comparative inhibition studies with β-D-glucosylbenzene and the cationic β-D-glucosylpyridinium ion were used to confirm the observation of Sinnott *et. al.*[139] that inhibitors bearing a permanent positive charge were bound to a lesser extent than their neutral analogues. The differences in active site structure and charge requirements of glycosidases is exemplified by these results and with those with β-D-glucosidase from *E. coli*[132] in which cationic inhibitors were bound no more tightly than their neutral analogues and β-D-glucosidase from *Aspergillus wentii*[140] in which the cationic species were bound $10^{2.5}$-$10^{3.5}$ times more tightly than directly comparable neutral analogues. However, in all cases the basic inhibitors were bound more tightly than their cationic and oxygenated counterparts. *N*-alkylation of the basic species was found to enhance the binding of inhibitors to β-glucosidaes from *E. coli.*[132] Conversely, *N*-arylation and *N*-acylation significantly lowered the binding,[138] as this reduced the basicity of the glycosyl nitrogen.

From these results it is clear that one of the requirements for tight binding of glycosylamines in an enzyme active site is the presence of an amino group at C-1 of the sugar ring that can be protonated in the active site. The importance of this requirement is

indicated by the weak binding of 2-amino-2-deoxy-β-D-glucose[138,140] in which the positively charged amino group is removed from C-1 by only one bond.

Examples of glycosylamines of 5-amino-5-deoxy sugars and their inhibition of glycosidases are reviewed in Chapter 1.

3.1.iii α-Glycosylamines

Specificity for the aglycon as well as the glycon of the natural substrate is evident in many glycosidases.[29] Studies using glycosylamines, therefore, are complicated by the spontaneous hydrolysis of many of these compounds. For studies in which information regarding the aglycon specificity is required, again glycosylamines cause complications due to their rapid mutarotation in solution. For example, α-galactosylamine, isolated as the ammonium complex was found to rapidly mutarotate into >95% of the β-anomer at pH's lower than 8,[141] a mechanism for which has been postulated.[126] α- and β- p-Tolyl-D-glucosylamine have been shown to mutarotate in methanol[142] to give a mixture consisting primarily of the β-anomer. D-Galactosylpiperidine, on the other hand, was found to mutarotate in aqueous solution to give predominantly the α-anomer, although originally present as the β-anomer.[132]

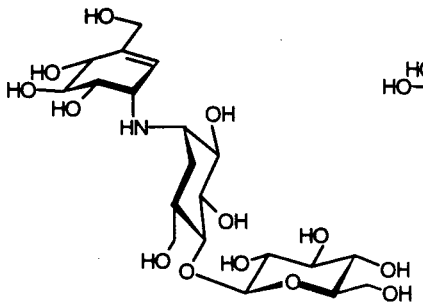
Where an equilibrium exists between the axial (α) and equatorial (β) anomers, the latter are thought to be more stable thermodynamically due to steric effects. This preference is opposed by the anomeric effect[124,143] for systems which possess an electronegative substituent at the anomeric position, which favours the α-anomer. When

the glycosylamines are protonated, as they are assumed to be upon binding to the enzyme, the so-called "reverse anomeric effect"[144] has been suggested to further increase the preference of the β-anomer, although this has been disputed by several workers.[145]

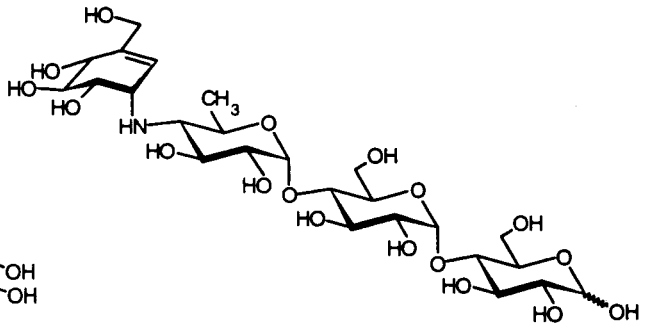
Thus, in most cases the synthesis of α-glycosylamines is difficult due to the significant thermodynamic preference for the β-configuration.

3.1.iv Pseudo-Sugar[146] Analogues

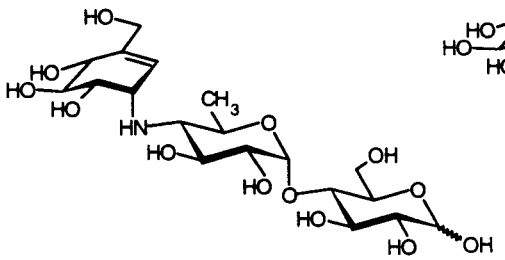
A new class of compounds, pseudo-oligosacharides, were isolated from various strains of *Streptomyces* during the 1970s and 80s, primarily by the Bayer AG group[14] and others,[147] which showed remarkable enzyme inhibitory activity. These included validamycin A[148] **3.8**, acarbose[149] **3.9**, amylostatin[149] **3.10**, adiposin[150] **3.11** and trestatins[150] **3.12** (Figure 3.2). These compounds all contain a similar structural feature that is essential for their inhibitory activity that consists of a cyclitol unit, bearing substituents resembling α-D-glucopyranose, and a 4-amino-4-deoxy sugar (or pseudo-sugar in the case of validamycin) of varying structure. The remainder of the structures contain different numbers of α-D-glucopyranose residues. In terms of an analogue of α-D-glucopyranose, the cyclitol unit can be thought of as that in which the ring oxygen is replaced by a group with a double bond to C-6, and the α-glycosidic oxygen is replaced by an NH group. A number of analogous pseudo-disaccharides have also been isolated and/or obtained



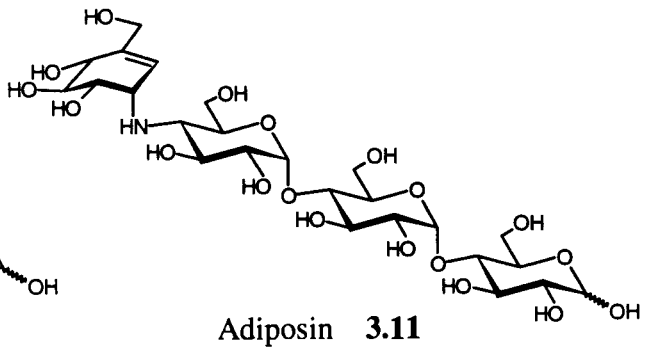
Validamycin A **3.8**



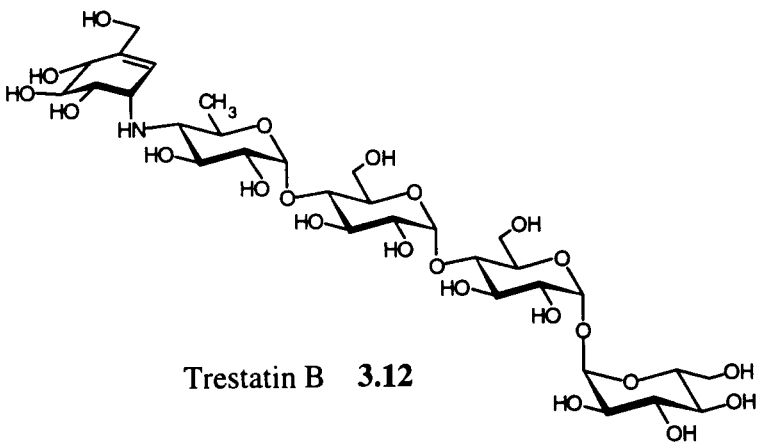
Acarbose **3.9**



Amylostatin XG **3.10**



Adiposin **3.11**

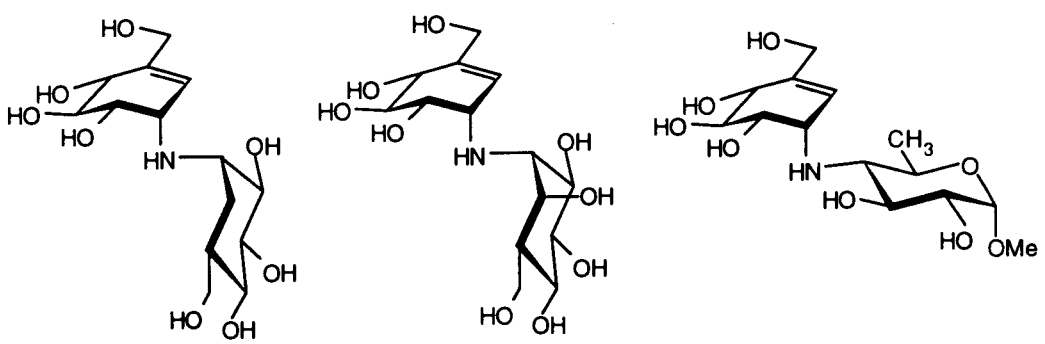


Trestatin B **3.12**

Figure 3.2 Structure of pseudo-oligosaccharides isolated from various strains of *Streptomyces*

chemically and include the validoxylamines A **3.13** and B **3.14**,[151] and acarviosin **3.15**[152] (Figure 3.3).

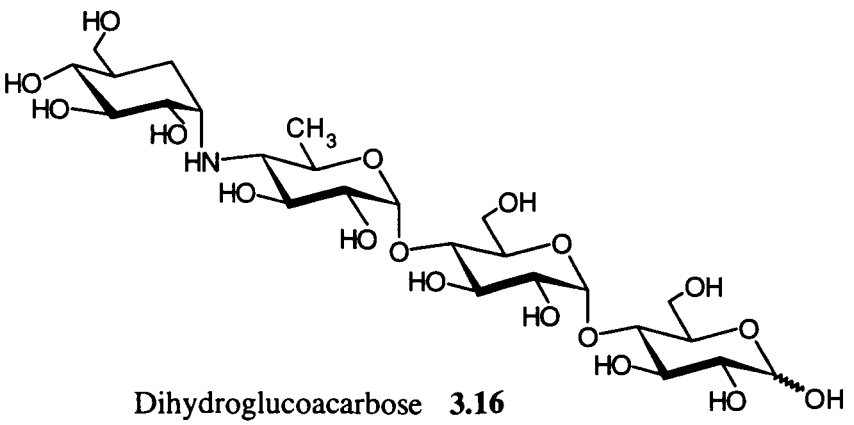
All of these compounds show interesting biological properties. For example, the validamycin complex is a mixture of seven structurally similar pseudo-oligosaccharides, each containing two pseudo-sugar residues, of which Validamycin A **3.8** is the major component. Validamycin A, the synthesis of which has been described,[150] is a potent antibiotic. Amylostatin XG **3.10** exhibits α-glucosidase activity[153] and adiposin 1 **3.11** exhibits both α-glucosidase as well as antimicrobial activity. The efficacy of these compounds as inhibitors of various enzymes is thought to be a direct result of the resemblance of the unsaturated cyclitol ring towards the putative transition state structure of a sugar ring in an enzyme active site, as well as the complementarity of the basic amino substituent with the active site carboxylate group.



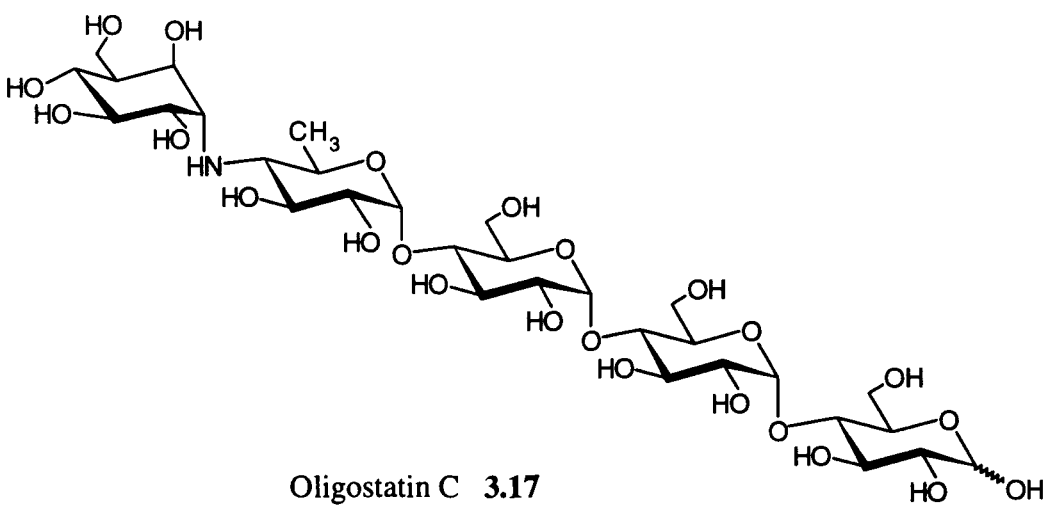
Validoxylamine A **3.13** Validoxylamine B **3.14** Acarviosin **3.15**

Figure 3.3 Biologically active pseudo-disaccharides

The most extensively studied[14] of these pseudo-oligosaccharides is acarbose **3.9**, the synthesis of which has recently been reported.[154] This compound was isolated from culture filtrates[14,148] as one component of a mixture of homologous compounds and was found to show very strong inhibitory properties against α-amylase, glucoamylase and intestinal sucrase.



Dihydroglucoacarbose **3.16**



Oligostatin C **3.17**

Figure 3.4 Biologically active saturated pseudo-oligosaccharides

Interestingly, with regard to the importance of the unsaturated pseudo-sugar residue towards the efficiency of acarbose as an enzyme inhibitor, hydrogenation[14,155] of acarbose yielded dihydroglucoacarbose **3.16** (Figure 3.4) as one of two isomeric products; its inhibitory activity towards intestinal sucrase was not dramatically reduced compared to acarbose.[14] Displacement titration calorimetry has shown[57] that dihydroglucoacarbose binds to glucoamylase more than four orders of magnitude more weakly than acarbose, but was still found to be a strong inhibitor.[57,155] Clearly, the structural resemblance of the pseudo-sugar component of acarbose to the proposed half-chair transition state structure is an important feature for strong binding. These results also show that the basic inter-ring NH in acarbose is, in part, responsible for the tight binding in that it can be protonated to give a charge complementary to that of the carboxylate group in the enzyme active site. It has also been shown[57] that acarviosin **3.15** is a much weaker inhibitor of glucoamylase, suggesting that the glucose units of acarbose also interact strongly with the enzyme or that they strongly influence the binding of the first two rings.[57]

The oligostatins,[152a,156] of which oligostatin C **3.17** (Figure 3.4) is an example, also have a saturated pseudo-sugar residue, and exhibit potent α-amylase and antimicrobial activity. Interestingly, the pseudo-disaccharide acarviosin **3.15**, formed by either the methanolysis of oligostatin C[152a] or acarbose,[152b] is a stronger inhibitor of α-amylase than acarbose, suggesting that structural modifications leading towards a better fit in the aglycon binding site for this enzyme are feasible.

The argument as to whether shape or charge of transition state inhibitors is the more important for enhanced binding to enzymes is very prevalant in the literature. Many

transition-state mimics have been reported that are used to probe this important question (see Chapter 1).

3.2 Research Objectives

Chapter 2 described the synthesis of novel heteronuclear disaccharide analogues containing sulphur and/or selenium as potential glycosidase inhibitors. This chapter describes other analogues in the series, namely those containing nitrogen in place of oxygen in the interglycosidic linkage. Compounds containing nitrogen in place of the ring oxygen (see Chapter 1) and in place of the glycosidic oxygen (glycosylamines) typically show enhanced enzyme inhibitory properties when compared with the corresponding sulphur analogues. This difference is attributed to the ability of the nitrogen analogues to be protonated in the enzyme active site, thereby increasing the binding of the substrate to the enzyme due to the complementarity of charge of the protonated analogue and the active site carboxylate group. Although glycosylamines commonly exhibit strong inhibition, these *O,N*- acetals often suffer from rapid hydrolysis[132] to give the free amine and the free sugar. Disaccharides containing NH in the interglycosidic linkage have been reported by Barker *et al.*[157a] in 1961 and subsequently by Micheel *et al.*[157b] in 1965, albeit in a preliminary form. To the best of our knowledge, these types of compounds have not been reinvestigated since the original reports and, therefore, no proof of structure has ever been obtained be modern NMR methods, for example. Furthermore, the biological activity of these compounds was not reported. To date, the only fully characterised

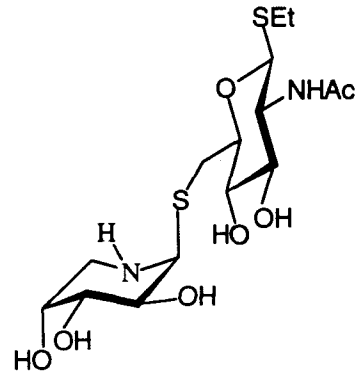
di/oligosaccharide analogues containing an NH-linkage are compounds containing pseudo-sugars, as described in Section **3.1.iv**.

The synthesis of α-anomers of *N*-linked disaccharides is especially difficult due to the overwhelming thermodynamic preference for the β-configuration. This has also been a problem in the synthesis of α *N*-linked glycopeptides, although a solution to this problem has recently been published.[158] In contrast to the *O,N*-acetals, the S,*N*-acetals have been shown to contain significant proportions of the axial isomer, at least within the parent thiacyclohexane system.[124] This has been attributed to the reduced steric effects and enhanced *exo*- and *endo*-anomeric orbital interactions with *S,N*-acetals compared to *O,N*-acetals.[124]

The synthesis of *S,N*-acetals of monosaccharides has been reported;[159] various crystalline aryl 5-thio-D-xylopyranosylamines have been obtained from the acid catalysed reaction of 5-thio-D-xylopyranose and the appropriate aryl amine. The anomeric configurations of these compounds were not determined but all showed large negative optical rotations,[159] suggesting the presence of the β-anomers. No comment as to their stability towards hydrolysis was specifically mentioned although the 5-thio-xylopyranosylamines were said to be "quite stable at 25°C".[159] Attempts at synthesising the corresponding alkylamines gave "dark-colored syrups" and their investigation was discontinued.[159]

O-Glycosides of azasugars (5-deoxy-5-amino sugars) containing the free NH group, which are also *O,N*-acetals, are easily hydrolysed in aqueous solution,[160] and thus far, the synthesis of azapyranosyl disaccharides has remained elusive. The stability of *S,N*-

acetals towards hydrolysis has been utilised by Suzuki and Hashimoto[161] who have recently reported the synthesis of an azapyranosyl disaccharide with a thioglycosidic linkage. However, this compound was found to be stable in strong acid but hydrolyses rapidly at pH>5.[162] The synthetic strategy relied upon the acid promoted (tosic acid; 1 equiv.) condensation of an *N*-protected aza-sugar and a 6-thiosugar to yield exclusively the 1,2-*cis*-linked thioglycoside **3.18** (Figure 3.5). The stereoselectivity of the glycosylation reaction was suggested to be controlled by the anomeric effect.



3.18

Figure 3.5 Azapyranosyl disaccharide with a thioglycosidic linkage

Based upon the assumption that α *N*-linked disaccharides containing a 5-thio sugar as the non-reducing residue would likely be favoured over the β *N*-linked disaccharide to a greater extent than with the analogous oxygen analogues, we focused our attention on the synthesis of the methyl maltoside analogue **5α** in which the oxygen in the non reducing ring is replaced by sulphur. The likelihood of the added stability of the *S,N*-analogue

towards hydrolysis was also recognised. The similarity in structure of **5α** and acarviosin **3.15**, a potent α-amylase inhibitor, was noted.

3.3 Results and Discussion

Initial attempts towards the synthesis of the *S,N*-maltoside analogue were made using more traditional glycosylation methodology.[101,163] Thus, the direct displacement of 2,3,4,6-tetra-O-acetyl-5-thio-α-D-glucopyranosyl bromide[164] **45** with methyl 4-deoxy-4-amino-α-D-glucopyranoside[165] **46** (synthesised in 5 steps from methyl α-D-galacto-pyranoside) in DMF at 40°C was attempted, but no reaction took place. The addition of silver triflate/collidine to the reaction mixture to activate the bromide was unsuccessful (Figure 3.6). The reaction was repeated with the β-bromide[164] **47** using pyridine as solvent and with the addition of mercuric cyanide as promoter and again, no reaction was observed. Heating this mixture to 60°C resulted in the breakdown of the bromide (Figure 3.6).

We next turned our attention to the condensation of the free amino-sugar with 5-thioglucopyranose. The synthesis of glycosylamines is typically performed by dissolving the free sugar in methanol containing an excess of the amine.[126,141] For our systems, in which the use of large excesses of amine are not feasible, we found that heating the free sugar (5-thioglucopyranose) with a two fold excess of the amine in methanol, containing a catalytic amount of acetic acid gave rise to the desired products. Thus, methyl 5'-thio-4-N-α-maltoside **5** was synthesised and was found to be stable towards hydrolysis but to be in equilibrium with its β-anomer analogue, methyl 5'-thio-4-N-α-cellobioside **48**. This

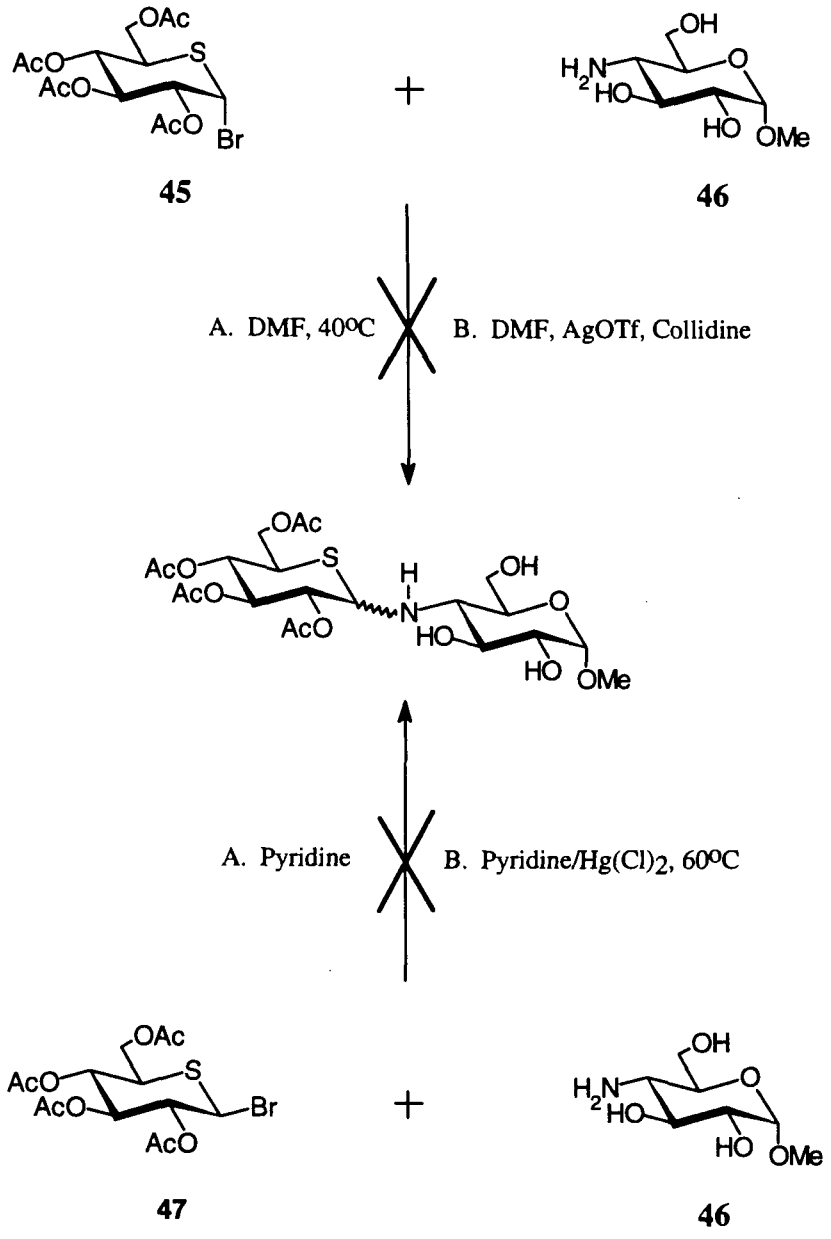


Figure 3.6 Failed attempts at synthesising the S,N-maltoside analogue **5**

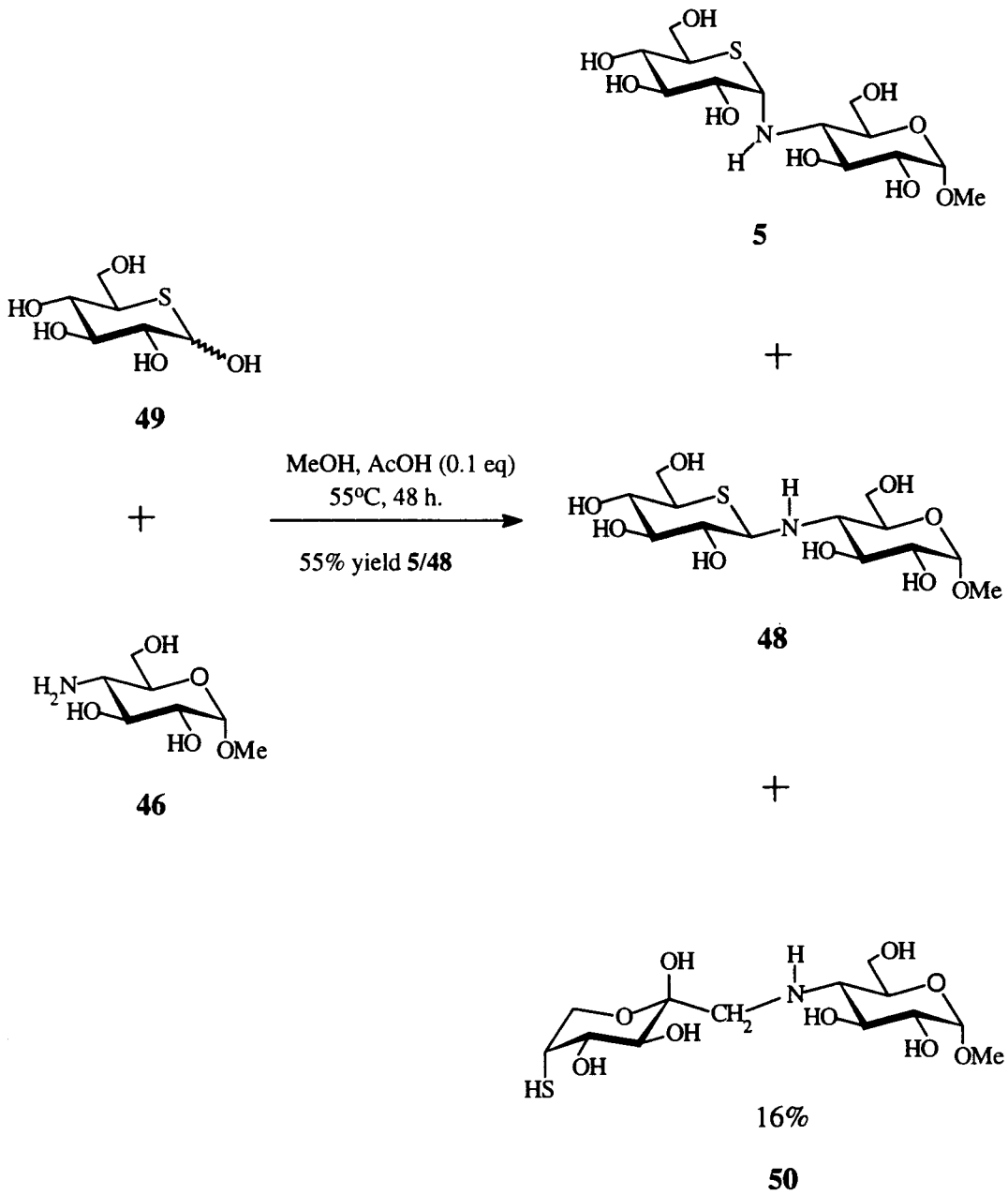
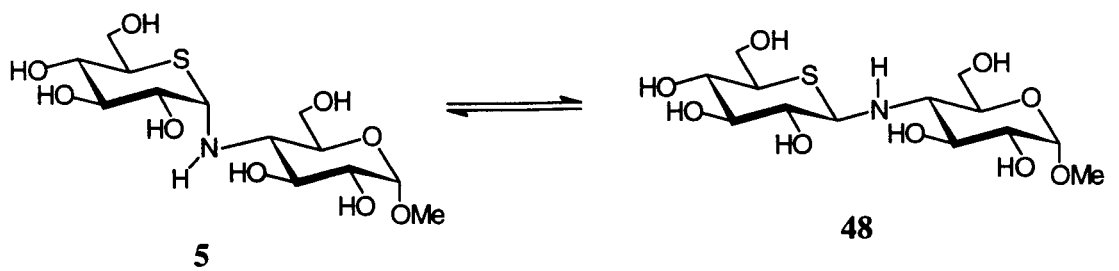


Figure 3.7 Synthesis of methyl maltoside and methyl cellobioside analogues

result was not particularly surprising since glycosylamines are known to mutarotate.[126]

Reaction of 5-thioglucopyranose **49** with methyl 4-amino-4-deoxy-α-D-gluco-pyranoside (**46**)[165] (2 equiv.) in methanol containing glacial acetic acid (0.1 equiv. relative to 5-thioglucopyranose) at 55° C for 48 h yielded a mixture of **5/48** (55%, or 72% based on recovered 5-thioglucopyranose) and a side product **50** (16%) which appears, by comparison of ^{1}H-NMR data[166] for Amadori-rearrangement products, to be the result of an Amadori-rearrangement (a 1-amino-1-deoxy-D-fructose derivative) (Figure 3.7). The use of higher concentrations of acid or higher reaction temperatures gave rise to larger amounts of the Amadori-rearrangement product. Pure **5/48** was obtained by careful flash chromatography. An almost anomerically pure sample of **5** obtained from early chromatography fractions was found to anomerise in D_2O (^{1}H-NMR) to an equilibrium mixture of **5/48** (~1:2.5) in about 3 days (Figure 3.8). No hydrolysis of the mixture in D_2O was observable after several weeks.



S,N maltoside analogue

S,N cellobioside analogue

Figure 3.8 Anomerisation of **5** and **58**

The ^{1}H-NMR spectrum of **5/48** (Figure 3.9), although the ring-proton resonances were severely overlapped, was useful in determining the outcome of the reaction. Based upon the intensities of the proton resonances it was clear that two compounds were present. The furthest upfield resonances were two triplets (2.81 ppm and 2.76 ppm), one each from the major and minor products, which were assigned to H-4α and H-4β, respectively, due to the expected relative shielding of protons adjacent to the 4-amino substituent. COSY (Figures 3.9, 3.10) and NOESY spectra of **5/48** allowed the assignment of all spin systems in the two disaccharides, whose stereochemical integrity was confirmed by observation of the $^1J_{H1',H2'}$ coupling constants (4.2 Hz and 9.5 Hz for **5** and **48**, respectively). The $^1J_{C1',H1'}$ coupling constants are routinely used to assess the stereochemistry of the anomeric linkage; typical values for sugars are in the order of 170 Hz for α and 160 Hz for β.[167] Wolfe *et al*[168] have described the origins of stereoelectronic effects which account for the observation that in cyclohexane and six-membered rings having one or more heteroatoms of the first row, the one-bond C-H coupling constants ($^1J_{CH}$) for equatorial hydrogens is always larger than for the corresponding axial hydrogens. The effects have been designated as Perlin effects and have been attributed to the dominance of $n_x \rightarrow \sigma^*_{C-H}$ orbital interactions.[168] The difference was observed to decrease when the carbon carrying the hydrogen of interest was attached to a second row atom, and the trend was observed to reverse when the carbon atom was attached to two heteroatoms from the second row. The reversal has been attributed to the dominance of orbital interactions of the $\sigma_{C-H} \rightarrow \sigma^*_{C-S}$ type. For the *S,N*-acetals **5** and **48**, the $^1J_{C1',H1'}$ coupling constants in the $^{13}C\{^1H\}$ spectrum are the same (153 Hz), a fact that, therefore,

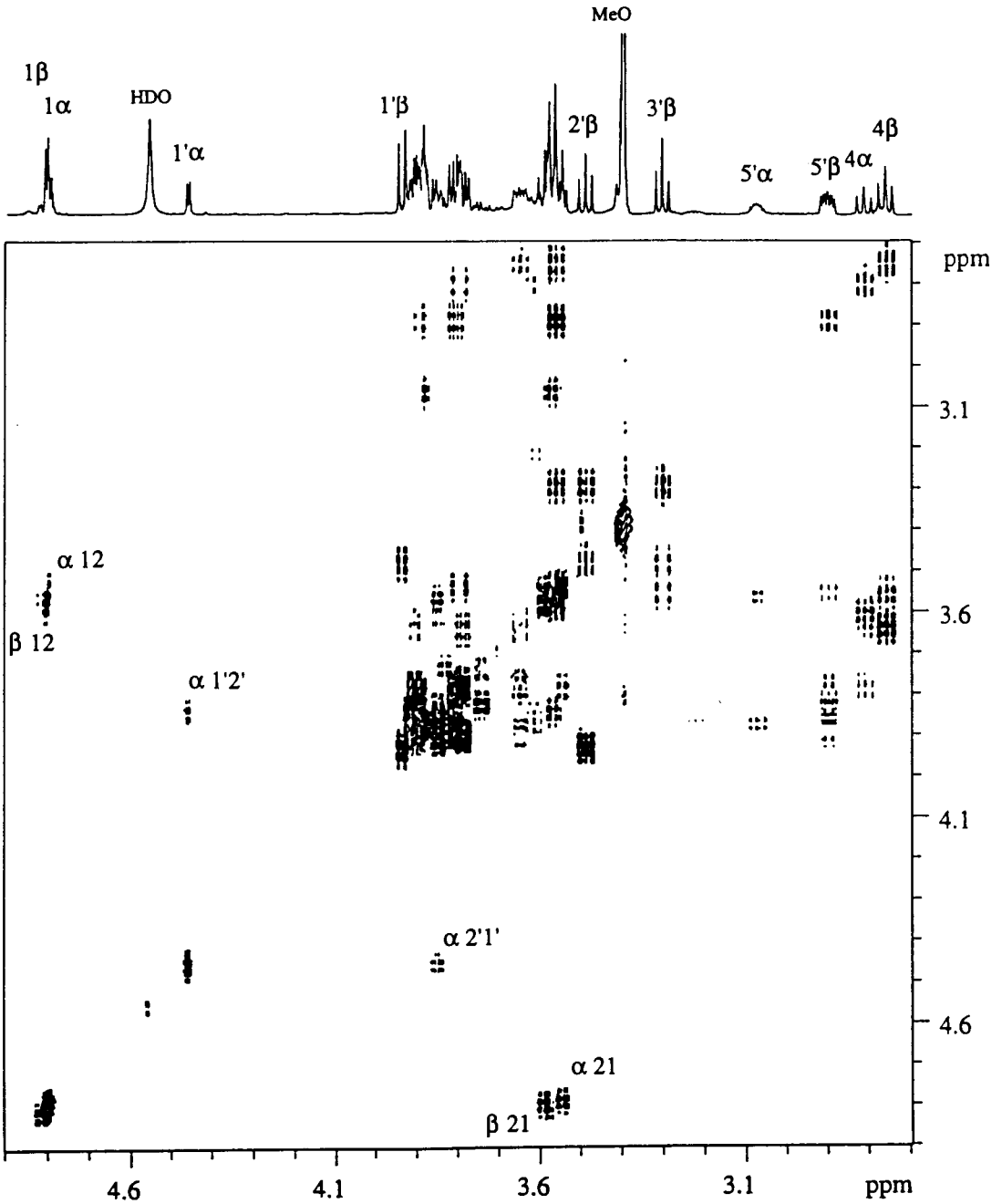


Figure 3.9 COSY ^{1}H NMR spectrum of **5/48**

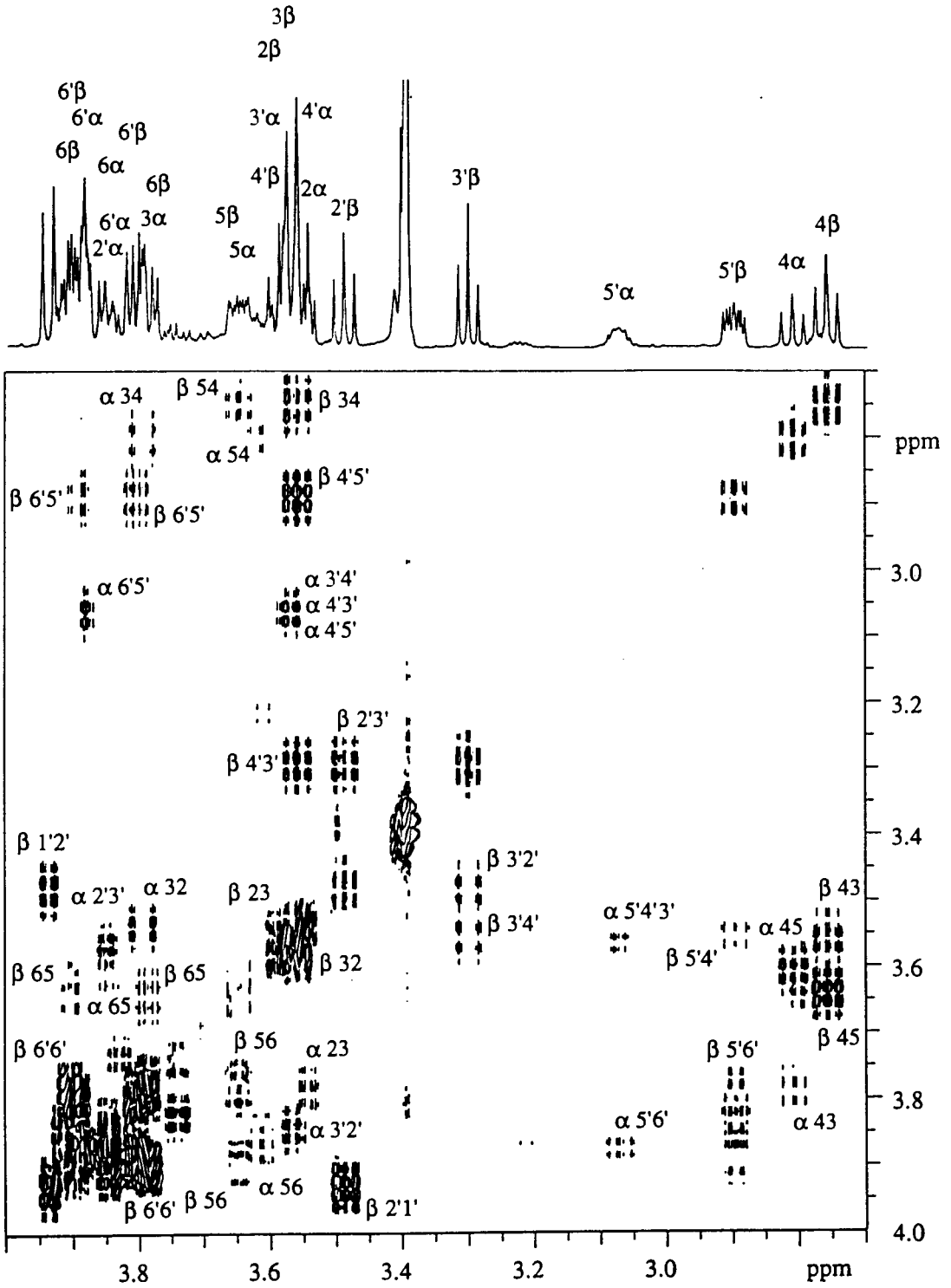


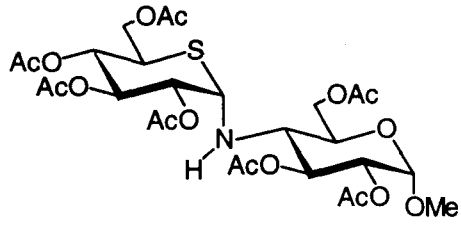
Figure 3.10 Expansion of the COSY ^{1}H NMR spectrum of **5/48**

likely reflects a balance of opposing orbital interactions associated with the Perlin effect.[168]

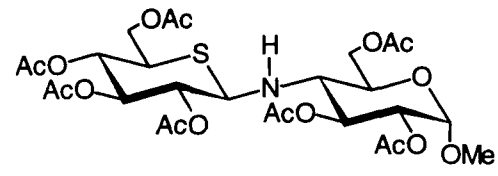
The assignment of the ^{13}C resonances for **5** and **48** was made on the basis of a ^{1}H-^{13}C-inverse detected NMR spectrum. The N-H resonances were absent from the ^{1}H-NMR spectra of **5** and **48** due to fast exchange in D_2O.

For compound **50** (Figure 3.7), which was not isolateable, the characteristic peaks in the ^{1}H-NMR spectrum that identified it as a product arising from an Amadori rearrangement were those for the diasterotopic H-1s (2.90, 3.04 (2H, AB quartet, $J_{H1a,H1b}$ = 12.5 Hz, CH_2-NH-R)).

Compounds **5** and **48** were also characterised as the acetylated compounds **51** and **52** (Figure 3.11) in order to unequivocally assign their structures. Fortunately, **51** and **52** were separable by careful chromatography and were found not to anomerise. For **51** and **52** the N-H resonance and the coupling between the interglycosidic N-H and H-4 and H-1' were now observable in the ^{1}H-NMR spectra (**51**: $J_{NH,4}$ = 10.0 Hz; **52**: $J_{NH,4}$ = 4.5 Hz, $J_{NH,1'}$ = 12.5 Hz). The addition of D_2O to the NMR solvent resulted in the disappearance of the N-H resonances due to exchange with deuterium.



51



52

Figure 3.11 Structures of the acetylated *S,N* maltoside and cellobioside analogues

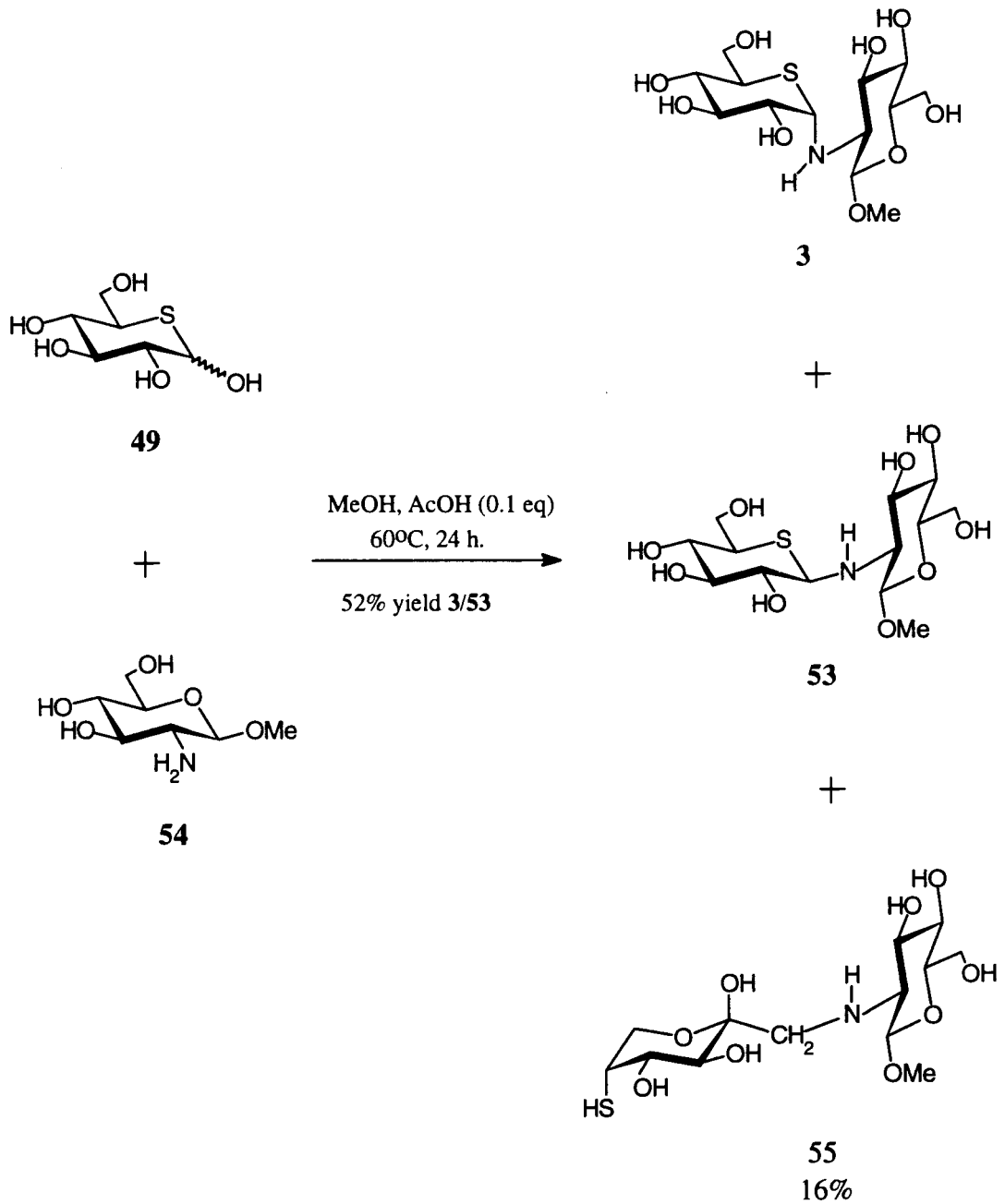
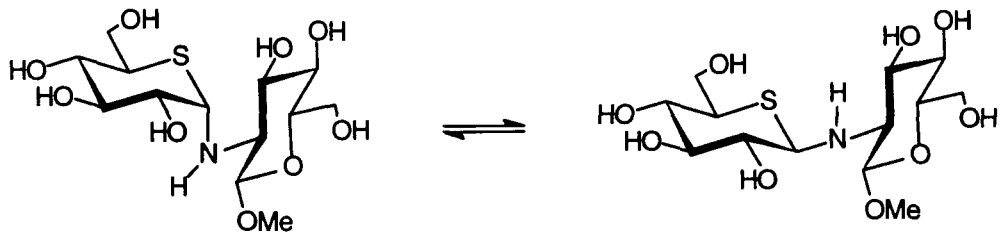


Figure 3.12 Synthesis of *S,N*-analogues of methyl kojibioside and methyl sophoroside

Due to the success of this method for the synthesis of this new class of disaccharide, we now wanted to check the generality of this method by synthesising the corresponding kojibioside analogue. Thus, methyl 5'-thio-2-N-β-kojibioside **3** was synthesised by the same approach and, not surprisingly, was found to be in equilibrium with the β-anomer, 5'-thio-2-N-β-sophoroside **53**. Reaction of 5-thioglucopyranose **49** with methyl 2-amino-2-deoxy-β-D-glucopyranoside **54**[169] (synthesised *via* the corresponding *N*-phthalimido derivative,[170] followed by deprotection using hydrazine monohydrate in 100% ethanol to give the free amine (92%), whose physical data agreed with that reported[169]) under the precisely described conditions for 24 h yielded a mixture of **3/53** (52%, or 70% based on recovered 5-thioglucopyranose) and a compound that appears to be the corresponding Amadori-rearrangement product **55** (15%) (Figure 3.12). As before, pure samples of **3/53** were obtained by careful flash chromatography, although purification was simplified by acetylation of the reaction mixture to isolate the pure α-anomer **56** (Figure 3.16), and deacetylation using MeOH:H_2O:Et_3N (5:1:1, 16 h)[171] to



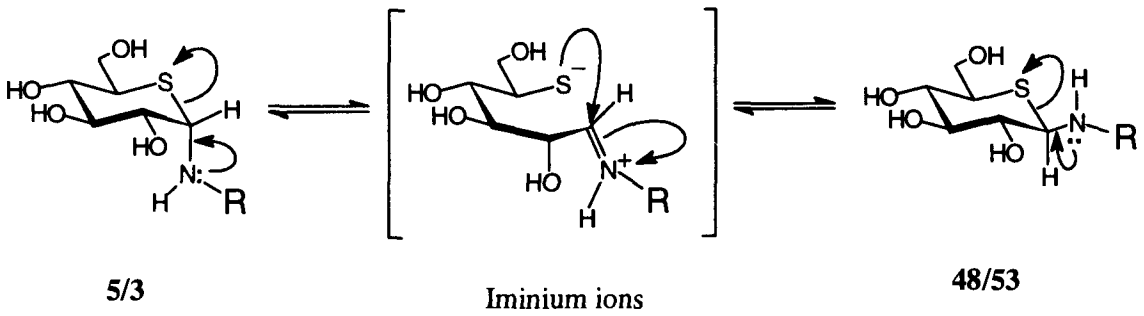
3 *S,N*-kojibioside analogue

53 *S,N*-sophoroside analogue

Figure 3.13 Anomerisation of **3** and **53**

give pure **3/53** (81% yield), which also equilibrated in D_2O over about 3 days to give **3/53** (~1:2.5; ^{1}H-NMR) (Figure 3.13).

Due to the fact that the deprotected sugars **5/48** and **3/53** do not hydrolyse appreciably in aqueous solution, we propose that anomerisation 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 (Figure 3.14).



5/3 Iminium ions **48/53**

Figure 3.14 Proposed mechanism for anomerisation of **5/48** and **3/53**

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-$COO^-$.[172] 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 $k_{RS^-}/k_{H2O} = 280$.[172]

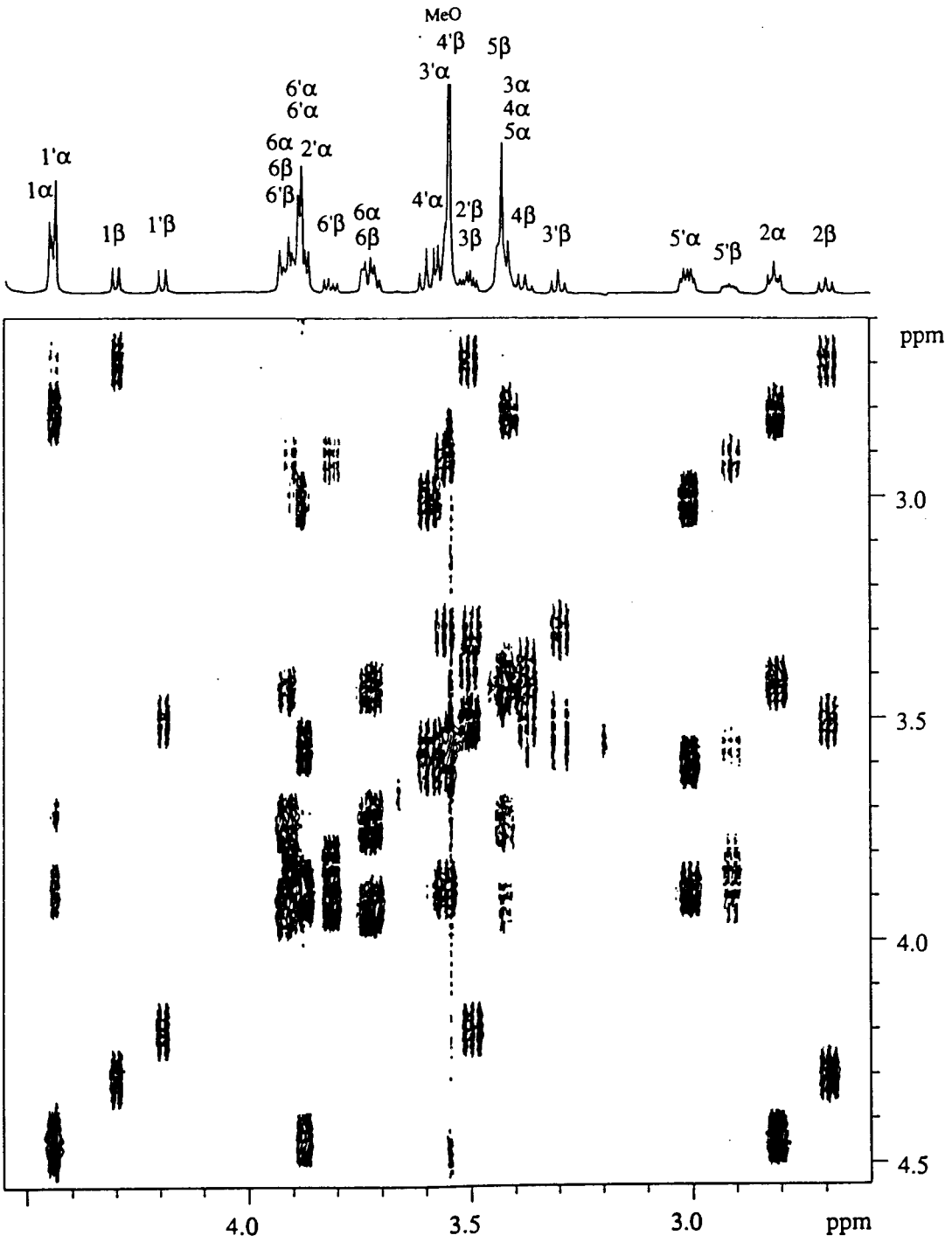


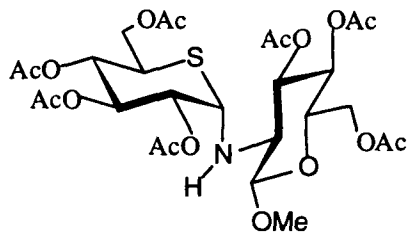
Figure 3.15 COSY ^{1}H NMR spectrum for **3/53**

The ^{1}H-NMR spectrum (Figure 3.15) of **3/53** appeared to be similar to that of **5/48** except that the two upfield resonances (2.81 ppm; multiplet and 2.70 ppm; doublet of doublets) were due to H-2α and H-2β, respectively. The N-H resonances were again not visible in the ^{1}H-NMR spectrum due to fast exchange. COSY (Figure 3.15) and NOESY spectra allowed the assignment of all spin systems. The stereochemical integrity of the two compounds was confirmed by observation of the $^1J_{H1',H2'}$ coupling constants, 4.9 Hz for **3** and 9.8 Hz for **53** (*cf.* 4.2 Hz for **5**, 9.5 Hz for **48**). The assignment of the ^{13}C resonances for **3** and **53** was made on the basis of a ^{1}H-^{13}C-inverse-detected NMR spectrum which allowed the correct assignment of the $^1J_{C1',H1'}$ coupling constants in the $^{13}C\{^1H\}$ spectrum, 154 Hz for **3** and 152 Hz for **53**. As with **5** and **48**, the $^1J_{C1',H1'}$ coupling constants could not be used with confidence in these compounds for the unequivocal evaluation of the stereochemistry about the interglycosidic linkage due to their similar values. The characteristic ^{1}H resonances for **55** [2.09, 3.05 (2H, AB quartet, $J_{H1a,H1b}$ = 12.5 Hz, CH_2-NH-R)] were very similar to those for **50** and were used to identify this by-product as a compound arising from an Amadori rearrangement.

Compounds **3/53** were also characterised as the acetylated compounds **56** and **57** and were easily separated by chromatography although **57** was slightly contaminated with an unknown product. Compound **56** fully characterised, whilst **57**, due to inseparable impurities, was fully characterised with the exception of elemental analyses and optical rotation. The N-H resonance for **57** was visible in the ^{1}H-NMR spectrum (1.37 ppm), although it was severely broadened such that no coupling information could be extracted. The N-H for **56** was still undergoing fast exchange even in dry non-acidic solvent

(CD_2Cl_2), and was therefore not visible in the ^{1}H-NMR spectrum, although the appropriate N-H stretch (3324 cm^{-1}) was observable in the IR spectrum.

The observation of vicinal proton-proton coupling constants J_{CHNH} is exceptional, and to the best of my knowledge is unprecedented. Using a modification of the Karplus equation Fraser *et al.*[172b] have defined a relationship between J_{CHOH} vicinal coupling constants and dihedral angles. As a first approximation, therefore, we have used the same modified Karplus relationship (J_{HCOH} = 10.4 $\cos^2\phi$ - 1.5 $\cos\phi$ + 0.2) to determine the Φ and ψ angles (for a definition of Φ and ψ angles see Chapter 4) of **51** and **52** based on the observed J_{CHNH} coupling constants. Thus, for the *S,N*-maltoside analogue **51**, $J_{C1'H1'NH}$ = ~0 Hz and J_{C4H4NH} = 10.0 Hz which give rise to dihedral angles of approximately 90° and 150°, respectively, and by the use of a 3-D model were found to correlate to Φ and ψ angles of approximately 180° and 0°, respectively. The unusual Φ angle of 180° still allows the antiperiplanar alignment of the nitrogen lone-pair and the C1'-S5' bond, a manifestation of the *exo*-anomeric effect that has been associated with *S,N*-acetals.[124] Similar calculations with the *S,N*-cellobioside **52** ($J_{C1'H1'NH}$ = 12.5 Hz and J_{C4H4NH} = 4.5 Hz) indicate approximate Φ and ψ angles of 180° and -45°, respectively. This Φ angle value also allows the activation of a strong *exo*-anomeric effect. Inspection of the 3-D models did not indicate any likely strong hydrogen bonding interactions between the N-H and acetate carbonyl oxygens in either ring that could account for the slow exchange of the N-H. The observation of the interglycosidic N-H vicinal coupling constants allows for the first time the calculation of an approximate 3-D solution conformation of disaccharides by this method.



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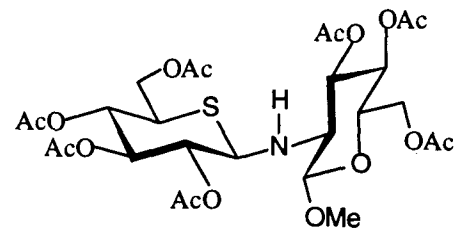

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Figure 3.16 Structures of the acetylated S,N kojibioside and sophoroside analogues

3.3.i Biological Activity of Analogue 5

All inhibition studies on glucoamylase were carried out by Dr. B. Svensson and T. P. Frandsen at the Carlsberg Laboratory in Denmark. Glucoamylase from *Aspergillus niger* catalyses the hydrolysis of maltose and related compounds with the release of β-D-glucose.[173] Compound **5** was tested,[174] therefore, as an inhibitor of maltose hydrolysis by glucoamylase G2. An equilibrated mixture of **5/48** (~1/2.5) was used in the kinetic studies[175] in which **5** was found to be a competitive inhibitor of maltose binding by glucoamylase G2, with a K_i value of 4 μM ±0.3. The corresponding K_m values for maltose and 4-nitrophenyl α-D-glucopyranoside are 1.2 mM and 3.7 mM, respectively. The cellobioside analogue **48** did not appear to be a substrate for the enzyme. These results are in agreement with preliminary transferred NOE studies of **5/48** with glucoamylase G1 which suggested that **5** was bound by the enzyme while **48** was not (see Chapter 4). Furthermore, when compared with the biological activity for the maltose derivatives containing sulfur and selenium, which gave only mM inhibition of

glucoamylase G2 (see Chapter 2), these results indicate the importance of a basic group attached to C-1 of the sugar ring for tight binding to the active site. Compound **5** is a substrate or ground state inhibitor; the replacement of the interglycosidic oxygen atom by NH, or the ring oxygen atom by sulfur does not render these compounds transition state analogues, i.e. they do not resemble the putative transition state within the enzyme active site. The K_i value of **5**, therefore, is significant. Compound **5** is a stronger inhibitor of glucoamylase G2 than the transition-state inhibitor 1-deoxynojirimycin **1.1**, which inhibits glucoamylase G2, with a K_i value of 96 μM.[14] More significantly, compound **5** is almost as potent an inhibitor as the pseudo-disaccharide acarviosin **3.15**, which was found to be two orders of magnitude stronger than 1-deoxynojirimycin **1.1**[57] (approximately 1 μM). Acarbose **3.9**, nevertheless, is far more potent than **5**, with a K_i (K_d) value of $<6 \times 10^{-12}$ M^{-1} [56] against glucoamylase G2. This is attributed, in part, to the extended surface of acarbose within the active site.[176] These inhibition results are summarised in Table 3.1.

Table 3.1 Comparison of the inhibition of glucoamylase by **5** and other known inhibitors

Inhibitor	K_i (μM)	Reference
methyl 5'-thio-4-N-α-maltoside **5**	4.0	this work
1-deoxynojirimycin **1.1**	96	14
acarviosin **3.13**	~1	57
acarbose **3.9**	0.0006	56

3.3.ii Biological Activity of Analogue 3

Compound **3** was tested[119] 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 glucosidase II. Compound **3** was determined to be a weak competitive inhibitor of glucosidase I, with a K_i value of 3 mM, and a potent competitive inhibitor of glucosidase II, with a K_i value of 30 μM. The corresponding K_m value for α-D-Glc-(1-2)-α-D-Glc-(1-3)-α-D-Glc-$O(CH_2)_8$-CO_2CH_3 was 1.28 mM with glucosidase I. The stronger inhibition of glucosidase II compared with glucosidase I by compound **3** is comparable to results obtained with propyl 2-S-β-kojibioside **1** (Chapter 2), although the inhibition of glucosidase II is far stronger with **3** than with **1** (K_i = 1.0 mM). The kojibioside analogues **1** and **3** are better inhibitors of glucosidase II, which hydrolyse internal α 1-3 linked glucose units, than for glucosidase I, which hydrolyses distal α 1-2 units. The greater inhibitory activity of the analogues with nitrogen in the interglycosidic linkage compared with those with sulfur in the interglycosidic linkage is clearly due to the presence of the basic group at C-1 of the ring, thereby increasing the interaction with active site carboxyl groups.

3.4 Conclusions

This work describes the first syntheses of a new class of disaccharide with sulfur in the non-reducing ring and NH in the interglycosidic linkage for evaluation as potential novel glycosidase inhibitors. Disaccharide analogues **5/48** and **3/53** have been synthesised

but were found to anomerise. As initially assumed, the thermodynamic preference for the α-configuration is more pronounced in these S,N-acetals, thereby, allowing appraisal of the α-anomer as a specific α-glucosidase inhibitor. Furthermore, these compounds appear to be very stable towards hydrolysis, in contrast to the behaviour of the disaccharide **3.18** with N in the ring and S in the interglycosidic linkage.[163] The synthesis of higher homologues of these disaccharides as probes for the structural requirements of the aglycon binding site is feasible using these methods.

Compound **5** is a competitive inhibitor of maltose hydrolysis by glucoamylase G2, with a K_i value of 4 μM, and compound **3** is a competitive inhibitor of glucose release from glucosidase I and II, with K_i values of 3 mM and 30 μM, respectively. These results, when compared with corresponding analogues with sulfur in the interglycosidic linkage, indicate the importance of a basic group at C-1 of the inhibitor for enhanced biological activity.

3.5 Suggestions for Further Research

The inhibitory potency of acarbose **3.9** is significantly greater (approximately five orders of magnitude)[57] than that found for acarviosin **3.15**, suggesting that, as an inhibitor of glucoamylase, the extended surface of bound acarbose[176] is, in part, responsible for the potent inhibitory activity. Since the inhibitory activity of **5** has been found to be similar to that of acarviosin **3.15**, it would, therefore, be of interest to synthesise a tetrasaccharide of similar structure as acarbose, but incorporating the disaccharide **5** in place of the pseudo-disaccharide of acarbose. Such a compound, with an extended binding surface, might be a

very potent inhibitor of glucoamylase, and comparison of its biological properties with those of acarbose would be of interest.

CHAPTER 4

CONFORMATIONAL ANALYSIS OF DISACCHARIDE HETEROANALOGUES

4.1 Conformational Analysis of Methyl 4,5'-Dithio-α-Maltoside Using A Combined NMR Spectroscopic/Molecular Mechanics Approach

The conformational analysis described here was carried out at Simon Fraser University in collaboration with Dr. T. Weimar who performed all transient and transferred NOE studies, and with Dr. U. C. Kreis who performed all molecular mechanics studies. A detailed understanding of the binding between protein and carbohydrate requires knowledge of the topographies presented by different oligosaccharides. Such information could be utilised in the design of superior inhibitors. X-ray crystallographic studies allow the absolute determination of compound conformations but suffer from the disadvantage that crystal packing forces may alter the solution conformation of the compound upon crystallisation, with the result that the conformation present in the crystal may not be representative of that in solution. An appreciation of the conformational features of possible importance can be gained by detailed NMR spectroscopy experiments through determination of conformationally dependent parameters such as NOEs. Molecular mechanics calculations can also been used to predict conformational preferences of compounds, and when combined with NMR spectroscopy, offer a method by which the solution conformations of carbohydrates may be determined with a reasonable degree of certainty. These methods, however, suffer from the limitations of

being based on the free ligands. Although it is logical that the low-energy conformations bind to the active sites of the enzymes, it is nevertheless possible that the energy required to produce conformational changes will be supplied by the free energy of binding. The direct examination of ligand conformations when bound by the enzymes by transferred NOE experiments[177] is, therefore, desirable. X-ray crystallography of ligand-protein complexes is the method of choice for the determination of substantial amounts of accurate data, but suffers from a number of setbacks. Apart from the possibility of altered conformations due to crystal packing forces, the growing of suitable protein-ligand complex crystals is very difficult and time consuming.

This section of Chapter 4 describes the use of selective 1D-transient-NOE experiments,[178] in conjunction with molecular mechanics calculations[179] using the PIMM91[180] force field to compute minimum energy conformations, to analyse the conformational behaviour of methyl 4,5'-dithio-α-maltoside **4** in solution. The protocol consists of the comparison of calculated theoretical NOE curves based on Boltzmann-averaged structures from grid searches and experimental (NMR) NOE curves determined from the initial rates of transient-NOE build up. A close fit of theoretical NOE curves to the experimentally derived NOE data confirms the validity of the molecular mechanics-derived three dimensional conformation, measured in terms of Φ, ψ and ω angles (Figure 4.1).

It is known that maltose and maltose derivatives show two different minimum energy regions on potential energy maps.[181] In maltose derivatives, the NOE H-1'/H-4 is a sensor for the global minimum energy conformation and the NOEs H1'/H3 and especially

H1'/H5 for a local minimum energy conformation (see Figure 4.2), although the NOE H1'/H3 is also possible to a small extent in the global minimum conformation.

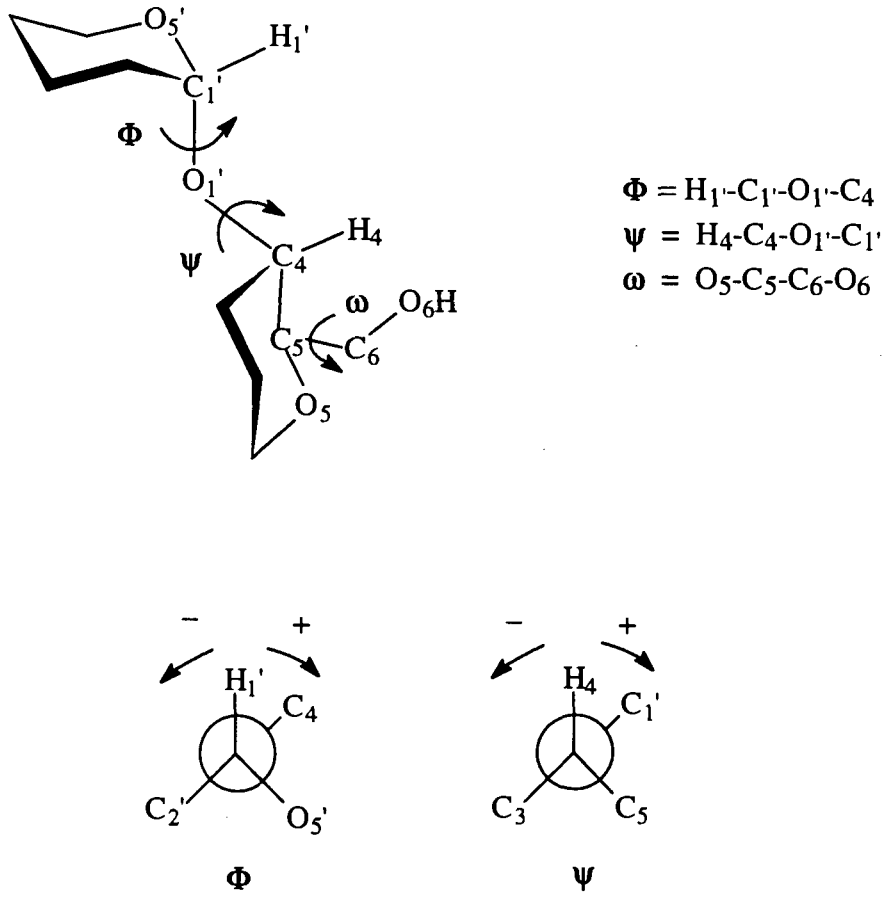

Figure 4.1 Definition of Φ, ψ and ω angles for glycosidic linkages

As expected, 1D transient NOE experiments for **4** show the NOE effects H-1'/H-4 (spectra b and e), H-1'/H-5 (spectra b and c) and H-1'/H-3 (spectrum b) (Figure 4.3), proving the existence of the two conformational families for this molecule in aqueous solution. Molecular mechanics calculations for maltose and the dithiomaltoside analogue

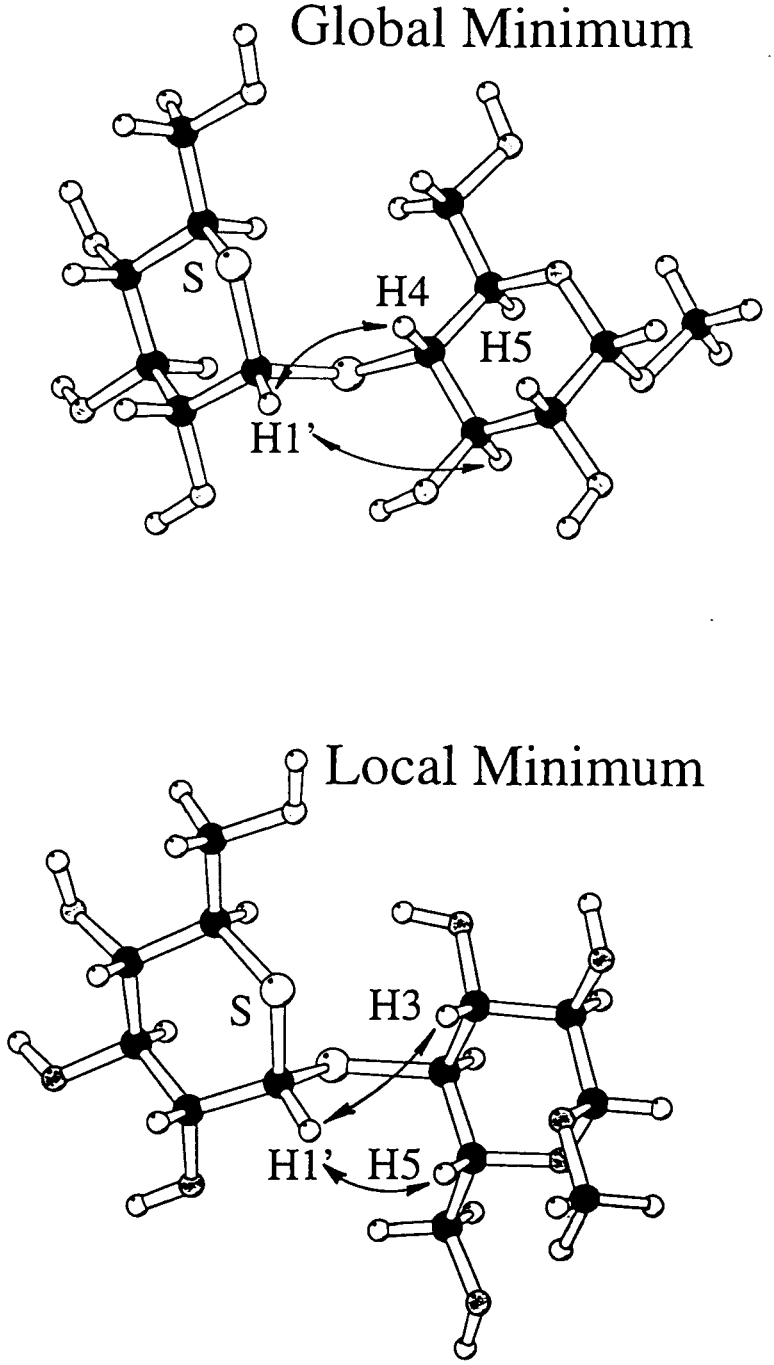

Figure 4.2 Ball and stick representations of the energy minima in the two conformational areas A and C found for **4**

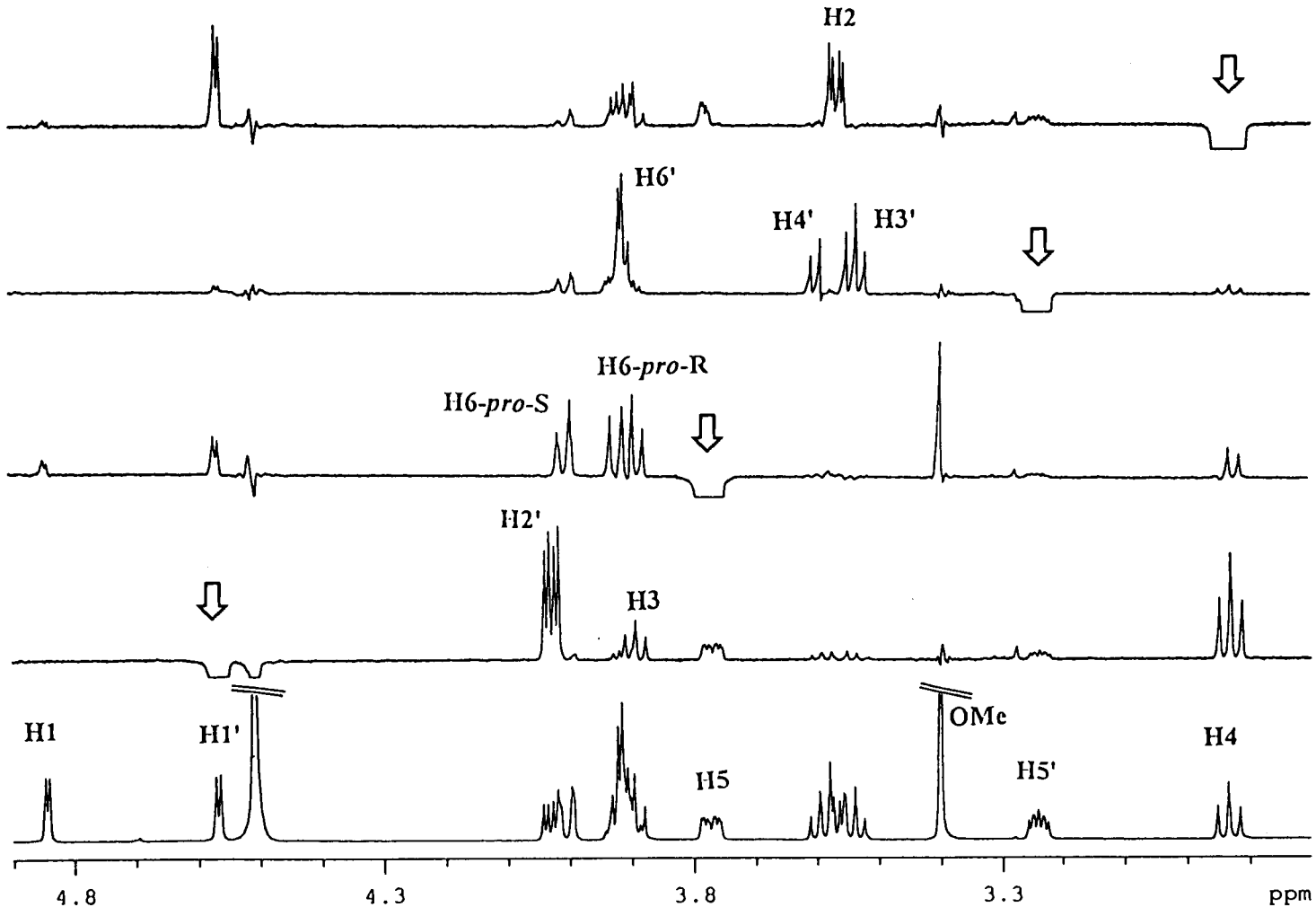

Figure 4.3 a) ^{1}H NMR spectrum of compound **4**. b)-e) Transient NOE spectra with ⇩ marking the selectively irradiated proton resonance in each spectrum

4 also show different minimum energy conformations of the 1-4 glycosidic linkages. A contour plot of all possible Φ and ψ angles for **4** is shown in Figure 4.4. The conformational family corresponding to the global minimum is represented by the region A (Φ/ψ ~-40°/-20°) in the contour map and a local minimum represented by the region C (Φ/ψ ~40°/180°). Region B (Φ/ψ ~40°/-20°) is not quantitatively distinguishable from region A in transferred NOE experiments. The longer C-S bond length in the interglycosidic linkage in comparison to a C-O bond length enhances the flexibility of the dithiomaltoside analogue **4** relative to the 5'-thio-α-maltoside analogue[107] with oxygen in the interglycosidic linkage. In accord with this conclusion, the calculated probability for conformations in region C increases from 1% for 5'-thio-α-maltoside to 14% in compound **4**.[181e,182] In order to determine if, in fact, the NOE H1'/H3 is a reasonable sensor for a local minimum, a comparison of the relative value of this NOE contact (as well as the NOE H1'/H5) observed for the series of analogues with O, S and Se in the interglycosidic linkage was made. Since it has been shown by molecular mechanics calculations that the percentage of a local minimum energy conformation (region C) increases from O to S (and can be presumed to increase further for the Se analogue), then for the above prediction to be correct, a corresponding increase in the NOE H1'/H3 (and H1'/H5) should be observed. A direct comparison of measured NOE values from different compounds is best accomplished by comparison of the intensity of the relevant NOEs in the compounds relative to a fixed NOE that is indicative of a "ruler" distance. Such a distance should be conserved within all of the structures, for example, the distance between protons within a ring, thus permitting a valid quantitative comparison of NOEs from different compounds.

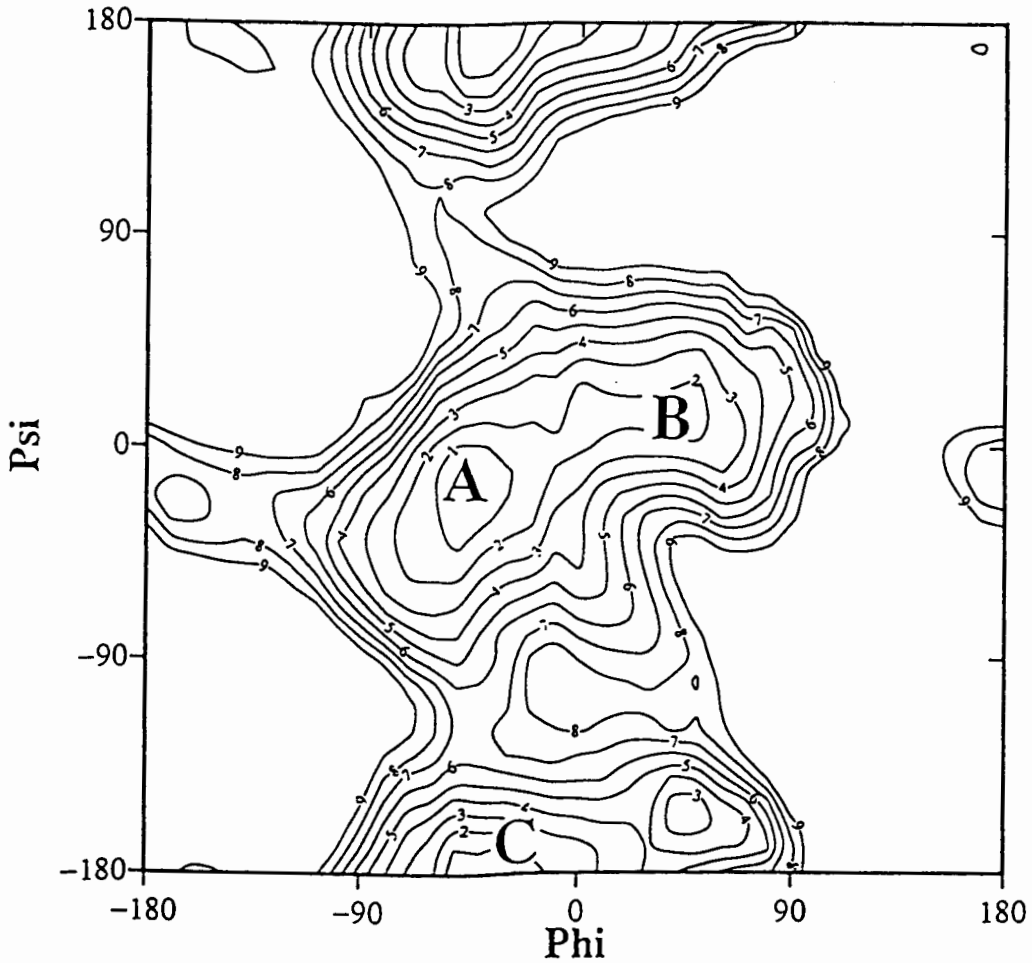

Figure 4.4 Φ / ψ contour map of **4**

In the NOE spectra of all the maltoside derivatives (S S, S O and S Se) the NOE H5'/(H3' + H4') is well resolved (for **4** see Figure 4.3, spectrum d) and was used to calculate relative NOEs by dividing the NOEs for H1'/H3, H1'/H4 and H1'/H5 by those for H5'/(H3'+ H4'). By comparison of these relative NOEs for the three compounds (Figure 4.5) it is apparent that the NOEs H1'/H3 and H1'/H5 increase in the order O to S to Se indicating a higher population of conformations in region C and, therefore, a higher degree of flexibility in these compounds. The NOE H1'/H4 (corresponding to the global minimum conformation) was, as expected, observed to decrease as the population of a local minimum conformation (region C) increased. The NOE H1'/H3 is, therefore, more significant in a local minimum conformation than in the global minimum and can be used as a sensor, along with the NOE H1'/H5, for the former conformation.

A comparison of the calculated and experimental NOE curves (Figure 4.6) shows a good match, indicating that the model presented for the dithiomaltoside analogue **4** appears to describe the various conformational states of **4** in aqueous solution. The global minimum structure of compound **4**, region A (Φ/ψ = ~-40°/-20°) coincides with the structure derived from X-ray crystallography[182] (see Figure 4.7 and Table 4.1). The X-ray-derived conformation indicates that, in accord with expectations based on the anomeric effect the S_5-C_1-S_1 bond angle is greater than tetrahedral[183] and that the torsional preference about the Φ angle is consistent with expectations based on the exo-anomeric effect.[184]

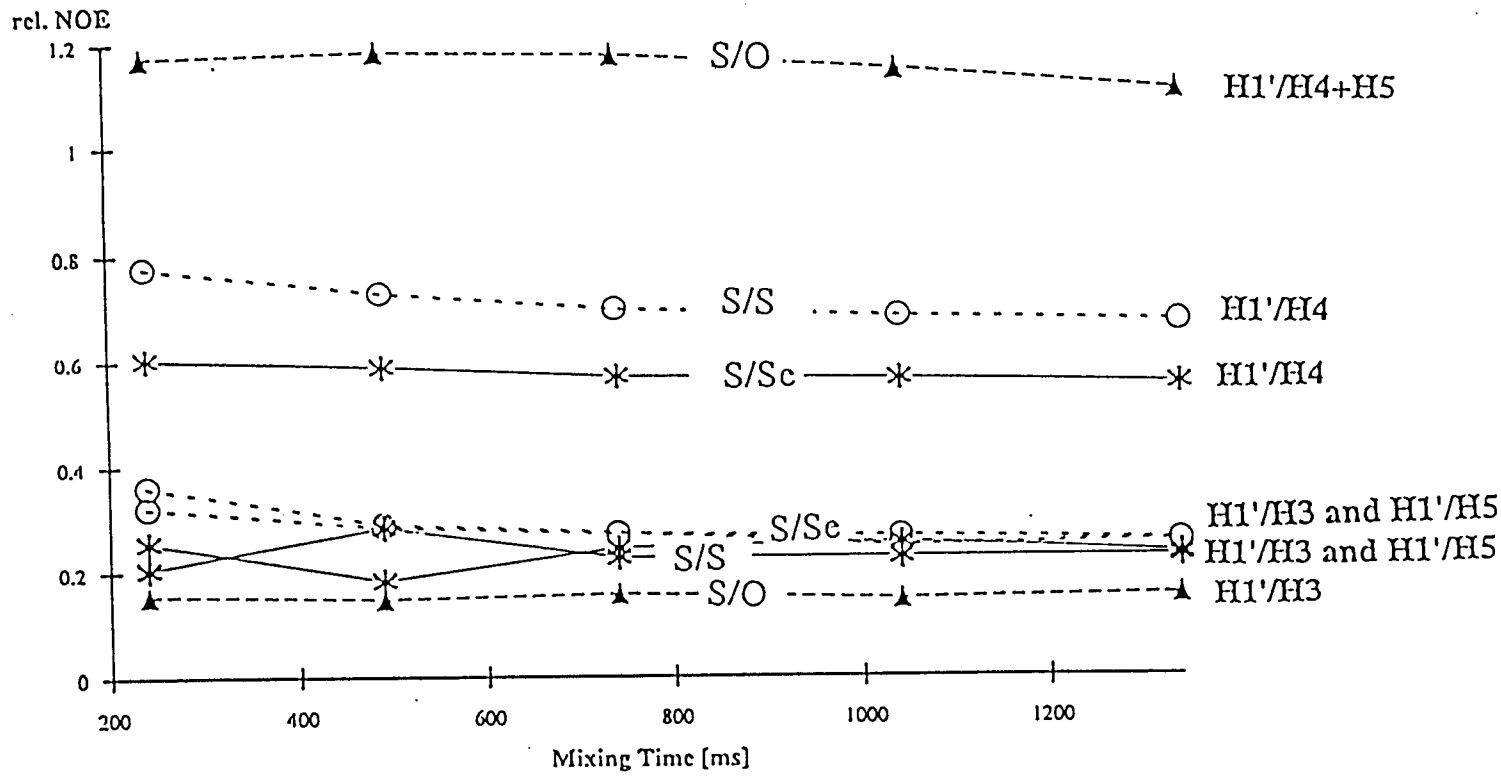

Figure 4.5 Comparison of NOEs H1'/H3, H1'/H4 and H1'/H5 for 4, methyl 5'-thio-α-maltoside and methyl 4-seleno-5'-thio-α-maltoside, relative to H5'/(H3' + H4')

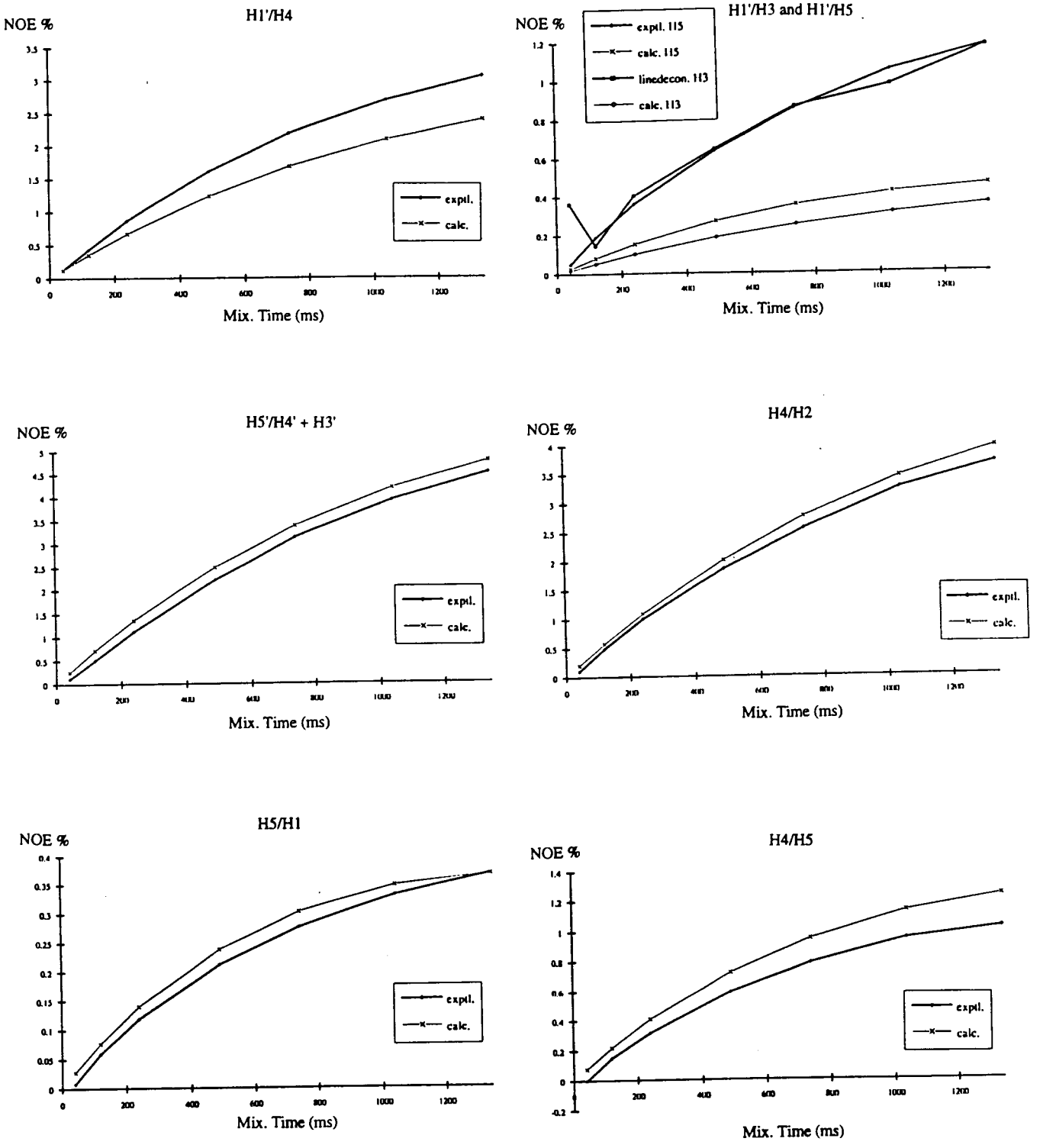

Figure 4.6 Comparison of theoretical and experimental NOE build-up curves for selected proton pairs for **4**

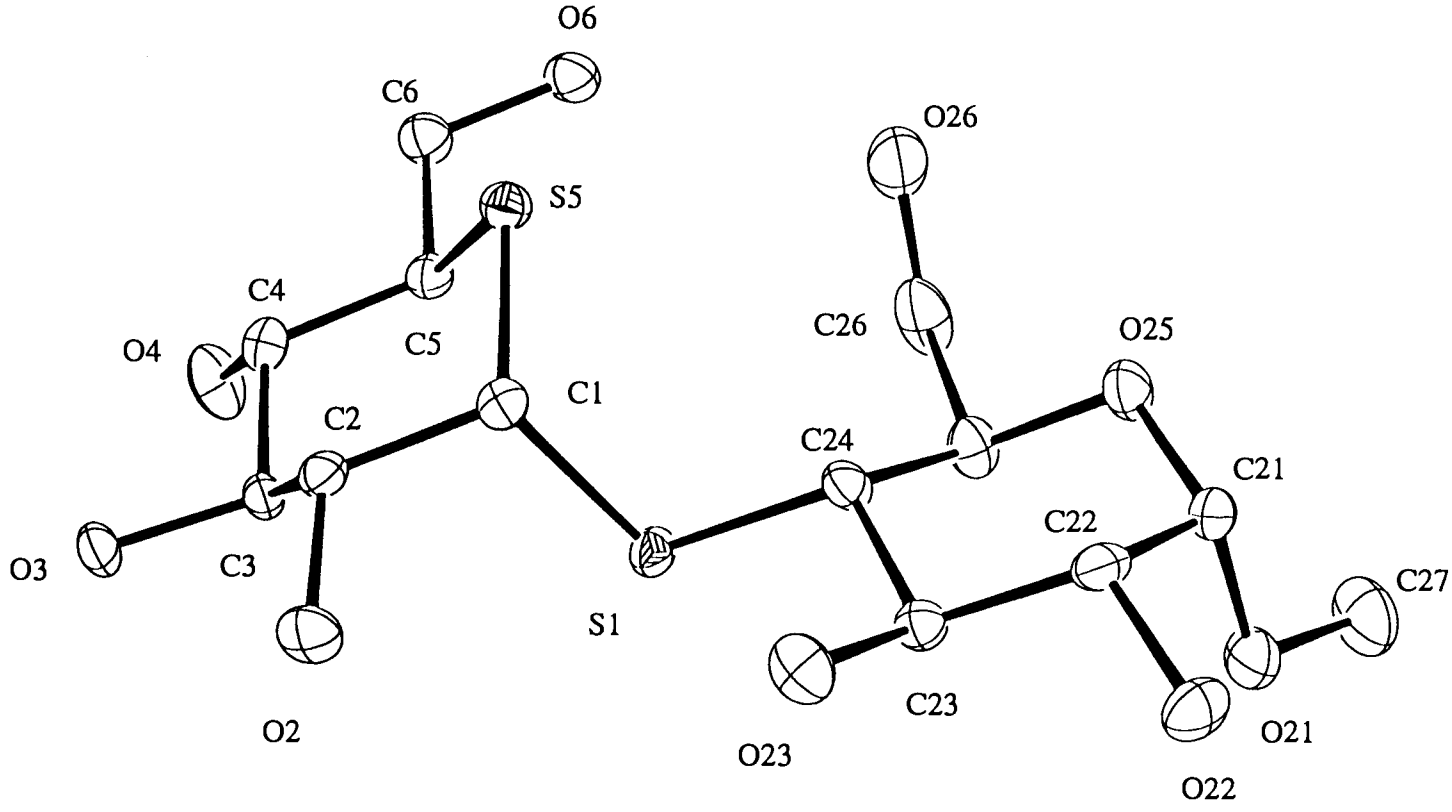

Figure 4.7 Conformation of **4** derived from X-ray crystallographic studies

Table 4.1 Selected structural features of methyl 4,5'-dithio-α-maltoside **4**

	H_1-C_1-S_1-C_{24}	-36
Φ (°)	C_2-C_1-S_1-C_{24}	-154.9 (2)
	S_5-C_1-S_1-C_{24}	+80.7 (1)
	C_1-S_1-C_{24}-H_{24}	-6
ψ (°)	C_1-S_1-C_{24}-C_{23}	+111.5 (2)
	C_1-S_1-C_{24}-C_{25}	-125.8 (2)
ω_1 (°)	S_5-C_5-C_6-O_6	+59.5 (2)
ω_2 (°)	O_{25}-C_{25}-C_{26}-O_{26}	-59.8 (2)
bond angles (°)	C_6-C_5-S_5	107.58 (21)
	C_5-S_5-C_1	99.15 (13)
	S_5-C_1-S_1	113.72 (15)
	C_1-S_1-C_{24}	100.44 (14)
bond lengths (Å)	C_5-S_5	1.818 (3)
	S_5-C_1	1.8199 (25)
	C_1-S_1	1.821 (3)
	S_1-C_{24}	1.826 (3)

4.1.i Transferred NOE NMR Studies of Methyl 4,5'-Dithio-α-Maltoside Bound By Glucoamylase G1[185]

In order to gain an insight into the conformation of the dithiomaltoside analogue **4** when bound by the enzyme glucoamylase G1, transferred NOE experiments[177,186] were performed[178] on mixtures of **4** and glucoamylase G1. For a system in which a ligand (inhibitor) is in equilibrium with a macromolecule (enzyme) (Figure 4.8), conformational information on the bound ligand may be obtained by measurement of NOEs on the easily

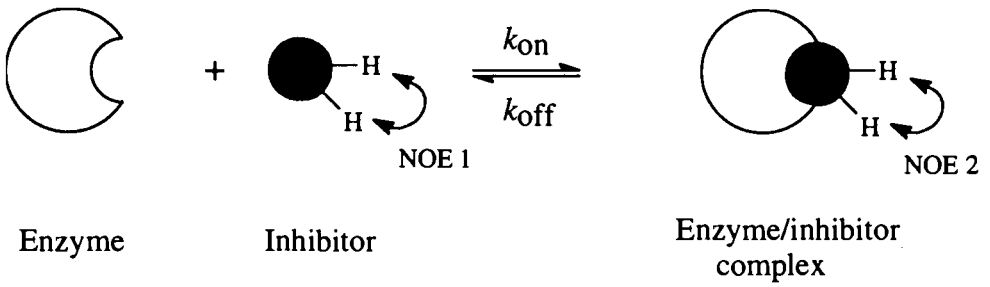

Figure 4. 8 Schematic representation of an enzyme and inhibitor in equilibrium

detectable, averaged ligand resonances through transferred NOE experiments. The technique takes advantage of the increased rate of NOE build up of the ligand in the slowly tumbling bound state (NOE 2), compared to the NOE build up in the rapidly tumbling free state (NOE 1), such that the NOE for the bound state dominates the observed NOE. This information is detected on the averaged ligand resonances and reflects the geometry of the ligand in its bound state. The correlation time of the macromolecule is long compared to the ligand and, therefore, its ^{1}H NMR resonances are often broad allowing observation of the time-averaged ligand resonances. The off-rate for the inhibitor-enzyme complex is an important factor in observing transferred NOEs and can be optimised by variation of the temperature and inhibitor:enzyme ratio.

Thus, a sample of glucoamylase G1 was titrated with 4,5'-dithio-α-maltoside **4** (enzyme:ligand ratio = 1:25) in sodium acetate buffer (pH 4.5) at a temperature of 284 K. Comparison of the NOE spectra of free **4** (Figure 4.9, spectrum b) with the TRNOE spectra of bound **4** (Figure 4.9) shows that the NOE H1'/H4 (characteristic of the global minimum) is observable (spectra c and e), but the NOEs H-1'/H-5 and H1'/H3

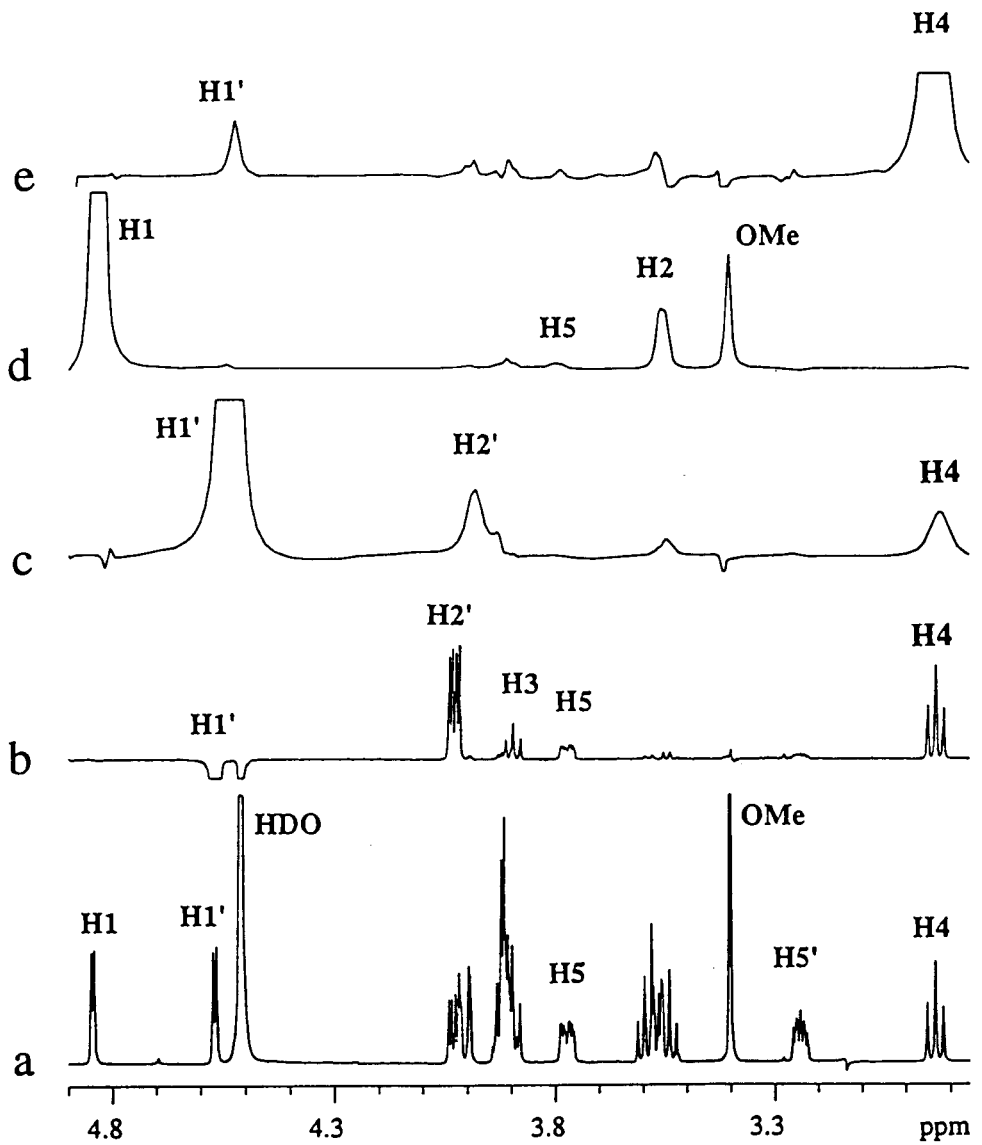

Figure 4.9 ^{1}H NMR spectra for free **4** and for a mixture with glucoamylase G1: a) 1D spectrum of free **4**. b) 1D-transient-NOE spectrum after selective inversion of H1'. c, d, e) Projections of the TRNOESY spectra of **4** at the resonances of H1', H1 and H4, respectively.

(characteristic of a local minimum structure) are no longer observable for the bound species. Therefore, glucoamylase G1 selects a conformation of **4** in the area of the global minimum.

4.2 Transferred NOE NMR Studies of Methyl 5'-Thio-4-N-α-Maltoside Bound to Glucoamylase G1[125]

The fungus *Aspergillus niger* produces two different forms of glucoamylase, glucoamylase G1 and G2. Whereas the G1 form has both the catalytic domain and starch-granule binding domain, the G2 form contains only the catalytic domain.[187,188] The enzyme catalyses the hydrolysis of both α-1,4- and α-1,6- glucosidic bonds from the non reducing end of starch and related saccharides, although the 1-4 linkages are hydrolysed significantly faster than the 1,6 linkages.[189] The pseudosugar, acarbose (**3.9**), has been shown to be an extremely potent inhibitor of glucoamylase,[14] with a K_i value of 10^{-10} M^{-1}. [56] The *S,N*-maltoside analogue **5** is a potential glucoamylase inhibitor and the solution conformation of this compound bound to glucoamylase is of interest in determining the surface topographies of the bound inhibitor; such information may be used to guide the genetic engineering of enzyme active sites, and in determining the active site topography of the enzyme to aid in the design of superior inhibitors. The conformational analysis of **5/48** in PBS (phosphate-buffered saline, pH 7.2) by NMR spectroscopy was, therefore, examined in our laboratory[178] The NOE H-1'/H-4 is a sensor for the global minimum conformation and the NOEs H-1'/H-5 and H1'/H3 sensors for a local minimum conformation (see Figure 4.10). The global and local minimum conformations depicted in

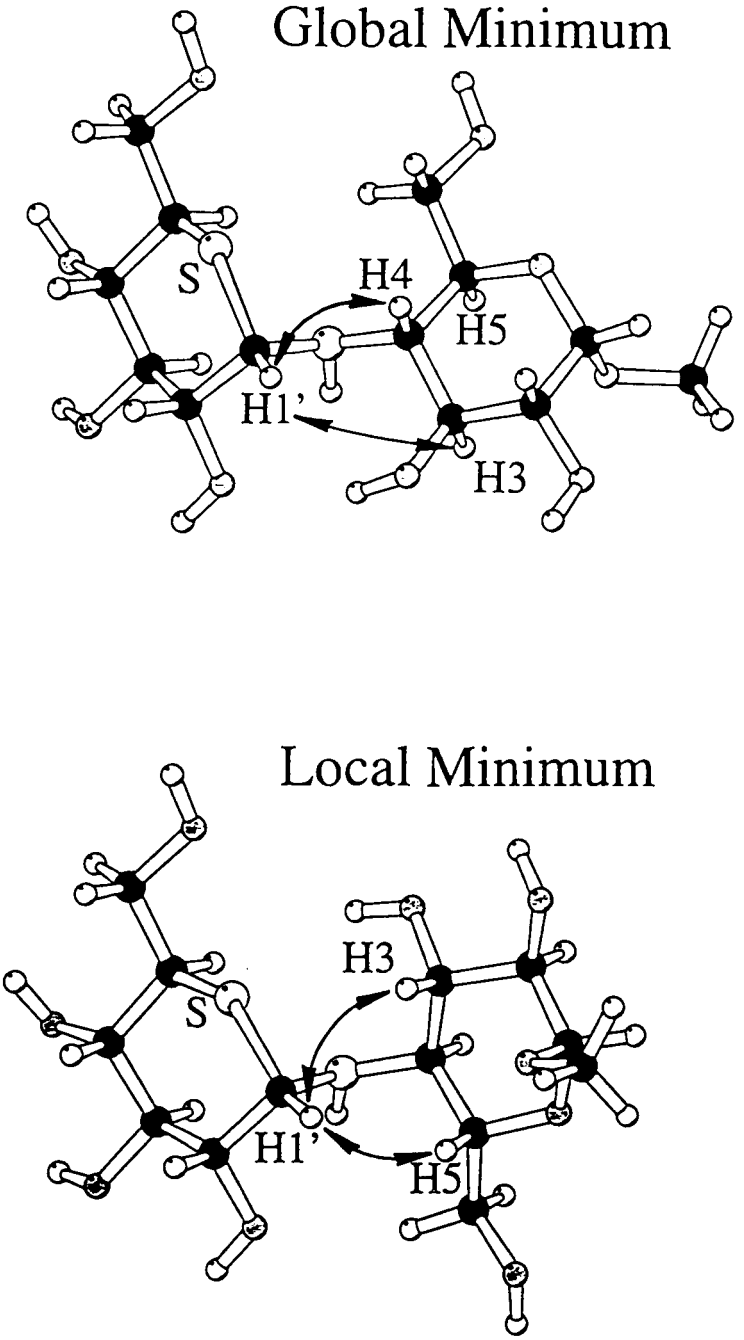

Figure 4.10. Ball and stick representations of the energy minima in the two conformational areas found for **5**

Figure 4.10 were generated by calculations performed with Sybyl 6.1 and the standard Amber force field on an SGI Personal Iris. Energy minimisations were started from minimum energy conformations found for methyl maltoside, after replacement of the appropriate oxygen atoms with sulfur and NH. The Amber force field is not specifically paramaterised for the S-C-NH bond sequence. The ball and stick models are, therefore, taken as crude representations of the two conformational families. One does not expect that minor changes in bond lengths and angles resulting from an exact parameterisation, will result in changes in the overall arguments advanced here. Given the flexibility of the glycosidic linkages, one attempts here to identify distinctly different conformational families for **5/48**. With this limitation in mind, the values obtained for the Φ and ψ angles and the relative potential energies are: global minimum: Φ -27°; ψ -26°; local minimum: Φ -31°; ψ 180°; relative potential energies: $PE_{local} = PE_{global} + 0.5$ kcal/mol. The energy difference between the two minimum energy conformations is the same as values obtained for maltose.[190] The H-4 and H-5 signals in the spectrum of **5** are first order and the NOE contact H-1'/H-5 can be used to access information about this local minimum. As expected, 1D transient NOE experiments for **5** show the NOE effects H-1'/H-4, H-1'/H-5 and H-1'/H-3 (Figure 4.11), proving the existence of the two conformational families for this molecule in aqueous buffer.

The conformations of **5/48** bound by glucoamylase G1 were next probed by transferred NOE (TRNOE) experiments.[177,186] Thus, a sample of glucoamylase G1 was titrated with the mixture **5/48** at 290 K. At this temperature, all carbohydrate resonances (see Figure 4.11) were broadened, but the resonances of **5** were broadened to the extent

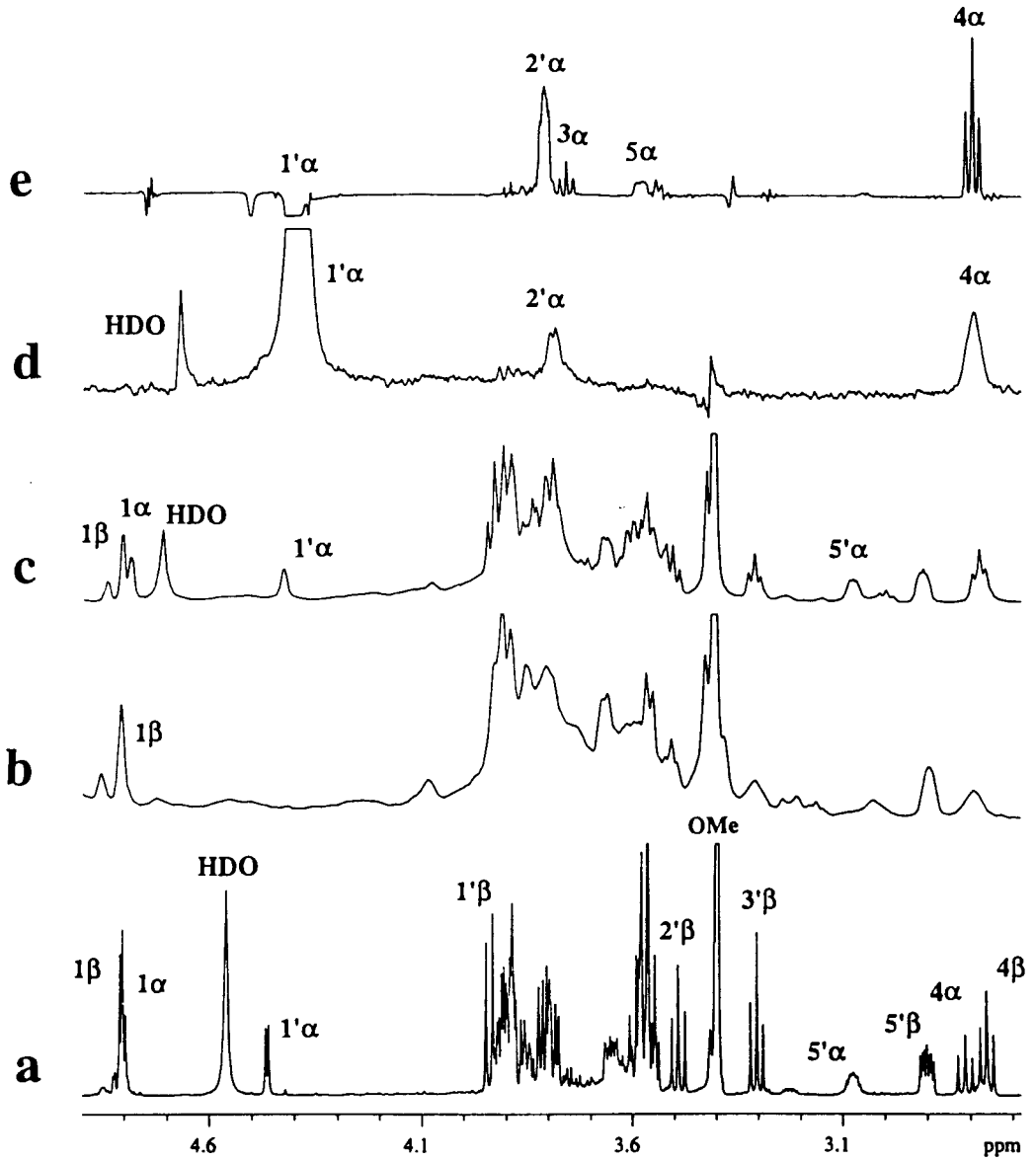

Figure 4.11 ^{1}H NMR spectra for free **5/48** and for a mixture with glucoamylase G1: a) 1D spectrum of free **5/48**. b) 1D spectrum of the mixture at low ligand concentration (~4:1; **5** : glucoamylase G1). c) 1D spectrum of the mixture at high ligand concentration (~25:1; **5** : glucoamylase G1). d, e) Projections of TRNOESY and NOESY spectra, respectively, at the resonance of H-1'α

that they were not visible at ligand:**5** ratios of up to 5:1. At higher temperatures and ligand:enzyme ratios, the signals of **5** were observable but were still broadened more than the corresponding resonances of **48**. TRNOE experiments of **5/48** at different mixing times performed with ~25 equivalents of **5** (and ~85 equivalents of **48**) showed TRNOEs only for the **5** resonances. In experiments with short mixing times, the resonances of **5** showed an NOE behaviour expected for a free disaccharide at this temperature (very small positive NOEs interfered by zero quantum signals).

Comparison of the NOE spectra of free **5** with the TRNOE spectra of bound **5** shows that the NOE H-1'/H-5 (characteristic of a local minimum structure) is no longer observable for the bound species. Our results suggest that although the compound populates both global and local minimum conformations, it is bound by glucoamylase G1 in a conformation in the area of the global minimum of the free disaccharide (see Figure 4.10). Our experimental data also suggest that **5** is bound by glucoamylase G1 and that it may, therefore, be an inhibitor of the enzyme. Indeed, as described in Chapter 3, compound **5** was found to be a competitive inhibitor of maltose binding by glucoamylase G2, with a K_i value of 4.4 μM ±0.3.[125]

Acarbose **3.9** and dihydroglucoacarbose **3.16**, both potent inhibitors of glucoamylase, have also been shown[155] by NOE experiments and molecular modelling studies to populate similar global and local energy minima as observed for maltose and maltose analogues. In addition, the conformational preferences about the imino linkage were shown to be strongly dependent on pH.[155] At high pH, the acarbose conformer with Φ/ψ angles of -51/-10° preponderates (corresponding to the global minimum in maltose),

while at low pH the alternative conformation with Φ/ψ angles of -26/-176° is prevalent; similar conformational preferences were shown for dihydroglucoacarbose.[155] Analysis of a crystal structure of a complex of acarbose and a very closely related glucoamylase, at pH 6, has revealed that acarbose is bound by the enzyme in a conformation in the area of the global energy minimum of free maltose (Φ/ψ = -16/6.5°),[176] and is similar to the conformation of acarbose at low pH.[155] The importance of the imino-linked moiety of acarbose to the binding affinity is suggested in the finding that the unsaturated ring of acarbose has, by far, the most interactions with the protein.[176] The crystal structure of dihydroglucoacarbose-glucoamylase complex has recently revealed that the first two residues of dihydroglucoacarbose bind at positions nearly identical to those of acarbose.[191] We assume that the binding interactions of methyl 5'-thio-4-N-α-maltoside **5** with glucoamylase are similar because the bound conformation is similar to that observed with acarbose and dihydroglucoacarbose.

Regarding the potential criticism that the transferred NOE studies and the enzyme kinetics were performed on the two different forms of glucoamylase, G2 and G1, respectively, it has been shown that acarbose has a high affinity for the catalytic site and a low affinity for the starch-binding domain,[56] whereas the opposite is true for β-cyclodextrin.[56] In addition, recent displacement titration calorimetry studies[57] have shown that the binding of 1-deoxynojirimycin **1.1** and acarbose **3.9** to glucoamylase from *Aspergillus niger* showed essentially the same affinities for the G1 form as for the G2 form. Based on the similarity in structure of **5** and acarbose **3.9** (especially the valienamine moiety) it is reasonable to assume that binding observed in the transferred

NOE experiments and the results obtained from the enzyme kinetics are primarily associated with the catalytic site in the G1 and G2 forms of glucoamylase, respectively. Our choice of glucoamylase G1 for the NMR studies was dictated by the requirement for large quantities of the enzyme; glucoamylase G2 was not readily available at the time.

These results represent the initial investigations of this inhibitor-enzyme system. Since it has been shown that the conformations of acarbose and dihydroglucoacarbose are pH dependent, it would be of interest to investigate the conformations of the free and bound *S,N*-maltoside analogue **5** at both high and low pH.

CHAPTER 5

EXPERIMENTAL

5.1 General Experimental

5.1.i Synthesis

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 deuterochloroform or deuterium oxide. Chemical shifts are given in ppm downfield from TMS for those spectra measured in deuterochloroform, and downfield 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. All new compounds were characterised by either microanalysis or electrospray mass spectrometry.

All new compounds were also fully characterised by the use of routine ^{1}H, ^{13}C, ^{1}H-homonuclear and ^{1}H-^{13}C inverse-detected NMR spectra. The ^{1}H-homonuclear chemical-shift correlated (COSY) spectra[192] were acquired using a pulse sequence d1-90°-d0-45°-FID with quadrature detection in both dimensions. The initial data sets of 1024 x 512 data points were zero-filled once in the F_1 direction to give a final data set of 1024 x 1024 real data points. For the inverse detection experiments a four-pulse sequence was used for the $^{1}H\{^{13}C\}$-^{13}C correlation.[193] The data sets of 2048 x 512 data points were

zero-filled once in the F_1-direction, to give a final data set of 1024 x 1024 real data points.

Analytical thin-layer chromatography (TLC) was performed on aluminium 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 5% sulfuric acid in ethanol, and heated at 150°C. All compounds were purified by medium-pressure 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.

Reactions performed under nitrogen were also carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

Microanalyses were obtained by M. Yang at the SFU Microanalytical Laboratory, and electrospray mass spectra were measured by Dr. J. Banoub, Department of Fisheries, Newfoundland.

5.1.ii Molecular Modelling[179]

All calculations were performed by Dr. U. C. Kreis on SGI Personal Iris 4D/35 and SGI 380 Power series computers. The molecular mechanics programme PIMM91[181] was used. The PIMM91 force field was parameterised for sulfur-containing sugars (*O,S*- and *S,S*-acetals) by consideration of bond lengths, bond angles, torsion angles and intramolecular hydrogen bonding patterns, derived from X-ray crystal structures of the following compounds: methyl 1,5-dithio-α-ribopyranoside, methyl 1,5-dithio-β-

ribopyranoside, methyl 5-thio-α-ribopyranside, methyl 5-thio-β-ribopyranside, methyl 1-thio-α-ribopyranside. In order to enhance the anomeric effect, torsion angles involving the heteroatoms in the acetal unit employ a potential that is linearly dependent on the polarisation of the adjacent bonds: $E_{IJKL} = \alpha\, \Delta q_{I,J}\, \Delta q_{J,K}\, (1 + \cos(2\theta))$, where I, J, K, L are the atoms which define the torsion (dihedral) angle. The hydrogen bond search routine was disabled for all computations to eliminate artificial stabilisations due to hydrogen bonding that would not be observed in an aqueous environment. First a grid search was performed by permuting both of the ω angles and all of the hydroxyl group torsion angles to 60, 180 and 360 degrees to give 2187 fully optimised structures. Contour searches were performed in which the intersaccharidic torsion angles Φ and ψ, i.e., H1'-C1'-S(N)1'-C2 and C1'-S(N)1'-C2-H2, were permuted starting from 0 as well as from 360 degrees, with an increment or decrement of 10 degrees. These two angles were fixed to particular values while the rest of the molecule was allowed to relax fully. For each of the nine possible conformations of the two ω angles, i.e., S(O)5-C5-C6-O6, the contour search was performed independently. The starting orientation of the hydroxyl groups was chosen according to the predominant conformation, i.e., highest percentage distribution according to Boltzmann averaging. Thus, 21393 structures were calculated, and for each Φ/ψ combination, the one lowest in energy was selected for the contour plot, independent of the orientation of the hydroxymethyl group. The population was determined for each of these conformations according to Boltzmann averaging and those structures were selected that exceeded 0.01%. This procedure resulted in 531 structures which were used to calculate averaged theoretical proton-proton distances on the basis of $\langle r^{-3} \rangle$,[194] with

weighting of each conformer according to its percentage contribution. The program CROSREL[195] which uses a full matrix relaxation approach[196] was used to calculate theoretical NOE values, assuming isotropic motion and neglecting effects of strong scalar coupling[197] and cross-relaxation[198] with an overall retention time τ_c of 50 ps and a leakage rate of 0.05 Hz.[199] A qualitative comparison of theoretical with experimental NOE date was made by visual inspection of the curves.

5.1.iii NOE and Transferred NOE Experiments[178]

Transient and transferred NOE studies were performed by Dr. T Weimar. Compounds **4** and **5** (10 mg) were lyophilised twice from 1.0 mL D_2O (99.9%, ISOTEC INC.) and then dissolved in 0.5 mL of D_2O (99.9%, ISOTEC INC.). The samples were degassed by repeated evacuation and inflation with argon and were sealed under argon. NOE spectra were recorded non spinning at 600 MHz, at 316K, with a spectral width of 3.8 ppm. One dimensional spectra were collected with 8 K data points and were zero-filled to 16 K prior to Fourier transformation. 2D NOESY experiments were acquired in phase-sensitive mode using TPPI[193c] with 48 scans per increment, preceded by 32 dummy scans, a relaxation delay of 3.5 s and a mixing time of 800 ms. 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 2 K (F_1 x F_2) data points. ID transient NOE experiments and data treatment were performed as described.[182d,200] 80 ms Gaussian-shaped[201] 180° pulses with 2 K data points and a truncation level of 1% were used for selective inversion of specific resonances. For each

1D transient NOE spectrum, 256 scans preceded by 32 dummy scans were acquired. The relaxation delay was 4.3 s. Corrected mixing times were 41, 120, 240, 490, 700, 1040 and 1304 ms for all experiments. Processing of spectra and user-defined line deconvolutions were performed with standard Uxnmr (Bruker) software.

For transferred NOE experiments CD_3COOD/CD_3COONa buffer (pD 4.3) and PBS/D_2O (pD 7.2) (99.9%; lyophilised five times from D_2O and finally degassed) were used as solvents for compounds **4** and **5**, respectively. All spectra were recorded on an AMX 600 spectrometer (Bruker) at temperatures of 314 K for free disaccharides **4** and **5**, and 290 K for bound disaccharides **4** and **5**, respectively. Slight shifts of some resonances in free and time-averaged species are due to the differences in temperature. For the NOESY spectrum, the standard NOESY pulse sequence was used. TRNOE spectra used water presaturation and a 180° pulse in the middle of the mixing time followed by a 5 ms homospoil pulse to suppress residual HDO. An 18 ms $T_{1\rho}$ filter[202] was used to relax the protein resonances during the TRNOE experiments.

5.1.iv X-ray Crystallography[203]

The crystal structure of compound **4** was determined by Drs. R. Batchelor and F. W. B. Einstein, on a single crystal (0.15 mm x 0.25 mm x 0.25 mm) at 200 K. The data was refined to give an R value of 0.028.

5.1.v Enzyme Inhibition Assays

5.1.v.a Glucoamylase G2[122,174]

Glucoamylase inhibition assays were performed by Dr. B. Svensson and T. P. Frandsen. The initial rates of glucoamylase (*Aspergillus niger* glucoamylase G2[204]-catalysed hydrolysis of maltose (up to eleven different substrate concentrations in the range 0.2 - 26 mM) were followed in the presence of the different inhibitors (five 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 analysed 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 µl or 300 µl.[175] The inhibitors were all competitive and the constant of inhibition was 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,[205] and [I] is the concentration of inhibitor. With 5-thio-D-glucose, the glucose oxidase had ≤1% of the activity towards D-glucose and neither 5-thio-D-glucose or the glucoamylase inhibitors tested were inhibitors of the glucose oxidase. The progress of substrate hydrolysis was analysed by NMR spectroscopy using a reported technique.[206]

5.1.v.b Glucosidase I and II[176]

Glucosidase I and II inhibition assays were performed by Dr. M. Palcic and C. Scaman. Glucosidase I activity was monitored using α-D-Glc-(1-2)-α-D-Glc-(1-3)-α-D-Glc-$O(CH_2)_8COOCH_3$. Reactions were initiated by addition of enzyme solution to lyophilised substrate in 500 μL microfuge tubes. Tubes were vortexed, briefly microfuged and incubated at 37°C for 1h. The tubes were again briefly microfuged and the reaction quenched with Tris, using 1.25 M Tris-HCl, pH 7.6. The reaction mixture was then transferred to a well on a microassay plate and 250 μL of developing solution, containing glucose oxidase (5 units/mL), horseradish peroxidase (1 purpurogallin unit/mL) and *o*-dianisidine dihydrochloride (40 mg/mL) in 1 M Tris-HCl, pH 7.2, was added. The solutions were protected from light and left to develop for 30 min at 37°C. Absorbances were read at 450 nm-650 nm in a microplate reader. A typical background absorbance reading for the glucosidase I reaction was 0.02 A.U. One unit if activity is defined as the amount of enzyme required to release 1 mmol of glucose/min at 37°C.

Glucosidase II activity was determined by incubating 5 mM *p*-nitrophenyl α-D-glucopyranoside with enzyme in a 500 μL microfuge tube for 15 min to 2 h at 37°C in phosphate buffer, pH 6.8. A typical reaction volume was 20 μL, composed of 5 μL of 20 mM *p*-nitrophenyl-α-D-glucopyranoside and 15 μL enzyme solution in buffer. Reactions were terminated by briefly microfuging the tubes, transferring the reaction mixture to a well on a microassay plate, and addition of 280 μL of 0.2 M sodium carbonate. The absorbance of each cell at 405-650 nm was read in a Molecular Devices Thermomax microplate reader. A typical background absorbance reading for the glucosidase II

reaction was 0.02 A.U. One enzyme unit is defined as the amount of enzyme which catalyses the cleavage of 1 mmol substrate/min (ε for *p*-nitrophenol was 1.77 x 10^4 M^{-1} cm^{-1}).

5.2 Experimental

***Allyl 3,4,6-tri-O-benzyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranoside* (10).**

The *title compound* was synthesised from the corresponding 2-hydroxy derivative **9**[98] by the method of Pavilak *et al.*[99] Purification by column chromatography using hexane:ethyl acetate (4:1; R_f 0.35) as eluent gave **10** as a yellow syrup (82%) which was used directly in the following reaction. $[\alpha]_D^{22}$ -37.0° (c 1.0 in CH_2Cl_2).

^{1}H NMR ($CDCl_3$) δ 7.39-7.12 (15H, m, 3OCHC$_6$*H*$_5$), 5.88 (1H, m, OCH$_2$C*H*CH$_2$), 5.31 (1H, m, J_{trans} = 17.5 Hz, CH$_2$CHCH$_Z$*H*$_E$), 5.22 (1H, m, J_{cis} = 10.5 Hz, OCH$_2$CHC*H*$_Z$H$_E$), 5.17 (1H, d, $J_{1,2}$ = 2.8 Hz, H-2), 4.87 (1H, d, $J_{A,B}$ = 11.5 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.83 (1H, d, $J_{A,B}$ = 10.5 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.63-4.51(5H, m, 4OC*H*$_2$C$_6$H$_5$, H-2), 4.42 (1H, m, OC*Ha*HbCHCH$_2$), 4.10 (1H, m, OCHa*Hb*CHCH$_2$), 3.78-3.70 (3H, m, H-4, H-6a, H-6b), 3.68 (1H, dd, $J_{2,3}$ = 2.8 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.45 (1H, m, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.2 Hz, H-5).

***Allyl 3,4,6-tri-O-benzyl-2-thioacetyl-β-D-glucopyranoside* (11).**

The triflate **10** (3.1 g, 4.98 mmol) and potassium thioacetate (0.816 g; 7.14 mmol) were dissolved in freshly distilled DMF (25 mL). The mixture was stirred under nitrogen

at room temperature for 5 min at which time tlc (hexane: ethyl acetate 4:1) indicated the reaction to be complete. The DMF was removed *in vacuo* and the orange syrup was dissolved in CH_2Cl_2 (100 mL) and washed successively with H_2O (3 x 20 mL) and aqueous sodium chloride solution. The organic layer was dried (Na_2SO_4) and concentrated to give an orange syrup that was chromatographed using hexane:ethyl acetate (4:1; R_f 0.35) as eluent to yield **11** as a light yellow syrup (2.12 g; 81%). $[\alpha]_D^{22}$ -22.0° (c 1.0 in CH_2Cl_2). Anal. Calcd for $C_{32}H_{36}O_6S$: C, 70.05; H, 6.61. Found: C, 70.04; H, 6.55%.

^{1}H NMR ($CDCl_3$) δ 2.30 (3H, s, SCOC*H*$_3$), 3.50 (1H, ddd, $J_{4,5}$ = 8.5 Hz, $J_{5,6a}$ = 2.4 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 3.60 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.67 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, H-4), 3.70-3.78 (3H, m, H-3, H-6a, H-6b), 4.09 (1H, m, OCHa*Hb*CHCH$_2$), 4.34 (1H, m, OC*Ha*HbCHCH$_2$), 4.56 (1H, d, $J_{A,B}$ = 12 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.57 (1H, d, $J_{A,B}$ = 11 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.63 (1H, d, $J_{A,B}$ = 12 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.82-4.74 (3H, m, 3OC*H*$_2$C$_6$H$_5$), 5.16 (1H, m, J_{cis} = 10.5 Hz, OCH$_2$CHC*H*$_Z$H$_E$), 5.28 (1H, m, J_{trans} = 17.0 Hz, CH$_2$CHCH$_Z$*H*$_E$), 5.88 (1H, m, OCH$_2$C*H*CH$_2$), 7.35-7.15 (15H, m, 3OCH$_2$C$_6$*H*$_5$),

^{13}C NMR ($CDCl_3$) δ 30.6 (SCO*C*H$_3$), 50.4 (C-2), 69.0 (C-6), 69.8 (O*C*H$_2$CHCH$_2$), 73.5, 74.7, (O*C*H$_2$C$_6$H$_5$), 75.0 (C-5), 75.3, (O*C*H$_2$C$_6$H$_5$), 79.4 (C-4), 81.8 (C-3), 100.0 (C-1), 117.0 (OCH$_2$CH*C*H$_2$), 128.3-127.5 (3OCH$_2$*C*$_5$C$_{1(ipso)}$H$_5$), 134.0 (OCH$_2$*C*HCH$_2$), 138.2, 138.1 (3OCH$_2$C$_5$*C*$_{1(ipso)}$H$_5$), 193.6 (S*C*OCH$_3$).

Allyl 3,4,6-tri-O-benzyl-2-thio-β-D-glucopyranoside (**12**).

A freshly prepared, oxygen-free (freeze-thaw) 0.1 M NaOMe solution (1.4 mL, 0.14 mmol) was cooled to O°C and transferred dropwise, by means of a cannula, to a cooled (O°C) solution of the thioacetate **11** (0.075 g, 0.137 mmol) in freshly distilled oxygen-free methanol (2 mL). The reaction mixture was stirred at O°C and checked by tlc (hexane:ethyl acetate 3:1) which indicated the reaction to be complete after approximately 5 min. The reaction was quenched with cold 1 M HCl solution (2 mL), extracted into CH_2Cl_2 (3 x 10 mL) and washed with water (2 x 10 mL). The organic layer was dried ($MgSO_4$) and concentrated to a light green/yellow syrup which was dried over P_2O_5 under vacuum to give a waxy solid (0.065 g; 97%) that was used without further purification. The thiol could be purified if necessary by column chromatography (silica gel) using hexane:ethyl acetate (6:1; R_f 0.45) as eluent. $[\alpha]_D^{22}$ +6.0° (c 1.08 in CH_2Cl_2). Anal. Calcd for $C_{30}H_{34}O_5S$: C, 71.12; H, 6.76. Found: C, 71.31; H, 6.88%.

^{1}H NMR ($CDCl_3$) δ 2.05 (1H, d, J = 3.0 Hz, S-H), 3.05 (1H, ddd, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.5 Hz, $J_{2,SH}$ = 3.0 Hz, H-2), 3.43 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 8.8.Hz, H-3), 3.44 (1H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.0 Hz, H-5), 3.62 (1H, dd, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.66 (1H, dd, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6b), 3.68 (1H, dd, $J_{5,6a}$ = 2.5 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6a), 4.16 (1H, m, OCHa*Hb*CHCH$_2$), 4.27 (1H, d, $J_{1,2}$ = 8.5 Hz, H-1), 4.47 (1H, d, $J_{A,B}$ = 12 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.61 (1H, d, $J_{A,B}$ = 12 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.48 (1H, d, $J_{A,B}$ = 11 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.80 (1H, d, $J_{A,B}$ = 11 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.81 (1H, d, $J_{A,B}$ = 11 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.83 (1H, d, $J_{A,B}$ = 11 Hz,

$OCH_AH_BC_6H_5$), 5.21 (1H, m, J_{cis} = 10.5 Hz, $OCH_2CHCH_ZH_E$), 5.27 (1H, m, J_{trans} = 17.0 Hz, $CH_2CHCH_ZH_E$), 5.88 (1H, m, OCH_2CHCH_2), 7.38-7.16 (15H, m, $3OCH_2C_6H_5$).

^{13}C NMR ($CDCl_3$) δ 46.3 (C-2), 68.9 (C-6), 70.2 (OCH_2CHCH_2), 75.3 (C-5), 75.6, 74.9, 73.4 ($OCH_2C_6H_5$), 79.2 (C-4), 84.9 (C-3), 102.9 (C-1), 117.7 (OCH_2CHCH_2), 130.3-126.1 ($3OCH_2C_6H_5$).

Allyl 3,4,6-tri-O-benzyl-2-S-(2',3',4',6'-tetra-O-benzyl-α-D-glucopyranosyl)-2-thio-β-D-gluco-pyranoside (**15**).

The thiol **12** (0.159 g; 0.313 mmol) and 2,3,4,6-tetra-O-benzyl-β-D-gluco-pyranosyl trichloroacetimidate **14**[101] (0.214 g; 0.313 mmol) were dissolved in dry CH_2Cl_2 (1.5 mL) containing 4Å molecular sieves in a Schlenk tube under nitrogen and the mixture was cooled to -40°C. Triethylsilyl trifluoromethanesulfonate (4.95 μL; 0.07 eq.) was added to the stirred solution at -40°C. The reaction mixture was warmed to -10°C over 1.5 h at which time tlc (hexane:ethyl acetate 3:1) indicated the absence of the trichloroacetimidate. The reaction mixture was quenched at -10°C with triethylamine (30 μL). The evaporated residue was purified by column chromatography using hexane:ethyl acetate (5:1) as eluent (R_f 0.25) to yield **15** (225 mg; 70%) and the amide **16** (43 mg; 20%). Compound **15**: $[\alpha]_D^{23}$ +61.8° (c 1.12 in CH_2Cl_2). Anal. Calcd for $C_{64}H_{68}O_{10}S$: C, 74.68; H, 6.66. Found C, 74.71; H, 6.80%.

^1H NMR ($CDCl_3$) δ 3.01 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.0 Hz, H-2), 3.37 (1H, dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.40 (2H, m, H-6'a, H-6'b), 3.46 (1H, ddd, $J_{4,5}$ =

9.5 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.0 Hz, H-5), 3.60 (1H, dd, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.67 (1H, m, H-4'), 3.73 (2H, m, H-6a, H-6b), 3.88 (2H, m, H-2', H-3'), 4.12 (1H, m, OCHa*Hb*CHCH$_2$), 4.23 (1H, dt, $J_{4',5'}$ = 10.0 Hz, $J_{5',6'a}$ = $J_{5',6'b}$ = 2.5 Hz, H-5'), 4.46 (1H, m, OC*Ha*HbCHCH$_2$), 4.51 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.27-4.95 (m, 14H, 7OC*H*$_2$C$_6$H$_5$), 5.15 (1H, m, J_{cis} = 10.5 Hz, OCH$_2$CHC*H*$_Z$H$_E$), 5.30 (1H, m, J_{trans} = 17.0 Hz, CH$_2$CHCH$_Z$*H*$_E$), 5.95 (1H, m, OCH$_2$C*H*CH$_2$), 6.03 (1H, d, $J_{1',2'}$ = 4.5 Hz, H-1'), 7.38-7.04 (m, 35H, 7OCH$_2$C$_6$*H*$_5$).

^{13}C NMR ($CDCl_3$) δ 48.1 (C-2), 68.4 (C-6'), 69.0 (C-6), 70.3 (O*C*H$_2$CHCH$_2$), 70.7 (C-5'), 71.4, 73.3, 73.5, 74.8, 74.9, 75.5, 76.6 (7O*C*H$_2$C$_6$H$_5$), 74.9 (C-5), 77.4 (C-4'), 79.3 (C-2' or C-3'), 79.7 (C-4), 82.3 (C-3), 82.5 (C-2' or C-3'), 82.9 [^{1}J(^{13}C-^{1}H) 170 Hz (C-1')], 104.6 [^{1}J(^{13}C-^{1}H) 164 Hz (C-1)], 117.4 (OCH$_2$CH*C*H$_2$), 127.3-128.4 (7OCH$_2$*C*$_5$C$_{1(ipso)}$H$_5$), 134.1 (OCH$_2$*C*HCH$_2$), 137.9-138.9 (7OCH$_2$C$_5$*C*$_{1(ipso)}$H$_5$).

Propyl 2-S-(α-D-glucopyranosyl)-2-thio-β-D-glucopyranoside (**1**).

A solution of **15** (56 mg; 0.054 mmol) in 80% aq. acetic acid (1 mL) and 80% aq. tetrahydrofuran (1 mL) containing 10% palladium on activated carbon (Degussa type, 50% H_2O; 0.110 g) was treated with hydrogen at a pressure of 50 p.s.i. After 3 days, tlc (ethyl acetate:methanol:water 7:3:1) analysis indicated that the reaction was complete. The solution was filtered through celite and codistilled with 100% ethanol to remove acetic acid. Purification by flash chromatography using dichloromethane-methanol (2:1) as eluent (R_f 0.3) gave **1** as a glass (10.8 mg; 50%). $[\alpha]_D^{19}$ +164.8° (c 0.54 in MeOH). Anal. Calcd for $C_{15}H_{28}O_{10}S$: C, 44.99; H, 7.05. Found C, 44.65; H, 7.11%.

^{1}H NMR (D_2O) δ 0.91 ($OCH_2CH_2CH_3$), 1.61 ($OCH_2CH_2CH_3$), 2.60 (1H, t, $J_{1,2}$ = $J_{2,3}$ = 10.0 Hz, H-2), 3.40 (2H, m, H-4, H-4'), 3.41 (2H, m, H-3, H-5'), 3.61 ($OCH_2CH_2CH_3$), 3.63 (1H, m, C-3'), 3.69 (1H, dd, $J_{5',6'}$ = 5.5.Hz, $J_{6'a,6'b}$ = 12.3 Hz, H-6'b), 3.76 (1H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 3.79 (1H, m, H-6a), 3.80 (1H, m, C-2'), 3.88 (2H, m, H-6'a, $OCH_2CH_2CH_3$), 4.08 (1H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.8 Hz, H-5), 4.61 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 5.74 (1H, d, $J_{1',2'}$ = 5.4 Hz, H-1').

^{13}C NMR (D_2O) δ 12.6 ($OCH_2CH_2CH_3$), 25.0 ($OCH_2CH_2CH_3$), 52.3 (C-2), 63.3 (C-6), 63.6 (C-6'), 72.3 (C-4), 73.5 (C-4'), 73.7 (C-2'), 75.0 (C-5), 75.3 ($OCH_2CH_2CH_3$), 75.6 (C-5'), 76.2 (C-3'), 78.3 (C-3), 86.8 [$^1J(^{13}C\text{-}^1H)$ 171 Hz (C-1')], 106.9 [$^1J(^{13}C\text{-}^1H)$ 162 Hz (C-1)].

Allyl 2-S-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)-3,4,6-tri-O-benzyl-2-thio-β-D-glucopyranoside (**19**) and ***allyl 2-S-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-3,4,6-tri-O-benzyl-2-thio-β-D-glucopyranoside*** (**20**).

The thiol **12** (0.075 g; 0.148 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate **18**[101] (0.115 g; 0.223 mmol) were dissolved in dry CH_2Cl_2 (2 mL) containing 4Å molecular sieves in a Schlenk tube under nitrogen. Triethylsilyl trifluoromethanesulfonate (7.0 μL; 0.031 mmol; 0.14 eq.) was added to the stirred solution at room temperature. Tlc (hexane-ethyl acetate 2:1) indicated the reaction to be complete after 30 min. The reaction mixture was quenched with triethylamine (30 μL) and concentrated to a syrup that was chromatographed using hexane-ethyl acetate

(5:3) as eluent. Chromatography yielded starting thiol **12** (and disulfide **17**) (0.012 g) and **19** and **20** (0.074 g; 59% or 75% based on recovered thiol); α/β ratio 1:2.3 as estimated by ^{1}H NMR. The α/β isomers were separable by further chromatography. α-isomer **19**: $[\alpha]_D^{22}$ +103.6° (c 0.55 in CH_2Cl_2). Anal. Calcd for $C_{44}H_{52}O_{14}S$: C, 63.14; H, 6.26. Found: C, 63.15; H, 6.26%. β-isomer **20**: $[\alpha]_D^{22}$ -18.8° (c 1.04 in CH_2Cl_2). Anal. Calcd for $C_{44}H_{52}O_{14}S$: C, 63.14; H, 6.26. Found: C, 63.36; H, 6.43%.

19: ^{1}H NMR ($CDCl_3$) δ 2.04, 2.01, 2.00, 1.96 (12H, 4s, $COCH_3$), 2.89 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.5, H-2), 3.33 (1H, dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 3.42 (1H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 3.0 Hz, H-5), 3.62 (1H, dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 3.73 (2H, m, H-6a, H-6b), 3.89 (2H, m, H-6'a, H-6'b), 4.08 (1H, m, OCHaHbCHCH$_2$), 4.33 (2H, m, H-5', OCHaHbCHCH$_2$), 4.41 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.55 (1H, d, $J_{A,B}$ = 12.5 Hz, OCH$_A$$H_BC_6H_5$), 4.59 (1H, d, $J_{A,B}$ = 11 Hz, OCH$_A$$H_BC_6H_5$), 4.63 (1H, d, $J_{A,B}$ = 12.5 Hz, OCH_AH$_B$C$_6$H$_5$), 4.79 (1H, d, $J_{A,B}$ = 11 Hz, OCH$_A$$H_BC_6H_5$), 4.83 (1H, d, $J_{A,B}$ = 11 Hz, OCH_AH$_B$C$_6$H$_5$), 4.92 (1H, d, $J_{A,B}$ = 11 Hz, OCH_AH$_B$C$_6$H$_5$), 4.98 (1H, dd, $J_{3',4'}$ = 9.8 Hz, $J_{4',5'}$ = 10.0 Hz, H-4'), 5.06 (1H, dd, $J_{1',2'}$ = 6.0 Hz, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.17 (1H, m, J_{cis} = 10.5 Hz, OCH$_2$CHCH_ZH$_E$), 5.27 (1H, m, J_{trans} = 17.5 Hz, CH$_2$CHCH$_Z$$H_E$), 5.37 (1H, dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.8 Hz, H-3'), 5.87 (1H, m, OCH$_2$CHCH$_2$), 5.98 (1H, d, $J_{1',2'}$ = 6.0 Hz, H-1'), 7.49-7.14 (15H, m, 3OCH$_2$C$_6$$H_5$).

^{13}C NMR ($CDCl_3$) δ 20.7, 20.6 (4COCH_3), 48.3 (C-2), 61.5 (C-6'), 67.6 (C-5'), 68.3 (C-4'), 68.8 (C-6), 70.4 (OCH$_2$CHCH$_2$), 70.7 (C-2'), 70.8 (C-3'), 73.6 (CH$_2$C$_6$H$_5$), 75.0 (C-5, CH$_2$C$_6$H$_5$), 76.4 (CH$_2$C$_6$H$_5$), 79.9 (C-4), 81.1 [^{1}J(^{13}C-^{1}H) 176 Hz (C-1')], 82.0 (C-3), 103.9 [^{1}J(^{13}C-^{1}H) 164 Hz (C-1)], 117.5 (OCH$_2$CHCH$_2$), 128.5-127.6

($3OCH_2C_5C_{1(ipso)}H_5$), 134.0 (OCH_2CHCH_2), 138.2, 138.0 ($3OCH_2C_5C_{1(ipso)}H_5$), 170.0, 169.4, 169.3 ($4COCH_3$).

20: ^{1}H NMR ($CDCl_3$) δ 2.04, 2.01, 1.99, 1.82 (12H, 4s, $COCH_3$), 3.06 (1H, dd, $J_{1,2}$ = 8.7 Hz, $J_{2,3}$ = 10.7 Hz, H-2), 3.45 (2H, m, H-3, H-5), 3.62 (2H, m, H-4, H-5'), 3.72 (2H, m, H-6a, H-6b), 4.08 (1H, dd, $J_{5',6'a}$ = 2.5 Hz, $J_{6'a,6'b}$ = 12.5 Hz, H-6'a), 4.15(1H, m, $OCHaHbCHCH_2$), 4.20 (1H, dd, $J_{5',6'b}$ = 5.0 Hz, $J_{6'a,6'b}$ = 12.5 Hz, H-6'b), 4.42 (1H, m, $OCHaHbCHCH_2$), 4.47 (1H, d, $J_{1,2}$ = 8.7 Hz, H-1), 4.54 (1H, d, $J_{A,B}$ = 12 Hz, $OCH_AH_BC_6H_5$), 4.57 (1H, d, $J_{A,B}$ = 11 Hz, $OCH_AH_BC_6H_5$), 4.82 (1H, d, $J_{A,B}$ = 11 Hz, $OCH_AH_BC_6H_5$), 4.62 (1H, d, $J_{A,B}$ = 12 Hz, $OCH_AH_BC_6H_5$), 4.81-4.76 (m, 2H, $OCH_2C_6H_5$), 4.96 (1H, d, $J_{1',2'}$ = 10.0 Hz, H-1'), 5.00 (1H, m, H-2'), 5.09 (1H, m, H-4'), 5.11 (1H, m, H-3'), 5.22 (1H, m, J_{cis} = 10.5 Hz, $OCH_2CHCH_ZH_E$), 5.34 (1H, m, J_{trans} = 17.0 Hz, $CH_2CHCH_ZH_E$), 5.97 (1H, m, OCH_2CHCH_2), 7.35-7.12 (15H, m, $3OCH_2C_6H_5$).

^{13}C NMR ($CDCl_3$) δ 20.6, 20.5 ($4COCH_3$), 55.2 (C-2), 62.3 (C-6'), 68.3 (C-4'), 68.8 (C-6), 70.0 (OCH_2CHCH_2), 71.7 (C-2'), 74.1 (C-3'), 73.5 ($OCH_2C_6H_5$), 75.9 (C-5'), 75.0 (C-5), 74.9, 76.3 ($OCH_2C_6H_5$), 79.4 (C-4),)], 83.3 (C-3), 83.6 [$^1J(^{13}C\text{-}^1H)$ 162 Hz (C-1'), 102.3 [$^1J(^{13}C\text{-}^1H)$ 156 Hz (C-1)], 117.5 (OCH_2CHCH_2), 128.4-127.6 ($3OCH_2C_5C_{1(ipso)}H_5$), 134.1 (OCH_2CHCH_2), 138.1, 138.0 ($3OCH_2C_5C_{1(ipso)}H_5$), 169.3, 170.1, 170.6 ($4COCH_3$).

***(Benzyl-2-thio-β-D-glucopyranos-2-yl)-3,4,6-Tri-O-acetyl-1,2-α-D-glucopyranosyl orthoacetate* (21).**

The thiol **12** (0.103 g; 0.203 mmol) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate **18** (0.158 g; 0.305 mmol) were dissolved in dry CH_2Cl_2 (3 mL) containing 4Å molecular sieves in a Schlenk tube and the mixture was cooled to -40°C. Triethylsilyl trifluoromethanesulfonate (4.8 μL; 0.021 mmol; 0.07 eq.) was added to the stirred solution at -40°C. Tlc (hexane-ethyl acetate 2:1, developed twice) after 2 h indicated the reaction to be complete. The reaction was quenched at -25°C with triethylamine (60 μL) and concentrated to a syrup that was chromatographed using hexane-ethyl acetate 1.9:1 (+ 1% triethylamine) as eluent to yield **21** as a clear syrup (0.135 g; 79%). $[\alpha]_D^{22}$ -28.4° (c 1.04 in CH_2Cl_2). Anal. Calcd for $C_{44}H_{52}O_{14}S$: C, 63.14; H, 6.26. Found: C, 62.89; H, 6.36%.

^{1}H NMR ($CDCl_3$) δ 1.95 (3H, s, C*H*$_3$.orthoester), 2.08, 2.07, 1.96, (9H, 3s, 3COC*H*$_3$), 2.91 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 3.31 (1H, dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.40 (1H, ddd, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 4.0 Hz, H-5), 3.63 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 3.67-3.74 (2H, m, H-6a, H-6b), 3.88 (1H, dt, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a}$ = $J_{5',6'b}$ = 4.5 Hz, H-5'), 4.12(1H, m, OCHa*Hb*CHCH$_2$), 4.14 (2H, d, $J_{5',6'a}$ = $J_{5',6'b}$ = 4.5 Hz, H-6'a, H-6'b), 4.26 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.42 (1H, ddd, $J_{1',2'}$ = 5.2 Hz, $J_{2',3'}$ = 2.6 Hz, $J_{2',4'}$ = 1.0 Hz, H-2'), 4.49 (1H, m, OC*Ha*HbCHCH$_2$), 4.56 (2H, d, $J_{A,B}$ = 12 Hz, OCH$_A$*H*$_B$C$_6$H$_5$, $J_{A,B}$ = 10.5 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.63 (1H, d, $J_{A,B}$ = 12 Hz, OC*H*$_A$H. $_B$C$_6$H$_5$), 4.78 (1H, d, $J_{A,B}$ = 10.5 Hz, OC*H*$_A$H$_B$C$_6$H$_5$), 4.80 (1H, ddd, $J_{2',4'}$ = 1.0 Hz, $J_{3',4'}$ = 1.9 Hz, $J_{4',5'}$ = 9.5 Hz, H-4'), 4.88 (1H, d, $J_{A,B}$ = 11 Hz, OCH$_A$*H*$_B$C$_6$H$_5$), 4.92 (1H, d, $J_{A,B}$ =

11 Hz, $OCH_AH_BC_6H_5$), 5.09 (1H, dd, $J_{2',3'}$ = 2.6 Hz, $J_{3',4'}$ = 1.9 Hz, H-3'), 5.19 (1H, m, J_{cis} = 10.5 Hz, $OCH_2CHCH_ZH_E$), 5.34 (1H, m, J_{trans} = 17.0 Hz, $CH_2CHCH_ZH_E$), 5.67 (1H, d, $J_{1',2'}$ = 5.2 Hz, H-1'), 5.96 (1H, m, OCH_2CHCH_2), 7.36-7.13 (15H, m, $3OCH_2C_6H_5$).

^{13}C NMR ($CDCl_3$) δ 20.7 ($3COCH_3$), 28.9 (OCH_3-orthoester), 51.6 (C-2), 63.4 (C-6'), 68.4 (C-4'), 69.0 (C-6), 69.6 (C-3'), 70.6 (OCH_2CHCH_2), 72.9 (C-2'), 73.5 ($OCH_2C_6H_5$), 74.8 (C-5), 75.0, 76.4 ($OCH_2C_6H_5$), 77.0 (C-5'), 79.7 (C-4), 83.6 (C-3), 97.4 [$^1J(^{13}C$-$^1H)$ 183 Hz (C-1')], 102.6 [$^1J(^{13}C$-$^1H)$ 164 Hz (C-1)], 116.2 (*C*-orthoester), 117.8 (OCH_2CHCH_2), 128.3-127.4 ($3OCH_2C_5C_{1(ipso)}H_5$), 134.0 (OCH_2CHCH_2), 138.0, 138.2, 138.5 ($3OCH_2C_5C_{1(ipso)}H_5$), 170.6, 169.5, 168.8 ($4OCOCH_3$),

Rearrangement of the orthoester 21. A sample of the pure orthoester **21** (16 mg; 0.019 mmol) was dissolved in dry CH_2Cl_2 (0.5 mL) containing freshly activated 4Å molecular sieves in a Schlenk tube under nitrogen and the mixture was cooled to 0°C. Triethylsilyl triflate (3 μL of a 20% solution in CH_2Cl_2, 0.003 mmol) was added to the stirred solution at 0°C. The reaction was warmed to room temperature over 30 min at which time tlc (hexane-ethyl acetate 2:1) indicated that the reaction was complete. The reaction was cooled to -78°C and quenched with triethylamine (30 μL). The evaporated residue was purified by column chromatography using hexane-ethyl acetate (1.75:1, R_f 0.35) to yield 6.5 mg (40%) of a 1:8 mixture of **19** : **20** as determined by ^1H NMR spectroscopy.

***Propyl 2-O-acetyl-3,4,6-tri-O-benzoyl-β-D-mannopyranoside* (26).**

To a solution of allyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-mannopyranoside **23**[98] (0.567 g; 1.06 mmol) in methanol/80% acetic acid (5/3; 8 mL) was added dry 10% palladium on carbon (0.250 g) and the mixture stirred under nitrogen at a pressure of 50 p.s.i. for 24 h. The mixture was filtered through Celite and concentrated, and the acetic acid removed by repeated codistillation with 100% EtOH. The resulting syrup was dissolved in pyridine (5 mL) and cooled to 0°C. Benzoyl chloride (0.65 mL; 5.60 mmol) was added dropwise to the stirred mixture which was then heated to 60°C. After 1 h tlc (hexane-ethyl acetate 3:1) indicated that the reaction was complete. Ice was added to the stirred solution to hydrolyse excess benzoyl chloride and the mixture was stirred for a further 20 min. Pyridine was removed by codistillation with toluene to yield a yellow syrup that was dissolved in CH_2Cl_2 and washed consecutively with H_2O, 10% HCl, aq. $NaHCO_3$ and H_2O. The organic layer was dried (Na_2SO_4) and the solvent evaporated. The resulting syrup was chromatoghraphed using hexane-ethyl acetate (3:1) as eluent (R_f 0.35) to yield 0.486 g of **26** (85%). The pure syrup was crystallised from methanol (mp. 125-127 °C). $[\alpha]_D^{22}$ -58.49° (c 1.06 in CH_2Cl_2). Anal. Calcd for $C_{32}H_{32}O_{10}$: C, 66.66; H, 5.59. Found: C, 66.68; H, 5.55%.

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, $OCH_2CH_2CH_3$), 1.62 (2H, m, $OCH_2CH_2CH_3$), 2.19 (3H, s, $COCH_3$), 3.53 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.88 (1H, m, $OCH_aCH_bCH_2CH_3$), 4.08 (1H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$= 6.0 Hz, H-5), 4.53 (1H, dd, $J_{5,6b}$ = 6.0 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4,65 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.85 (1H, d, $J_{1,2}$ = 1.0 Hz, H-1), 5.50 (1H, dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.0 Hz, H-

3), 5.73 (1H, dd, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.82 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 7.32-8.04 (15H, m, aromatic).

^{13}C NMR ($CDCl_3$) δ 10.3 ($OCH_2CH_2\mathit{C}H_3$), 20.7 ($CO\mathit{C}H_3$), 22.7 ($OCH_2\mathit{C}H_2CH_3$), 63.8 (C-6), 67.7 (C-4), 69.2 (C-2), 71.9 (C-3, $O\mathit{C}H_2CH_2CH_3$), 72.5 (C-5), 98.9 (C-1), 128.3-133.4 (3*C*OPh), 165.5, 166.1 (3*C*OPh), 169.9 (*C*OCH_3).

Propyl 3,4,6-tri-O-benzoyl-β-D-mannopyranoside (**27**).

Compound **26** (2.5 g; 4.34 mmol) was dissolved in an anhydrous solution of 1% HCl in methanol (25 mL) containing dry CH_2Cl_2 (5 mL) and the mixture was stirred at 22°C under nitrogen. Tlc (hexane-ethyl acetate 3:1) after 16 h indicated the reaction to be complete. The reaction was diluted with CH_2Cl_2 and the solution made basic with solid $NaHCO_3$ and washed consecutively with H_2O and saturated aq. NaCl. After drying with Na_2SO_4 the solvent was evaporated and the resulting syrup crystallised from methanol to give **27** (2.05 g, 89%). Mp. 164-165°C. $[\alpha]_D^{22}$ +-54.37° (c 1.03 in CH_2Cl_2). Anal. Calcd for $C_{30}H_{30}O_9$: C, 67.41; H, 5.66. Found: C, 67.19; H, 5.61%.

^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, $OCH_2CH_2C\mathit{H}_3$), 1.67 (2H, m, $OCH_2C\mathit{H}_2CH_3$), 2.45 (1H, broad s, OH), 3.57 (1H, m, $OC\mathit{H}_aCH_bCH_2CH_3$), 3.92 (1H, m, $OCH_aC\mathit{H}_bCH_2CH_3$), 4.03 (1H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$= 5.5 Hz, H-5), 4.38 (1H, dd, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 4.51 (1H, dd, $J_{5,6b}$ = 5.5 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4,63 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.78 (1H, d, $J_{1,2}$ = 1.0 Hz, H-1), 5.38 (1H, dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.95 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 7.31-8.04 (15H, m, aromatic).

^{13}C NMR ($CDCl_3$) δ 10.3 ($OCH_2CH_2CH_3$), 22.7 ($OCH_2CH_2CH_3$), 63.7 (C-6), 67.4 (C-4), 69.3 (C-2), 71.6, 72.4, 73.9 (C-3, C-5, $OCH_2CH_2CH_3$), 99.5 (C-1), 128.3-133.3 (3*C*OPh), 165.4, 166.0, 166.2 (3*C*OPh).

Propyl 3,4,6-tri-O-benzoyl-2-thioacetyl-β-D-glucopyranoside (**29**).

To a stirred solution of **27** (1.635 g; 3.06 mmol) in dry CH_2Cl_2 (20 mL) containing pyridine (0.57 mL; 7.04 mmol) at -15°C under nitrogen was added over a period of 1 h, *via* a dropping funnel, triflic anhydride (1.03 mL; 6.18 mmol) in CH_2Cl_2 (15 mL). The reaction was heated to room temperature and after 1 h tlc (hexane: ethyl acetate 3:1) indicated that the reaction was complete. The reaction was diluted with CH_2Cl_2 and extracted consecutively with ice cold H_2O, saturated $NaHCO_3$ soln., H_2O, saturated NaCl soln. and dried with Na_2SO_4 to yield, after removal of solvent, a light yellow foam **28** which was pure by tlc and was used immediately in the following reaction.

To the triflate **28** (0.40 g; 0.60 mmol) in dry DMF (5 mL) was added potassium thioacetate (0.089 g; 0.78 mmol) and stirred at room temperature for 1 h. Tlc (hexane-ethyl acetate 3:1) after this time indicated that the reaction was complete. The DMF was removed *in vacuo* and the resulting orange syrup diluted with CH_2Cl_2 and extracted consecutively with H_2O (x2), saturated NaCl soln. and dried over Na_2SO_4. After removal of the solvent, the syrup was chromatographed using hexane-ethyl acetate (4:1) as the eluent (R_f 0.35) to yield **29** (0.261 g; 74%), which was crystallised from the chromatography solvent. Mp. 142-143°C. $[\alpha]_D^{22}$ -49.07 ° (c 0.54 in CH_2Cl_2). Anal. Calcd for $C_{32}H_{32}O_9S$: C, 64.85; H, 5.44. Found: C, 64.49; H, 5.39%.

^{1}H NMR ($CDCl_3$) δ 0.40 (3H, t, $OCH_2CH_2CH_3$), 1.61 (2H, m, $OCH_2CH_2CH_3$), 2.25 (3H, s, $SCOCH_3$), 3.50 (1H, m, $OCHaCHbCH_2CH_3$), 3.85 (1H, m, $OCHaCHb$-CH_2CH_3), 3.88 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 4.08 (1H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$= 5.5 Hz, H-5), 4.48 (1H, dd, $J_{5,6b}$ = 5.5 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4,59 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.79 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 5.57 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.76 (1H, dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 7.03-8.04 (15H, m, aromatic).

^{13}C NMR ($CDCl_3$) δ 10.3 ($OCH_2CH_2CH_3$), 22.8 ($OCH_2CH_2CH_3$), 30.6 ($SCOCH_3$), 49.1 (C-2), 63.5 (C-6), 71.2, 71.9, 72.0, 72.1 (C-3, C-4, C-5, $OCH_2CH_2CH_3$), 101.2 (C-1), 128.3-133.3 (3*C*OPh), 163.5 (3*C*OPh), 193.2 ($SCOCH_3$).

Propyl 3,4,6-tri-O-benzoyl-2-thio-β-D-glucopyranoside (**30**).

A 0°C solution of **29** (0.45 g; 0.76 mmol) in anhydrous 2% HCl in methanol (4 mL, oxygen free) containing CH_2Cl_2 (1.5 mL) was stirred under nitrogen at room temperature. After 16 h tlc (hexane-ethyl acetate 3:1) indicated that the reaction was approximately 60% complete. The reaction was heated to 40°C and after 3 h tlc indicated that a small amount of the starting thioacetate was unreacted, and the formation of a more polar compound (probably due to the removal of the 6-benzoate). The reaction was quenched with ice and extracted with CH_2Cl_2 (x3). The organic layer was further extracted with cold aqueous NaCl soln. and dried over $MgSO_4$ and the solvent evaporated to yield a light yellow syrup. Chromatography with hexane-ethyl acetate (6:1) as eluent (R_f 0.36) yielded **30** (0.261 g; 64%) (or 74% yield based on recovered **29**) and **29** (0.063

g). **30** $[\alpha]_D^{22}$ -33.9° (c 0.87 in CH_2Cl_2). Anal. Calcd for $C_{30}H_{30}O_8S$: C, 65.44; H, 5.49. Found: C, 65.25; H, 5.48%.

^{1}H NMR ($CDCl_3$) δ 0.45 (3H, t, $OCH_2CH_2CH_3$), 1.68 (2H, m, $OCH_2CH_2CH_3$), 1.92 (1H, d, $J_{2,SH}$ = 4.8 Hz, SH), 3.31 (1H, ddd, $J_{2,SH}$ = 4.8 Hz, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 9.8 Hz, H-2),), 3.57 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.90 (1H, m, $OCH_aCH_bCH_2CH_3$), 4.07 (1H, ddd, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$= 5.5 Hz, H-5), 4.47 (1H, dd, $J_{5,6b}$ = 5.5 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4.56 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1), 4,58 (1H, dd, $J_{5,6a}$ = 3.3 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 5.53 (2H, m, H-3, H-4), 7.30-8.00 (15H, m, aromatic).

^{13}C NMR ($CDCl_3$) δ 10.5 ($OCH_2CH_2CH_3$), 22.8 ($OCH_2CH_2CH_3$), 45.1 (C-2), 63.5 (C-6), 71.0 (C-4), 72.2 (C-5, $OCH_2CH_2CH_3$), 74.9 (C-3), 104.2 (C-1), 128.3-133.2 (3*C*OPh), 165.4, 165.9 (3*C*OPh).

Propyl 2-S-(2',3',4',6'-tetra-O-acetyl-5'-thio-α-D-glucopyranosyl)-3,4,6-tri-O-benzoyl-2-thio-β-D-glucopyranoside (**31**) and ***2-S-(2',3',4',6'-tetra-O-acetyl-5'-thio-β-D-glucopyranosyl)-3,4,6-tri-O-benzoyl-2-thio-β-D-glucopyranoside*** (**32**).

The thiol **30** (0.200 g; 0.363 mmol) and O-(2,3,4,6-tetra-O-acetyl-5-thio-α-D-glucopyranosyl)-trichloroacetimidate[91] were dissolved in dry CH_2Cl_2 (2.5 mL) containing activated 4Å molecular sieves (0.135 g) in a Schlenk tube under nitrogen. Trimethylsilyl triflate (10.9 μL; 0.048 mmol) was added to the stirred solution at -78°C. The reaction mixture was stirred at -78°C for 1 h and then warmed to 0°C over 2 h at which time tlc (hexane-ethyl acetate 3:2) indicated that the reaction was complete. The reaction was warmed to room temperature for 20 min and then quenched at -78°C with triethylamine

(20 μL). The reaction mixture was filtered and the solvent evaporated, and the resulting syrup purified by chromatography using hexane-ethyl acetate (3:2) as eluent (R_f 0.33) to yield a mixture of **31** and **32** (one spot on tlc) (0.156 g; 72%) and **33** (0.023 g; 11%) and excess thiol (0.05 g). The ratio of **31** to **32** was determined by ^{1}H NMR spectroscopy to be 1.6:1. Fractional crystallisation of the mixture of **31** and **32** yielded pure fractions of **32**. Pure fractions of the α-anomer **31** were unattainable due to the preferential crystallisation of **32**, although a satisfactory elemental analysis for an almost pure sample of **31** was obtained. **31**: Anal. Calcd for $C_{44}H_{48}O_{16}S_2$: C, 58.92; H, 5.39. Found: C, 58.99; H, 5.31%. **32**: Mp. 164-165°C. $[\alpha]_D^{19}$ -14.4° (c 0.9 in CH_2Cl_2). Anal. Calcd for $C_{44}H_{48}O_{16}S_2$: C, 58.92; H, 5.39. Found: C, 58.83; H, 5.38%.

31: ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, $OCH_2CH_2CH_3$), 1.61 (2H, m, $OCH_2CH_2CH_3$), 1.82, 1.94, 2.03 (12H, 3s, $4COCH_3$), 3.09 (1H, m, H-5'), 3.45 (1H, dd, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 3.51 (1H, m, OC*Ha*HbCH2CH3), 3.52 (1H, dd, $J_{5',6'a}$ = 2.4 Hz, $J_{6'a,\ 6'b}$ = 12.0 Hz, H-6'a), 3.81 (1H, m, OCHa*Hb*CH2CH3; 1H, dd, $J_{5',6'b}$ = 4.0 Hz, $J_{6'a,\ 6'b}$ = 12.0 Hz, H-6'b), 4.03 (1H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$ = 5.5 Hz, H-5), 4.48 (1H, dd, $J_{5,6a}$ = 5.5 Hz, $J_{6a,\ 6b}$ = 12.0 Hz, H-6a), 4.58 (1H, dd, $J_{5,6b}$ = 3.5 Hz, $J_{6a,\ 6b}$ = 12.0 Hz, H-6b), 4.69 (1H, d, $J_{1,2}$ = 8.8 Hz, H-1), 4.91 (1H, d, $J_{1',2'}$ = 4.5 Hz, H-1'), 5.12 (2H, m, H-3', H-4'), 5.26 (1H, dd, $J_{1'2'}$ = 4.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2'), 5.52 (1H, dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.59 (1H, t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4).

^{13}C NMR ($CDCl_3$) δ 10.4 ($OCH_2CH_2CH_3$), 20.5-20.6 ($4COCH_3$), 22.6 ($OCH_2CH_2CH_3$), 39.0 (C-5'), 49.9 (C-2), 50.9 (C-1'), 60.3 (C-6'), 63.3 (C-6), 70.9 (C-4),

71.1 (C-3), 71.2, 71.5 (C-3', C-4'), 71.9 (C-5), 72.2 ($OCH_2CH_2CH_3$), 73.9 (C-2'), 104.9 (C-1), 128.3-133.4 (3*C*OPh), 165.3-166.1 (3*C*OPh), 169.2-170.4 (4*C*OCH_3).

32: ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, $OCH_2CH_2CH_3$), 1.68, 1.89, 1.98, 2.07 (12H, 4s, 4$COCH_3$), 1.72 (2H, m, $OCH_2CH_2CH_3$), 3.16 (1H, ddd, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a}$ = 3.5 Hz, $J_{5',6'b}$ = 5.5 Hz , H-5'), 3.35 (1H, dd, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 3.61, 3.91 (2H, 2m, $OCH_2CH_2CH_3$), 4.02 (1H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$ = 5.5 Hz, H-5), 4.10 (1H, dd, $J_{5',6'a}$ = 3.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 4.19 (1H, dd, $J_{5',6'b}$ = 5.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b), 4.29 (1H, d, $J_{1',2'}$ = 10.8 Hz, H-1'), 4.48 (1H, dd, $J_{5,6b}$ = 5.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.58 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.59 (1H, d, $J_{1,2}$ = 8.5 Hz, H-1), 4.78 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3'), 5.04 (1H, dd, $J_{1',2'}$ = 10.8 Hz, $J_{2',3'}$ = 9.5 Hz, H-2'), 5.17 (1H, dd, $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 10.5 Hz, H-4'), 5.45 (1H, dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.55 (1H, t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4).

^{13}C NMR ($CDCl_3$) δ 10.6 ($OCH_2CH_2CH_3$), 20.1, 20.3, 20.4, 20.5 (4$COCH_3$), 22.9 ($OCH_2CH_2CH_3$), 44.9 (C-5'), 48.0 [$^1J(^{13}C$-$^1H)$ 156 Hz (C-1')], 52.0 (C-2), 61.4 (C-6'), 63.4 (C-6), 70.8 (C-4), 71.9 (C-4'), 72.0 (C-5), 72.5 ($OCH_2CH_2CH_3$), 72.9 (C-3), 74.6 (C-3'), 74.9 (C-2'), 104.1 [$^1J(^{13}C$-$^1H)$ 164 Hz (C-1)], 128.3-133.4 (3*C*OPh), 165.4, 165.8, 166.1 (3*C*OPh), 168.8, 169.2, 169.4, 170.3 (4*C*OCH_3).

33: ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, t, $OCH_2CH_2CH_3$), 1.44 (2H, m, $OCH_2CH_2CH_3$), 1.96, 2.02, 2.13 (9H, 3s, 3$COCH_3$), 3.13 (1H, dt, $J_{4',5'}$ = 10.5 Hz, $J_{5',6'a}$ = $J_{5',6'b}$ = 3.0 Hz, H-5'), 3.35 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.5 Hz, H-2), 3.55 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.58 (1H, dd, $J_{5',6'b}$ = 3.0 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b), 3.87 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.97 (1H, dd, $J_{5',6'a}$ = 3.0 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 4.02 (1H, ddd,

$J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$ = 5.5 Hz, H-5), 4.48 (1H, dd, $J_{5,6b}$ = 5.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.59 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.73 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.97 (1H, broad s, H-1'), 5.44 (1H, d, $J_{2',4'}$ = 2.5 Hz, H-2'), 5.47 (1H, dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 5.58 (1H, obscured m, H-4'), 5.59 (1H, obscured m, H-4), 7.03-8.00 (15H, m, 3COPh).

^{13}C NMR ($CDCl_3$) δ 10.4 ($OCH_2CH_2CH_3$), 20.5, 20.7 ($3COCH_3$), 22.8 ($OCH_2CH_2CH_3$), 37.7 (C-5'), 48.1 [$^1J(^{13}C$-$^1H)$ 156 Hz (C-1')], 52.2 (C-2), 61.1 (C-6'), 63.4 (C-6), 68.5 (C-4), 71.1 (C-4',C-5), 72.0, 72.1 (C-3, $OCH_2CH_2CH_3$), 105.0 [$^1J(^{13}C$-$^1H)$ 163 Hz (C-1)], 119.7 (C-2'), 128.3-133.4 (3*C*OPh), 146.7 (C-3'), 165.3, 165.6, 166.1 (3*C*OPh), 168.0, 170.0, 170.3 ($4COCH_3$).

Propyl 2-thio-2-S-(5'-thio-α-D-glucopyranosyl)-β-D-glucopyranoside (**2**) and ***Propyl 2-thio-2-S-(5'-thio-β-D-glucopyranosyl)-β-D-glucopyranoside*** (**34**).

A mixture of **31/32** (0.098 g; 0.109 mmol) in 0.1 M NaOMe was stirred under nitrogen. After 12 h tlc (ethyl acetate-methanol-water 4:2:0.5) indicated that the reaction was complete. The reaction mixture was diluted with MeOH (~10 mL) and neutralised with Rexyn 101 (H^+) resin. After washing the resin with MeOH, the solvent was removed and the syrupy residue was purified by column chromatography using dichloromethane-methanol (2:1) as eluent (R_f 0.38-0.55). The tlc plates were run in ethyl acetate-dichloromethane-methanol-water (1:1:1:0.4) (R_f 0.35) and were visualised as usual. Chromatography yielded a total of 0.039 g (85%) of **2/34** of which the initial and final fractions contained pure **34** and **2**, respectively. **2**: $[\alpha]_D^{19}$ +230.8° (c 0.52 in MeOH).

Anal. Calcd for $C_{15}H_{28}O_9S_2$: C, 43.26; H, 6.78. Found: C, 43.63; H, 6.37%. **34**: $[\alpha]_D^{19}$ +26.03° (c 0.73 in MeOH). Anal. Calcd for $C_{15}H_{28}O_9S_2$: C, 43.26; H, 6.78. Found: C, 43.71; H, 6.41%.

2: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, t, $OCH_2CH_2CH_3$), 1.60 (2H, m, $OCH_2CH_2CH_3$), 2.96 (1H, t, $J_{1,2} = J_{2,3} = 9.0$ Hz, H-2), 3.32 (1H, m, H-5'), 3.39 (1H, m, H-4), 3.40 (2H, m, H-3, H-5), 3.57 (2H, m, H-3', H-4'), 3.60 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.68 (1H, dd, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.85 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.88 (3H, m, H-6b, H-6'a, H-6'b), 4.04 (1H, m, H-2'), 4.63 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 4.68 (1H, d, $J_{1',2'}$ = 4.5 Hz, H-1').

^{13}C NMR ($CDCl_3$) δ 12.7 ($OCH_2CH_2CH_3$), 25.0 ($OCH_2CH_2CH_3$), 46.5 (C-5'), 54.3 (C-2), 55.2 [^{1}J(^{13}C-^{1}H) 159 Hz, (C-1')], 62.8 (C-6'), 63.8 (C-6), 73.7 (C-4), 75.4 (C-5, $OCH_2CH_2CH_3$), 76.5, 77.2 (C-3', C-4'), 77.5 (C-2'), 78.4(C-3), 107.2 [^{1} J(^{13}C-^{1}H) 161 Hz, (C-1)].

34: ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, $OCH_2CH_2CH_3$), 1.63 (2H, m, $OCH_2CH_2CH_3$), 2.84 (1H, dd, $J_{1,2}$ = 9,0 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 2.98 (1H, ddd, $J_{4',5'}$ = 10.5 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 6.0 Hz, H-5'), 3.25 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.0 Hz, H-3'), 3.34 (1H, dd, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.40 (1H, m, H-5), 3.45 (1H, dd, $J_{1',2'}$ = 10.5 Hz, $J_{2',3'}$ = 9.0 Hz, H-2'), 3.46 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.53 (1H, dd, $J_{3',4'}$ = 9.0 Hz, $J_{4',5'}$ = 10.5 Hz, H-4'), 3.62 (1H, m, $OCH_aCH_bCH_2CH_3$), 3.67 (1H, dd, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.67(1H, dd, $J_{5',6'a}$ = 6.0 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 3.83-3.89 (3H, m, H-6b, H-6'b, $OCH_aCH_bCH_2CH_3$), 4.08 (1H, d, $J_{1',2'}$ = 10.5 Hz, H-1'), 4.52 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1).

^{13}C NMR ($CDCl_3$) δ 12.6 ($OCH_2CH_2\underline{C}H_3$), 25.0 ($OCH_2\underline{C}H_2CH_3$), 51.6 (C-5'), 51.7 [$^1J(^{13}C\text{-}^1H)$ 156 Hz, (C-1')], 55.4 (C-2), 63.0 (C-6'), 63.7 (C-6), 73.5 (C-4), 75.3 ($O\underline{C}H_2CH_2CH_3$), 75.8 (C-4'), 77.3 (C-3), 78.4(C-5), 79.6 (C-2'), 80.5 (C-3'), 105.2 [$^1J(^{13}C\text{-}^1H)$ 161 Hz, (C-1)].

Propyl 3,4,6-tri-O-benzoyl-2-thiocyanato-β-D-glucopyranoside

To a solution of the triflate (0.750 g; 1.125 mmol) in dry DMF (10 mL) was added potassium thiocyanate (0.164 g; 1.688 mmol) and the mixture was heated at 70°C for 3 h, after which time tlc (hexane-ethyl acetate 3:1) indicated that the reaction was complete. The DMF was removed *in vacuo* and the resulting syrup diluted with CH_2Cl_2 and extracted consecutively with H_2O (x2), saturated NaCl soln. and dried over Na_2SO_4. After removal of the solvent the syrup was chromatographed using hexane-ethyl acetate (3:1) as the eluent (R_f 0.43) to yield the *title compound* (0.496 g) in 77% yield.

^1H NMR ($CDCl_3$) δ 0.98 (3H, t, $OCH_2CH_2C\underline{H_3}$), 1.70 (2H, m, $OCH_2C\underline{H_2}CH_3$), 3.31 (1H, dd, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 11.5 Hz, H-2), 3.65, 3.95 (2H, 2m, $OC\underline{H_2}CH_2CH_3$), 4.11 (1H, m, H-5), 4.48 (1H, dd, $J_{5,6b}$ = 5.5 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4,60 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.81 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 5.59 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.77 (1H, dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 7.03-8.00 (15H, m, aromatic).

Propyl 3,4,6-tri-O-benzoyl-2-selenocyano-β-D-glucopyranoside (**35**).

To a solution of the triflate **28** (0.571 g; 0.857 mmol) in dry DMF (4 mL) was added potassium selenocyanate (0.185 g; 1.285 mmol) and the mixture was heated at 70-80°C for 16 h, after which time tlc (hexane-ethyl acetate 3:1) indicated that the reaction was complete. The DMF was removed *in vacuo* and the resulting syrup diluted with CH_2Cl_2 and extracted consecutively with H_2O (x2), saturated NaCl soln. and dried over Na_2SO_4. After removal of the solvent the syrup was chromatographed using hexane-ethyl acetate (4:1) as the eluent (R_f 0.35) to yield the *title compound* (0.456 g) in 86% yield.

^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t, $OCH_2CH_2CH_3$), 1.70 (2H, m, $OCH_2CH_2CH_3$), 3.51 (1H, dd, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 3.63, 3.94 (2H, 2m, $OCH_2CH_2CH_3$), 4.11 (1H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$= 5.5 Hz, H-5), 4.49 (1H, dd, $J_{5,6b}$ = 5.5 Hz $J_{6a,6b}$, = 12.0 Hz, H-6b), 4,60 (1H, dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.84 (1H, d, $J_{1,2}$ = 8.5 Hz, H-1), 5.59 (1H, dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.8 Hz, H-4), 5.77 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 7.32-8.00 (15H, m, aromatic).

^{13}C NMR ($CDCl_3$) δ 10.4 ($OCH_2CH_2CH_3$), 22.8 ($OCH_2CH_2CH_3$), 49.0 (C-2), 63.0 (C-6), 70.8 (C-4), 71.8 (C-5), 72.2 (C-3), 72.7 ($OCH_2CH_2CH_3$), 101.3 (C-1).

Propyl 2-dideoxy-4,6-di-O-benzoyl-β-D-erythro-hex-2-enopyranoside (**36**).

To a solution of the selenocyanate **35** (0.186 g; 0.299 mmol) in THF/EtOH (6:1; 6 mL; oxygen free) at 0°C was added sodium borohydride (0.054 g; 1.41 mmol) and the reaction mixture was warmed to room temperature while stirring. After 10 min tlc

(hexane-ethyl acetate 3:1) indicated that no more starting material was present. The reaction was diluted with Et_2O (25 mL; oxygen free), cooled to 0°C and acidified with 5% HCl soln. (10 mL). The solution was further diluted with Et_2O and extracted with H_2O (x2) and dried ($MgSO_4$) and the solvent removed to yield a syrup (single spot on tlc) which was filtered through silica gel using hexane-ethyl acetate (5:1) as eluent (R_f 0.5) to yield **36** (0.095 g; 80%).

^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, $OCH_2CH_2CH_3$), 1.60 (2H, m, $OCH_2CH_2CH_3$), 3.48, 3.86 (2H, 2m, $OCH_2CH_2CH_3$), 4.37 (1H, m, $J_{4,5} = J_{5,6a} = J_{5,6b}$= 5.8 Hz, H-5), 4.56 (2H, d, H-6a, H-6b), 5.22 (1H, m, H-1), 5.57 (1H, dddd, $J_{1,4} = J_{2,4}$ = 1.2 Hz, $J_{3,4}$ = 3.8 Hz, $J_{4,5}$ = 6.0 Hz, H-4), 6.03 (1H, ddd, $J_{2,3}$ = 10.0 Hz, $J_{1,2} = J_{2,4}$ = 1.2 Hz, H-2), 6.13 (1H, ddd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.6 Hz, $J_{1,3}$ = 1.5 Hz, H-3), 7.39-8.07 (10H, m, aromatic).

Methyl 4-S-(2',3',4',6',-tetra-O-acetyl-5'-thio-α-D-glucopyranosyl)-2,3,6-tri-O-benzoyl-4-thio-α-D-glucopyranoside (**41**) and ***methyl 4-S-(2',3',4',6',-tetra-O-acetyl-5'-thio-β-D-glucopyranosyl)-2,3,6-tri-O-benzoyl-4-thio-α-D-glucopyranoside*** (**42**).

O-(2,3,4,6-Tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl)-trichloroacetimidate **22** (0.150 g; 0.2949 mmol) and the thiol **39**[82] (0.308 g; 0.5899 mmol) were dissolved in dry CH_2Cl_2 (2 mL) containing 4Å molecular sieves in a Schlenk tube under nitrogen. Triethylsilyl triflate (16.7 mL; 0.0737 mmol) was added to the stirred solution at -50°C. The reaction mixture was heated to -10°C over 1 h at which time tlc indicated that the reaction was complete. The reaction mixture was heated to 20°C for 10 min and then cooled to -78°C and quenched with collidine (60 μL). The reaction was filtered, excess

collidine was removed by repeated codistillation with toluene, and the resulting syrup purified by column chromatography with hexane-ethyl acetate (3:2 R_f 0.28) as eluent to yield 0.137 g of **41** (53%) and 0.0038g of the β-disaccharide **42** (α/β = 36/1). In addition, 0.018 g of the glucal **43** and 0.198 g of unreacted thiol **39** were also isolated. **41**: $[\alpha]_D^{21}$ +265.0° (c 0.6 in CH_2Cl_2). Anal. Calcd for $C_{42}H_{44}O_{16}S_2$: C, 58.06; H, 5.10%, Found: C, 58.20; H, 5.12%. **42**: $[\alpha]_D^{22}$ +132.4° (c 1.28 in CH_2Cl_2). Anal. Calcd for $C_{42}H_{44}O_{16}S_2$: C, 58.06; H, 5.10%, found: C, 58.30; H, 5.21%. ES-MS calcd 868, found 891 (M + Na).

41: ^{1}H NMR($CDCl_3$) δ 1.65, 1.91, 1.99, 2.02 (3H, s, $COCH_3$), 3.39, (1H, t, $J_{3,4} = J_{4,5}$ = 11.0 Hz, H-4), 3.45 (3H, s, OCH_3), 3.61 (1H, ddd, $J_{5',6'a}$ = 2.9 Hz, $J_{5',6'b}$ = 2.5 Hz, H-5'), 3.95 (1H, dd, $J_{5',6'a}$ = 2.9 Hz, $J_{6',6'b}$ = 12.5 Hz, H-6'a), 4.19 (1H, ddd, $J_{5,6a}$ = 5.5. Hz, $J_{5,6b}$ = 2.5 Hz, H-5), 4.39 (1H, dd, $J_{6'a,6'b}$ = 12.5 Hz, H-6'b), 4.60 (1H, d, $J_{1',2}$ = 4.8 Hz, H-1'), 4.72 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.80 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, H-6b), 5.08-5.23 (5H, complex m, H-1, H-2, H-2', H-3', H-4'), 6.16 (1H, t, $J_{2,3} = J_{3,4}$ = 10.2 Hz, H-3), 7.14-8.12 (15H, complex m, $3COC_6H_5$).

^{13}C NMR($CDCl_3$) δ 20.0, 20.5 ($4COCH_3$), 40.2 (C-5'), 46.8 (C-4), 51.9 [^{1}J(^{13}C-^{1}H) 158 Hz (C-1')], 55.6 (OCH_3), 60.8 (C-6'), 64.3 (C-6), 68.4 (C-5), 70.8, 71.8, 73.0, 73.3 (C-2, C-2', C-3', C-4'), 73.2 (C-3), 97.2 [^{1}J(^{13}C-^{1}H) 174 Hz (C-1')], 128.3, 128.6, 129.0, 129.6, 129.8, 129.9, 133.3, 133.5 ($3COC_6H_5$), 165.3, 165.9, 166.2, 169.0, 169.4, 170.3 ($3OCOCH_3$, $3COC_6H_5$).

42: ^{1}H NMR ($CDCl_3$) δ 1.87, 1.91, 1.95, 2.0 (3H, s, $COCH_3$), 2.74 (1H, ddd, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 4.5 Hz, H-5'), 3.44 (3H, s, OCH_3), 3.44, (1H, t, $J_{3,4} = J_{4,5}$ = 11.2 Hz, H-4), 3.79 (1H, dd, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 4.06 (1H, dd, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b),

4.07 (1H, d, $J_{1',2'}$ = 11.0 Hz, H-1'), 4.31 (1H, ddd, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ =1.8 Hz, H-5), 4.71 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.80 (1H, t, $J_{2',3'} = J_{3',4'}$ = 18.8 Hz, H-3'), 4.86 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.98 (1H, dd, $J_{2',3'}$ = 9.9 Hz, H-2'), 5.11 (1H, t, $J_{3',4'} = J_{4',5'}$ = 20.5 Hz, H-4'), 5.18 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.24 (1H, dd, $J_{2,3}$ = 9.8 Hz, H-2), 5.88 (1H, dd, $J_{3,4}$ = 11.0 Hz, H-3), 7.35-8.15 (15H, m, $3COC_6H_5$).

^{13}C NMR ($CDCl_3$) δ 43.1 (C-5'), 46.6 (C-4), 47. [$^1J(^{13}C-^1H)$ 155 Hz (C-1')], 60.6 (C-6'), 63.8 (C-6), 68.5 (C-3), 69.8 (C-5), 71.2 (C-4'), 73.0 (C-2), 73.2 (C-2'), 74.4 (C-3'), 97.0 (C-1).

Methyl 4-thio-4-S-(5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**4**).

The disaccharide **41** (0.091 g; 0.1047 mmol) was dissolved in 0.2 M NaOMe in MeOH (4 mL) and stirred at room temperature under nitrogen. After 2.5 h tlc (ethyl acetate-methanol-water 6:2.5:1.0) indicated that the reaction was complete. The reaction mixture was diluted with methanol (20 mL) and neutralised using Rexyn (H^+) resin, filtered, concentrated, and purified by column chromatography (silica gel) with ethyl acetate-methanol-water (6:2:0.9) as eluent. Further purification by Sephadex LH20 filtration yielded 0.036 g of **4** (89%) which was crystallised from hot methanol (mp. 217-220°C). $[\alpha]_D^{22}$ +361.5° (c 1.0 in MeOH). Anal. Calcd for $C_{13}H_{24}O_9S_2$: C, 40.20; H, 6.23%, found: C, 40.47; H, 6.21%. ES-MS calcd 388, found 411 (M + Na).

^1H NMR(D_2O) δ 2.91 (1H, t, $J_{3,4} = J_{4,5}$ = 22.5 Hz, H-4), 3.22 (1H, dt, $J_{4',5'}$ = 10.0 Hz, $J_{5',6'a} = J_{5',6'b}$ = 3.2 Hz, H-5'), 3.37 (3H, S, OCH_3), 3.50 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3'), 3.54 (1H, dd, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.57 (1H, t, $J_{3',4'} = J_{4',5'}$ = 10.0

Hz, H-4'), 3.77 (1H, ddd, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 5.0 Hz, H-5), 3.85-3.92 (4H, m, 3.88, H-3; 3.89, H-6'a, H-6a; 3.90, H-6'b), 3.98 (1H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.01 (1H, dd, $J_{1',2'}$ = 4.5 Hz, $J_{2',3'}$ = 9.5 Hz, H-2'), 4.56 (1H, d, $J_{1',2'}$ = 4.5 Hz, H-1'), 4.82 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1).

^{13}C NMR(D_2O) δ 47.4 (C-5'), 51.1 (C-4), 56.0 (C-1', $^1J_{C,H}$ = 155 Hz), 57.8 (OCH_3), 62.7 (C-6'), 64.5 (C-6), 73.5 (C-5), 75.0 (C-2), 75.9 (C-3), 76.3 (C-4'), 77.3 (C-3'), 77.7 (C-2'), 102.11 (C-1).

Methyl 4-thio-4-S-(5'-thio-β-D-glucopyranosyl)-α-D-glucopyranoside (**44**).

The disaccharide **42** (0.025 g; 0.000 mmol) was dissolved in 0.1 M NaOMe in MeOH (4 mL) and the mixture was stirred at room temperature under nitrogen. After 16 h tlc (ethyl acetate-methanol-water; 6:2:1) indicated that the reaction was complete. The reaction was diluted with methanol (20 mL) and neutralised using Rexyn (H^+) resin, filtered, concentrated, and purified by column chromatography (silica gel) with dichloromethane-methanol 5:3 as eluent to yield **44** (0.095 g, 85%). $[\alpha]_D^{19}$ +87.5° (c 0.80 in MeOH). Anal. Calcd for $C_{13}H_{24}O_9S_2$ C, 40.20; H, 6.23%, Found: C, 40.41; H, 6.17%.

^{1}H NMR(D_2O) δ 2.83 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 10.4 Hz, H-4), 2.98 (1H, ddd, $J_{4',5'}$ = 10.0 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 6.0 Hz, H-5'), 3.28 (1H, t, $J_{2',3'}$ = $J_{3',4'}$ = 9.0 Hz, H-3'), 3.36 (3H, S, OCH_3),), 3.42 (1H, dd, $J_{1',2'}$ = 10.5 Hz, $J_{2',3'}$ = 9.0 Hz, H-2'), 3.56 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4'), 3.57 (1H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.64 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 3.77 (1H, dd, $J_{5',6'b}$ = 6.0 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b), 3.83

(1H, ddd, $J_{4,5}$ = 10.5 Hz, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 5.0 Hz, H-5), 3.87 (1H, dd, $J_{5',6'a}$ = 3.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 3.92 (1H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 3.93 (1H, d, $J_{1',2'}$ = 10.5 Hz, H-1'), 4.00 (1H, dd, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.81 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1).

^{13}C NMR(D_2O) δ 50.6 (C-4), 51.0 [$^1J(^{13}C\text{-}^1H)$ 153Hz (C-1')], 51.6 (C-5'), 58.0 (OCH_3), 63.0 (C-6'), 64.4 (C-6), 79.2 (C-3), 74.7 (C-5), 75.2 (C-2), 76.0 (C-4'), 78.7 (C-2'), 80.8 (C-3'), 102.2 [$^1J(^{13}C\text{-}^1H)$ 170Hz (C-1)].

Methyl 4-amino-4-deoxy-4-N-(5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside **(5) and *methyl 4-amino-4-deoxy-4-N-(5'-thio-β-D-glucopyranosyl)-α-D-glucopyranoside*** **(48).**

A mixture of 5-thioglucopyranose (0.199 g; 1.10 mmol) and methyl 4-amino-α-D-glucopyranoside **46** (0.381 g; 2.03 mmol) in methanol (4 mL) containing glacial acetic acid (5.8 μL; 0.101 mmol) was heated at 55-58°C. After 48 h tlc (ethyl acetate-methanol-water 4:2:1) indicated that no more products were being formed. The reaction was quenched with triethylamine (14 μL) and the solvent evaporated to give a syrup that was purified by chromatography using dichloromethane-ethyl acetate-methanol- water (1:1:0.9:0.2) as eluent (R_f 0.2) to yield a mixture of **5/48** (0.207 g; 55%, or 72% based on recovered 5-thio-glucopyranose [0.048 g]) and an impurity **50** that appears to be the result of an Amadori rearrangement (1-amino-1-deoxy-D-fructose derivative; 0.094 g; 16%). **5/48**: Anal. Calcd for $C_{13}H_{25}O_9SN$ C, 42.04; H, 6.79; N, 3.77%. Found: C, 41.98; H, 6.93; N, 4.00%.

5/48: ^{1}H NMR (600 MHz, D_2O) δ 2.76 (1H, t, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4β), 2.81 (1H, t, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4α), 2.90 (1H, ddd, $J_{4',5'} = 10.5$ Hz, $J_{5',6'a} = 3.2$ Hz, $J_{5',6'b} = 5.8$ Hz, H-5'β), 3.07 (1H, m, H-5'α), 3.30 (1H, t, $J_{2',3'} = J_{3',4'} = 9.0$ Hz, H-3'β), 3.38 (3H, s, OCH_3), 3.49 (1H, t, $J_{1',2'} = J_{2',3'} = 9.5$ Hz, H-2'β), 3.55-3.85 (10H, m, 3.55, H-2α; 3.56 H-3β, H-4'β; 3.57, H-3'α, H-4'α; 3.58, H-2β; 3.61, H-5α; 3.65, H-5β; 3.80, H-3α; 3.85, H-2'α), 3.94 (1H, d, $J_{1',2'} = 9.5$ Hz, H-1'β), 4.46 (1H, d, $J_{1',2'} = 4.2$ Hz, H-1'α), 4.80 (1H, d, $J_{1,2} = 3.5$ Hz, H-1α), 4.81 (1H, d, $J_{1,2} = 3.5$ Hz, H-1β).

^{13}C-NMR (D_2O; 150 MHz): δ 45.9 (C-5'α), 48.8 (C-5'β), 57.8 (OCH_3), 59.9 (C-4β), 60.3 (C-4α), 62.8-64.5 (C-6α, C-6'α, C-6β, C-6'β), 65.6 [^{1}J(^{13}C,^{1}H) 153 Hz, (C-1'α)], 66.2 [^{1}J(^{13}C,^{1}H) 153 Hz, (C-1'β)], 75.3 (C-5β), 73.4-76.4 (C-2α, C-5α, C-3'α, C-4'α, C-2β, C-3β, C-4'β), 77.4, (C-3α), 77.8 (C-2'α), 79.7 (C-2'β), 80.4 (C-3'β), 102.1 [^{1}J(^{13}C,^{1}H) 170 Hz, (C-1α, C-1β)].

Methyl 4-amino-4-deoxy-4-N-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**51**) and **methyl *4-amino-4-deoxy-4-N-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-2,3,6-tri-O-acetyl-α-D-glucopyranoside*** (**52**).

A mixture of 5-thio-glucopyranose **49** (0.059 g; 0.30 mmol) and methyl 4-amino-α-D-glucopyranoside **46** (0.116 g; 0.60 mmol) in methanol (1 mL) containing glacial acetic acid (1.7 μL; 0.03 mmol) was heated to 55°C for 24 h. The solvent was removed and the residue dissolved in pyridine (1.5 mL) and acetic anhydride (1.5 mL) and stirred for 16 h. After removal of the Ac_2O/Py by co-distillation with toluene the residue was purified by careful column chromatography using hexane-ethyl acetate (1:1 containing 1%

triethylamine) as eluent (R_f 0.25) to completely separate the α/β anomers to yield **51** (0.054 g) and **52** (0.031 g) in a total yield of 38% (or 70% based on recovered 5-thio-glucopyranose (0.025 g)). The ratio of **51/52** was determined by ^{1}H NMR spectroscopy to be 1.75:1). **51** $[\alpha]_D^{20}$ +99.1° (c 0.54 in CH_2Cl_2). **52** $[\alpha]_D^{22}$ +95.9° (c 0.98 in CH_2Cl_2).

51: ^{1}H NMR (CD_2Cl_2) δ 1.84 (1H, d, $J_{NH,4}$ = 10.0 Hz, N-*H*), 1.95, 1.96, 1.99, 2.01, 2.02, 2.03, 2.10 (21H, 7s, 7COC*H*$_3$), 3.23 (1H, q, $J_{3,4} = J_{4,5} = J_{4,NH}$ = 10.0 Hz, H-4), 3.40 (3H, s, OC*H*$_3$), 3.42 (1H, m, H-5'), 3.74(1H, m, $J_{4,5}$ = 10.5 Hz, $J_{5,6a} = J_{5,6b}$ = 3.6 Hz, H-5), 4.00 (1H, dd, $J_{5',6'a}$ = 3.0 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6'a), 4.31 (2H, m, H-6a, H-6b), 4.34 (1H, dd, $J_{5',6'b}$ = 4.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6'b), 4.42 (1H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 4.78 (1H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.85 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.17 (1H, dd, $J_{1',2'}$ = 4.0 Hz, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.22 (1H, t, $J_{3',4'} = J_{4',5'}$ = 10.0 Hz, H-4'), 5.28 (1H, t, $J_{2',3'} = J_{3',4'}$ = 10.5 Hz, H-3'), 5.38 (1H, t, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3).

^{13}C-NMR (CD_2Cl_2) δ 20.7–21.1 (7COC*H*$_3$), 39.0 (C-5'), 54.4(C-4), 55.4 (OC*H*$_3$), 61.0 [^{1}J(^{13}C,^{1}H) 154 Hz, (C-1')], 61.3 (C-6'), 63.9 (C-6), 70.0 (C-5), 70.6 (C-3'), 72.0 (C-2), 72.4 (C-4'), 74.2 (C-2'), 74.4 (C-3), 97.2 [^{1}J(^{13}C,^{1}H) 176 Hz, (C-1)], 170.0-171.1 (7*C*OCH$_3$).

52: ^{1}H-NMR (CD_2Cl_2) δ 1.50 (1H, dd, $J_{NH,1'}$ = 12.5 Hz, $J_{NH,4}$ = 4.5 Hz, N-*H*), 1.96, 1.99, 2.02, 2.03, 2.04, 2.09, 2.10 (21H, 7s, 7COC*H*$_3$), 2.92 (1H, dt, $J_{NH,4}$ = 4.5 Hz, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4), 3.13 (1H, ddd, $J_{4',5'}$ = 10.5 Hz, $J_{5',6'a}$ = 3.5 Hz, $J_{5',6'b}$ = 5.4 Hz, H-5'), 3.35 (3H, s, OC*H*$_3$), 3.64 (1H, m, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 5.2 Hz, H-5), 3.91 (1H, m, $J_{1',NH}$ = 12.5 Hz, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.12 (1H, dd, $J_{5,6a}$ = 5.2 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.13 (1H, dd, $J_{5',6'a}$ = 3.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 4.20 (1H, dd,

$J_{5',6'b}$ = 5.4 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b), 4.48 (1H, dd, $J_{5,6b}$ = 2.0 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6b), 4.83 (2H, m, H-1, H-2), 5.02 (2H, m, H-2', H-3'), 5.07 (1H, m, H-3), 5.17 (1H, m, $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 10.5 Hz, H-4').

^{13}C-NMR (CD_2Cl_2): δ 20.6–21.6 (7$COCH_3$), 42.3 (C-5'), 55.4 (OCH_3), 57.9(C-4), 62.0 (C-6'), 62.8 (C-6), 64.6 [$^1J(^{13}C,^1H)$ 153 Hz, (C-1')], 70.6 (C-3), 71.4 (C-5), 71.5 (C-2), 72.7 (C-4'), 73.9, 74.8 (C-2', C-3'), 97.1 [$^1J(^{13}C,^1H)$ 169 Hz, (C-1)], 169.7-172.2 (7$COCH_3$).

Methyl 2-amino-2-deoxy-2-N-(5'-thio-α-D-glucopyranosyl)-β-D-glucopyranoside (**3**) and ***methyl 2-amino-2-deoxy-2-N-(5'-thio-β-D-glucopyranosyl)-β-D-glucopyranoside*** (**53**).

A mixture of 5-thioglucopyranose **49** (0.134 g; 0.68 mmol) and methyl 2-amino-β-D-glucopyranoside (0.263 g; 1.36 mmol) in methanol (3 mL) containing glacial acetic acid (4 μL; 0.068 mmol) was heated at 60-70°C. After 24 h, tlc (ethyl acetate- methanol-water 4:2:1) indicated that no more products were being formed. The reaction was quenched at -30°C with triethylamine (4 μL) and the solvent evaporated to give a syrup that was purified by chromatography using ethyl acetate-methanol-water (6:2:1) as eluent (R_f 0.35) to yield a mixture of **3/53** (0.132 g; 52% yield, or 70% based on recovered 5-thio-glucopyranose **49**) and an impurity **55** that appears to be the result of an Amadori rearrangement (1-amino-1-deoxy-D-fructose derivative; 0.038 g; 15%). Pure **3/53** was obtained by acetylation of the above mixture (acetic anhydride/pyridine) followed by

decetylation of the pure α anomer **3** (see later). **3/53** Anal. Calcd for $C_{13}H_{25}O_9SN$ C, 42.04; H, 6.79; N, 3.77%. Found: C, 42.04; H, 7.00; N, 3.67%.

^{1}H-NMR (D_2O; 600 MHz) δ 2.70 (1H, dd, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 9.5 Hz, H-2β), 2.81 (1H, m, $J_{1,2} = J_{2,3}$ = 8.3 Hz, H-2α), 2.91 (1H, m, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a}$ = 3.3 Hz, $J_{5',6'b}$ = 5.8 Hz, H-5'β), 3.01 (1H, m, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a} = J_{5',6'b}$ = 4.4 Hz, H-5'α), 3.30 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.0 Hz, 3'β), 3.37 (H-4β), 3.4 (H-3α, H-4α, H-5α, H-5β), 3.50 (H-2'β), 3.51 (H-3β), 3.54 (2OCH_3), 3.56 (H-4'β, H-3'α), 3.60 (H-4'α), 3.73 (H-6aα, H-6aβ), 3.82 (1H, m, $J_{5',6'a}$ = 6.1 Hz, $J_{6'a,6'b}$ = 11.9, H-6'bβ), 3.88 (H-2'α, H-6'aα, H-6'bα), 3.91 (H-6'bβ), 3.92 (H-6bα, H-6bβ), 4.19 (1H, d, $J_{1',2'}$ = 9.8 Hz, H-1'β), 4.30 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1β), 4.44 (2H, m, $J_{1',2'}$ = 4.9 Hz, H-1'α; $J_{1,2}$ = 8.1 Hz, H-1α).

^{13}C-NMR (D_2O; 150 MHz) δ 44.9 (C-5'α), 48.7 (C-5'β), 60.0 (2O*C*H$_3$), 62.1 (C-2α), 63.0 (C-6'α), 63.1 (C-6'β), 63.6 (C-6α, C-6β), 64.1 (C-2β), 64.7 [^{1}J(^{13}C-^{1}H) 154 Hz (C-1'α)], 66.4 [^{1}J(^{13}C-^{1}H) 152 Hz (C-1'β)], 72.7 (C-5α, C-4β, C-5β), 76.3 (C-2'β), 76.4 (C-4α, C-3'α, C-4'α), 77.1 (C-2'α), 78.6 (C-3α), 79.2 (C-3β), 79.8 (C-4'β), 80.2 (C-3'β), 105.9 [^{1}J(^{13}C-^{1}H) 159 Hz (C-1β)], 108.1 [^{1}J(^{13}C-^{1}H) 162 Hz (C-1α)].

Methyl 2-amino-2-deoxy-2-N-(2',3',4',6'-tetra-O-acetyl-5'-thio-α-D-glucopyranosyl)-3,4,6-tri-O-acetyl-β-D-glucopyranoside (**56**) and ***Methyl 2-amino-2-deoxy-2-N-(2',3',4',6'-tetra-O-acetyl-5'-thio-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-β-D-glucopyranoside*** (**57**).

To a sample of **3/53** (0.125 g; 0.35 mmol), containing the Amadori rearrangement product, in pyridine (2 mL) was added Ac_2O (2 mL) and the mixture was stirred for 16 h.

After co-distillation with toluene the residue was purified by chromatography using hexane: ethyl acetate (5:4) as eluent (R_f 0.23) to yield pure **56** (0.088 g) and **57**(0.024 g; containing an inseparable impurity) and a mixture of **56** and **57** (0.042 g). Total yield of acetylated product is 70%. **56**: $[\alpha]_D^{22}$ +115.9° (c 0.37 in CH_2Cl_2). Anal. Calcd for $C_{27}H_{39}O_{16}SN$: C, 48.72; H, 5.91; N, 2.10. Found: C, 49.00; H, 6.02; N, 2.16%. IR (nujol) 3324 cm^{-1}, NH.

56: ^{1}H- NMR (400 MHz, $CDCl_3$) δ 1.98–2.09 (21H, 7s, 7COCH_3), 3.13 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.35 (1H, ddd, $J_{4',5'}$ = 10.6 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 4.5 Hz, H-5'), 3.45 (3H, s, OCH_3), 3.64 (1H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 4.00 (1H, dd, $J_{5',6'a}$ = 4.2 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'a), 4.12 (1H, dd, $J_{5,6a}$ = 2.5 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.23 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.29 (1H, dd, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.38 (1H, dd, $J_{5',6'b}$ = 4.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6'b), 4.67 (1H, d, $J_{1',2'}$ = 3.5 Hz, H-1'), 4.97 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.05 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.20-5.31 (3H, m, H-2', H-3', H-4').

^{13}C-NMR (100 MHz, $CDCl_3$) δ 20.4–20.6 (7COCH_3), 37.7 (C-5'), 56.8 (OCH_3), 57.8 (C-2), 59.4 [^{1}J(^{13}C,^{1}H) 156 Hz, (C-1')], 61.4 (C-6'), 62.1 (C-6), 69.2 (C-4), 70.8 (C-3'), 71.8 (C-5), 72.6 (C-4'), 73.2 (C-3), 74.5 (C-2'), 106.1 [^{1}J(^{13}C,^{1}H) 157 Hz, (C-1)], 168.9-170.5 (7*C*OCH$_3$).

57: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.37 (1H, broad m, N–*H*), 1.98–2.10 (21H, 7s, 7COCH_3), 3.01 (1H, m, H-2), 3.09 (1H, ddd, $J_{4',5'}$ = 10.5 Hz, $J_{5',6'a}$ = 3.5 Hz, $J_{5',6'b}$ = 5.5 Hz, H-5'), 3.52 (3H, s, OCH_3), 3.66 (1H, m, H-5), 4.07 (1H, dd, $J_{5',6'a}$ = 3.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6'a), 4.07 (1H, broad m, H-1'), 4.11 (1H, dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.2

Hz, H-6a), 4.14 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.27 (1H, dd, $J_{5',6'b}$ = 5.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6'b), 4.28 (1H, dd, $J_{5,6b}$ = 3.0 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.93 (1H, dd, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 10.0 Hz, H-3'), 4.97 (2H, m, H-3, H-4), 5.08 (1H, t, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.22 (1H, dd, $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 10.5 Hz, H-4').

^{13}C-NMR ($CDCl_3$) δ 20.2–20.8 (7$COCH_3$), 42.2 (C-5'), 57.2 (OCH_3), 59.5 (C-2), 61.8 (C-6'), 62.1 (C-6), 63.9 [$^1J(^{13}C,^1H)$ 145 Hz, (C-1')], 68.9 (C-4), 72.1 (C-5), 72.4 (C-4'), 74.0 (C-3'), 74.7 (C-2'), 76.4 (C-3), 102.7 [$^1J(^{13}C,^1H)$ 160 Hz, (C-1)], 169.3-170.5 (7$COCH_3$).

Deprotection of **56**.

A pure sample of **56** (0.062 g; 0.093 mmol) was dissolved in a mixture of MeOH: H_2O: Et_3N (5:1:1) and stirred for 16 h at room temperature. After this time tlc (EtOAc: MeOH: H_2O 4:2:1) indicated that the reaction was complete. The solvents were removed and the residue purified by chromatography using CH_2Cl_2: MeOH (5:3) as eluent to yield a mixture of methyl 5'-thio-2-N-β-kojibioside **3** and methyl 5'-thio-2-N-β-sophoroside **53** (0.028g, 81%).

REFERENCES

1. Horowitz, M. I.; Pigman, W. *The Glycoconjugates*, **1977-1982**, vol I-IV, Academic Press, New York.
2. Hakamori, S. *Ann. Rev. Biochem.* **1981**, *50*, 733, and references therein.
3. Li, Y.-T.; Li, S.-C. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 235.
4. Varki, A. *Glycobiology* **1993**, *3*, 97.
5. Feizi, T.; Childs, R. A. *Biochem. J.* **1987**, *245*, 1.
6. Phillips, M. L.; Nudelman, E.; Gaeta, C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130, and references therein.
7. a) Kameyama, A.; Ishida, C.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, C1.

   b) Nicolaou, K. C.; Hummel. C. W.; Backovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870.
8. a) Dumas, D. P.; Ichikawa, Y.; Wong, C.-H. *Biomed. Lett.* **1991**, *1*, 425.

   b) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.
9. a) Kornfeld, R.; Kornfeld, S. *Ann. Rev. Biochem.* **1985**, *54*, 631.

   b) Datema, R.; Oloffson, S.; Romero, P. A.; *Pharmacol. Ther.* **1987**, *33*, 221.

   c) Elbein, A. D. *FASEB J.* **1991**, *5*, 3055.
10. Feizi, T.; Larkin, M. *Glycobiology* **1990**, *1*, 17.

11. von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418.

12. Humphries, M. J.; Matsumota, K.; White, S. L.; Olden, K. *Cancer Res.* **1986**, *46*, 5215.

13. Evans, S. V.; Fellows, L. E.; Shing, K. T. M.; Fleet, G. W. J. *Phytochemistry* **1985**, *24*, 1953.

14. Truscheit, E.; Frommer, W.; Junge, B.; Muller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744, and references therein.

15. Gallo, R. C. *Sci. Amer.* **1987**, *256*, 47.

16. Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chameret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vesinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.

17. a) Klatzmann, D.; Barre-Sinoussi, F.; Nugeyre, M. T.; Dauguet, C.; Vilmer, E.; Griscelli, C.; Vesinet-Brun, F.; Rouzioux, C.; Gluckman, J. C.; Chermann, J. C.; Montagnier, L. *Science* **1984**, *225*, 59.

b) Dagleish, A. G.; Beverly, P. C.; Clapham, P. R.; Crawford, D. H.; Greaves, M. F.; Weiss, R. A. *Nature* **1984**, *312*, 763.

c) Lifson, J. D.; Rays, G. R.; McGrath, M. S.; Stein, B. A.; Engleman, E. G. *Science* **1986**, *232*, 1123.

d) Sosroski, J.; Goh, W. C.; Rosen, C. A.; Campbell, A.; Haseltine, W. A. *Nature* **1986**, *322*, 470.

e) McDougal, J. S.; Kennedy, M. S.; Sligh, J. M.; Cort, S. P.; Mawle, A.; Nicholson, J. K. A. *Science* **1986**, *231*, 382.

f) Robey, E.; Axel, R. *Cell* **1990**, *60*, 697.

18. Laskey, L. A.; Groopman, J. E.; Fennie, C. W.; Benz, P. M.; Capon, D. J.; Dowbenko, D. J.; Nakamura, G. R.; Nunes, W. M.; Renz, M. E.; Berman, P. W. *Science* **1986**, *233*, 209.

19. a) Karlsson, G. B.; Butters. T. D.; Dwek, R. A.; Platt, F. M. *J. Biol. Chem.* **1993**, *268*, 570.

b) Pal, R.; Hoke, G. M.; Sarngadharan, M. G. *Proc. Natl. Acad. Sci.* **1989**, *86*, 3384.

20. For recent reviews see: a) Hughes, A. B.; Rudge, A. J. *Nat. Prod. Reports* **1994**, 135.

b) van den Broek, L. A. G. M.; Vermaas, D. J.; Heskamp, B. M.; van Boeckel, C. A. A.; Tan, M. C. A. A.; Bolscher, J. G. M.; Ploegh, H. L.; van Kemenade, F. J.; de Goede, R. E. Y.; Miedema, F. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 82.

21. Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; de Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. *Nature* **1987**, *330*, 74.

22. Walker, B. D.; Kowalski, M.; Goh, W. C.; Kozarski, K.; Krieger, M.; Rosen, C.; Rohrschneider, L.; Haseltine, W. A.; Sodroski, J. *Proc. Nat. Acad. Sci. U.S.A.* **1987**, *84*, 8120.

23. Schweden, J.; Borgmann, C.; Legler, G.; Bause, E. *Arch. Biochem. Biophys.* **1986**, *248*, 335.

24. a) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128.

b) Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9229.

25. Asano, N.; Kizu, H.; Oseki, K.; Tomioka, E.; Matsui, K.; Okamoto, M.; Baba, M. *J. Med. Chem.* **1995**, *38*, 2349.

26. Comber, R. N.; Friedrich, J. D.; Dunshee, S. L. P.; Petty, S. L.; Secrist III, J. A. *Carbohydr. Res.* **1994**, *262*, 245.

27. a) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182, and references therein.

b) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, Jr., J. A.; Wong, C.-H. *J. Am. Chem . Soc.* **1991**, *113*, 6187, and references therein.

c) Fuhrmann, U.; Bause, E.; Ploegh, H. *Biochim. Biophys. Acta* **1985**, *825*, 95, and references therein.

28. Fischer, E. *Untersuchungen über Kohlenhydrate und Ferment*, Verlag Berlin, **1909**, Vol. I, 843.

29. Sinnott, M. L. *Chem. Rev.* **1990**, *90* , 1171, and references therein.

30. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319, and references therein.

31. McCarter, J. D.; Withers, S. G. *Curr. Opin. Str. Biol.* **1994**, *4*, 855.

32. Koshland, D. E. *Biol. Rev.* **1953**, *28*, 416.

33. a) Phillips, D. C. *Sci. Am.* **1966**, *215*, 78.

b) Blake C. C. F.; Mair, G. A.; North, A. T. C.; Phillips, D. C.; Sarma, V. R. *Proc. Roy. Acad. Sci. (London), Ser. B* **1967**, *167*, 378.

34. Qian, M.; Haser, R.; Buisson, G.; Duee, E.; Payan, F. *Biochemistry* **1994**, *33*, 6284.

35. Svensson, B.; Clarke, A. J.; Svendsen, I.; Moller, H. *Eur. J. Biochem.* **1990**, *188*, 29.

36. Strynadka, N. C. J.; James, M. N. G. *J. Mol. Biol.* **1991**, *220*, 401.

37. Kuroki, R.; Weaver, L. H.; Matthews, B. W. *Science* **1993**, *262*, 2030.

38. a) Review: Frank, R. W. *Bioorg. Chem.* **1992**, *20*, 77.

b) Fleet, G. W. J. *Tetrahedron Lett.* **1985**, *26*, 5073.

c) Post, C. B.; Karplus, M. *J. Am. Chem. Soc.* **1986**, *108*, 1317.

39. Sinnott, M. L. *Bioorg. Chem.* **1993**, *21*, 34.

40. Wang, Q.; Graham, R. W.; Trimbur, D.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 11594.

41. Pauling, L. *Chem. Eng. News.* **1946**, *24*, 1375.

42. a) Laglerie, P.; Legler, G.; Yon, J. M. *Biochemie.* **1982**, *64*, 977.

b) Saul, R.; Molyneux, R. L.; Elbein, A. D. *Arch. Biochem. Biophys.* **1984**, *230*, 668.

43. Conchie, J.; Hay, A. J.; Strachan, I.; Levvy, G. A. *Biochem.* **1967**, *102*, 929.

44. Beer, D.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 267.

45. Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *23*, 2125.

46. Ermert, P.; Vasella, A.; Weber, M.; Rupitz, K.; Withers, S. G. *Carbohydr. Res.* **1993**, *250*, 113.

47. Tong. M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137.

48. Ganem, B.; Papandreou, G. *J. Am. Chem. Soc.* **1991**, *113*, 8984.

49. Hoos, R.; Naughton, A. B.; Thiel, W.; Vasella, A.; Weber, W. *Helv. Chim. Acta* **1993**, *76*, 2666.

50. Ermert, P.; Vasella, A. *Helv. Chim. Acta* **1991**, *74*, 2043.

51. a) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280.

b) Dumas, D. P.; Kajimoto, T.; Liu, K. K.-C.; Wong, C.-H. *Biorg. Med. Chem. Lett.* **1992**, *2*, 33.

c) Wang, Y.-F.; Dumas, D. P.; Wong, C.-H. *Tetrahedron Lett.* **1993**, *34*, 403.

52. Jespersen, T. M.; Dong. W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, *M. Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1788.

53. a) Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett.* **1995**, *36*, 1767.

b) Ichikawa, M.; Igarashi, Y. *Tetrahedron Lett.* **1995**, *36*, 4585.

54. von Itzstein, M.; Wu, W.-Y.; Jin, B. *Carbohydr. Res.* **1994**, *259*, 301.

55. Holzer, C. T.; von Itzstein, M.; Jin, B.; Pegg, M. S.; Stewart, W. P.; Wu, W.-Y. *Glycoconj. J.* **1993**, *10*, 40.

56. Svensson, B.; Sierks, M. R. *Carbohydr. Res.* **1992**, *227*, 29.

57. Sigurskjold, B. W.; Berland, C. R.; Svensson, B. *Biochemistry* **1994**, *33*, 10191.

58. Ermert, P.; Vasella, A. *Helv. Chim. Acta* **1993**, *76*, 2687.

59. Hashimoto, S.; Fujimori, T.; Yuasa, H. *J. Carbohydr. Chem.* **1990**, *9*, 683.

60. Whistler, R. L.; Lake, W. C. *Biochem. J.* **1972**, *130*, 919.

61. Capon, R. J.; MacLeod, J. K. *J. Chem. Soc., Chem. Commun.* **1987**, 1200.

62. Daniel, J. R.; Whistler, R. L.; Zingaro, R. A. *Phosphorous and Sulfur* **1979**, *7*, 31.

63. See for example, a) Feather. M. S.; Whistler, R. L. *Tetrahedron Lett.* **1962**, 667.

b) Chmielewski, M.; Whistler, R. L. *J. Org. Chem.* **1975**, *40*, 639.

c) Anisuzzaman, A. K. M.; Whistler, R. L. *Carbohydr. Res.* **1977**, *55*, 205.

d) Hasegawa, A.; Kawai, Y.; Kasugai, H.; Kiso, M. *Carbohydr. Res.* **1978**, *63*, 131.

e) Shin, J. E. N.; Perlin, A. S. *Carbohydr. Res.* **1979**, *76*, 165.

f) Yuasa, H.; Izukawa, Y.; Hashimoto, H. *J. Carbohydr. Chem.* **1989**, *8*, 753.

g) Hashimoto, H.; Fujimori, T.; Yuasa, H. *J. Carbohydr. Chem.* **1990**, *9*, 683.

64. a) Hoffman, D. J.; Whistler R. L. *Biochemistry* **1968**, *7*, 4479.

b) Whistler, R. L.; Lake, W. C. *Biochem. J.* **1972**, *130*, 919.

65. Hellman, B.; Lernmark, A.; Sehlin, J.; Taljedal, J. B.; Whistler, R. L. *Biochem. Pharmacol.* **1973**, *22*, 29.

66. Zysk, J. R.; Bushway, A. A.; Whistler, R. L.; Carlton, W. W. *J. Reprod. Fert.* **1975**, *45*, 69.

67. Bamford, C.; Capon, B.; Overend, W. G. *J. Chem. Soc.* **1962**, 5138.

68. Blanc-Meusser, M.; Vigne, L.; Driguez, H.; Lehmann, J.; Steck, J.; Urbahns, K. *Carbohydr. Res.* **1992**, *224*, 59.

69. Lee, Y. C. *Ciba Found. Symp.* **1989**, *145*, 80.

70. Orgeret, C.; Seillier, E.; Gautier, C.; Defaye, J.; Driguez, H. *Carbohydr. Res.* **1992**, *224*, 29.

71. Schou, C.; Rasmussen, G.; Schulein, M.; Henrissat, B.; Driguez, H. *J. Carbohydr. Chem.* **1993**, *12*, 743.

72. Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron Asymm.* **1994**, *5*, 2351.

73. a) Cohen, G. N.; Monod, J. *Bacteriol. Rev.* **1957**, *21*, 169.

b) Okada, H.; Halvorson, H. O. *Biochim. Biophys. Acta* **1964**, *82*, 538.

74. Defaye, J.; Gelas, J. in *Studies in Natural Products Chemistry*, Atta-ur-Rahman (Ed.), Elsevier, Amsterdam, **1991**, *8*, 315.

75. Boos, W.; Schaedel, P.; Wallenfels, K. *J. Bacteriol.* **1976**, *1*, 382.

76. Defaye, J.; Driguez, H.; Poncet, S.; Chambert, R.; Petit-Glatron, M.-F. *Carbohydr. Res.* **1984**, *130*, 299.

77. Rho, D.; Desrochers, M.; Jurasek, L.; Driguez, H.; Defaye, J. *J. Bacteriol.* **1982**, *149*, 47.

78. Defaye, J.; Driguez, H.; John, M.; Schmidt, J. Ohleyer, E. *Carbohydr. Res.* **1985**, *139*, 123.

79. a) Tomino, A.; Paigen, K. in *The lac operon*, Zipser, D.; Beckwite, J. (Eds.) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, **1970**, 233.

b) Steers, Jr., E.; Cuatrecasas, P.; Pollard, H. B. *J. Biol. Chem.* **1971**, *264*, 196.

c) van Tilbeurg, H.; Bhikhabhai, R.; Pettersson, L. G.; Claeyssens, M. *FEBS Lett.* **1984**, *169*, 215.

80. Hutson, D. H. *J. Chem. Soc. (C)* **1967**, 442.

81. Wang, L.-X.; Sakairi, N.; Kuzuhara, H. *J. Chem. Soc., Perkin Trans. I* **1990**, 1677.

82. Reed, L. A.; Goodman, L. *Carbohydr. Res.* **1981**, *94*, 91.

83. Blanc-Meusser, M.; Defaye, J.; Driguez, H. *J. Chem. Soc., Perkin Trans. I* **1982**, 15.

84. Blanc-Meusser, M.; Driguez, H. *J. Chem. Soc., Perkin Trans. I* **1988**, 3345.

85. Miljkovic, M.; Gligorijevic, M.; Glisin, D. *J. Org. Chem.* **1974**, *39*, 3223.

86. Vos, J. N.; van Boom, J. H.; van Boeckel, C. A. A.; Beetz T. *Carbohydr. Res.* **1984**, *3*, 117.

87. Wong, C.-H.; Krach, T.; Gautheron-le Narvor, C.; Ichikawa, Y.; Look, G. C.; Gaeta, F.; Thompson, T.; Nicolaou, K. C. *Tetrahedron Lett.* **1991**, *32*, 4867.

88. Wong, C.-H.; Ichikawa, Y.; Krach, T.; Gautheron-le Narvor, C.; Dumas, D. P.; Look, G. C *J. Am. Chem. Soc.* **1991**, *113*, 8137.

89. Yuasa, H.; Hindsgaul, O.; Palcic, M. M. *J. Am. Chem. Soc.* **1992**, *114*, 5891.

90. Hashimoto, H.; Kawanishi, M. *XVIth Int. Carbohydr. Symp.*, Paris, France, July 1992, Abstr. A132.

91. Mehta, S.; Pinto, B. M. *Tetrahedron Lett.* **1992**, *33*, 7675.

92. Hashimoto, H.; Izumi, M. *Tetrahedron Lett.* **1993**, *34*, 4949.

93. Spohr, U.; Bach, M.; Spiro, R. G. *Can. J. Chem.* **1993**, *71*, 1928.

94. Andrews, J. S.; Pinto, B. M. *Carbohydr. Res.* **1995**, *270*, 51.

95. Hamacher, K. *Carbohydr. Res.* **1984**, *128*, 291.

96. Kochetkov, N. N.; Bochkov, A. F. *Methods Carbohydr. Chem.* **1972**, 480.

97. Kochetkov, N. N.; Dimitriev, B. A.; Chizhof, O. S.; Klimov, E. M.; Malysheva, N. N.; Chernyak, Y. A.; Bayramova, N. E.; Torgov, V. I. *Carbohydr. Res.* **1974**, *33*, C5.

98. Srivastava, V. K.; Scheurch, C. *Tetrahedron Lett.* **1979**, *35*, 3269.

99. Pavliak, V.; Kovac, P. *Carbohydr. Res.* **1991**, *210*, 333.

100. Li, Z.-J.; Liu, P.-L.; Li, Z.-J.; Qui, D.-X.; Cai, M.-S. *Syn. Commun.* **1990**, *20*, 2169.

101. Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212, and references therein.

102. Hoffmann, M. G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1985**, 2403.

103. Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, 1249.

104. Hallgren, C. *J. Carbohydr. Chem.* **1992**, *11*, 527.

105. Defaye, J.; Guillot, J.-M. *Carbohydr. Res.* **1994**, *253*, 185.

106. Defaye, J.; Guillot, J.-M.; Biely, P.; Vrsanska, M. *Carbohydr. Res.* **1992**, *228*, 47.

107. Mehta, S.; Andrews, J. S.; Johnston, B. D.; Pinto, B. M. *J. Am. Chem. Soc.* **1994**, *116*, 1569.

108. For example: a) Hughes, N. A.; Wood, C. J. *J. Chem. Soc., Perkin Trans. I* **1986**, 695.

b) Al-Masoudi, N. A. L.; Hughes, N. A. *J. Chem. Soc., Perkin Trans. I* **1987**, 2061.

c) Hashimoto, H.; Yuasa, H. *Tetrahedron Lett.* **1988**, *29*, 1939.

109. 5-Thioglucose was synthesised by Dr. Blair D. Johnston, Simon Fraser University.

110. Pavliak, V.; Kovac, P. *Carbohydr. Res.* **1991**, *210*, 333.

111. Endo, T.; Oda, K.; Mukaiyama, T. *Chem. Lett.* **1974**, 443.

112. Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.* **1994**, *259*, 293.

113. a) Lichtenthaler, F. W.; Cuny, E.; Weprek, S. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 891.

b) Lichtenthaler, F. W.; Kläres, U.; Lergenmüller, M.; Schwidetzky, S. *Synthesis* **1992**, 179.

c) Lichtenthaler, F. W.; Kaji, E.; Weprek, S. *J. Org. Chem.* **1985**, *50*, 3505.

114. Renson, M. in *The Chemistry of Organic Selenium and Telurium Compounds,* Patai, S.; Rappoport, Z. (Eds.), John Wiley & Sons Ltd., **1986**, *1*, 399.

115. Mehta, S.; Andrews, J. A.; Johnston, B. D.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.*, in press.

116. Williams, J. M.; Richardson, A. C. *Tetrahedron* **1967**, *23*, 1369.

117. Reist, E. J.; Spencer, R. R.; Calkins, D. F.; Baker, B. R.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 2312.

118. Lépine, C.; Roy, C.; Delorme, D. *Tetrahedron Lett.* **1994**, *35*, 1843.

119. Kojibioside analogues were tested by M. M. Palcic and C. Scaman, Department of Chemistry, University of Alberta.

120. Mehta, S.; Jordan, K. L.; Weimar, T.; Kreis, U. C.; Batchelor, R. J.; Einstein, F. W. B.; Pinto, B. M. *Tetrahedron Asymm.* **1994**, *5*, 2367.

121. Weill, C. E.; Burch, R. J.; Van Dyck, J. W. *Cereal Chem.* **1954**, *31*, 150.

122. Maltoside analogues were tested by Dr. Birte Svensson, Department of Chemistry, Carlsberg Laboratory, Denmark.

123. Jagannadham, V.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 8465.

124. Pinto, B. M.; Leung, R. Y. N. in *The Anomeric Effect and Associated Stereoelectronic Effects*, Thatcher, G. R. J. (Ed.), American Chemical Society, Washington, DC. Chapter 8, **1993**.

125. Andrews, J. S.; Weimar, T.; Frandsen, T. P.; Svensson. B.; Pinto. B. M. *J. Am. Chem. Soc.* accepted for publication.

126. Isbell, H. L.; Frush, H. L. *J. Org. Chem.* **1958**, *23*, 1309.

127. Hodge, J. E.; Moy, B. F. *J. Org. Chem.* **1963**, *28*, 2784.

128. a) Linek, K.; Alföldi, J.; Defaye, J. *Carbohydr. Res.* **1987**, *164*, 195.

b) Linek, K.; Alföldi, J.; Defaye, J. *Carbohydr. Res.* **1993**, *247*, 329-335.

129. Isbell, H. L.; Frush, H. L. *Methods Carbohydr. Chem.* **1980**, *8*, 255.

130. a) Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1.

b) Kallin, E.; Lönn, H.; Norberg, T.; Elofsson, M. J. *Carbohydr. Chem.* **1992**, *8*, 597.

c) Urge, L.; Otvos, l., Jr.; Lang, E.; Wroblewski, K.; Laczko, I.; Hollosi, M. *Carbohydr. Res.* **1992**, *235*, 83.

131. Hodge, J. E.; Rist, C. E. *J. Am. Chem. Soc.* **1952**, *74*, 1494.

132. Legler, G.; Herrchen, M. *Carbohydr. Res.* **1983**, *116*, 95.

133. Schiff, H. *Ann.* **1870**, *154*, 30.

134. a) Kuhn, R.; Weygand, F. *Ber.* **1937**, *70*, 769.

b) Kuhn, R.; Ströbele, R. *Ber.* **1937**, *70*, 773.

c) Kuhn, R.; Birkofer, L. *Ber.* **1938**, *71*, 621.

d) Honeyman, J.; Tatchel, A. R. *J. Chem. Soc.* **1950**, 967.

e) Ellis, G. P.; Honeyman, J. *J. Chem. Soc.* **1952**, 1490.

135. a) Weygand, F. *Ber.* **1939**, *72*, 1663.

b) Weygand, F. *Ber.* **1940**, *73*, 1259.

b) Adachi, S. Carbohydr. Res. **1969**, *10*, 165.

136. Lai, H.-Y. L.; Axelrod, B. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 463.

137. Phillips, D. C. *Proc. Nat. Acad. Sci. U. S. A.* **1967**, *57*, 484.

138. Legler, G. *Biochimica et Biophysica Acta* **1978**, *524*, 94.

139. Loeffler, R. S. T.; Sinnott, M. L.; Whiting, M. C. *Chem. Commun.* **1974**, 984.

140. Legler, G.; Sinnott, M. L.; Withers, S. G. *J. Chem. Soc, Perkin Trans. II* **1980**, 1376.

141. Frush, H. L.; Isbell, H. S. *J. Res. Natl. Bur. Stand.* **1951**, *47*, 239.

142. Paulsen, H.; Pflughaupt, K.-W. in *The Carbohydrates* **1980**, V1B, 881.

143. Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer, New York, **1983**.; Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019.

144. Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2205.

145. a) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. I* **1990**, 747.

b) Perrin, C. L.; Armstrong, K. B. *J. Am. Chem. Soc.* **1993**, *115*, 6825.

c) Fabian, M. A.; Perrin, C. L.; Sinnott, M. L. *J. Am. Chem. Soc.* **1994**, *116*, 8398.

146. McCasland, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1966**, *32*, 1516.

147. Iwasa, T.; Yamamoto, H.; Shibata, M. *J. Antibiot.* **1970**, *32*, 595.

148. Schmidt, D. D.; Frommer, W.; Junge, B.; Müller, L.; Wingender, W Truscheit, E.; Schäfer, D. *Naturwissenschaften* **1977**, *64*, 535.

149. a) Murao, S.; Ohyama, K. *Agric. Biol. Chem.* **1975**, *39*, 2271.

b) Murao, S.; Ohyama, K.; Ogura, S. *Agric. Biol. Chem.* **1977**, *41*, 919.

c) Murao, S.; Ohyama, K. *Agric. Biol. Chem.* **1979**, *43*, 679.

150. Suami, T. in *Carbohydrates: Synthetic Methods and Applications in Medicinal Chemistry*, Eds. Ogura, H.; Hasegawa, A.; Suami,T., VCH, **1992**, 136.

151. Horii, S.; Kameda, Y.; Kawahara, K. *J. Antibiot.* **1972**, *25*, 48.

152. a) Omoto, S.; Itoh, J.; Ogino, H.; Iwamatsu, K.; Nishizawa, N.; Inouye, S. *J. Antibiot.* **1981**, *34*, 1429.

b) Junge, B.; Heiker, F.; Kurz, J.; Müller, L.; Schmidt, D. D.; Wunsche, C. *Carbohydr. Res.* **1984**, *128*, 235.

153. Fukuhara, K.; Murai, S.; Murao, S. *Agric. Biol. Chem.* **1982**, *46*, 1941.

154. Ogawa, S.; Shibata, Y. *J. Chem. Soc., Chem. Commun.* **1988**, 605.

155. Bock, K.; Meldal, M.; Refn, S. *Carbohydr. Res.* **1991**, *221*, 1.

156. Itoh, J.; Omoto, S.; Shomura, T.; Ogino, H.; Iwamatsu, K.; Inouye, S. *J. Antibiot.* **1981**, *34*, 1429.

157. a) Barker, S. A.; Murray, K.; Stacey, M. *Nature* **1961**, *191*, 142.

b) Micheel, P.; Heinemann, K. H.; Schwieger, K. H.; Frowein, A.; *Tetrahedron Lett.* **1965**, *42*, 3769.

158. a) Ratcliffe, A. J.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1990**, *112*, 5665.

b) Ratcliffe, A. J.; Konradsson, P.; Fraser-Reid, B. *Carbohydr. Res.* **1991**, *216*, 323.

159. van Es, T.; Whistler, R. L. *J. Org. Chem.* **1964**, *29*, 1087.

160. Paulsen, H.; Todt, K. *Adv. Carbohydr. Chem.* **1968**, *23*, 115.

161. Suzuki, K.; Hashimoto, H. *Tetrahedron Lett.* **1994**, *35*, 4119.

162. Hashimoto, H. private communication.

163. a) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *21*, 155.

b) Schimdt, R. R. *Chem., Int. Ed. Engl.* **1986**, *25*, 212.

c) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

164. Joseph, B.; Rollin, P. *J. Carbohydr. Chem.* **1993**, *12*, 719.

165. Reist, E. J.; Spencer, R. R.; Calkins, D. F.; Baker, B. R.; Goodman. L. *J. Org. Chem.* **1965**, *30*, 2312.

166. For example: a) Altena, J. H.; van den Ouweland, G. A. M.; Teunis, C. J.; Tjan, S. B. *Carbohydr. Res.* **1981**, *92*, 37.

b) Röper, H.; Röper, S.; Heyns, K.; Meyer, B. *Carbohydr. Res.* **1983**, *116*, 183.

c) Mossine, V. V.; Glinsky, G. V.; Feather, M. S. *Carbohydr. Res.* **1994**, *262*, 257.

167. a) Perlin, A. S.; Casu, B. *Tetrahedron Lett.* **1969**, 2921.

b) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, 1037.

c) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans.* II **1974**, 293.

d) Bock, K.; Pedersen, C. *Acta. Chem. Scand., Ser. B* **1975**, *29*, 258.

168. Wolfe, S.; Pinto, B. M.; Varma, V.; Leung, R. Y. N. *Can. J. Chem.* **1990**, *68*, 1051.

169 Tsuchiya, T.; Usui, T.; Kamiya, T.; Umezawa, S. *Carbohydr. Res.* **1979**, *77*, 267.

170. Kochetkov, N. K.; Byramova, N. E.; Tsvetkov, Yu. E.; Backinowsky, L. V. *Tetrahedron* **1985**, *41*, 3363.

171. Tsuzuki, K.; Nakajima, Y.; Watanabe, T.; Yanagiya, M.; Matsumoto, T. *Tetrahedron Lett.* **1978**, *11*, 989.

172. Eldin, S.; Digits, J. A.; Huang, S.-T.; Jencks, W. P. *J. Am. Chem. Soc.* **1995**, *117*, 6631.

173. Weill, C. E.; Burch, R. J.; Van Dyke, J. W. *Cereal Chem.* **1954**, *31*, 150.

174. Enzyme kinetic studies were performed by T. P. Frandsen and B. Svensson, Department of Chemistry, Carlsberg Laboratory, Denmark.

175. a) Fox, J.; Robyt, J. F. *Anal. Biochem.* **1991**, *195*, 93.

b) Palcic, M. M.; Skrydstrup, T.; Bock, K.; Le, N.; Lemieux, R. U. *Carbohydr. Res.* **1993**, *250*, 87.

c) Frandsen, T. P.; Dupont, C.; Lehmbeck, J.; Stoffer, B.; Sierks, M. R.; Honzatko, R. B.; Svensson, B. *Biochemistry* **1994**, *33*, 13808.

176. Aleshin. A. E.; Firsov, L. M.; Honzatko, R. B. *J. Biol. Chem.* **1994**, *269*, 15631.

177. Gronenborn, A. M.; Glore, G. M. *Biochem. Pharmacol.* **1990**, *40*, 115.

178. All NOE and transferred NOE NMR experiments were performed by Dr. Thomas Weimar at SFU.

179. All molecular mechanics calculations were performed by Dr. Uwe C. Kreis at SFU.

180. a) Kroeker, M.; Lindner, H. J. Pi SCF Molecular Mechanics PIMM91, University of Darmstadt **1991**.

b) Smith, A. E.; Lindner, H. J. *J. Comput. Aided. Mol. Des.* **1991**, *5*, 235.

c) Kroeker, M. *Ph.D. Thesis*, Technical University Darmstadt, **1993**.

181. For example: a) Ha, S. N.; Madsen, L. J.; Brady, J. W. *Biopolymers* **1988**, *27*, 1927.

b) Tran, V.; Buleon, A.; Imberty, A.; Pérez, S. *Biopolymers* **1989**, *28*, 679.

c) Bock , K.; Meldal, M.; Refn, S. *Carbohydr. Res.* **1991**, *221*, 1.

d) Weimar, T.; Meyer, B.; Peters, T. *J. Biomol. NMR* **1993**, *3*, 399.

e) Weimar, T.; Kreis, U. C.; Pinto, B. M. 3rd International Satellite Symposium on the Conformational Analysis of Carbohydrates and Protein/Carbohydrate Interactions, Abstr. No. 39, Val Morin, Québec, Canada. July1994.

182. Weimar, T.; Kreis, U. C.; Andrews, J. S.; Batchelor, R. J.; Einstein, F. W. B.; Pinto, B. M., manuscript in preparation.

183. a) Wolfe, S.; Pinto, B. M.; Varma, V.; Leung, R. Y. N. *Can. J. Chem.* **1989**, *68*, 1051.

b) Pinto, B. M.; Schlegel, H. B.; Wolfe, S. *Can. J. Chem.* **1987**, *65*, 1658.

184. Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933.

185. Weimar, T.; Andrews, J. S.; Svensson, B.; Pinto, B. M., Manuscript in preparation.

186. a) Albrand, J. P.; Birdsall, B.; Feeney, J.; Roberts, G. C. K.; Burgen, A. S. V. *Int. J. Biol. Macromol.* **1979**, *1*, 37.

b) Balaram, P.; Bothner-By, A. A.; Breslow, E. J. *J. Am. Chem. Soc.* **1972**, *94*, 4017.

c) Balaram, P.; Bothner-By, A. A.; Dadok, J. *J. Am. Chem. Soc.* **1972**, *94*, 4015.

d) Clore, G. M.; Gronenborn, A. M. *J. Magn. Reson.* **1982**, *48*, 402 and **1983**, *53*, 423.

187. Svensson, B.; Larsen, K.; Svendsen, I.; Boel, E. *Carlsberg. Res. Commun.* **1983**, *48*, 529.

188. Svensson, B.; Larsen, K.; Gunnarsson, A. *Eur. J. Biochem.* **1986**, 154, 497.

189. Hiromi, K.; Kawai, M.; Ono, S. *J. Biochem. (Tokyo)* **1966**, *59*, 476.

190. Dowd, M. K.; Zeng, J.; French, A. D.; Reilly, P. J. *Carbohydr. Res.* **1992**, *230*, 223.

191. Stoffer, B.; Aleshin. A. E.; Firsov, L. M.; Svensson, B.; Honzatko, R. B. *FEBS Lett.* **1995**, *358*, 57.

192. Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.

193. a) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301.

b) Bax, A.; Subramaniam, S. *J. Magn. Reson.* **1986**, *67*, 565.

c) Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.

194. Kessler, H.; Griesinger, C.; Lautz, J.; Müller, A.; Van Gunsteren, W. F.; Berendsen, H. L. *J. Am. Chem. Soc.* **1988**, *110*, 3393.

195. Leeflang, B. R.; Kroon-Batenburg, L. M. J. *J. Biomol. NMR* **1992**, *2*, 495.

196. a) Boelens, R.; Koning, T. M. G.; Van der Marel, G. A.; Van Boom, J. H.; Kaptein, R. J. *J. Magn. Reson.* **1989**, *82*, 290.

b) Borgias, B. A.; James, T. L. *Methods Enzymol.*; Academic Press: New York. 1989; vol. 176, pp 169.

197. Kay, L. E.; Scarsdale, J. N.; Hare, D. R.; Prestegard, J. H. *J. Magn. Reson.* **1986**, *68*, 515.

198. a) Bull, T. E. *J. Magn. Reson.* **1987**, *72*, 379.

b) Krishnan, V. V.; Kumar, A. *J. Magn. Reson.* **1991**, *92*, 293.

199. Bock, K.; Lonn, H.; Peters, T. *Carbohydr. Res.* **1990**, *198*, 375.

200. Peters, T.; Weimar, T. *J. Biomol. NMR* **1994**, *4*, 97.

201. Bauer, C.; Freeman, R.; Frenkiel, T.; Keeler, J.; Shaka, A. *J. Magn. Reson.* **1984**, *58*, 442.

202. Scherf, T.; Anglister, J. *Biophys. J.* **1993**, *64*, 754-761.

203. X-ray crystallographic studies were performed by Dr. Raymond Batchelor at Simon Fraser University.

204. Svensson, B.; Pedersen, T. G.; Svendsen, I.; Sakai, T.; Ottesen, *Carlsberg. Res. Commun.* **1983**, *48*, 529.

205. Leatherbarrow, R. J. *Enzfitter, a non-linear regression data analysis program for IBM PC*, Elsevier Science Publishers BV, Amsterdam, The Netherlands. 1987.

206. a) Bock, K.; Refn, S. *Acta. Chem. Scand.* **1989**, *43*, 373.

b) Bock, K.; Sigurskjold, B. W. *Eur. J. Biochem.* **1989**, *178*, 711.