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ABSTRACT

Glycomimetics of α -D-mannose were synthesized by derivatizing it at the 1-, 2-, and 6-positions. The synthesis of a series of known aryl α -D-mannopyranosides and novel aryl thio- α -D-mannopyranosides using Lewis acid catalysis is described. The method affords the desired products in very good yields (61-76%). Synthesis of the corresponding, novel arylsulfinyl α -D-mannopyranosides and arylsulfonyl α -D-mannopyranosides by conventional *m*CPBA oxidation is also described. The inhibitory effect by the C-1 modified compounds mentioned above on the binding of *concanavalin A* to yeast mannan was evaluated by ELLA and is reported. Aryl thio- α -D-mannopyranosides proved to be better inhibitors than the analogous aryl α -D-mannopyranosides. Arylsulfinyl α -D-mannopyranosides and arylsulfonyl α -D-mannopyranosides are better inhibitors than the corresponding aryl α -D-mannopyranosides but poorer inhibitors than the corresponding sulfides, except for *p*-methoxyphenylsulfonyl 1-thio- α -D-mannopyranoside which proved to be the most potent inhibitor of all the above tested compounds. Derivatization of methyl α -D-mannopyranoside to methyl 6-amino-6-deoxy- α -D-mannopyranoside, methyl 2-*O*-alkyl- α -D-mannopyranosides (alkyl = Me and Bn) and methyl 6-amino-2-*O*-alkyl-6-deoxy - α -D-mannopyranosides (alkyl = Et and Bn) is also described. The inhibitory effect of the first and latter series on the binding of *Concanavalin A* to yeast mannan was evaluated by ELLA and is reported. Modification of α -D-mannose at the 2- and 6-positions decreased the inhibitory effect of the binding of *Concanavalin A* to yeast mannan.

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List of Abbreviations

ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
Ac	acetyl
BF ₃ .Et ₂ O	borontrifluoride etherate
br	broad
Br ₂	bromine
BSA	bovine serum albumin
CH ₂ Cl ₂	dichloromethan
Con A	concanavalin A
COSY	H-H correlation spectroscopy
d	doublet
dd	doublet of doublet
DEAD	diethylazodicarboxylate
decomp.	decomposed
DEPT	distortionless Enhanced Polarization Transfer
DMF	dimethylformamide
ELLA	enzyme linked lectin assay
equiv.	equivalents
Et ₃ N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FAB-MS	fast atom bombardment ionization mass spectrometry
Hex	hexanes

HRMS	High Resolution Mass Spectrometry
hrs	hours
Leu	leucine
m	multiplet
m/z	mass-to-charge ratio
Man	mannose
mCPBA	meta-chloroperbenzoic acid
Me	methyl
mM	millimolar
N₂	nitrogen
NMR	nuclear magnetic resonance
O.D.	optical density
Ph	phenyl
Ph₃P	triphenyl phosphine
ppm	parts per million
Quant.	quantitative
s	singlet
SD	standard deviation
t	triplet
^tBu	tert-butyl
TFA	trifluoroacetic acid
TLC	thin layer chromatography

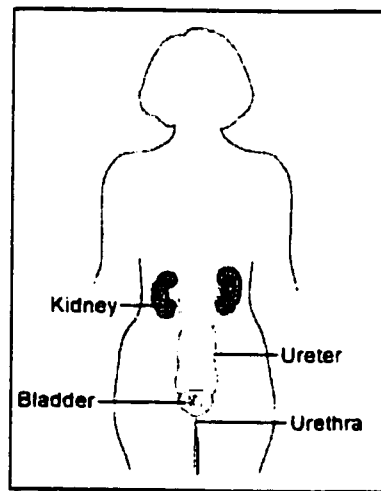
TPP	triphenyl phosphine
Tyr	tyrosine
UTI	urinary tract infection

CHAPTER I

INTRODUCTION

1.1 Urinary Tract Infection

Urinary tract infection (UTI) is among the most common diseases that occur worldwide. Patients of UTI include both genders of different ages, but 90% of these patients are women.¹ It accounts for 8 million doctor visits per year, in the United States alone. Its symptoms may include burning on urination, urgency and frequency, appearance of smell or color in urine, lower abdominal pain and fever.²



The Urinary Tract

Figure 1. 1. 1 Constituents of the urinary tract

The urinary tract consists of the kidneys, the ureters, the bladder and the urethra. Infection can occur in any constituent of the urinary tract, depending on where the bacteria colonize. Generally, urine is sterile containing only salts, water, and waste products, but no bacteria. However, urine is an excellent medium for bacterial growth. Bacteria can enter the urinary tract either from above (from the blood stream to the kidneys and then down the urinary tract) or from below (from the urethra and upwards). Hence, there are two broad types of UTI: Upper UTI, which happens when either the

kidneys or the ureters get infected, and Lower UTI, which happens when the bladder or the urethra gets infected. Bladder infection, however, is the most common type of UTI.²

UTI is usually treated with antibiotics such as ampicillin and amoxicillin. The choice of the antibiotic and the length of treatment depend on the severity of the case. UTI can be cured with 1-2 day treatment, provided the infection is not complicated, but doctors often recommend that patients take antibiotics for a week or two.²

What causes UTI?

E. coli is by far the most common pathogen to cause UTI. It accounts for almost 80% of UTI cases. Figure 1.1.2 illustrates the different causes of UTI.³ The strains of *E. coli* that cause UTI differ from others in the fact that they can adhere to the cell surface. Adhesion is the first step in bacterial infection. It is necessary to prevent the bacteria from being flushed away by the normal cleansing mechanisms that operate on mucosal surfaces, such as urinary flow. It is also important to allow the bacteria to colonize the epithelia and initiate infection.⁴ Adhesion is mediated by hair-like surface appendages, commonly known as fimbriae. Fimbriae are lectins (a class of proteins) that consist of two distinct assemblies: a thick cylindrical, polymeric rod, joined to a thin, helical, carbohydrate-specific binding tip.⁵

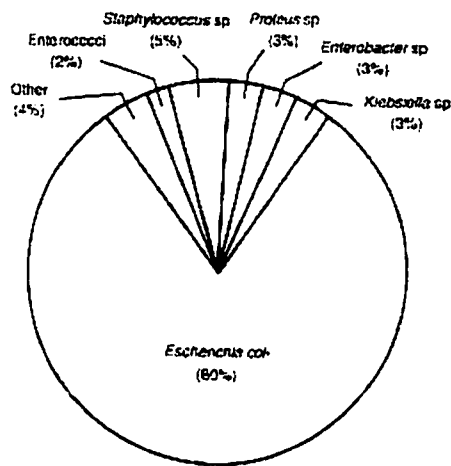


Figure 1.1.2 Common Causes of UTI

Strains of *E. coli* that are most commonly responsible for UTI are Types 1, P and S, each specific to a certain sugar. Type 1 fimbriae are mannose-specific and are responsible for bladder infection.⁴ The binding site on the fimbrial unit is located at the tip of each fimbriae and is believed to be in the form of a pocket.⁶

1.2 Mannopyranosides as potential preventative drugs for UTI

In 1979, Sharon and Ofek conducted a study, which demonstrated that the presence of sugars that specifically bind to the bacterial-surface lectins markedly decreases the incidence of urinary tract infections.⁷ The group injected Type 1 fimbriated *E. coli* in the urinary bladder of mice in the presence of Me α -mannoside and in its absence. They found that the presence of the mannopyranoside compound decreased the incidence of bacterial infection by a factor of 3, compared to the control group. Figure 1.2.1 illustrates the finding. In addition, the group investigated the inhibitory effect of a series of aromatic α -mannopyranosides and found that such compounds are even better

inhibitors than the aliphatic Me α -mannopyranoside.⁸ For example, phenyl α -mannopyranoside was found to be 40 times better inhibitor than Me α -mannopyranoside, as can be seen in table 1.2.1. Based on these results, the group concluded that the rate of infection is decreased due to the binding of the mannopyranoside compounds to the surface-lectins of the bacteria and, consequently, inhibiting their adherence to epithelial cells and preventing infection. The better binding properties of the aromatic mannopyranosides was explained by proposing that there exists a hydrophobic-binding site adjacent to the sugar-binding site, which maximizes the interactions.

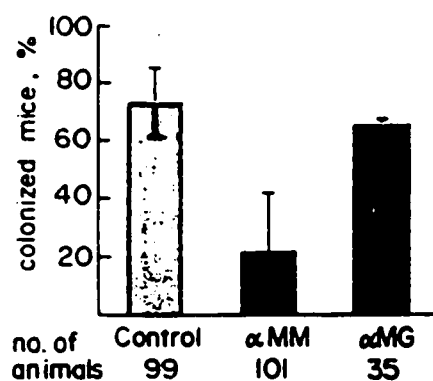


Figure 1.2.1 Sugar inhibitors of type 1 fimbriated *E. coli*.

α MM, methyl alpha-mannoside, α MG, methyl alpha-glucoside

The results from these studies demonstrate the great potential of carbohydrates in preventing infections caused by bacteria expressing surface lectins. It is therefore possible to develop a new class of therapeutics, antiadhesive agents, based on carbohydrates. This is much needed especially in an age where antibiotic resistance is on the rise.

Table 1.2.1 Inhibition by aryl α -mannopyranosides of yeast agglutination by *E. coli* O25

Aglycon	Relative Inhibitory Activity \pm SD ^a
Methyl	1
Phenyl	40 \pm 7 ^b
p-Nitrophenyl	69 \pm 4 ^b
p-Bromophenyl	72
p-Ethylphenyl	77
p-Methoxyphenyl	140
p-Ethoxyphenyl	154
p-Nitro-o-chlorophenyl	717
4-Methylumbelliferyl	600

^a The inhibitory activities of the various sugars are relatively to that of the concentration (0.43 mM) of Methyl mannopyranoside causing 50% inhibition, which was arbitrarily set as 1.

^b Average of three sets of experiments.

1.3 Lectins and *Concanavalin A*

Lectins are a class of proteins that bind specifically and reversibly to carbohydrates. They are found in most organisms ranging from viruses and bacteria to plants and animals. Typically, the lectins and the complementary carbohydrates are located on the surfaces of opposing cells, which could be of the same type or of different types. The affinity of lectins to monosaccharides is often weak, with an association constant in the millimolar range, yet it is often highly selective. Many lectins, however, tolerate variations at C-2 of the pyranose ring. For example those of the mannose specificity group may bind its epimeric glucose as well.⁶

Concanavalin A (Con A), which is isolated from Jack bean (*canavalia ensiformis*), was the first lectin to be recognized.⁹ It is the prototype of the legume family, which belongs to the class simple lectins. It is fairly specific in its binding, but not as specific as the lectins constituting the fimbriae. It generally binds to saccharides containing α -D-mannose and α -D-glucose, but it might also recognize oligosaccharide sequences that lack these units. Under physiological pH, Con A exists as a tetramer, but it can also exist as a monomer and a dimer depending on the pH and temperature conditions. Each subunit, which consists of 237 residues, has a carbohydrate-binding site, a transition-metal binding site, predominantly Mn^{2+} , and a metal binding site, preferentially Ca^{2+} .¹⁰ Evidence suggests that both metal sites have to be occupied before carbohydrate binding can occur.¹¹

Con A, and many other lectins, are often used to study the molecular basis for carbohydrate-protein interactions. Lectins are ideal model systems for such studies, especially since they are easily purified and their interactions with various sugars have been very well documented by microcalorimetry, X-ray crystallography and NMR.¹²

1.4 Mannopyranosides- *Concanavalin A* interactions parallel mannopyranosides- *E. coli* interactions.

Loontjens *et al.* studied the binding of a series of *para*-substituted phenyl mannopyranosides to Con A.¹³ Among the mannopyranosides investigated were those listed in table 1.4.1. As can be seen from the table, aryl mannopyranosides with *para*-substituted electron donating groups bind better to Con A than aryl mannopyranosides

with *para*-substituted electron withdrawing groups. *p*-alkoxy derivatives, for example, bind 4 times better than the *p*-nitro derivative. It was, hence, suggested that there exists an electron-deficient site adjacent to the carbohydrate-binding site, which enhances the binding of electron-donating *p*-substituted phenyl mannopyranosides.

Table 1.4.1 Relative Inhibition of *p*-substituted phenyl α -D-Mannopyranosides to the binding of Con A to yeast Mannan

Para-substituent	IC ₅₀ X 10 ⁻⁵ M
Methoxy	3.498
Ethoxy	2.841
Methyl	5.618
Ethyl	4.552
Bromo	5.953
Nitro	12.05

This binding trend of mannopyranosides to Con A parallels the binding of mannopyranosides to bacterial-surface lectins (See table 1.2.1). This, furthermore, verifies the use of Con A as a model lectin to study the inhibitory effect of mannopyranosides on type 1 fimbriated *E. coli*. The results obtained from studying the interactions between mannopyranosides and Con A, can be used to predict the interactions between mannopyranosides and type 1 fimbriated *E. coli*.

1.5 *co*-Crystal Structures of Con A and mannopyranosides

Mannopyranoside- Con A interactions have been very well established by X-ray crystallography.^{12,14,15} The first *co*-crystal structure to provide detailed information about

the interactions of legume lectins with monosaccharides was that of Con A complexed to Me α -D-mannopyranoside, published in 1989.¹⁴ Mannopyranosides bind to Con A by a network of hydrogen bonds and electrostatic interactions (Figure 1.5.2). Virtually all hydroxyl groups on the sugar are involved in hydrogen bonding, either cooperative bonding (where the OH group acts as both H-bond donor and acceptor) or bidentate bonding (where two adjacent hydroxyl groups H-bond to the same residue on the lectin).

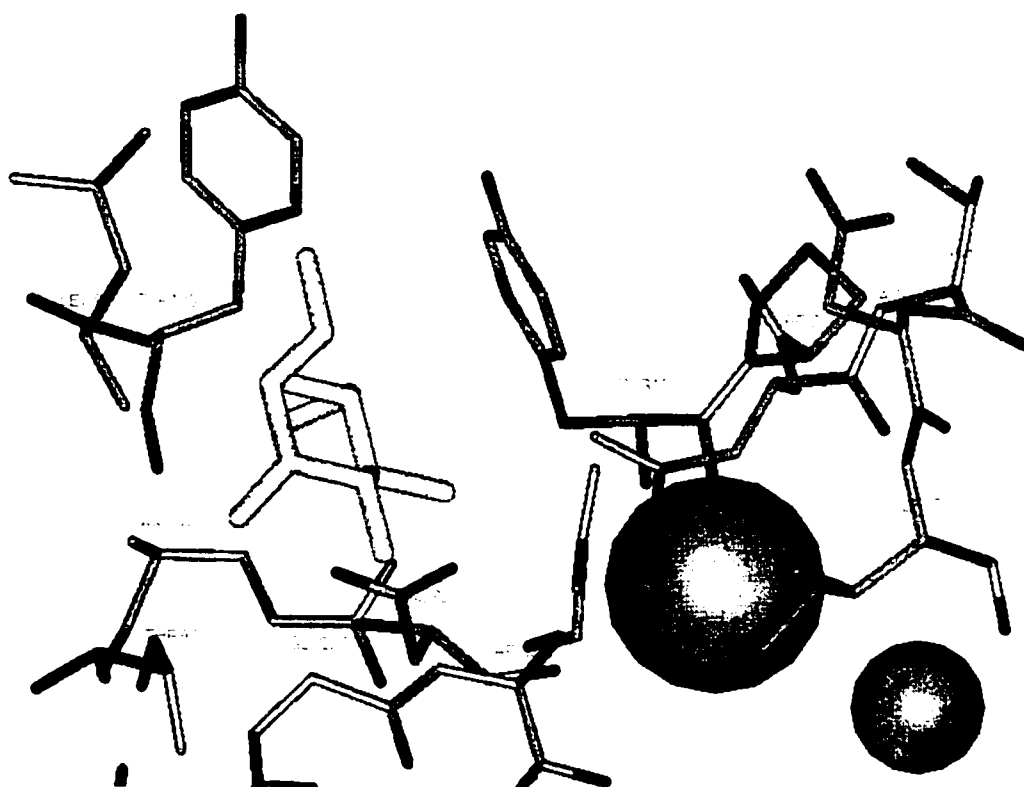


Figure 1.5.1 Crystal structure of methyl α -D-mannopyranoside in the binding site of *Concanavalin A*

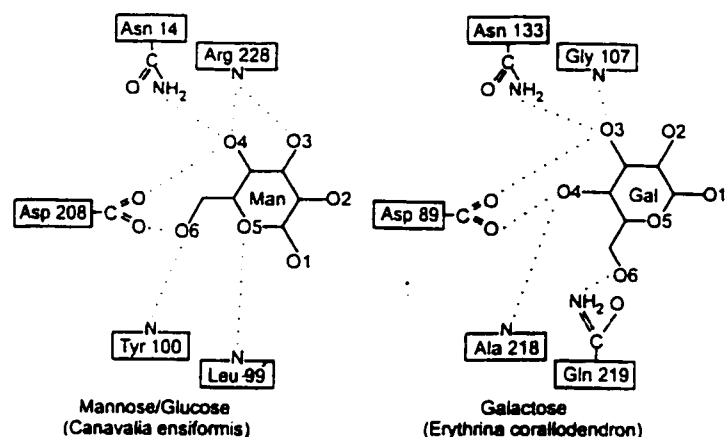


Figure 1.5. 2 Schematic representation of the binding of mannose to Con A

The most common scheme of H-bonding is as follows: $(\text{NH})_n \rightarrow \text{OH} \rightarrow \text{O}=\text{C}$, where NH is a hydrogen bond donor and $n=1$ or 2 , OH is a sugar, non-anomeric hydroxyl group which can be both donor and acceptor, and $\text{O}=\text{C}$ is a hydrogen bond acceptor and can be either a carboxylate or a carbonyl group.⁵ Few essential interactions are responsible for holding the sugar moiety in the combining site of the lectin. *a)* An aspartic acid (Asp208) H-bonds to hydroxyls 4 and 6 on the sugar and is bridged by a water molecule to Ca^{2+} in the metal-binding site. Demetallization of the lectin has been shown to deprive it from its sugar-binding ability, which explains the metal ion requirement for sugar binding.⁶ Asp208 is properly oriented to H-bond by a preceding, rare, *cis*-peptide. *b)* An asparagine (Asn14) H-bonds to hydroxyl 4. Asn14, also interacts directly with the calcium ion and the backbone NH of Arg228. *c)* Arg228 H-bonds to both hydroxyls 3 and 4. *d)* A Tyrosine residue (Tyr100) H-bonds to hydroxyl 6. *e)* The backbone peptide of a Leucine residue (Leu99) H-bonds to the sugar-ring oxygen. Figure 1.5.3 illustrates the interactions of Me Mannopyranoside with Con A.⁶

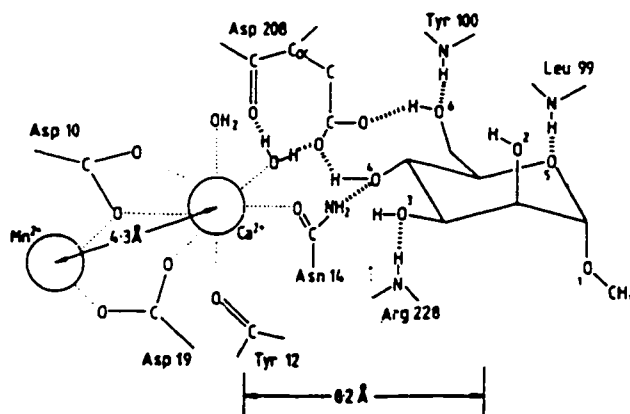


Figure 1.5.3 Methyl α -mannoside in the combining site of Con A

It is known that the presence of an aromatic residue at the anomeric position significantly enhances the binding of mannopyranosides to Con A. Hydrophobic mannopyranosides bind 10- to 50-fold more strongly than non-hydrophobic ones.¹² A close examination of the interactions of *p*-nitrophenyl mannopyranoside and Con A reveals that there exists a hydrophobic binding site adjacent to the carbohydrate-binding site. Figures 1.5.4 and 1.5.5 show the interactions of Con A with *p*-nitrophenyl α -D-mannopyranoside. The nitrophenyl group hydrogen bonds to the side-chain hydroxyl of Tyr100 which facilitates hydrophobic interactions between the aromatic moiety and the side chain atoms of Tyr12, Leu99 and Tyr100. As a result, the aglycon moiety is bound in a hydrophobic cavity formed by the aforementioned three residues. Tyr12 stacks against one face of the nitrophenyl ring forming π - π interactions, as does the phenyl ring of Tyr100, which stacks against the same face of the nitrophenyl ring. This supports the earlier prediction of Loontjens of a hydrophobic-binding site adjacent to the carbohydrate-binding site.

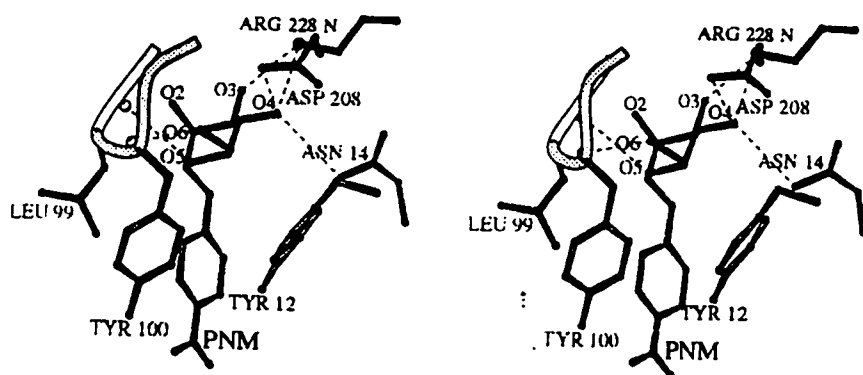


Figure 1.5.4 Stereoview of the hydrophobic binding site of *Concanavalin A* adjacent to the saccharide binding site

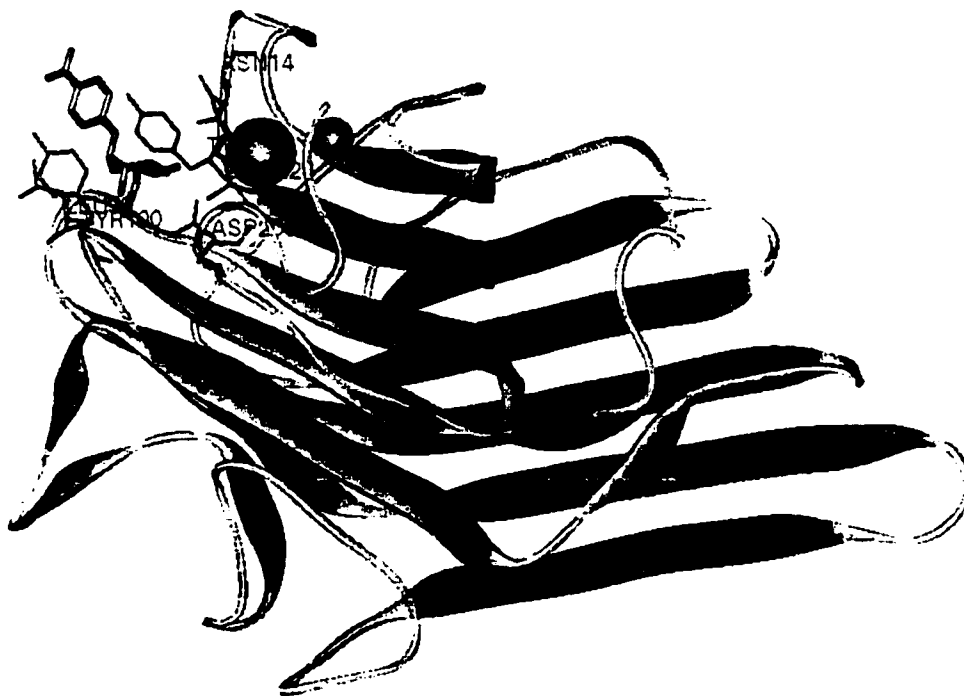


Figure 1.5.5 Crystal structure of *p*-nitrophenyl α -D-mannopyranoside in the binding site of *Concanavalin A*

1.6 Focus and Strategy

Our goal for this project was to develop an effective therapeutic for the prevention of urinary tract infections. An effective therapeutic would be one where the lectin-mannopyranoside interactions are maximized. In our rational approach to design an effective drug, we carefully examined the co-crystal structure of *p*-nitrophenyl mannoside and Con A and decided that we can modify mannose at the C1, C2 and C6 positions. Figure 1.6.1 summarizes the proposed modifications to *O*-aryl mannoside.

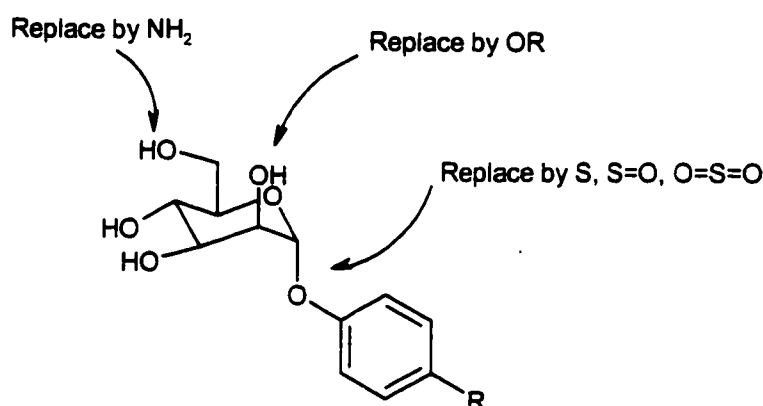


Figure 1.6.1 Proposed modifications of mannose.

Modification at C1

Based on the studies conducted by Sharon *et al.*, we decided that it is essential to retain the aromatic residue at the anomeric position, but were curious to see the effect of replacing the oxygen by sulfur to yield *S*-mannopyranosides. An advantage doing so is that *S*-mannopyranosides resist hydrolysis by mannosyltransferase, a common problem encountered with *O*-mannopyranosides, which hydrolyze readily once in the body. In addition, replacing oxygen by sulfur would have electronic and geometrical

consequences. Sulfur is a bigger atom with its lone pairs less tightly bound than oxygen. As a result the bond distances around the anomeric sulfur would be expected to be longer than in *O*-mannopyranosides. C1-S in *t*-butyl dimethyl silyl protected α -phenyl thiomannopyranoside¹⁶, for example, is 1.803Å compared to 1.437Å for C1-O in *p*-nitrophenyl mannopyranoside.¹⁷ The bond angle around the anomeric sulfur, on the other hand, would be expected to be smaller, 103.529° in *t*-butyl dimethyl silyl protected α -phenyl thiomannopyranoside compared to 119.706° in *p*-nitrophenyl mannopyranoside. This is easily explained by VSEPR theory. The lone pairs on the sulfur atom occupy a larger volume than in the case of oxygen, pushing the bonding-pair closer together and resulting in a smaller angle. Geometrical changes around the anomeric position will affect the position of the aglycon moiety with respect to the hydrophobic-binding site on the lectin, and in turn will affect the binding properties of the compounds.

Oxidation of *S*-mannopyranosides to the corresponding sulfoxides and sulfones will also effect electronic and geometrical changes around the anomeric sulfur, as well as changes in the polarity of the resulting compounds. The sulfoxide and sulfone derivatives would have higher electron density around the anomeric position due to the presence of oxygen atoms. The bond distances around the anomeric sulfur would be expected to lengthen for the sulfoxides compared to the sulfides, then shorten for the sulfones. This can be demonstrated in the following bond distances (Å) for diphenyl derivatives:¹⁸



Bond angles would also be affected for the sulfoxide and sulfone derivatives. Experimental data collected for a number of analogous sulfides, sulfoxides and sulfones

show that the C-S-C bond angle decreases for sulfoxides compared to sulfones, but then increases for sulfides compared to sulfoxides.¹⁹ While the decrease in the C-S-C bond angle for sulfoxides compared to sulfones is consistent with VSEPR considerations, it would have been expected that this bond angle should further decrease for sulfides. This anomaly was explained by proposing that not only should the bond angle variation be considered, but also the angle of the lone pairs.¹⁸

The polarity of the sulfoxide derivatives is expected to be higher than the corresponding sulfones, which have higher polarity than the parent sulfides. The hydrogen bonding properties of the three derivatives is expected to be different. Sulfoxide derivatives are known to be strong hydrogen bond acceptors compared to the corresponding sulfides and sulfones. This in turn should influence the binding abilities of the different derivatives to the lectin.

Modification at C2

Toone *et al.* conducted a study in which they measured the binding constants for a series of C2-alkylated Me-mannopyranosides.²⁰ The group suggested that since OH-2 points towards a threonine residue, which creates a pocket, an alkyl group on C2 could productively occupy the pocket and enhance the binding. The group found that C2-alkylated mannosides exhibit better binding to Con A than the unsubstituted one. In particular, methyl 2-*O*-ethyl mannoside binds significantly better to Con A (an increase of 1 kcal/mol in the binding energy). This led us to modify the 2-position by alkylating it with different alkyl groups.

Modification at C6

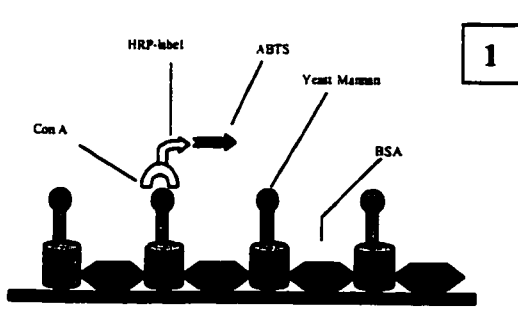
The hydroxyl group on C6 hydrogen bonds to the carboxylate of aspartic residue 208. We thought that replacing the hydroxyl on C6 with an amine might strengthen the sugar-Con A interactions. A primary amine has a pKa value of 9.24, and hence would be expected to protonate under physiological pH to form ammonium cation. As a result, the interaction of the amine residue on C6 with the carboxylate of Asp208 will become ionic interaction, which is much stronger than H-bond interactions. Hydrogen bonds for neutral molecules have bond energies in the range of 10-65 kJmol⁻¹.²¹ This value can rise up to 190 kJmol⁻¹ when one of the hydrogen-bond pair is charged.²¹ Ionic interactions, on the other hand, have bond energies in the order 400-500 kJmol⁻¹.²² Based on this, we expect that replacing hydroxyl-6 with an amine residue will enhance the carbohydrate-protein binding.

1.7 Enzyme Linked Lectin Assay

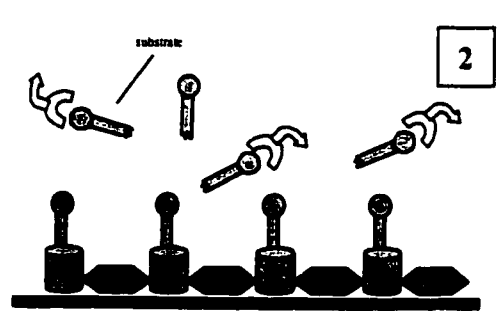
Enzyme Linked Lectin Assay (ELLA) is a quantitative competitive assay that measures the minimal concentration of a substrate required to cause 50% inhibition in binding of the lectin to a standard. The choice of the lectin and the standard is dependent on the substrate being tested. In case of mannopyranosides, Con A is the choice for lectin and yeast mannan is the choice for the standard.

The assay is performed in a 96-well plate, coated with yeast mannan. Any bare spots on the plate that are not coated by yeast mannan are blocked with an inactive

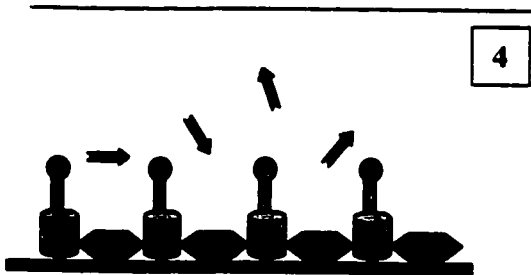
material, BSA. The substrate and the lectin are incubated together for 1 hr in a separate plate, then transferred into the standard-coated plate. Con A used in ELLA is typically labeled with horseradish peroxidase (HRP) to facilitate its detection. The three-mix is then incubated for another hour and then washed with a phosphate buffer solution containing Tween (PBS/Tween). If the substrate is a better binder to the lectin, the couple stays bound together and is washed off the plate with PBS. Once the plate is washed, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), which recognizes the HRP label, is added. If the HRP-labeled Con A was washed off the plate along with the substrate, no color change in the ABTS is observed. If HRP-labeled Con A remained bound to the yeast mannan, ABTS changes color from colorless to green. Optical density is then measured and is used to determine the concentration of substrate required to cause 50% inhibition. Figure 1.7.1 illustrates the principle of the ELLA test.



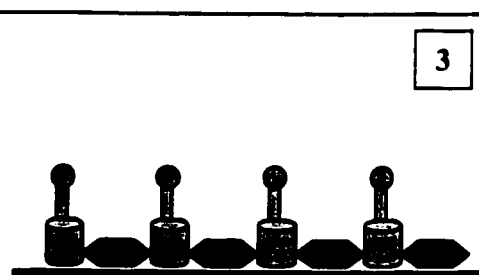
In the absence of a competitor substrate, HRP-Con A binds to Yeast mannan and is recognized by ABTS.



In the presence of a better-binding substrate, Con A binds to the substrate.



When ABTS is added, no color change is observed, since no HRP-labeled Con A to bind to.



When the plate is washed, HRP-labeled Con A is washed off along with the bound substrate.

Figure 1.7.1 Schematic representation of the principal of the ELLA test.

CHAPTER II

SYNTHESIS AND INHIBITORY EFFECTS OF

ARYL *O*-MANNOPYRANOSIDES, ARYL *S*-

MANNOPYRANOSIDES AND THE

CORRESPONDING SULFOXIDES AND

SULFONES

2.1 Introduction

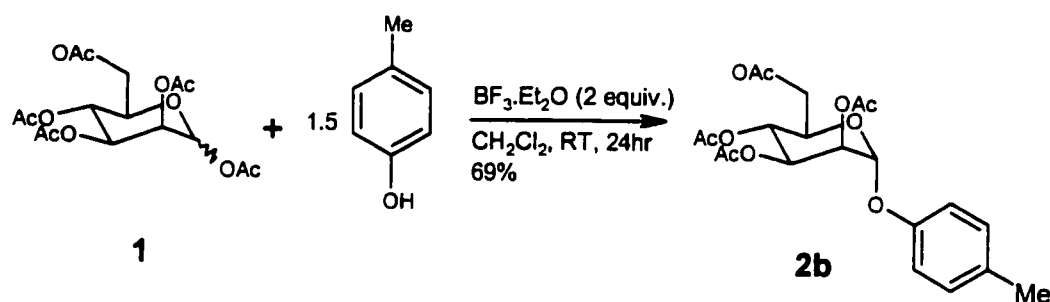
As was mentioned in chapter I, Sharon *et al.* reported that aryl α -D-mannopyranosides are powerful inhibitors for the adhesion of type 1 *E. coli* to the intestinal epithelial cells. Since *O*-glycosides are easily hydrolyzed once in the body, there is growing interest in developing new types of glycosides such as *C*-, and *S*-glycosides. These types of compounds have been shown to resist hydrolysis and maintain the integrity of the glycoside. As a result, we focused our interest on *S*-mannopyranosides and were curious to determine how aryl α -thiomannopyranosides and their corresponding sulfoxides and sulfones compare in bioactivity to *O*-mannopyranosides. To determine this we re-synthesized a series of *O*-mannopyranosides; synthesized the analogous, novel *S*-mannopyranosides and their corresponding sulfoxides and sulfones.

This chapter will focus on the synthetic methods used to obtain these compounds and on their biological activity determined by ELLA.

2.2 Synthesis of Aryl α -*O* and *S*-Mannopyranosides

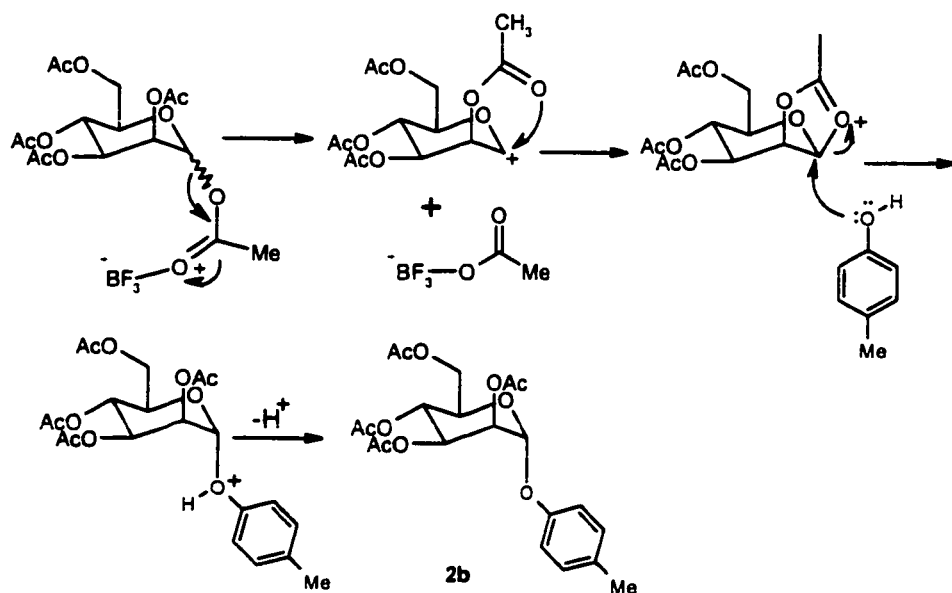
De Bruyne and Vervoort previously reported the synthesis of a series of aryl α -D-mannopyranosides by the Helfrich method in which α -D-mannose pentaacetate (1) is fused with the appropriate phenol in the presence of zinc chloride.²³ This method requires the melting of the reactants and heating under diminished pressure at high temperature to yield the desired products in poor yields (26-53%). In our attempt to remake these compounds we sought an easier, more efficient method. Three different methods were investigated, using *p*-cresol as a model phenol.

First, we investigated the direct coupling of α -D-mannose pentaacetate (**1**) with *p*-cresol in dichloromethane in the presence of a Lewis acid catalyst, $\text{BF}_3 \cdot \text{Et}_2\text{O}$.²⁴ The reaction proceeded at room temperature under N_2 atmosphere over a period of 24 hrs. Aqueous work up of the reaction mixture followed by crystallization from ethanol yielded the desired *p*-methylphenyl α -mannopyranoside (**2b**) in 77% yield, compared to 53% by De Bruyne *et al.* Scheme 2.2.1 illustrates this synthetic method.



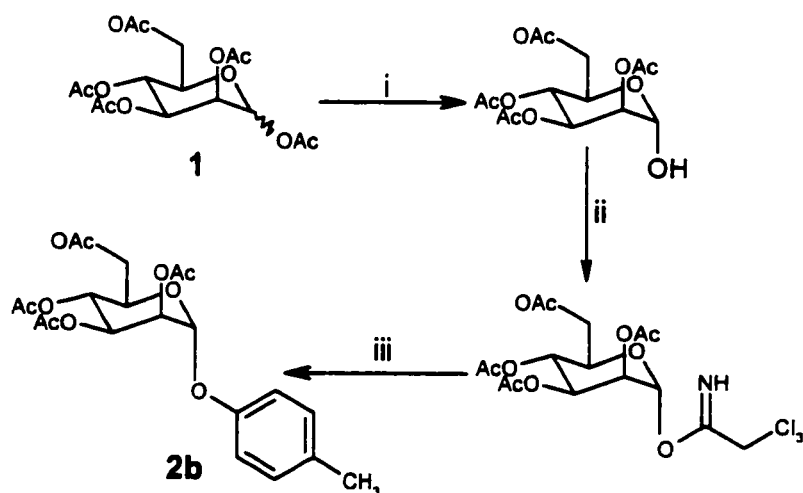
Scheme 2.2.1 Synthesis of *O*-glycosides using Lewis acid catalysis

Boron trifluoride etherate catalyzes the reaction and facilitates the departure of the anomeric acetate, allowing the nucleophilic phenol to attack. Scheme 2.2.2 depicts the reaction mechanism.



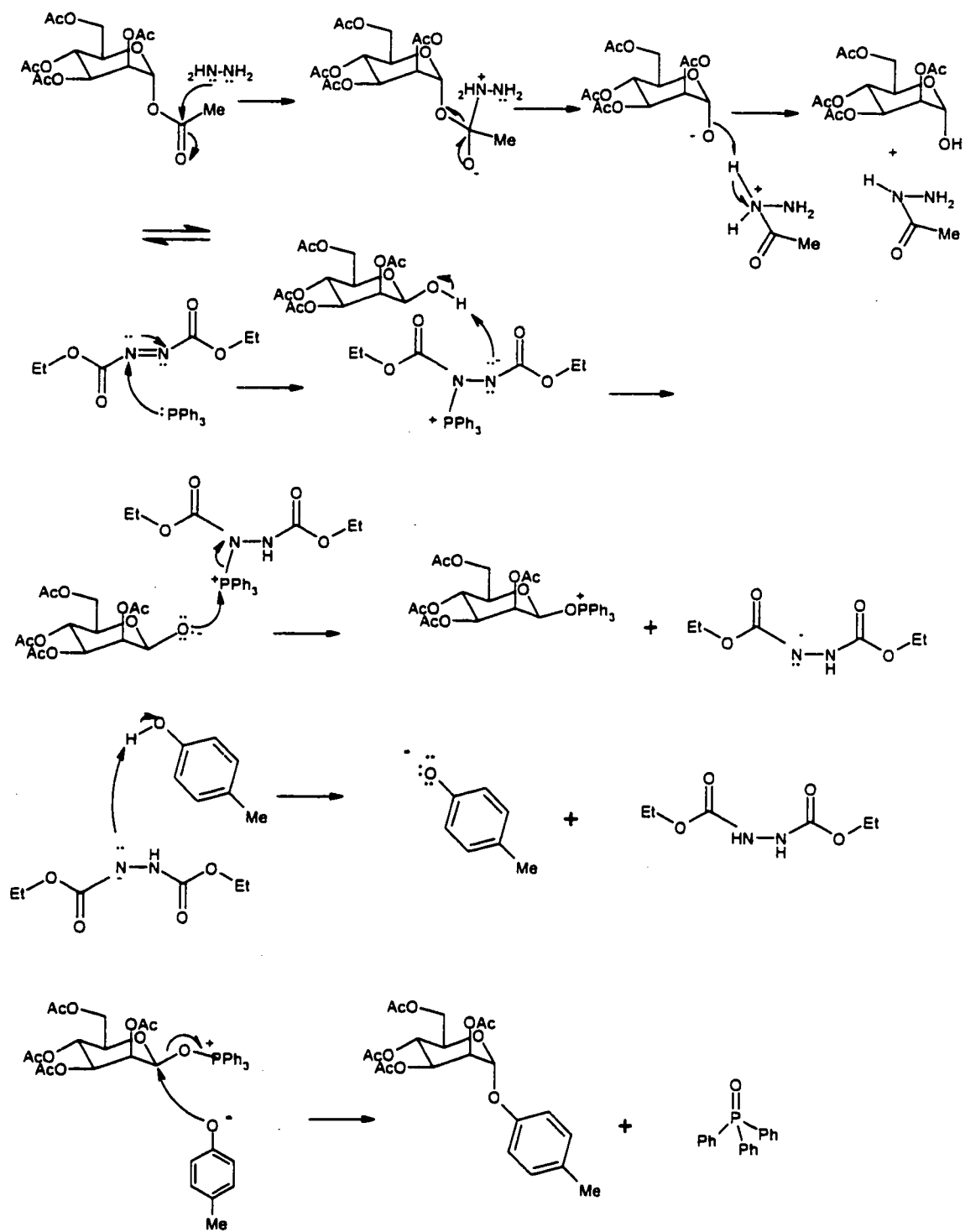
Scheme 2.2.2 Mechanism for the synthesis of 2b using $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as a catalyst

Second, the classical Schmidt reaction was investigated. This was a three-step reaction that proceeded via an anomeric trichloroacetimidate²⁵ derivative and required chromatographic purification to give an overall yield of 31%. Reaction steps and the corresponding yields are illustrated in scheme 2.2.3.



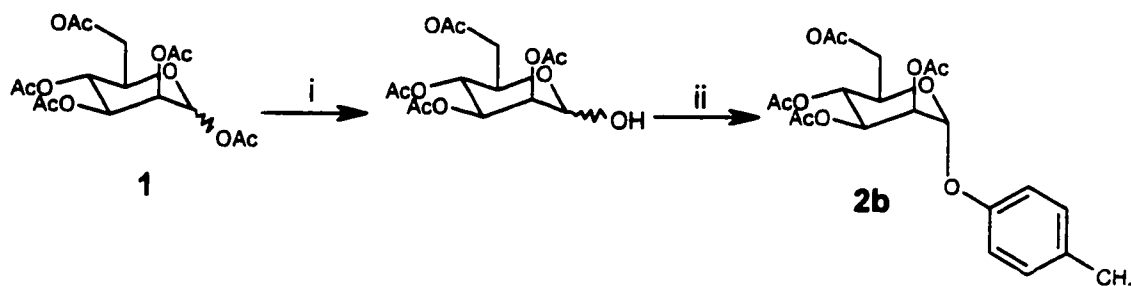
Scheme 2.2.3 Schmidt reaction for the synthesis of aryl *O*-mannopyranosides Reagents and conditions: i) AcOH/HNH_2 (1.3 equiv.), DMF, N_2 , 50°C , 10 min, 65%; ii) NCCl_3 , DBU, CH_2Cl_2 , N_2 , 0°C , 67%; iii) *p*-resol (1.1 equiv.), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, -40 – $(-50)^\circ\text{C}$, N_2 , 76%

Finally, preparing the desired *p*-methylphenyl α -D-mannopyranoside (**2b**) was achieved via the Mitsunobu reaction. This was a two-step reaction that required selective deprotection of the anomeric position of D-mannose pentaacetate (**1**) then coupling the product with *p*-cresol in the presence of TPP and DEAD at 0 °C, under N₂ atmosphere for 24 hrs. The reaction mechanism is illustrated in scheme 2.2.4.



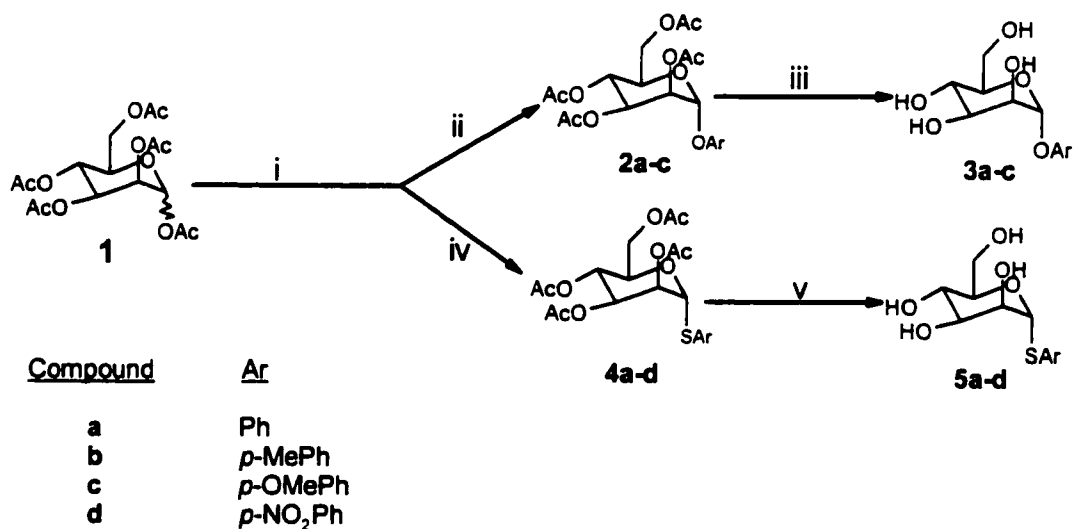
Scheme 2.2.4 Mechanism of the Mitsunobu Reaction

Chromatographic purification of the crude mixture yielded the desired product in 16%, overall yield. Scheme 2.2.5 illustrates the reaction.



Scheme 2.2.5 Mitsunobu reaction for the synthesis of aryl α -*O*-mannopyranosides. Reagents and conditions: i) AcOH/ HNH_2 (1.3 equiv.), DMF, N_2 , 50 °C, 10 min, 65%; *p*-cresol (1.2 equiv.), TPP (1.4 equiv.), DEAD (1.6 equiv.), CH_2Cl_2 , 0 °C, 20%

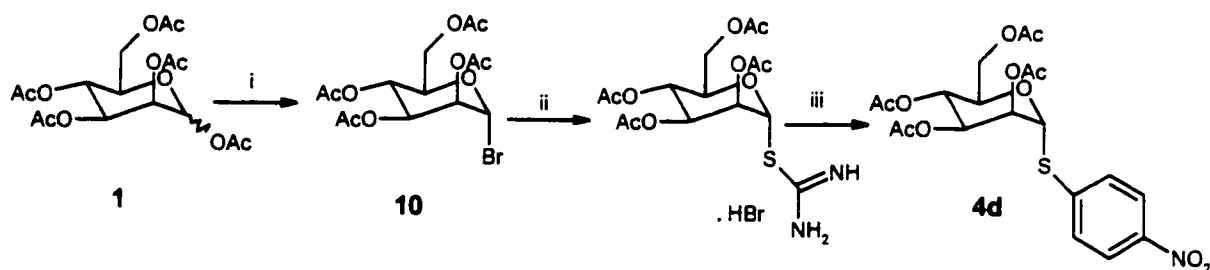
Based on the results obtained from these experiments, the first method was the one of choice. It considerably increased the yield of the *O*-mannopyranosides compared to that of the Helfrich method, described by De Bruyne and Vervoort, and yielded *S*-mannopyranosides in relatively good yields. Scheme 2.2.6 depicts the synthesis of the desired *O*- and *S*-mannopyranosides.



Scheme 2.2.6 Synthesis of *O*- and *S*- aryl mannopyranosides. Reagents and conditions: i) $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 equiv.), CH_2Cl_2 , 24hrs, RT; ii) ArOH, 61-77%; iii) 1N NaOMe, MeOH, RT, <45 min, 85-93%; iv) ArSH (1.5 equiv.), 68-76%; v) 1N NaOMe, MeOH, RT, <45 min, 75-100%

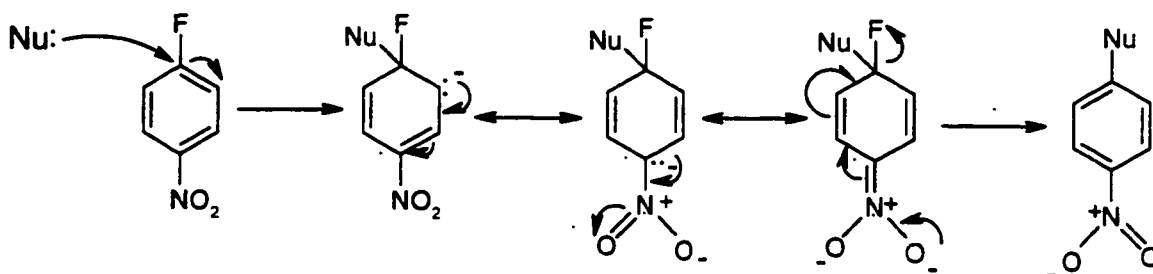
This method, however, failed to effectively provide *p*-nitrophenyl 2,3,4,6-tetra-*O*-acetyl- α -*S*-*D*-mannopyranoside (**4d**). The reaction proceeded only with longer reaction time (5-6 days) to give the desired product in a very poor yield (<10%). We reasoned that this is because of the electron-withdrawing properties of the nitro group. Electron-withdrawing substituents on a phenol pull the electrons, through mesomeric effect, away from the hydroxyl oxygen, reducing its nucleophilicity and in turn decreasing the rate of the reaction.²⁶ In order to obtain **4d**, we exploited a different method.

Scheme 2.2.7 illustrates the synthesis of **4d**. This procedure involved the synthesis of **10** (bromo 2,3,4,6-tetra-*O*-acetyl- α -*D*-mannopyranoside) first followed by its reaction with an equimolar amount of thiourea in anhydrous acetone. The resulting salt was subsequently reacted with *p*-fluoronitrobenzene to yield the desired compound **4d**, in an overall yield of 75%.^{27, 28}



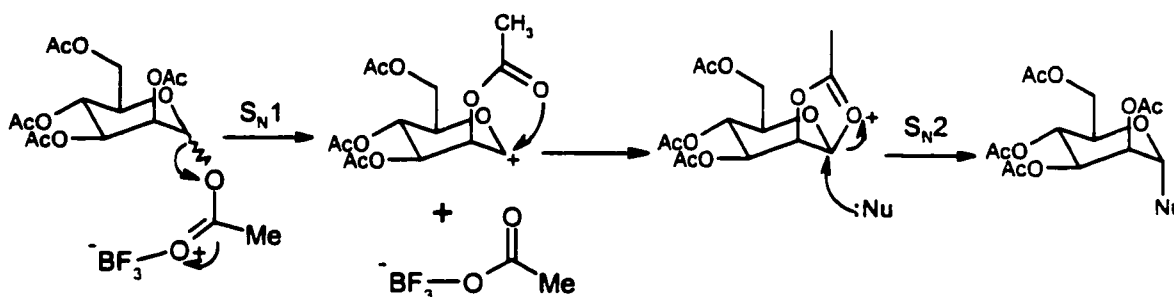
Scheme 2.2.7 Synthesis of *p*-nitrophenyl 2,3,4,6-tetra-*O*-acetyl α -*D*mannopyranoside (**4d**); i) 45% HBr in acetic acid, CH₂Cl₂, 1.5hr, 94%; ii) thiourea, acetone, 2hrs; iii) *p*-fluoronitrobenzene, K₂CO₃, acetone, RT, 24 hrs, 80%.

The reaction of the precursor of **4d** with fluoronnitrobenzene is facilitated by the electron withdrawing properties of the nitro group as shown in scheme 2.2.8.



Scheme 2.2.8 Mechanism for step iii in scheme 2.2.7.

All the reactions attempted to make the *O*- and *S*-mannopyranosides produced the α -anomer, exclusively. This was concluded from the NMR spectra obtained, particularly, ^{13}C NMR spectra. $^1J_{\text{C1-H1}}$ for all the *O*- and *S*-mannopyranosides had a value that was consistent with that of α -mannopyranosides ($^1J_{\text{C1-H1}} = 169\text{-}174\text{Hz}$). Alpha anomers usually have $^1J_{\text{C1-H1}}$ values >165 Hz, whereas beta anomers have $^3J_{\text{C1-H1}}$ values <165 Hz.²⁹ This selective production of one anomer can be easily explained by the neighboring-group effect. As the acetate-leaving group at C1 departs, the oxygen atom of the ester group on C2 stabilizes the formed carbocation by forming an oxonium ion. Since the nucleophilic substitution step is an $\text{S}_{\text{N}}2$ reaction, the nucleophile (phenol or thiophenol) can only attack from below resulting in inversion of configuration and giving rise to the alpha anomer (scheme 2.2.9).²²



Scheme 2.2.9 Neighboring group participation results in exclusive formation of the alpha anomer

The prepared *S*-mannopyranosides were fully characterized by the various spectroscopic techniques. Figures 2.2.1 and 2.2.2 show the ¹H and ¹³C NMR spectra, respectively, of sulfide **4c**. These spectra represent typical spectra of the sulfide series. Aromatic protons of the sulfide series resonate in the region between δ 6.83- 8.13 ppm. Protons of the sugar moiety resonate in the region between δ 4.06- 5.67 ppm. Acetate protons of all sulfides have almost identical chemical shifts and resonate in the region between δ 1.98- 2.18 ppm. The effect of electron-donating, electron-withdrawing properties of the aryl substituent can be clearly seen in the ¹H NMR spectra. Compound **4d**, having an electron-withdrawing substituent (-NO₂), has its aromatic protons most deshielded and resonating at δ 7.57 and 8.13 ppm. Compound **4c**, having an electron-donating substituent (-OMe), has its aromatic protons resonating at 6.83 and 7.40 ppm. This effect of shielding and deshielding is also seen with the anomeric proton. H-1 in **4d** resonates at δ 5.67 ppm, whereas, H-1 in **4c** resonates at δ 5.29 ppm. Table 2.2.1 lists the chemical shift ranges for the characteristic signals of the sulfide series.

Table 2.2.1 Chemical shift range for protons of the sulfide series (500MHz, CDCl₃, ppm)

	4a	4b	4c	4d
Aromatic	7.44, 7.27	7.35, 7.10	7.40, 6.83	8.13, 7.57
H1	5.47	5.38	5.29	5.67
H1-H6	5.47- 4.09	5.46- 4.06	5.45- 4.07	5.67- 4.10
Acetates	2.18- 1.98	2.11- 1.98	2.10- 1.98	2.17- 2.01

The protected thiomannopyranosides show similar fragmentation pattern in the FAB mass spectra. The molecular ion peak can be clearly seen in the spectra of all the sulfides, except for **4d**, with intensity ranging between 3.7%-100%. The acetylated sugar

fragment is very pronounced for all the sulfides, where it is either the base peak (**4a** and **4d**) or a high intensity peak (50.8% for **4c** and 92.8% for **4b**). The *S*-aryl fragment can also be seen for all the sulfides with low to high intensity (8.0-56.0%).

The synthesized *O*- and *S*-mannopyranosides were fully deprotected under standard Zemplén conditions to yield the free sugars in excellent yields (75%-quantitative). The physical and spectroscopic data for the known *O*-mannopyranosides were consistent with those in the literature,²² and hence will not be discussed.

The ¹H NMR spectra of the sulfides, compounds **5a-5d**, show the disappearance of the acetate peaks in the 2.0 ppm region. Aromatic protons resonate in the region between δ 7.06-8.12 ppm. Protons of the sugar moiety resonate in the region between δ 3.79-5.75 ppm. There is an obvious upfield shift for all protons relative to their protected counterparts. Table 2.2.2 lists the chemical shift ranges for protons in compounds **5a-5d**.

Table 2.2.2 Chemical shifts for protons of the free sugars in the sulfide series (500Mhz, D₂O, ppm)

	5a	5b	5c	5d
Aromatic	7.63, 7.47	7.55, 7.34	7.59, 7.06	8.12-7.65
H1	5.57	5.52	5.43	5.75
H1-H6	5.57- 3.79	5.52- 3.79	5.43- 3.91	5.75- 3.92

FAB mass spectra were obtained for all free thiomannopyranosides. The molecular ion peak can be clearly seen in all spectra with moderate to high intensity (1.3%-100%). The sugar fragment ($m/e = 163$) is very pronounced in all compounds giving rise to a peak with intensity ranging between 2.9%-100%

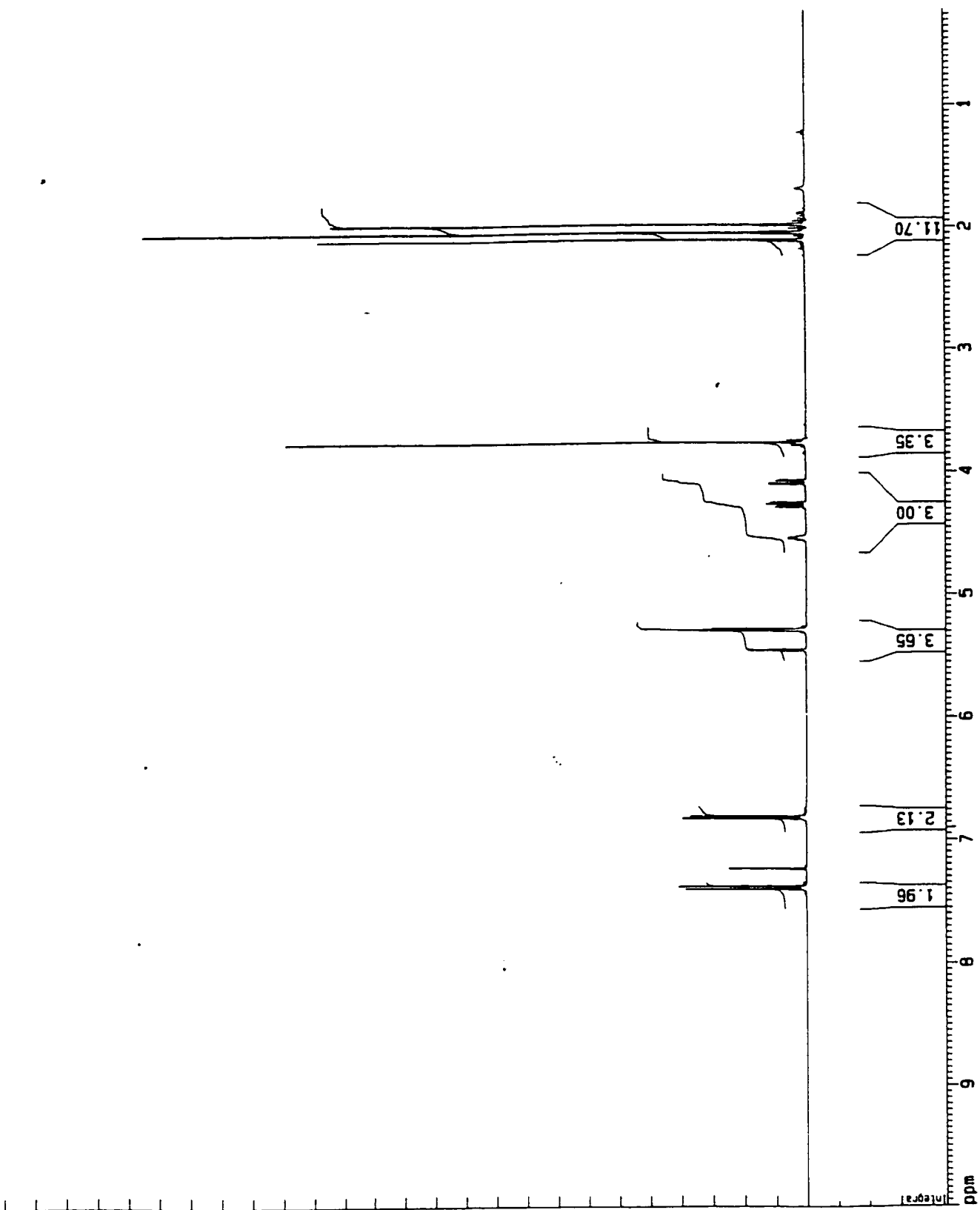


Figure 2.2.1 ¹H NMR (500MHz, CDCl₃) of 4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (4c)

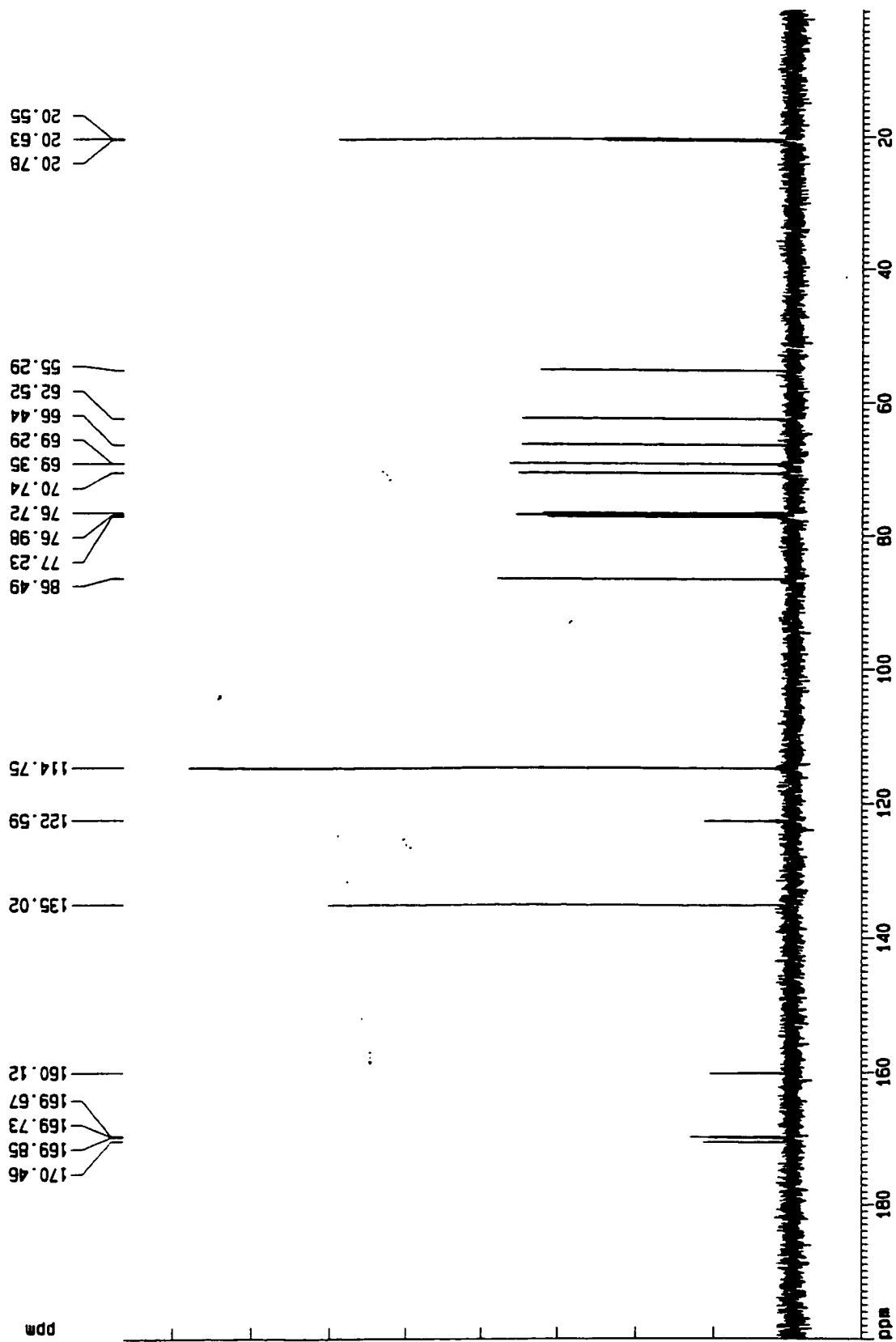


Figure 2.2.2 ^{13}C NMR (500MHz, CDCl_3) of 4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (4c)

Table 2.2.3 lists all the compounds prepared along with their yields and some physical properties.

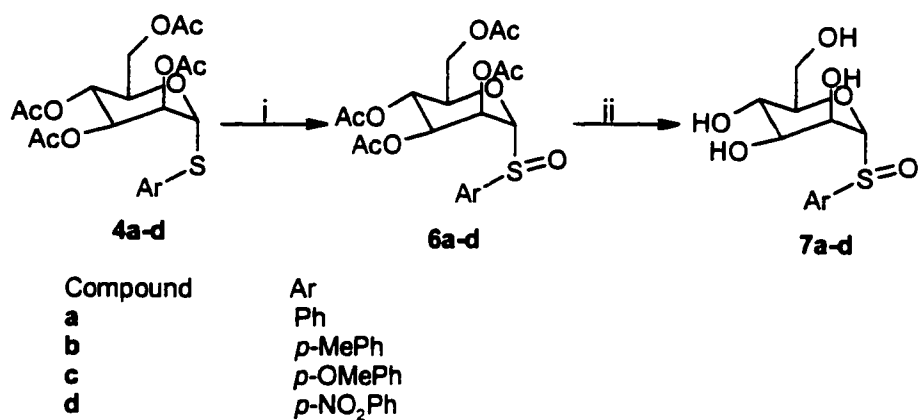
Table 2.2.3 Summary of physical properties of the prepared aryl *O*-mannopyranosides [2a-c (protected sugars) and 3a-c (free sugars)] and *S*-mannopyranosides [4a-d (protected sugars) and 5a-d (free sugars)]

Compound	Yield (%) [lit]	m.p. (°C) [lit.]	$[\alpha]_D^*$	Compound	Yield (%)	m.p. (°C)	$[\alpha]_D$
2a	74	95-96	+64.7	4b	69	oil	+97.2
2b	77 [53] ²²	87-89 [80-81] ²²	+71.8 [+69.5] ²²	4c	68	89-90	+103.4
2c	61 [35] ²²	98-99 [101-102] ²²	+71.4 [+95.9] ²²	4d	75	152 (decomp.)	+139.3
3a	92	112-114	+100.8	5a	Quant.	74-75	+209.6
3b	98 [88] ²²	165-166 [166-167] ²²	+122.0 [+122.9] ²²	5b	98	125-127	+177.9
3c	97 [83] ²²	150-151 [155-156] ²²	+120.2 [+122.6] ²²	5c	75	137-138	+265.0
4a	76	84-85	+109.4	5d	Quant.	162-164 (decomp.)	+285.4

*Solvents used for $[\alpha]_D$: MeOH for compounds **3a-c** and **5a-d**, CHCl₃ for compounds **2a-c** and **4a-d**

2.3 Synthesis of Aryl Sulfinyl α -D-Mannopyranosides

For the synthesis of aryl sulfinyl α -D-mannopyranosides, classical oxidation of the sulfides by *m*-chloroperbenzoic acid (*m*CPBA) was employed. The fully protected thiomannopyranosides were reacted with a slight excess of *m*CPBA in dichloromethane at -78 °C, as illustrated in scheme 2.3.1.



Scheme 2.3.1 Synthesis of aryl sulfinyl mannopyranosides Conditions and Reagents: i) *m*CPBA (1.1 equiv.), CH₂Cl₂, -78 °C, 63-76%; ii) NaOMe (1N), MeOH, RT, <45 min, 82-98%

The low temperature was necessary to prevent over oxidation of the sulfide to the sulfone. The reaction proceeded over a period of 4-6 hrs to stereoselectively yield a major sulfoxide (5:1 from the ¹H NMR integration). The reaction mixture was then treated with saturated Na₂SO₃ to destroy any excess *m*CPBA and washed with NaHCO₃ and water. The diastereomers were separated by preparative TLC. Column chromatography was found unsuitable for the separation since the minor isomer was always contaminated with the major one. Since the minor diastereomer was isolated only in minute quantities, only the major one was evaluated by ELLA.

Three other oxidation methods were attempted to obtain the sulfoxides. None successfully produced the desired product. Oxidation using Br₂ in the presence of hydrated silica³⁰ did not proceed and only appeared to destroy the starting material as was concluded from the TLC. Oxidation using NaIO₄ proceeded with very long reaction time (4 days) only to give a mixture of products. Oxidation with 30% H₂O₂ in

dichloromethane in the presence of silica gel and acetic anhydride³¹ required long reaction time (5 days) and produced only the sulfones.

The obtained sulfoxides were fully characterized by various spectroscopic techniques. ¹H NMR spectra give a clear indication of the formation of sulfoxides. Figures 2.3.1 and 2.3.2 show the ¹H NMR and ¹³C NMR spectra, respectively, of compound **6c**, protected *p*-methoxyphenylsulfinyl α -D-mannopyranoside. The effect of oxidation is most pronounced in the chemical shifts of the aromatic protons, which resonate downfield relative to the aromatic protons of the sulfide series. The aromatic protons of **6c** resonate at δ 7.60 and δ 7.05 ppm compared to δ 7.40 and δ 6.83 ppm for the corresponding sulfide, **4c**. The deshielding effect is also exerted on the aromatic OMe substituent. Protons of the methoxy substituent resonate at δ 3.83 ppm in **6c** compared to δ 3.76 ppm for the corresponding sulfide. The anomeric proton, however, shows a reversed trend. Instead of shifting downfield, it resonates upfield relative to the corresponding sulfide (δ 4.47 ppm, compared to δ 5.29 ppm). This reversed trend is seen for all the sulfoxide series.

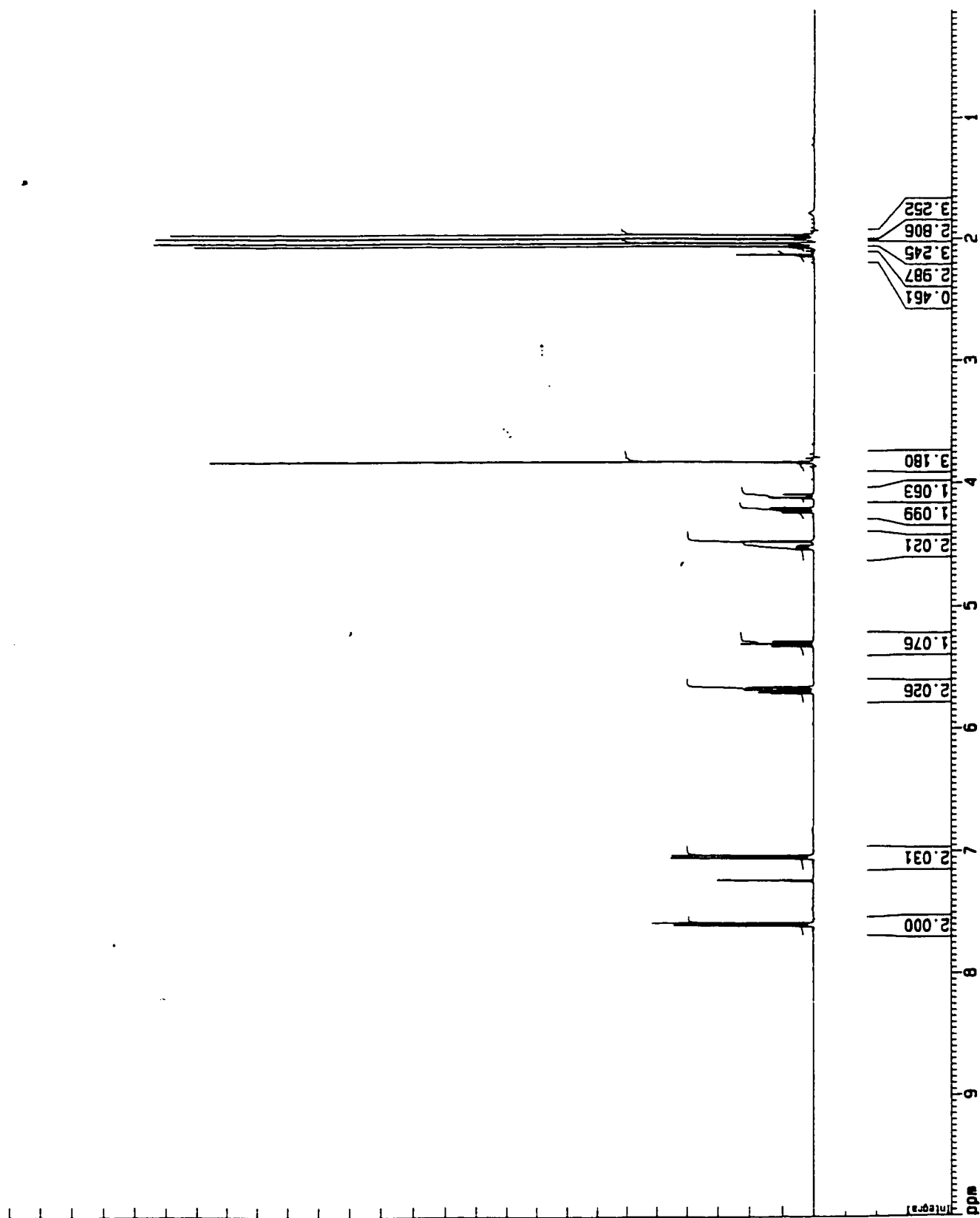


Figure 2.3.1 ¹H NMR (500MHz, CDCl₃) of 4-Methoxyphenylsulfinyl α-D-mannopyranoside (6c)

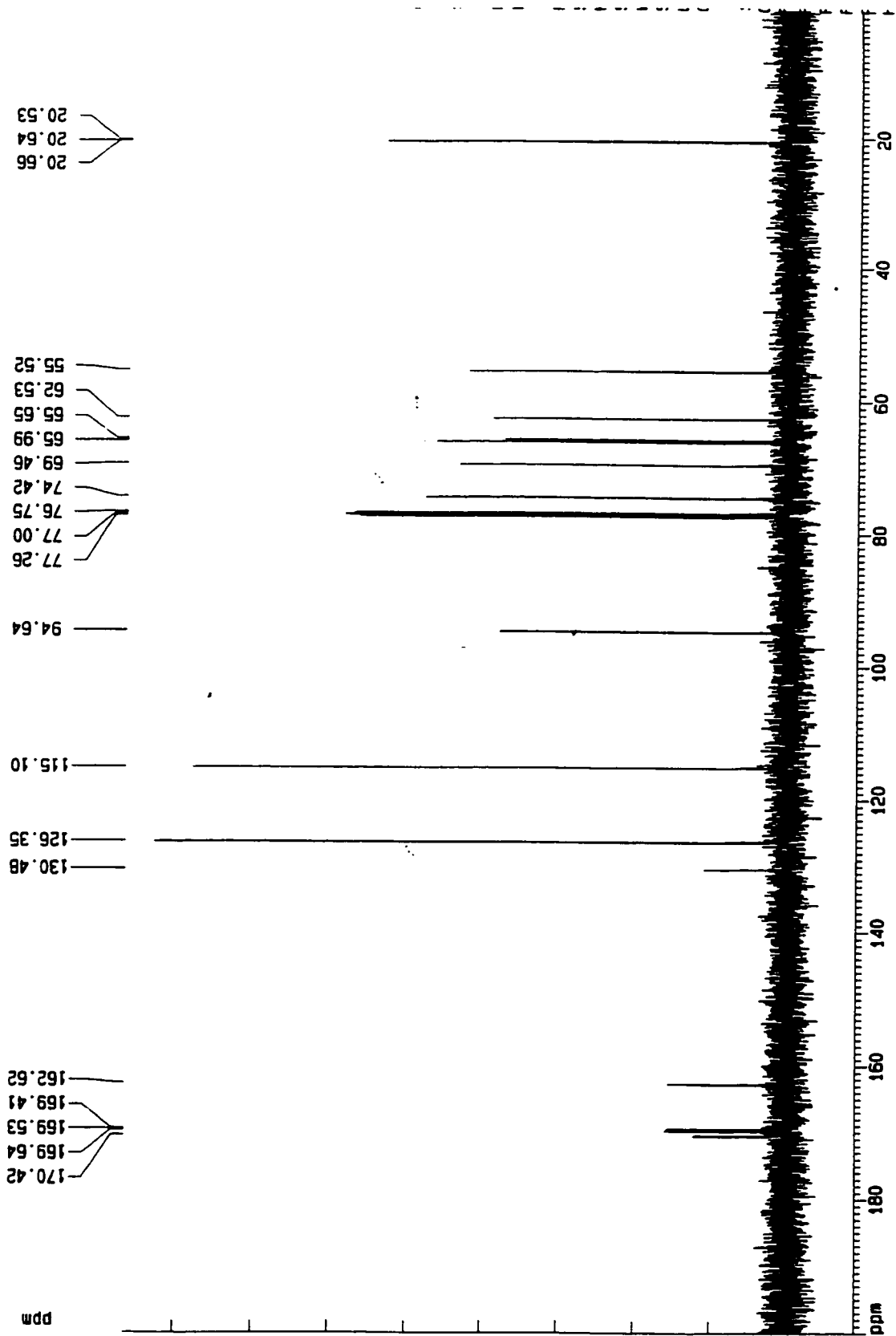


Figure 2.3.2 ¹³C NMR (500MHz, CDCl₃) of 4-Methoxyphenylsulfinyl α-D-mannopyranoside (6c)

IR spectroscopy proved useful in confirming the identity of the sulfoxide derivatives. Sulfoxides typically show a strong stretch around ν 1050 cm^{-1} . All compounds of the sulfoxide series gave rise to such a stretch.

FAB mass spectra of the protected sulfoxides showed similar fragmentation pattern. The molecular ion peak is present in the spectra of all sulfoxides, except for **6d** (*p*-nitrophenylsulfinyl derivative), with an intensity ranging from 13.6%-22.2%. Fragmentation of the sulfoxides gave rise to the same base peak in all four compounds at $m/e= 331$, which corresponds to the protected sugar skeleton.

Definite assignment of the configuration around the sulfinyl sulfur could not be attained, since X-ray quality crystals could not be obtained. However, based on Crich's work,³² the configuration around the sulfur in the isolated sulfoxides could be assumed to be R. Crich *et al.*³¹ have synthesized a series of sulfoxides using different oxidation methods and were able to obtain crystal structures of three sulfoxides obtained from three different oxidation methods. In all cases, the configuration around the sulfinyl sulfur was found to be R. Crich proposed, based on the Newman projection in Figure 2.3.3, that the *pro-R* lone pair is more easily accessible due to the conformation imposed on the α -thioglycoside by the *exo*-anomeric effect. Oxidation of the *pro-S* lone pair is rather difficult due to the steric hindrance of the lone pair by the pyranose ring.

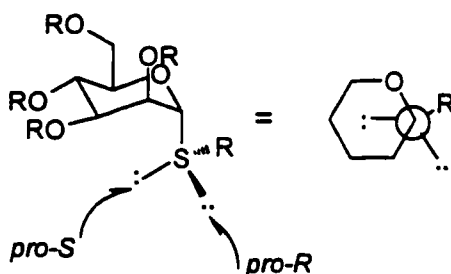


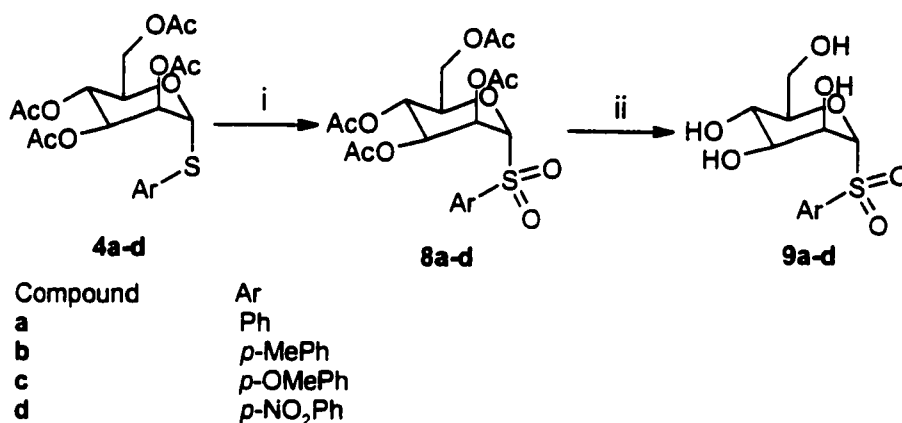
Figure 2.3.3 Newman projection of pyranose ring demonstrating how the *pro-R* lone pair is exposed for attack

Deprotection of the sulfoxides was achieved under standard Zemplén conditions to yield the free aryl sulfinyl mannopyranosides in excellent yields (82-98%), as was shown in scheme 2.3.1. NMR spectroscopy along with high resolution FAB mass spectrometry and IR were used to confirm the identity of the free sulfoxides. All sulfoxides showed a stretch around 1050 cm^{-1} in their IR spectra, characteristic of S=O functionality. The ^1H NMR spectra of the free sulfoxides, as was noted for the free sulfide series, show a general downfield shift for all protons relative to the protected sulfoxides. In the ^1H NMR spectrum of **7c** (*p*-methoxysulfinyl derivative), for example, the anomeric proton resonates at δ 4.79 ppm compared to δ 4.47 ppm for the protected sulfoxide. The aromatic protons give rise to two doublets at δ 7.81 and 7.27 ppm, compared to δ 7.60 and 7.05 ppm for the corresponding protected sulfoxide. The aromatic O-Me substituent resonates as a singlet at δ 3.97 ppm compared to δ 3.83 ppm in the NMR spectrum of the corresponding protected sulfoxide. The ^{13}C NMR spectra of all free sulfoxides agree with their structure. The anomeric carbon being the most deshielded resonates at around δ 98 ppm. C6, being the most shielded, resonates at around δ 60 ppm. The remaining carbons give rise to signals between δ 81.3 and 70.5

ppm. The molecular ion peak is present in the mass spectra of all the free sulfoxides with intensity ranging between 7.4%-79.9%. The fragment corresponding to the sugar skeleton ($m/e=163$) can be seen in all spectra with intensity ranging between 10.4% -100%.

2.4 Synthesis of Aryl Sulfonyl α -D-Mannopyranosides

Aryl sulfonyl α -D-mannopyranosides were readily prepared by oxidizing the fully protected thiomannopyranosides with two equivalents of *m*CPBA in dichloromethane at room temperature. The reaction proceeded over a period of 55min-24hrs to give the sulfones as the sole product in very good yields (75-95%). Purification of the products was achieved by column chromatography. Scheme 2.4.1 depicts the reaction conditions.



Scheme 2.4.1 Synthesis of aryl sulfonyl mannopyranosides Reagents and conditions: i) *m*CPBA (2.1 equiv.), CH₂Cl₂, RT, 55min-24hrs, 75-95%; ii) NaOMe (1N), MeOH, RT, <45 min, 66-85%

The identity of all sulfones was confirmed by various spectroscopic techniques. ¹H NMR spectra of all sulfones show a clear downfield shift for the aromatic protons, relative to the corresponding sulfides and sulfoxides. Table 2.4.1 compares the chemical

shifts in the ^1H NMR spectra of protected *p*-methylphenyl *S*-mannopyranoside (**4b**) and its corresponding sulfoxide and sulfone.

Table 2.4.1 Comparison of key ^1H chemical shifts in *p*-methylphenyl thiomannopyranoside and its corresponding sulfone and sulfoxide (500MHz, CDCl_3 , ppm)

Proton	4b	6b	8b
H1	5.38	4.50	4.75
H2	5.46	5.63	5.98
H3	5.29	5.70	5.70
H4	5.28	5.30	5.27
H5	4.52	4.56	5.79
H6a, H6b	4.26, 4.08	4.22, 4.13	4.19, 4.05
Ac	2.11-1.98	2.07-1.96	2.10-2.00
Ph-Me	2.29	2.39	2.44
Ar	7.35, 7.10	7.55, 7.35	7.81, 7.36

The aromatic protons resonate at δ 7.81 and 7.36 ppm for the sulfone derivative (**8b**) compared to δ 7.35 and 7.10 for the corresponding sulfide (**4b**) and δ 7.55 and 7.35 for the corresponding sulfoxide (**6b**). The anomeric proton is deshielded and shifted downfield relative to the sulfoxide derivative (δ 4.75 compared to 4.50 ppm, respectively), but as was with the sulfoxide it is more shielded relative to its sulfide counterpart (δ 4.75 compared to 5.38 ppm). Except for the anomeric proton, there is downfield shift for all protons relative to the corresponding sulfide. IR spectroscopy proved useful for the identification of the sulfone series. All sulfones gave rise to two characteristic stretches around ν 1360 cm^{-1} and 1136 cm^{-1} . These stretches correspond to the asymmetric, and symmetric stretches of the O=S=O functionality, respectively.

The free sulfones were obtained by deprotecting the acetylated sulfones under standard Zemplén conditions to yield the desired products in good to excellent yields (56%-89%). Since the anomeric arylsulfonyl residue is a good leaving group, we were concerned that treatment of the protected sulfones with NaOMe might result in its elimination. TLC and NMR, however, indicated no elimination of the anomeric substituents.

Spectroscopic data of the free sulfones were consistent with their structures. The IR spectra of all free sulfones showed two bands characteristic of O=S=O functionality at around ν 1316 cm^{-1} and 1136 cm^{-1} . No acetate peaks were present in the NMR spectra of the deprotected sulfones indicating the removal of the protecting groups. The ^1H NMR spectra showed the same trend observed for the free sulfides and sulfoxides where there is a general downfield shift relative to the corresponding protected sulfone. The aromatic protons in free sulfone **9b** (with a *p*-methyl substituent), for example, resonate at δ 7.91 and 7.60 ppm compared to δ 7.81 and 7.36 for the corresponding protected sugar (**8b**). The anomeric proton gives rise to a signal at δ 5.16 ppm compared to δ 4.75 ppm for the corresponding protected sulfone (**8b**). The aromatic methyl substituent experiences the same deshielding effect giving rise to a signal at δ 2.53 compared to δ 2.44 ppm in **8b**. The ^{13}C NMR spectra of the free sulfones are simple showing signals that account for all carbons in each molecule. The anomeric carbon resonates around δ 93.5 ppm, whereas C6 resonates around δ 62.5 ppm. The remaining carbons of the sugar moiety resonate in the region between δ 77.2 ppm and δ 65.3 ppm. The molecular ion is present in the

spectra of all free sulfones (except **9d**) with intensity ranging between 1.1% and 100%. In the case of **9d** an NO₂ fragment is readily lost to give rise to $m/e = 310$ with an intensity of 2.5%. The mass spectra of all free sulfones show a peak with $m/e = 136$, which corresponds to the sugar skeleton, with intensity ranging between 2.9% -53%

2.5 Inhibitory effect of *O*-mannopyranosides, *S*-mannopyranosides and the corresponding sulfoxides and sulfones

ELLA was used to evaluate the inhibition (for all synthesized compounds) of the binding of yeast mannan to Con A (table 2.5.1). Yeast mannan was used as the coating antigen in microtiter plates and Horseradish peroxidase-labeled Con A was used as the lectin. Methyl α -mannopyranoside was used as a standard. Stock solutions were prepared by dissolving 1-3mg of sample in 1mL PBS solution. Each sample underwent a 2-fold serial dilution and each dilution was tested in duplicate. Detailed description of the experimental procedure can be found in appendix 1.

Table 2.5.1 Inhibition results of the binding of Con A to yeast mannan by aryl mannopyranosides.

Compound	IC₅₀ (μM)	Relative Potency †	Compound	IC₅₀ (μM)	Relative Potency
Me Man	1.11	1			
3a	0.464	2.4	7a	0.261	4.4
3b	0.134	8.1	7b	0.078	14.3
3c	0.072	15.5	7c	0.099	11.2
3d	0.390	2.9	7d	0.166	6.7
5a	0.106	10.4	9a	0.198	5.6
5b	0.055	20.3	9b	0.051	21.8
5c	0.084	23.1	9c	0.033	33.5
5d	0.062	17.8	9d	0.218	5.1

† Relative potency is measured relative to methyl α-D-mannopyranoside

The inhibitory effect of aryl *O*-mannopyranosides was found consistent with that reported by Loontjens.¹³ Electron-donating substituted aryl *O*-mannopyranosides are better inhibitors than electron-withdrawing substituted aryl *O*-mannopyranosides. Figure 2.5.1 demonstrates these findings. The same trend was also observed for the *S*-mannopyranosides and their corresponding sulfoxides and sulfones.

S-mannopyranosides showed better inhibitory effect, overall, than their *O*-analogues. For example, *p*-methylphenyl *S*-mannopyranoside (**5b**) was found to be 20 times better inhibitor for the binding of Con A to yeast mannan than methyl mannopyranoside (IC₅₀ 0.055μM and 1.11μM, respectively). The corresponding *O*-mannopyranoside (**3b**) was only 8 times better inhibitor relative to methyl mannopyranoside (IC₅₀ 0.134μM and 1.11μM, respectively).

The inhibitory effect of the sulfoxides was found to be less than that of the sulfides. *p*-methylphenyl sulfinyl mannopyranoside (**7b**) (IC_{50} 0.78 μ M), for example is 11 times better relative to methyl mannopyranoside, whereas the corresponding sulfide (**5b**) is 20 times better (IC_{50} 0.055 μ M).

The inhibitory effect of the sulfones was found to be greater than the sulfoxides, but less than the sulfides (IC_{50} 0.051 μ M, 0.078 μ M, 0.055 μ M for **9b**, **7b**, **5b**, respectively). Sulfone **9c** (*p*-methoxy sulfonyl mannopyranoside), however, showed better inhibitory effect than its corresponding sulfoxides and sulfides. In fact, **9c** exhibited the best inhibitory effect overall with a potency 33 times greater than methyl mannopyranoside (IC_{50} 0.033 μ M compared to 1.11 μ M).

The inhibitory effect of the sulfoxide and sulfone series was found to be better than the *O*-mannopyranosides, except for **7c**. Figures 2.5.2 and 2.5.3 illustrate some of these conclusions.

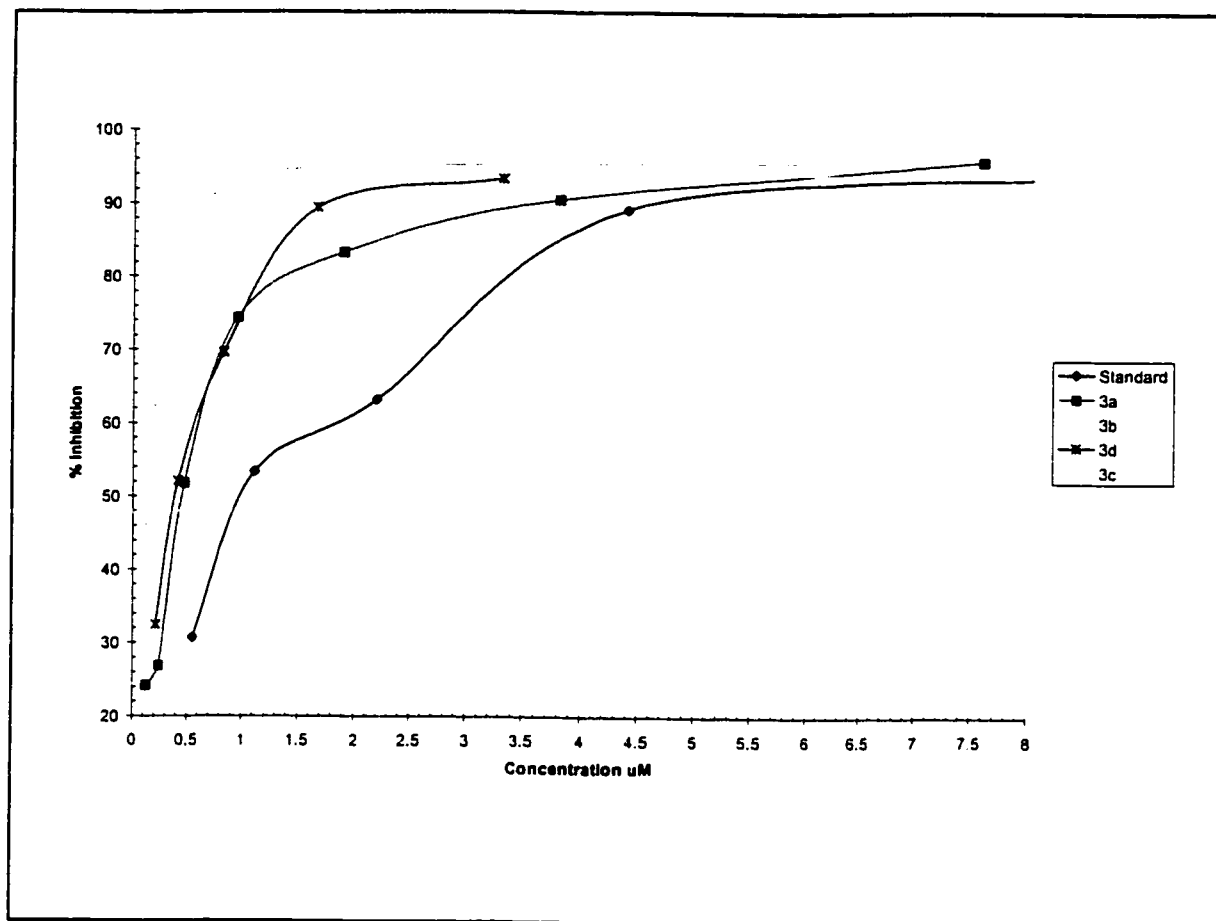


Figure 2.5.1 ELLA results for the inhibition of the binding of Con A to yeast mannan by aryl α -D-mannopyranosides (3a-3d)

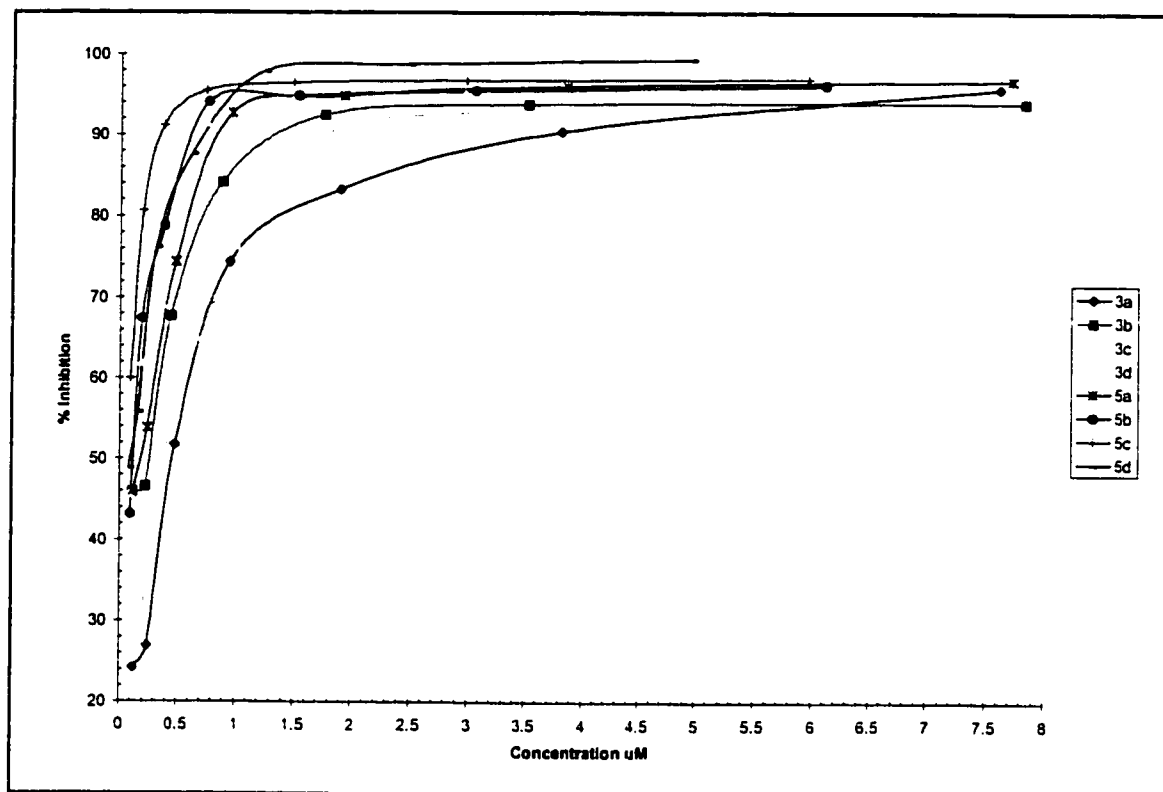


Figure 2.5.2 ELLA results demonstrating that Aryl *S*-mannopyranosides are better inhibitors than the corresponding aryl *O*-mannopyranosides

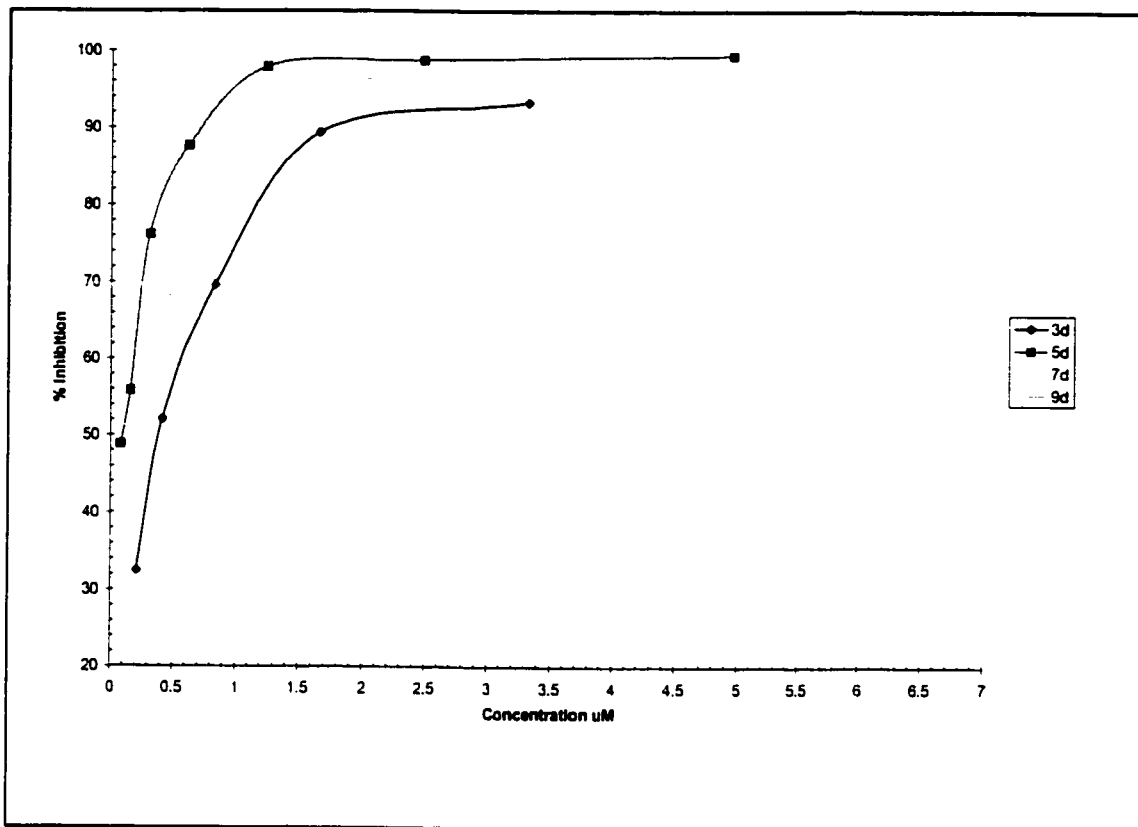


Figure 2.5.3 Inhibition curves comparing *p*-nitrophenyl α -D-mannopyranoside (3d), the analogous *S*-mannopyranoside (5d) and the corresponding sulfoxide (7d) and sulfone (9d)

2.6 Conclusions

Glycosylation of mannose can be easily achieved from mannose pentaacetate in the presence of a Lewis acid ($\text{BF}_3 \cdot \text{Et}_2\text{O}$). Aryl *O*- and *S*-mannopyranosides were prepared in high yields using this method. Aryl *S*-mannopyranosides were oxidized to the corresponding sulfoxides and sulfones in good yields using classical *m*CPBA oxidation.

The inhibitory effect of aryl *O*- and *S*-mannopyranosides and their corresponding sulfoxide and sulfones were measured by ELLA. Aryl *S*-mannopyranosides proved to be better inhibitors than the *O*-mannopyranosides analogues. Sulfoxides showed poorer inhibitory effects than the corresponding sulfides. In general, sulfones exhibited better inhibitory effect than the corresponding sulfoxides, but poorer inhibitory effect relative to the sulfides. Compound **9c** was found to be the best inhibitor of the C1-modified compounds (33 times more potent than Me-mannopyranoside) with an IC_{50} of 0.033 μM .

2.7 Experimental

Chemicals and general methods

Melting points were determined on a Gallenkamp and are not corrected. The ^1H and ^{13}C NMR spectra were obtained on a Bruker AMX NMR spectrometer or Gemini 200 spectrometer. The proton chemical shifts (δ) are given relative to internal chloroform (7.24 ppm) for CDCl_3 solutions, to internal HOD (4.65 ppm) for D_2O solutions. The carbon chemical shifts are given relative to CDCl_3 (77.0 ppm). The assignments are based on COSY, DEPT, and HMQC experiments. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and were measured at room temperature. FAB Mass spectra were recorded on Kratos IIIH instrument. Xenon was used as the neutral carrier atom. Infrared spectra were recorded on a BOMEM Michelson series FT-IR. All chemicals and solvents used were reagent grade. Further purification was performed, when necessary, following published procedures. Thin layer chromatography (TLC) was performed using silica gel 60 F-254 aluminum plates. Reagents used to develop the plates include ammonium molybdate (2.5% w/v) in 10% aqueous sulfuric acid and ninhydrine (10% w/v) in ethanol for the detection of primary amines. Plates were heated to ca. 150 $^\circ\text{C}$, when necessary. Column chromatography was performed on silica gel 60 (230-400 Mesh, E. Merck No. 9385). Solvents used for elution were reagent grade and used as supplied. Optical densities (O.D.) for the ELLA tests were obtained on a Dynatech MR 600 Microplate Reader. pH of aqueous solutions for the ELLA experiments were measured using a Fischer Scientific Model 805NP instrument fitted with a Fischer Scientific E-N5 pencil electrode. pH measurements for routine reactions were performed with Hydron test paper. The lectin from *Concanavalin ensiformis*, *Concanavalin A*

peroxidase-labeled, along with the yeast mannan from *Saccharomyces cerevisiae* were purchased from Sigma (cat. # L 6397 and M 7504, respectively). The ELLA test was performed as described in Appendix A.

General procedure for the synthesis of *O*- and *S*-mannopyranosides

1 was dissolved in anhydrous dichloromethane and the appropriate phenol or thiophenol (1.1 equivalents) was added (neat if liquid, as a dichloromethane solution if solid). Upon cooling the solution in an ice bath, $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 equiv.) was added drop-wise, under N_2 -atmosphere with continuous stirring. The reaction mixture was left to stir under ambient temperature and N_2 atmosphere overnight (ca. 16 hrs). The mixture was then diluted with dichloromethane and washed with 1M NaOH (1X), 5% HCl (1X), and H_2O (1X), dried over Na_2SO_4 and concentrated. Crystallization of the crude product from 95% EtOH afforded the desired product. Where applicable, the mother liquor was chromatographed on a silica column.

General procedure for the synthesis of sulfinyl mannopyranosides

A sample of appropriate *S*-mannopyranoside was dissolved in anhydrous dichloromethane and cooled to $-78\text{ }^\circ\text{C}$. *m*CPBA (1.1 equiv.) was added to the sugar solution and the mixture was left to stir at $-78\text{ }^\circ\text{C}$ until tlc indicated the completion of the reaction (4-6 hrs). The mixture was then filtered and the filtrate was washed with conc. Na_2SO_3 (2X), saturated NaHCO_3 (2X), H_2O (1X), and concentrated. The crude product was chromatographed on PTLC and the major sulfinyl diastereomer was isolated.

General procedure for the synthesis of Sulfonyl Mannopyranosides

A sample of the appropriate *S*-mannopyranoside was dissolved in anhydrous dichloromethane and cooled to 0 °C. *m*CPBA (2.1 equiv.) was added to the sugar solution and the mixture was left to warm up to room temperature and stir until TLC indicated the completion of the reaction (40min-24 hrs). The reaction mixture was then cooled to -78 °C, filtered and the filtrate washed with saturated Na₂SO₃ (2X), NaHCO₃ (1X), H₂O (1X) and concentrated. The crude product was chromatographed on a silica column to yield the desired product.

General procedure for the Zemplén reaction

To a solution of the appropriate protected sugar in MeOH was added, drop-wise, a solution of 1M NaOMe in MeOH until the pH is slightly basic. The reaction solution was left to stir under ambient conditions until TLC indicated completion of the reaction (10min-1 hr). Amberlite IR-120 resin was then added until pH is neutral. The solution was then filtered and the filtrate roto-evaporated to yield the desired compound as the sole product.

1,2,3,4,6-Penta-*O*-acetyl- α -D-mannopyranose (1)

D-(+)-mannose (36 g, 0.20 mol) was added to a mixture of pyridine (400 mL) and acetic anhydride (300 mL) at 0 °C. The reaction was left to warm to room temperature and stir until TLC indicated the completion of reaction (ca. 6 hrs) (EtOAc/ Hex 1:1). Methanol was added to destroy excess acetic anhydride and the solvents were then evaporated and co-evaporated with toluene under reduced pressure to yield a yellowish residue. The

color impurity was removed by dissolving the residue in methanol and adding charcoal. The solution was then filtered and evaporated to yield thick, colorless oil. The oil was dissolved in CH₂Cl₂, washed (2X) with an equal volume of saturated NaCl solution, dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The product was then crystallized from 95% EtOH to yield pure **1** (72.0 g) in 92% yield; m.p. 63-65 °C, [α]_D +53.1 (c.1.0, CHCl₃) [lit. m.p. 64°C, [α]_D +55.1 (c.1.0, CHCl₃)]

Phenyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (2a)

1 (1.05 g, 2.69 mmol) was dissolved in 10 mL of anhydrous CH₂Cl₂ and then reacted with phenol (460 mg, 4.89 mmol) and BF₃.Et₂O (0.63mL, 5.12 mmol) according to the general procedure. The crude product was chromatographed on a silica column (eluent EtOAc: Hex 1:1) to yield the title compound in 74% yield (805.2 mg, 1.90 mmol). m.p. 95-96 °C; [α]_D +64.7 (c 1.61, CHCl₃), ¹H NMR (500MHz, CDCl₃): δ = 7.25 (m, 2H, CH arom), 7.08 (m, 3H, CH arom.), 5.36 (d, 1H, H1), 5.27 (dd, 2H, H2), 5.22 (dd, 1H, H3), 4.20 (dd, 1H, H6a), 4.10 (m, 1H, H5), 3.82 (dd, 1H, H6b), 3.75 (t, 1H, H4), 2.10 (s, Ac, 3H), 2.08 (s, Ac, 3H), 1.98 (s, Ac, 3H), 1.96 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 169.9, 159.4, 129.9, 118.3, 97.3 (C1), 70.9 (C2, C3), 70.3 (C5), 68.3 (C4), 61.8 (C6), 20.5, 20.4; FAB-MS for C₂₀H₂₈O₁₄ Calc'd 424.1369, found 425.1320 (M+1, 24.1%)

4-Methylphenyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (2b)

1 (294.2 mg, 0.754 mmol) was dissolved in 5 mL of anhydrous CH₂Cl₂ and then reacted with *p*-cresol (219.1 mg, 2.0 mmol) and BF₃.Et₂O (0.185mL, 1.51 mmol) according to the general procedure. The crude product was crystallized from 95% EtOH to yield the

title compound in mg, 77% yield (lit. 53%); m.p. 80-81°C (lit. 87-89°C); $[\alpha]_D +71.8$ (c 1.08, CHCl₃) (lit. +69.5); ¹H NMR (500MHz, CDCl₃): δ = 7.06 (d, CH arom., 2H), 6.95 (d, CH arom., 2H), 5.55 (dd, H3, 1H), 5.45 (d, H1, 1H), 5.41 (dd, H2, 1H), 5.33 (t, H4, 2H), 4.26 (dd, H6a, 1H), 4.10 (m, H5, 1H), 4.06 (dd, H6b, 1H), 2.27 (s, Ph-CH₃, 3H), 2.16 (s, Ac, 3H), 2.02 (s, Ac, 3H), 2.00 (s, Ac, 3H), 1.99 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 170.5, 169.9, 169.8, 169.7, 153.5, 132.4, 129.9, 116.4, 96.0(C1), 69.5(C2), 68.9(C3), 68.8(C5), 66.0(C4), 62.1(C6), 20.9(Ph-CH₃), 20.6, 20.5, 20.4; FAB-MS for C₂₁H₂₆O₁₀ Calc'd 438.21, found 439.20 (M+1, 9.9%)

4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (2c)

1 (256.3 mg, 0.661 mmol) was dissolved in 5mL of anhydrous CH₂Cl₂ and then reacted with 4-methoxyphenol (126.8 mg, 1.02 mmol) and BF₃.Et₂O (0.163mL, 1.32 mmol) according to the general procedure. The crude product was crystallized from 95% EtOH to yield the title compound as white crystals in 52 % yield (156.9 mg). The mother liquor was chromatographed on a silica column (eluent EtOAc/Hex 2:3) to furnish another 183.8 mg of the title compound to give a total yield of 61%. (lit. 35%); m.p. 98-99 °C (lit. 101-102 °C); $[\alpha]_D +71.4$ (c.1.0, CHCl₃) (lit. +95.9); ¹H NMR (500MHz, CDCl₃): δ = 6.97 (d, CH arom., 2H), 6.78 (d, CH arom., 2H), 5.50 (dd, H3, 1H), 5.40 (dd, H2, 1H), 5.37 (d, H1, 1H), 5.32 (t, H4, 2H), 4.26 (dd, H6a, 1H), 4.07 (m, H5, 1H), 4.04 (dd, H6b, 1H), 3.73 (s, Ph-OCH₃, 3H), 2.15 (s, Ac, 3H), 2.02 (s, Ac, 3H), 2.00 (s, Ac, 3H), 1.99 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 170.5, 169.9, 169.8, 169.7, 155.4, 149.6, 117.8, 114.6, 96.6(C1), 69.4(C2), 68.9(C3), 68.8(C5), 66.0(C4), 62.2(C6), 55.6(OCH₃), 20.8, 20.6, 20.5; FAB-MS for C₂₁H₂₆O₁₁ Calc'd 454.21, found 454.19 (M+, 2.6%)

Phenyl α -D-mannopyranoside (3a)

2a (54.4 mg, 0.128 mmol) was treated with 1N NaOM in MeOH according to the general procedure to yield the title compound in 92% yield (30.0 mg, 0.117 mmol). m.p. 112-114°C; $[\alpha]_D +100.8$ (*c* 4.9, MeOH); ^1H NMR (250 MHz, D_2O): $\delta=7.36-7.29$ (m, 2H, CH arom.), 7.12-7.04 (m, 3H, CH arom.), 5.55 (s, 1H, H1), 4.73 (m, 4H), 4.10 (br s, 1H), 3.99 (br s, 1H), 3.69 (br s, 4H, OH); ^{13}C NMR (125MHz, D_2O): $\delta= 131.3, 124.6, 118.6, 118.5, 99.6$ (C1), 74.8, 71.8, 71.3, 68.0, 62.1 (C6); FAB-MS for $\text{C}_{12}\text{H}_{16}\text{O}_6$ Calc'd 256.0947, found 256.1138 (5.8%), 163.0776 (100%)

4-Methylphenyl α -D-mannopyranoside (3b)

2b (74.7 mg, 0.171 mmol) was treated with 1N NaOM in MeOH according to the general procedure to yield the title compound in 98% (45.4 mg, 0.168 mmol). m.p. 165-167 °C (lit. 166-167 °C), $[\alpha]_D +120.0$ (*c* 2.0, MeOH) (lit.+122.9, *c* 2.0, MeOH)

4-Methoxyphenyl α -D-mannopyranoside (3c)

2c (80.0 mg, 0.176 mmol) was treated with 1N NaOM in MeOH according to the general procedure to yield the title compound in 97% yield (49 mg, 0.171 mmol). m.p. 153-155°C, $[\alpha]_D +121.9$ (*c*.2.0, MeOH) [lit. m.p. 155-156 °C, $[\alpha]_D +122.6$ (*c*.2.0, MeOH)]

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio- α -D-mannopyranoside (4a)

1 (298.8 mg, 0.852 mmol) was dissolved in 15 mL of anhydrous CH_2Cl_2 and then reacted with benzenethiol (0.12 mL, 1.16 mmol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.19mL, 1.55 mmol) according to the general procedure. Crystallization of the crude product failed and isolation of **4a**

was achieved with silica column chromatography (EtOAc/Hex 2:3) to yield the title compound as a clear syrup. (282.9 mg, 76%); m.p. (oil); $[\alpha]_D +103.2$ (c. 1.03, CHCl₃); ¹H NMR (500MHz, CDCl₃): $\delta = 7.44$ (dd, CH arom., 2H), 7.27 (m, CH arom., 3H), 5.47 (s, H1,H2, 2H), 5.29 (t, H3, H4, 2H), 4.51 (m, H5, 1H), 4.29 (dd, H6a, 1H), 4.09 (dd, H6b, 1H), 2.18 (s, Ac, 3H), 2.04 (s, Ac, 3H), 2.02 (s, Ac, 3H), 1.98 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): $\delta = 132.6, 132.0, 129.1, 128.1, 85.6, 71.8, 70.9, 70.6, 65.8, 62.4, 20.8, 20.63, 20.61, 20.56$; FAB-MS: calc'd for C₂₀H₂₂O₉S= 438.44, found 439.17

4-Methylphenyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (4b)

1 (292.4 mg, 0.754 mmol) was dissolved in 15 mL of anhydrous CH₂Cl₂ and reacted with 4-thiocresol (185.3 mg, 1.49 mmol) and BF₃.Et₂O (0.19mL, 1.5 mmol) according to the general procedure. After work-up the amber-yellow crude product was chromatographed on SiO₂ (EtOAc/Hex 1:1) to yield **4b** as a clear syrup. (234.7 mg, 69%); m.p. (oil); $[\alpha]_D +97.2$ (c. 0.9 , CHCl₃); ¹H NMR (500MHz, CDCl₃): $\delta = 7.35$ (dd, CH arom., 2H), 7.10 (dd, CH arom., 2H), 5.46 (dd, H2, 1H), 5.38 (d, H1, 1H), 5.29 (d, H3, 1H), 5.28 (t, H4, 1H), 4.52 (m, H5, 1H), 4.26 (dd, H6a, 1H), 4.06 (dd, H6b, 1H), 2.29 (s, Ph-CH₃, 3H), 2.11 (s, Ac, 3H), 2.04 (s, Ac, 3H), 2.03 (s, Ac, 3H), 1.98 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): $\delta = 170.5, 169.8, 169.7, 169.6, 138.4, 132.57, 129.9, 128.75, 85.9(C1), 70.8(C2), 69.4(C5), 69.3(C3), 66.4(C4), 62.5(C6), 21.1, 20.8, 20.6, 20.5$; FAB-HRMS for C₂₁H₂₇O₉S Calc'd 455.1376, found 455.1307 (M+1 100 %)

4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (4c)

1 (298.6 mg, 0.852 mmol) was dissolved in 15 mL of anhydrous CH_2Cl_2 and reacted with 4-methoxybenzenethiol (0.21 mL, 1.70 mmol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.21 mL, 1.70 mmol) according to the general procedure. After work-up the crude product was crystallized from 95% EtOH to yield the title compound as white crystals (204.9 mg, 68%); m.p. 89-90 °C (decomp); $[\alpha]_D +103.4$ (c. 0.95, CHCl_3); $^1\text{H NMR}$ (500 MHz, CDCl_3): $\delta = 7.40$ (d, CH arom., 2H), 6.83 (d, CH arom., 2H), 5.45 (t, H2, 1H), 5.29 (m, H1, H3, H4, 3H), 4.55 (m, H5, 1H), 4.27 (dd, H6a, 1H), 4.07 (dd, H6b, 1H), 3.76 (s, O- CH_3 , 3H), 2.10 (s, Ac, 3H), 2.05 (s, Ac, 6H), 1.98 (s, Ac, 3H); $^{13}\text{C NMR}$ (125 MHz, CDCl_3): $\delta = 160.1$, 135.0, 122.6, 114.8, 86.5, 70.7, 69.4, 66.4, 62.5, 55.3, 20.78, 20.63, 20.55; FAB-HRMS Calcd for $\text{C}_{21}\text{H}_{27}\text{O}_{10}\text{S}$ 471.1325 ($\text{M}^+ + 1$), found 471.1342 ($\text{M} + 1$ 100 %)

4-Nitrophenyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (4d)

To a solution of **10** (1.57 g, 3.84 mmol) in anhydrous acetone (10 ml) was added a solution of thiourea (303.6 mg, 3.99 mmol) in acetone (15 ml). The reaction mixture was refluxed until TLC indicated the complete consumption of **10** (eluent EtOAc/Hex 1:1). 4-Fluoronitrobenzene (0.41 ml, 3.84 mmol), K_2CO_3 (767.4 mg) and H_2O (1.5 ml) were added to the reaction solution in the above mentioned sequence. The mixture was left to stir at RT for 24 hrs, after which it was diluted with CH_2Cl_2 , filtered and the filtrate evaporated. Compound **4d** was isolated by crystallization of the crude product from EtOH in 80% yield (1.45 g, 3.07 mmol); m.p. 152 °C (decomp.); $[\alpha]_D +139.3$ (c. 1.02, CHCl_3); $^1\text{H NMR}$ (500 MHz, CDCl_3): $\delta = 8.13$ (d, CH arom., 2H), 7.57 (d, CH arom., 2H), 5.67 (d, H1, 1H), 5.46 (dd, H2, 1H), 5.34 (t, H4, 1H), 5.27 (dd, H3, 1H), 4.40 (m,

H5, 1H), 4.28 (dd, H6a, 1H), 4.10 (dd, H6b, 1H), 2.17 (s, Ac, 3H), 2.05 (s, Ac, 3H), 2.01 (s, Ac, 6H); ^{13}C NMR (125MHz, CDCl_3): δ = 170.3, 169.7, 169.6, 142.5, 129.6, 124.0, 84.1(C1), 70.5(C2), 70.2(C5), 69.1(C3), 65.9(C4), 62.1(C6), 20.8, 20.6, 20.5; FAB-MS for $\text{C}_{20}\text{H}_{23}\text{O}_{11}\text{NS}$ Calc'd 485.17, found 487.2 (M+2 1.0%)

Phenyl 1-thio- α -D-mannopyranoside (5a)

4a (100.0 mg, 0.228 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 99.7 % yield (61.9mg, 0.227 mmol); m.p (74-75 °C); $[\alpha]_{\text{D}} +209.6$ (c 5.0, MeOH); ^1H NMR (500MHz, D_2O): δ = 7.63 (dd, CH arom., 2H), 7.47 (dd, CH arom., 3H), 5.57 (d, H1, 1H), 4.27 (dd, H2, 1H), 4.21 (m, H5, 1H), 3.93-3.82 (m, H3&H6a-b), 3H), 3.79(t, H4, 1H); ^{13}C NMR (125MHz, D_2O): δ = 132.0, 131.9, 128.9, 127.8, 87.7(C1), 73.1(C5), 71.0(C2), 70.6(C3), 66.5(C4), 60.2(C6); FAB-HRMS for $\text{C}_{12}\text{H}_{17}\text{O}_5\text{S}$ Calc'd 273.0797, found 273.0807 (M+1 18.3 %)

4-Methylphenyl 1-thio- α -D-mannopyranoside (5b)

4b (54.3 mg, 0.115 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 98% yield (34.3mg, 0.113mmol); m.p (125-127 °C); $[\alpha]_{\text{D}} +177.9$ (c 0.99, MeOH); ^1H NMR (500MHz, D_2O): δ = 7.55 (d, CH arom., 2H), 7.34 (d, CH arom., 2H), 5.52 (d, H1, 1H), 4.28 (dd, H2, 1H), 4.25 (m, H5, 1H), 3.96-3.82 (m, H3&H6(a-b), 3H), 3.79 (t, H4, 1H), 2.41 (s, Ph- CH_3 , 3H); ^{13}C NMR (125MHz, D_2O): δ = 138.7, 132.6, 129.6, 128.2, 88.0(C1), 73.1(C5), 71.0(C2), 70.6(C3), 66.6(C4), 60.3(C6), 19.7(Ph- CH_3); FAB-HRMS for $\text{C}_{13}\text{H}_{19}\text{O}_5\text{S}$ Calc'd 287.0953, found 287.1663 (M+1 19.2 %)

4-Methoxyphenyl 1-thio- α -D-mannopyranoside (5c)

4c (109.3 mg, 0.242 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 52.06 mg (0.182 mmol, 75 % yield); m.p (137-138 °C); $[\alpha]_D +265.0$ (c 7.2 , MeOH); $^1\text{H NMR}$ (500MHz, D_2O): $\delta = 7.59$ (d, CH arom., 2H), 7.06 (d, CH arom., 2H), 5.43 (d, H1, 1H), 4.27 (dd, H2, 1H), 4.25 (m, H5, 1H), 3.93-3.91 (m, H3&H6a, 2H), 3.89 (s, Ph-OCH₃, 3H), 3.83 (dd, H6b, 1H), 3.77(t, H4, 1H); $^{13}\text{C NMR}$ (125MHz, D_2O): $\delta = 158.9, 134.9, 122.7, 114.5, 88.4(\text{C}1), 73.0(\text{C}5), 70.9(\text{C}2), 70.5(\text{C}3), 66.6(\text{C}4), 60.2(\text{C}6), 55.0(\text{Ph-OCH}_3)$; FAB-HRMS for $\text{C}_{13}\text{H}_{19}\text{O}_6\text{S}$ Calc'd 303.0902, found 303.0859 (M+1 3.5 %)

4-Nitrophenyl 1-thio- α -D-mannopyranoside (5d)

4d (65.7 mg, 0.135 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in quantitative yield (43.2 mg, 0.135 mmol); m.p. 154 °C (decomp.); $[\alpha]_D +285.4$ (c. 1.0 , MeOH); $^1\text{H NMR}$ (200 MHz, D_2O): $\delta = 8.12$ (d, 2H, $J=9.06$ Hz, CH arom), 7.64 (d, 2H, $J= 9.10$ Hz, CH arom), 5.74 (d, 1H, $J_{\text{H}1-\text{H}2}= 1.59\text{Hz}$, H1), 7.96 (br s, 4H, OH), 4.18 (dd, 1H, $J_{\text{H}2-\text{H}3}= 1.65\text{Hz}$, H2), 3.99-3.96 (m, 1H, H5), 3.87-3.70 (m, 4H, H3, H4, H6a-b); $^{13}\text{C NMR}$ (125MHz, D_2O): $\delta = 139.6, 136.7, 133.3, 130.8, 8.6 (\text{C}1), 81.6, 76.4, 71.5, 63.4 (\text{C}6)$; FAB-MS for $\text{C}_{12}\text{H}_{15}\text{NO}_7\text{S}$ Calc'd 317.0569 found 317.0465

Phenylsulfinyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (6a)

4a (64.7 mg, 0.148 mmol) was reacted with 57-86% *m*CPBA (34.4 mg, 0.172 mmol) in 7mL CH_2Cl_2 according to the general procedure. TLC indicated the completion of the

reaction after 4.5 hrs (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on PTLC (EtOAc/Hex 1:1). The major sulfinyl isomer was isolated to give 76% yield (51.6 mg, 0.114 mmol); m.p. 154 °C (decomp.); $[\alpha]_D$ -34.6 (c.0.59 , CHCl₃); ¹H NMR (500MHz, CDCl₃): δ= 7.66 (dd, CH arom., 2H), 7.54 (dd, CH arom., 3H), 5.70 (dd, H3, 1H), 5.61 (dd, H2, 1H), 5.31 (t, H4, 1H), 4.59 (m, H5, 1H), 4.55 (d, H1, 1H), 4.24 (dd, H6a, 1H), 4.15 (dd, H6b, 1H), 2.08 (s, Ac, 3H), 2.05 (s, Ac, 3H), 1.97 (s, Ac, 6H); ¹³C NMR (125MHz, CDCl₃): δ= 170.4, 169.6, 169.5, 169.4, 129.5, 129.5, 125.0, 124.3, 94.6(C1), 74.5(C5), 69.4(C3), 65.8(C2), 65.6(C4), 62.5(C6), 20.7, 20.6, 20.5, 20.4; IR (neat, cm⁻¹); 1051.3; FAB- HRMS for C₂₀H₂₅O₁₀S Calc'd 457.1169, found 457.1166 (M+1 22.2%)

4-Methylphenylsulfinyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (6b)

4b (284.4 mg, 0.626 mmol) was reacted with 57-86% *m*CPBA (157.3 mg, 0.622 mmol) in CH₂Cl₂ (15 mL) according to the general procedure. TLC indicated the completion of the reaction after 3.5 hrs (ether/hex 9:1) The reaction was worked up as described and chromatographed on PTLC (ether/hex 9:1). The major sulfinyl isomer was isolated to give 161.4mg, 55%yield; m.p. 114-115 °C; $[\alpha]_D$ -92.0 (c. 0.49 , CHCl₃); ¹H NMR (500MHz, CDCl₃): δ = 7.55 (d, CH arom., 2H), 7.35 (d, CH arom., 2H), 5.70(dd, H3, 1H), 5.63 (dd, H2, 1H), 5.30 (t, H4, 1H), 4.56 (m, H5, 1H), 4.50 (d, H1, 1H), 4.22 (dd, H6a, 1H), 4.13 (dd, H6b, 1H), 2.39 (s, CH₃ arom., 3H), 2.07 (s, Ac, 3H), 2.04 (s, Ac, 3H), 1.98 (s, Ac, 3H), 1.96 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 142.5, 136.6, 130.2, 125.1, 94.6, 74.4, 69.4, 66.0, 65.7, 62.5, 21.4, 20.96, 20.63, 20.61, 20.57 ; IR

(neat, cm^{-1}); 1050.5; FAB-MS calc'd for $\text{C}_{21}\text{H}_{26}\text{O}_{10}\text{S}$ 470.21, found 471.1390 ($\text{M}+1$ 13.6 %)

4-Methoxyphenylsulfinyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (6c)

4c (339.6 mg, 0.722 mmol) was reacted with 57-86% *m*CPBA (160 mg, 0.801 mmol) in CH_2Cl_2 (10 mL) according to the general procedure. TLC indicated the completion of the reaction after 5 hrs (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on PTLC (EtOAc/Hex 1:1). The major sulfinyl isomer was isolated to give 59% yield (206.0 mg, 0.423 mmol); m.p. (135 °C decomp); $[\alpha]_{\text{D}}$ +97.2 (c. 0.9, CHCl_3); ^1H NMR (500MHz, CDCl_3): δ = 7.60 (dd, CH arom., 2H), 7.05 (dd, CH arom., 2H), 5.71 (dd, H3, 1H), 5.69 (dd, H2, 1H), 5.31 (t, H4, 1H), 4.53 (m, H5, 1H), 4.47 (d, H1, 1H), 4.22 (dd, H6a, 1H), 4.12 (dd, H6b, 1H), 3.83 (s, Ph-OCH₃, 3H), 2.07 (s, Ac, 3H), 2.04 (s, Ac, 3H), 2.01 (s, Ac, 3H), 1.97 (s, Ac, 3H); ^{13}C NMR (125MHz, CDCl_3): δ = 170.4, 169.6, 169.5, 169.4, 130.5, 126.4, 115.1, 94.6(C1), 74.4(C5), 69.5(C3), 65.9(C2), 65.7(C4), 62.5(C6), 55.5 (OCH₃), 20.7, 20.6, 20.5; IR (neat, cm^{-1}); 1052.3; FAB-HRMS for $\text{C}_{21}\text{H}_{26}\text{O}_{11}\text{S}$ Calc'd 487.1274 (M^++1), found 487.1297 (100 %)

4-Nitrophenylsulfinyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (6d)

4d (70.2 mg, 0.145 mmol) was reacted with *m*CPBA (31.8 mg, 0.159 mmol) in CH_2Cl_2 (5mL) according to the general procedure. TLC indicated the completion of the reaction after 45 min (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on PTLC (eluent EtOAc: Hex 1:1). The major sulfinyl isomer was isolated to give 61% yield (43.5 mg, 0.087 mmol); $[\alpha]_{\text{D}}$ +104.8 (c. 0.65, CHCl_3); ^1H

NMR (500MHz, CHCl₃): δ = 8.94 (d, CH arom., 2H), 7.92 (d, CH arom., 2H), 6.23 (dd, H2, 1H), 5.30 (d, H1, 1H), 5.22 (dd, H3, 1H), 5.07 (t, H4, 1H),), 4.53 (dd, H6a, 1H), 4.44 (dd, H6b, 1H), 3.96 (m, H5, 1H), 2.03 (s, Ac, 3H), 1.99 (s, Ac, 3H), 1.82 (s, Ac, 6H); ¹³C NMR (125MHz, CDCl₃): δ = 171.2, 169.8, 169.7, 169.1, 144.9, 142.9, 132.5, 131.7, 96.2(C1), 74.7(C5), 71.7(C3), 71.5(C4), 70.2(C2), 62.5(C6), 20.7, 20.6, 20.5; IR (neat, cm⁻¹); 1050.1; FAB-MS for C₂₀H₂₃NO₁₂S Calc'd 501.0940, found 472.1221 (M-NO₂+1, 2.5%)

Phenylsulfinyl α -D-mannopyranoside (7a)

6a (136.2 mg, 0.298 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 82% yield (77.9 mg, 0.245 mmol); $[\alpha]_D +2.33$ (c. 1.4, MeOH); ¹H NMR (500MHz, D₂O): δ = 7.68-7.65 (m, CH arom., 2H), 7.56-7.52 (m, CH arom., 3H), 5.11 (d, H1, 1H), 4.70 (br s, OH, 4H), 4.31 (dd, H2, 1H), 4.18 (dd, H6a, 1H), 4.07 (dd, H6b, 1H), 3.75 (t, H4, 1H), 3.63 (m, H5, 1H), 3.56 (dd, H3, 1H); ¹³C NMR (125MHz, D₂O): 141.8, 130.6, 127.9, 124.9, 98.2(C1), 80.32(C5), 77.8(C3), 74.6(C2), 70.5(C4), 60.3 (C6); IR (neat, cm⁻¹); 1050.4; FAB-HRMS for C₁₂H₁₆O₆S Calc'd 289.0746(M+1), found 289.0690 (M+1 7.4 %)

4-Methylphenylsulfinyl α -D-mannopyranoside (7b)

6b (29.8mg, 0.0634 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 69 % (13.2mg, 0.0437mmol); m.p. 150-151 °C; $[\alpha]_D -5.54$ (c. 0.74, MeOH); ¹H NMR (500MHz, D₂O): δ = 7.62 (dd, CH arom., 2H), 7.24 (dd, CH arom., 2H), 5.23 (d, H1, 1H), 4.75 (br s, OH, 4H), 4.50 (dd, H2, 1H), 4.28 (dd, H6a, 1H),

4.18 (dd, H6b, 1H), 3.89 (t, H4, 1H), 3.57 (m, H5, 1H), 3.64 (dd, H3, 1H), 2.32 (s, Ph-CH₃, 3H); ¹³C NMR (125MHz, D₂O): 141.3, 132.9, 130.0, 98.9(C1), 81.4(C5), 78.2(C3), 76.1(C2), 70.5(C4), 62.1(C6), 21.4(Ph-CH₃); IR (neat, cm⁻¹); 1051.6; FAB-HRMS for C₁₃H₁₈O₆S Calc'd 303.0902 (M+1), found 303.0926 (M+1 79.7 %)

4-Methoxyphenylsulfinyl α -D-mannopyranoside (7c)

6c (44.8 mg, 0.0921 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 86 % yield (25.3 mg, 0.0795 mmol); m.p. (115 °C); [α]_D +0.544 (c. 1.47, MeOH); ¹H NMR (500MHz, D₂O): δ = 7.81 (dd, CH arom., 2H), 7.27 (dd, CH arom., 2H), 4.79 (d, H1, 1H), 4.59 (dd, H2, 1H), 4.19 (dd, H3, 1H), 3.97 (s, Ph-OCH₃, 3H), 3.84 (t, H4, 1H), 3.79-3.76 (m, H5, H6a, 2H), 3.67 (dd, H6b, 1H); ¹³C NMR (125MHz, D₂O): δ = 162.6, 128.2, 127.7, 114.9, 96.7(C1), 78.4(C5), 70.0(C3), 66.5(C2), 65.4(C4), 60.2(C6), 55.2 (-OCH₃); IR (neat, cm⁻¹); 1051.8; FAB-HRMS for C₁₃H₁₈O₇S Calc'd 319.0852 (M+1), found 319.1055 (M+1 29.1 %)

4-Nitrophenylsulfinyl α -D-mannopyranoside (7d)

6d (30.0 mg, 0.059 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 75% yield (21.1mg, 0.044mmol); ¹H NMR (500MHz, D₂O): δ = 8.86 (dd, CH arom., 2H), 7.85 (dd, CH arom., 2H), 5.37 (br s, OH, 4H), 5.35 (d, H1, 1H), 4.61 (dd, H2, 1H), 4.39 (dd, H3, 1H), 4.35 (dd, H6a, 1H), 4.15(dd, H6b, 1H), 3.89(m, H4, H5, 2H), 3.57 (dd, H3, 1H); ¹³C NMR (125MHz, D₂O): δ = 144.4, 142.8, 131.7, 130.8, 98.2(C1), 80.3(C5), 77.8(C3), 74.6(C2), 70.5(C4), 60.2(C6); IR (neat,

cm⁻¹): 1050.3; FAB- HRMS for C₂₄H₁₅NO₈S Calc'd 477.0519, found 448.1618 (M-NO+1 6.3%)

Phenylsulfonyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (8a)

4a (92.8 mg, 0.212 mmol) was reacted with 57-86% *m*CPBA (140.4mg, 0.465 mmol) in CH₂Cl₂ (5mL) according to the general procedure. TLC indicated the completion of the reaction after 55min (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on a silica gel column (EtOAc/Hex 1:1) to yield the title compound in 89% yield (101.5 mg, 0.203mmol); m.p. (105-106 °C); [α]_D +31.1 (c.0.18 , CHCl₃); ¹H NMR (500MHz, CDCl₃): δ = 7.92 (dd, CH arom., 2H), 7.69 (td, CH arom., 1H), 7.57 (td, CH arom., 2H), 5.98 (dd, H2, 1H), 5.68 (dd, H3, 1H), 5.25 (t, H4, 1H), 4.77 (m, H1 &H5, 2H), 4.21 (dd, H6a, 1H), 4.18 (dd, H6b, 1H), 2.08 (s, Ac, 3H), 2.04 (s, Ac, 3H), 2.01 (s, Ac, 3H), 1.99 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 170.2, 169.6, 169.3, 169.2, 136.2, 134.6, 129.3, 129.1, 90.2(C1), 73.3(C5), 68.8(C3), 65.4(C2), 65.2(C4), 62.5(C6), 20.6, 20.5, 20.4, 20.3; IR (neat, cm⁻¹): 1316.1 (asym), 1140.3 (sym); FAB-MS for C₂₀H₂₄O₁₁S Calc'd 472.41, found 473.11 (M+1, 2.8%)

4-Methylphenylsulfonyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (8b)

4b (41.1 mg, 0.090 mmol) and *m*CPBA (65.6, 0.22 mmol) were reacted according the general procedure until TLC (EtOAc/ Hex 1:1) indicated completion of the reaction (1hr). The reaction mixture was worked up and purified according to the general procedure to afford **8b** (37.4mg, 85%); ¹H NMR (500MHz, CDCl₃): δ = 7.81 (d, CH arom., 2H), 7.36 (d, CH arom., 2H), 5.98(dd, H2, 1H), 5.71 (dd, H3, 1H), 5.27 (t, H4,

1H), 4.79 (m, H5, 1H), 4.75 (d, H1, 1H), 4.19 (dd, H6a, 1H), 4.05 (dd, H6b, 1H), 2.44 (s, CH₃ arom., 3H), 2.10 (s, Ac, 3H), 2.06 (s, Ac, 3H), 2.04 (s, Ac, 3H), 2.00 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ = 133.2, 130.0, 129.6, 129.2, 90.4 (C1), 73.3 (C5), 68.8 (C3), 65.5 (C2), 65.4 (C4), 62.6 (C6), 21.73, 20.65, 20.63, 50.51; IR (neat, cm⁻¹): 1317.1 (asym), 1139.2 (sym); FAB-MS: calc'd for C₂₁H₂₆O₁₁S= 486.49, found 525.3 [M+K⁺] (100%)

4-Methoxyphenylsulfonyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (8c)

4c (85.6 mg, 0.125 mmol) was reacted with 57-86% *m*CPBA (79.0 mg, 0.262 mmol) in CH₂Cl₂ (5 mL) according to the general procedure. TLC indicated the completion of the reaction after 40 min (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on a silica column to yield **8c** in 96% yield (60.2 mg, 0.120 mmol); m.p. (135-137°C (decomp.); [α]_D +36.9 (c.0.86, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ= 7.85 (dd, CH arom., 2H), 7.01 (dd, CH arom., 2H), 5.96 (dd, H2, 1H), 5.70 (dd, H3, 1H), 5.27 (t, H4, 1H), 4.76 (m, H5, 1H), 4.71 (d, H1, 1H), 4.19 (dd, H6a, 1H), 4.05 (dd, H6b, 1H), 3.87 (s, Ph-OCH₃, 3H), 2.09 (s, Ac, 3H), 2.05 (s, Ac, 3H), 2.04 (s, Ac, 3H), 1.99 (s, Ac, 3H); ¹³C NMR (125MHz, CDCl₃): δ= 170.3, 169.6, 169.3, 164.6, 131.4, 114.6, 90.4(C1), 73.2(C5), 68.8(C3), 65.5(C2), 65.4(C4), 62.6(C6), 55.7(OCH₃), 20.6, 20.5; IR (neat, cm⁻¹): 1319.5 (asym), 1143.7 (sym); FAB- HRMS for C₂₁H₂₇O₁₂S Calc'd 503.1223, found 503.1278 (M+1 100%)

4-Nitrophenylsulfonyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (8d)

4d (107.1 mg, 0.220 mmol) was reacted with 57-86% *m*CPBA (159.6 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) according to the general procedure. TLC indicated the completion of the reaction after 24 hrs (EtOAc/Hex 1:1) The reaction was worked up as described and chromatographed on a silica column to yield **8d** in 84% yield (95.7 mg); $[\alpha]_{\text{D}} +36.9$ (c.0.86, CHCl_3); ^1H NMR (500MHz, CDCl_3): δ = 8.97 (dd, CH arom., 2H), 8.03 (dd, CH arom., 2H), 5.80 (dd, H2, 1H), 5.67 (dd, H1, 1H), 5.18 (dd, H3, 1H), 5.06 (t, H4, 1H), 4.30(dd, H6a, 1H), 4.20 (dd, H6b, 1H), 3.73 (m, H5, 1H); ^{13}C NMR (125MHz, D_2O): δ = 171.5, 169.7, 169.6, 168.7, 132.2, 131.8, 90.9(C1), 75.3(C5), 72.6(C3), 72.1(C2), 65.3(C4), 62.1(C6), 20.6, 20.5; IR (neat, cm^{-1}): 1315.3 (asym), 1139.4 (sym)

Phenylsulfonyl α -D-mannopyranoside (9a)

8a (109.5 mg, 0.218 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 83 % yield (60.2 mg, 0.180 mmol); m.p. (63-65 $^{\circ}\text{C}$); $[\alpha]_{\text{D}} +81.8$ (c. 1.41, MeOH); ^1H NMR (500MHz, D_2O): δ = 8.01 (dd, CH arom., 2H), 7.88 (tt, CH arom., 2H), 7.75 (td, CH arom., 2H), 5.18 (d, H1, 1H), 4.75 (dd, H2, 1H), 4.29 (m, H5, 1H), 4.21 (dd, H3, 1H), 3.86 (dd, H6a, 1H), 3.81 (t, H4, 1H), 3.73 (dd, H6b, 1H); ^{13}C NMR (125MHz, D_2O): δ = 134.9, 134.2, 129.2, 128.3, 92.9(C1), 77.5(C5), 70.3(C3), 65.9-65.3(C2, C4), 60.5(C6); IR (neat, cm^{-1}): 1316.5 (asym), 1136.6 (sym); FAB-HRMS for $\text{C}_{12}\text{H}_{17}\text{O}_7\text{S}$ Calc'd 305.0695, found 305.0679 (M+1 100 %)

4-Methylphenylsulfonyl α -D-mannopyranoside (9b)

8b (81.0mg, 486.21mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 56 % (29.8mg, 0.935mmol); oil; $[\alpha]_D +91.8$ (c. 0.92, MeOH); ^1H NMR (500MHz, D_2O): $\delta = 7.91$ (d, CH arom., 2H), 7.60 (d, CH arom., 2H), 5.16 (d, H1, 1H), 4.73 (dd, H2, 1H), 4.28 (m, H5, 1H), 4.21 (dd, H3, 1H), 3.85 (dd, H6a, 1H), 3.81 (t, H4, 1H), 3.72 (dd, H6b, 1H), 2.53 (s, Ph- CH_3 , 3H); ^{13}C NMR (125MHz, D_2O): $\delta = 146.8, 131.0, 129.8, 128.3, 93.0(\text{C}1), 77.5(\text{C}5), 70.2(\text{C}3), 65.3, 65.2(\text{C}2 \& \text{C}4), 60.4(\text{C}6), 20.4(\text{Ph-CH}_3)$; IR (neat, cm^{-1}): 1317.3 (asym), 1137.5 (sym); FAB-MS for $\text{C}_{13}\text{H}_{18}\text{O}_7\text{S}$ Calc'd 318.14, found 319.13 (M+1 2.1 %)

4-Methoxyphenylsulfonyl α -D-mannopyranoside (9c)

8c (32.3mg, 0.0643mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 85 % (18.3 mg, 0.0548 mmol); m.p (68-69 °C); $[\alpha]_D +94.9$ (c. 0.64 , MeOH); ^1H NMR (500MHz, D_2O): $\delta = 7.95$ (d, CH arom., 2H), 7.27 (d, CH arom., 2H), 5.14 (d, H1, 1H), 4.73 (dd, H2, 1H), 4.28 (m, H5, 1H), 4.21 (dd, H3, 1H), 3.99 (s, Ph- OCH_3 , 3H), 3.86 (dd, H6a, 1H), 3.81 (t, H4, 1H), 3.72 (dd, H6b, 1H); ^{13}C NMR (125MHz, D_2O): $\delta = 164.1, 130.8, 125.5, 114.6, 93.7(\text{C}1), 77.4(\text{C}5), 70.2(\text{C}3), 65.4(\text{C}2), 65.3(\text{C}4), 60.5(\text{C}6), 55.4(\text{Ph-OCH}_3)$; IR (neat, cm^{-1}): 1318.5 (asym), 1139.3 (sym); FAB-MS for $\text{C}_{13}\text{H}_{18}\text{O}_8\text{S}$ Calc'd 334.14, found 335.15 (M+1 1.1 %)

4-Nitrophenylsulfonyl α -D-mannopyranoside (9d)

8d (38.0 mg, 0.0734 mmol) was treated with 1N NaOMe according the general procedure to yield the title compound in 89% yield (23.0 mg, 0.0659 mmol); (oil); $[\alpha]_D +22.5$ (c.

0.8 , MeOH); ^1H NMR (500MHz, D_2O): δ = 8.88 (dd, CH arom., 2H), 7.97 (dd, CH arom., 2H), 5.47 (d, H1, 1H), 5.04 (br s, OH, 4H), 3.96 (dd, H6a, 1H), 3.86-3.56 (m, H2, H4, H6b, 3H), 3.52 (dd, H3, 1H), 3.42-3.39 (m, H5, 1H); ^{13}C NMR (125MHz, D_2O): δ = 144.9, 139.3, 131.4, 130.9, 98.9(C1), 83.6 (C5), 78.3(C3), 77.0(C2), 70.5 (C4), 61.9(C6); IR (neat, cm^{-1}): 1316.2 (asym), 1136.4 (sym); FAB-MS for $\text{C}_{12}\text{H}_{15}\text{NO}_9\text{S}$ Calc'd 349.0467, found 310.7461 ($\text{M}^+ - \text{NO}_2$ 2.5 %)

2,3,4,6-tetra-*O*-acetyl mannopyranosyl bromide (10)

To a solution of 1 (307.7mg, 0.789 mmol) in dichloromethane (3mL) was added a solution of 45% HBr in dichloromethane (3.5 equiv. in 2mL) and the mixture was left to stir under ambient conditions for 1.5 hrs. The reaction solution was diluted with CH_2Cl_2 and poured into 100 ml of ice-cold saturated NaHCO_3 , with continuing stirring. Once the fizzing stopped, the aqueous layer was decanted and the organic layer was transferred into a separatory funnel, washed with H_2O (2X), saturated NaCl solution (2X), dried over Na_2SO_4 then evaporated to afford to the title compound in 95% yield (305.1 mg, 0.746 mmol).

CHAPTER III
SYNTHESIS AND INHIBITORY EFFECTS OF 2-,
6-, AND 2-, 6-MODIFIED METHYL
MANNOPYRANOSIDE

3.1 Introduction

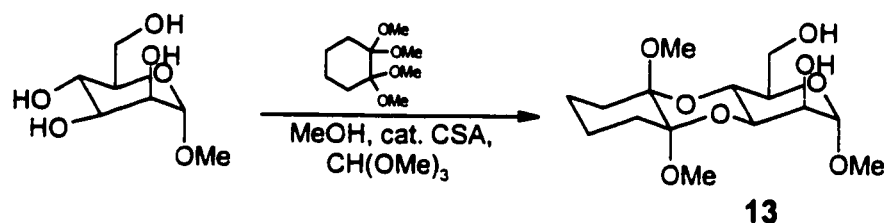
Chapter II concluded that *S*-mannopyranosides and their sulfoxide and sulfone derivatives are better inhibitors than the corresponding *O*-mannopyranosides. Ensuing our strategy outlined in chapter I, we next tackled the task of modifying mannose at the 2- and 6-positions. We exploited methyl α -D-mannopyranoside as our model compound that we derivatized.

Three series of molecules were prepared: *a*) only 6-modified, where methyl 6-amino-6-deoxy- α -D-mannopyranoside was prepared; *b*) only 2-modified, where methyl 2-*O*-alkyl- α -D-mannopyranosides were prepared; *c*) 2,6-modified, where methyl 2-*O*-alkyl-6-amino-6-deoxy- α -D-mannopyranosides were prepared. For the latter two series, we were interested in preparing a series of molecules with different alkyl groups (R= Me, Et, Pr, Bn), however, due to time limitations only two compounds in each series were prepared. This chapter will focus on the synthesis of the above mentioned compounds and on their inhibitory effects evaluated by ELLA.

Manipulation of saccharides often presents a challenge due to the presence of multiple, reactive hydroxyl groups. Although some preference in reactivity does exist, it is often insufficient for selective manipulation. The relative reactivity of the hydroxyl groups in α -D-mannose is OH-6 > OH-3 > OH-2 > OH-4.³³ OH-6 is distinct and is the most reactive since it is a primary alcohol and its manipulation is often achievable with no protection for the other hydroxyls. The order of reactivity of OH-2 relative to OH-3 is

rather surprising since OH-2 is expected to be more reactive due to its activation by the anomeric center. Its axial disposition, however, is believed to be the reason for its reduced reactivity compared to OH-3.³² The greater reactivity of OH-2 relative to OH-4 is difficult to explain and suggests that OH-2 experiences less unfavorable interactions, and/or is more activated by the anomeric center. Selective manipulation of any of the secondary hydroxyl groups requires protection.

Ley and co-workers³⁴ published a protection method for methyl α -D-mannopyranoside that protects hydroxyls 3 and 4, simultaneously, while leaving hydroxyls 2 and 6 free (scheme 3.1.1). This method was found ideal for our purposes since we were interested in 2,6-derivatization of methyl α -D-mannopyranoside.



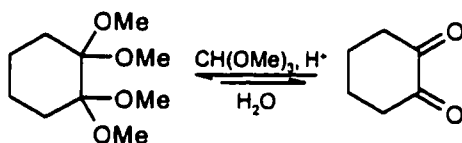
Scheme 3.1.1 Synthesis of cyclohexane-1,2-diacetal protected mannopyranoside

3.2 Synthesis of methyl cyclohexane-1,2-diacetal-protected mannopyranoside (13)

Synthesis of the protected saccharide, 13, was easily achieved by treating methyl α -D-mannopyranoside with 1.4 equivalents of 1,1,2,2-tetramethoxycyclohexane in boiling methanol in the presence of trimethyl orthoformate and catalytic amount of camphor sulfonic acid (CSA). The reaction proceeded over a period of 16 hours to yield

known **13** as the major product. Ley reported a yield of 48% for the title compound; however, we managed to increase the yield to 55% under the same reaction conditions. Purification was easily achievable by column chromatography.

Trimethyl orthoformate is used to ensure that the acetal reagent does not decompose by adventitious water (e.g. water of crystallization in the sugar substrate). In the presence of water and under acidic conditions, tetramethoxycyclohexane is in equilibrium with cyclohexane-1,2-dione (scheme 3.2.1).



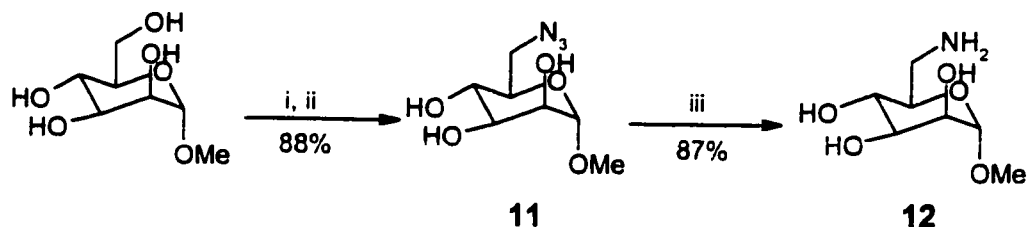
Scheme 3.2.1 Under acidic conditions 1,1,2,2-tetramethoxy cyclohexane is in equilibrium with cyclohexane-1,2-dione

3.3 Synthesis of methyl 6-amino-6-deoxy- α -D-mannopyranoside

The synthesis of methyl 6-amino-6-deoxy- α -D-mannopyranoside was achieved via a two-step reaction directly from methyl α -D-mannopyranoside. Protection of the sugar was not necessary since OH-6 is a primary alcohol, and hence the most susceptible to nucleophilic substitution.

Scheme 3.3.1 depicts the synthesis of the title compound. Methyl α -D-mannopyranoside is reacted with *N*-iodosuccinimide and triphenylphosphine in DMF to give a 6-iodo intermediate, which undergoes nucleophilic substitution reaction, by an

azido ion upon the addition of NaN_3 . The reaction yields the 6-azido derivative, **11** in an excellent yield (88%).³⁵ Hydrogenation of **11** yields the 6-amino derivative, **12**, as the sole product in 87% yield.



Scheme 3.3.1 Synthesis of methyl 6-aminomannopyranoside Reagents and conditions: i) Ph_3P , NIS, DMF, 2.5hrs; ii) NaN_3 , DMF, 2.5hrs; iii) H_2/Pd , MeOH, 1hr

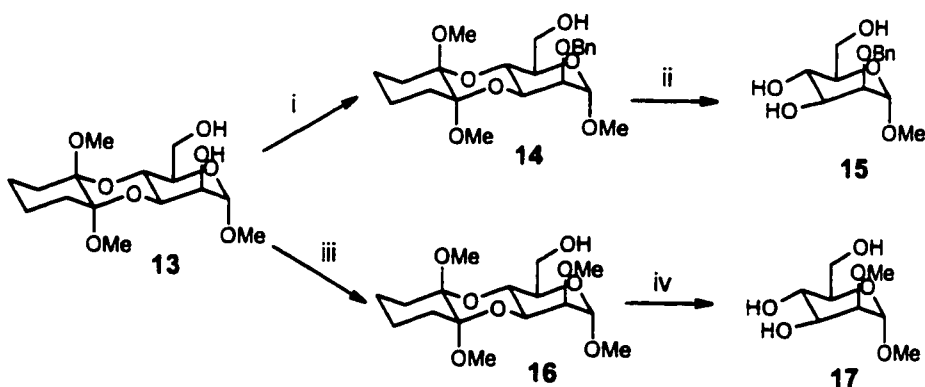
Another method that was investigated to prepare **11** was selective tosylation of OH-6 in pyridine followed by *in-situ* azide displacement.³⁶ Reaction time for this method was comparable to that of the NIS method; however, the overall yield of the reaction was poor (23%).

All the physical and spectroscopic data obtained for **11** and **12** were consistent with the literature.³⁷ The IR spectrum of **11** shows the characteristic N_3 stretch at 2101 cm^{-1} . This stretch disappears in the IR spectrum of **12**, where a stretch at 3400 cm^{-1} and a broad bend at 1600 cm^{-1} appear, indicating the presence of primary amine functionality. The NMR spectra of both compounds are consistent with their structures. The ^1H NMR spectra for both **11** and **12** are similar. The anomeric proton is the most deshielded for both compounds and resonates at δ 4.68 ppm and δ 4.53 ppm, respectively. The anomeric OMe gives rise to a singlet at δ 3.36 for **11** and δ 3.28 ppm for **12**. The remaining protons

in the azide derivative, 11, give rise to two signals, a multiplet resonating in the region between δ 3.82 and 3.64 ppm and a singlet at δ 3.50 ppm. The spectrum of the amine derivative, 12, on the other hand, is more resolved where distinct peaks could be seen for each proton.

3.4 Synthesis of methyl 2-*O*-alkylated- α -D-mannopyranosides

Ley *et al.*³¹ demonstrated that when the protected methyl mannopyranoside, 13, is treated with sodium hydride and one equivalent of benzyl bromide, selective alkylation at the 2-position occurs. This unexpected selectivity for the secondary alcohol over the primary alcohol was also reported for the benzylation of methyl 3,4-*O*-isopropylidene- α -D-galactopyranoside.³⁸ Based on these reports we embarked on the synthesis of methyl 2-*O*-alkylated- α -D-mannopyranosides, directly from 13 without further protection. Two such compounds were prepared: methyl 2-*O*-benzyl- α -D-mannopyranoside, 15, and methyl 2-*O*-methyl- α -D-mannopyranoside, 17 (scheme 3.4.1).



Scheme 3.4.1 Synthesis of methyl 2-*O*-alkylated mannopyranosides. Conditions: *i*) NaH (1.1 equiv.), BnBr (1.1 equiv.), DMF, 46%; *ii*) 40% TFA, 65%; *iii*) NaH (1.1 equiv.), MeI(1.1 equiv.), DMF, 47%; *iv*) 20:1 TFA-H₂O, 60%

Alkylation reactions of **13** were conducted in DMF using sodium hydride (1.1 equivalents) and the appropriate alkylating agent (1.1 equivalents). Benzylation of **13** using Benzyl bromide in DMF furnished **14** (the 2-*O*-benzyl derivative of **13**) in 46% yield along with the 2,6-di-*O*-benzylated derivative of **13** in 24% yield. As can be seen from the yields, there is a degree of selectivity for the alkylation of OH-2, but not as high as that reported by Ley *et al.*³² The group reported a much higher yield of 75% for the 2-*O*-benzylated derivative. To confirm that we did, indeed, obtain the 2-*O*-benzylated derivative, we compared the optical rotation and ¹H NMR spectra of **14** to those reported in the literature.³² The optical rotation for **14** (+37, *c* 0.85 in CHCl₃) was found consistent with that reported by Ley (+33, *c* 0.96 in CHCl₃). The ¹H NMR spectrum of **14** shows the aromatic protons of the benzyl substituent resonating in the region between δ 7.42 and 7.22 ppm. The signal integrates to five aromatic protons indicating that the product, **14**, is monoalkylated. The benzylic protons can be seen as two doublets at δ 4.94 ppm and δ 4.56 ppm. The anomeric proton gives rise to a doublet at δ 4.62 ppm. The two H6 protons appear as a multiplet between δ 4.34 and 4.06 ppm. The remaining protons of the sugar moiety resonate as a multiplet between δ 3.76 and 3.67 ppm. The anomeric methoxy substituent gives rise to a singlet at δ 3.26 ppm, whereas the methoxy substituents of the protecting unit give rise to two singlets at δ 3.20 and 3.21 ppm. The methylene protons of the cyclohexane ring of the protecting unit give rise to two multiplets that resonate in the region between δ 1.91 and 1.37 ppm. All the signals are consistent with those reported by Ley for the 2-*O*-benzylated derivative of **13**.³² Based on the consistency of the data obtained with that in the literature, we concluded that we did obtain the 2-*O*-benzylated derivative. Ley derived his conclusion that the product is the 2-*O*-benzylated derivative

based on the multiplicity of the hydroxy proton in the ^1H NMR and by observing a long range C-H correlation between the benzylic protons and C-2 of the sugar ring in the HMBC spectrum.

Deprotection of **14** to yield methyl 2-*O*-benzyl- α -D-mannopyranoside (**15**) was effected using 40% TFA. The reaction proceeded over a period of 24hrs to yield the title compound in modest yield (56%). The ^1H NMR spectrum of **15** agrees with its structure. The two-methoxy singlets of the protecting unit disappear indicating its removal, as do the multiplets in the δ 1.4-1.9 ppm region arising from the methylene protons of the cyclohexane ring. The aromatic protons of the benzyl moiety resonate in about the same region as the protected precursor (δ 7.34-4.26 compared to δ 7.42-7.22 ppm for **15** and **14**, respectively). The benzylic protons shift downfield in **15** compared to **14** (δ 5.20 and δ 5.03 ppm compared to δ 4.94 and δ 4.56 ppm, respectively). The anomeric OMe is also deshielded and resonates downfield in **15** relative to **14** (δ 3.47 ppm compared to δ 3.26 ppm, respectively). The anomeric proton has almost identical chemical shift for both compounds (δ 4.66 ppm and δ 4.62 ppm for **15** and **14**, respectively). As was in **14**, the remaining protons of the sugar ring do not give rise to distinct signals, rather they give rise to multiplets resonating in the region between δ 4.46 and δ 3.78 ppm.

Alkylation of **13** with methyl iodide in DMF afforded methyl 2-*O*-methyl- α -D-mannopyranoside, **16** in 47% yield. As was the case with the 2-*O*-benzylated derivative (**14**), some selectivity in alkylation was observed for **16**, but again the 2,6-di-*O*-methyl-derivative formed. The ^1H NMR spectrum of **16** agrees with its structure. Four Me

singlets can be seen in the spectrum: the anomeric OMe resonates at δ 3.42 ppm, the 2-O-Me resonates at δ 3.12 ppm and the two OMe substituents of the protecting unit resonate at δ 3.14 ppm and δ 3.12 ppm. The anomeric proton is the most deshielded proton and resonates at δ 4.67 ppm. The remaining protons of the sugar moiety give rise to multiplets in the region between δ 1.75 and 1.27 ppm. The ^{13}C NMR spectrum of **16** shows signals accounting for all carbon atoms. C1 is the most deshielded carbon and hence resonates downfield at δ 101.3 ppm. C6 is the least deshielded carbon of the sugar ring and resonates at δ 61.5 ppm. The other four carbons of the sugar ring resonate in the region between δ 99.2 ppm and δ 64.3 ppm. Carbons of the four O-CH₃ substituents resonate at δ 58.9, 54.7, 46.7 and 46.5 ppm for 1-OMe, 2-OMe, 1'-OMe and 2'-OMe, respectively. The methylene carbons of the cyclohexane ring of the protecting unit resonate in the region between δ 26.9 and 21.2 ppm. FAB-MS of **16** shows a molecular ion peak with a low intensity of 2.1%. The base peak in the spectrum, however, arises from the loss of an OMe residue as is seen in **13**. This fragmentation pattern was reported by Ley³² for the mannopyranoside compounds protected with tetramethoxycyclohexane.

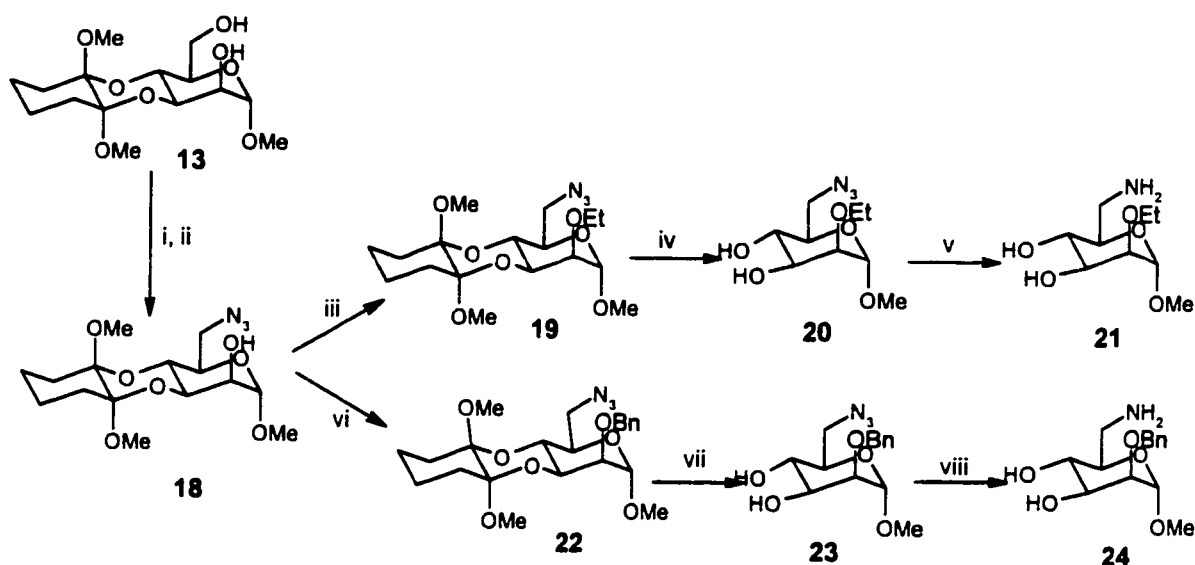
Deprotection of **16** to yield methyl 2-O-methyl- α -D-mannopyranoside, **17**, was effected using 20:1 TFA/H₂O. Ley investigated different H₂O/TFA mixtures for different compounds and reported what is most effective for each type of compound. The deprotection reaction was complete in a much shorter time period than **14** (30 minutes only). Isolation of **17** was achieved by column chromatography in 60% yield. The NMR spectra of **17** confirm its structure. ^1H NMR of the compound shows only two OMe singlets that correspond to the anomeric O-Me and the 2-O-Me substituents at δ 3.38 and

3.29 ppm. The methylene protons of the protecting cyclohexane ring disappear in the ^1H NMR spectrum of **17** confirming the complete removal of the protecting unit. The anomeric proton resonates at δ 4.59 ppm compared to δ 4.67 ppm for the protected compound, **16**. Only H2 can be seen as a clear doublet at δ 3.40 ppm, whereas the remaining protons of the sugar ring appear as a multiplet between δ 3.78 and 3.53 ppm. The ^{13}C NMR spectrum of **17** is fairly simple showing signals that account for all carbons in the molecule. The anomeric carbon is the most deshielded giving rise to a signal at δ 98.2 ppm. C6 is the most shielded carbon of the sugar ring, giving rise to a signal at δ 61.4 ppm. The remaining carbons of the sugar ring resonate in the region between δ 77.8 and 67.5 ppm. Carbons of the O-Me substituents resonate at δ 58.9 and 54.6 ppm. The molecular ion peak+1 is present in the FAB-MS spectrum of **17** with a modest intensity of 15.2%.

3.5 Synthesis of methyl 2-O-alkyl-6-amino-6-deoxy- α -D-mannopyranosides

Once methyl α -D-mannopyranoside was protected as described in section 3.2 to yield **13**, it was modified at the 6-position first, then the 2-position. This preferred sequence ensures that only one product is formed. Section 3.3 demonstrated that adding an azide at the 6-position is selective and no 2-azido forms. Section 3.4 demonstrated that manipulation at the 2-position without protection of the 6-position results in a mixture of products. Hence, once the azide is added to the 6-position, alkylation of the resulting molecule ensures that only one product forms, methyl 2-O-alkyl-6-azido-6-deoxy- α -D-mannopyranoside. Two molecules were synthesized in this series: methyl 6-azido-6-

deoxy-2-*O*-ethyl- α -D-mannopyranoside, **19**, and methyl 6-azido-6-deoxy-2-*O*-benzyl- α -D-mannopyranoside, **22**, as illustrated in Scheme 3.5.1.



Scheme 3.5.1- Synthesis of methyl 2-*O*-alkyl-6-amino-6-deoxy- α -D-mannopyranosides. Reagents and conditions: i) Ph_3P , NIS, DMF, 1.5hrs; ii) NaN_3 , DMF, 2.5hrs, 46%; iii) NaH , EtI, DMF, 24 hrs, 74%; iv) 20:1 TFA/ H_2O , 1hr, 49%; v) H_2 / Pd , EtOH, 24hrs, 98%; vi) NaH , BnBr , DMF, 81%; vii) 40% TFA, 24hrs, 45%; viii) H_2 / Pd , MeOH, 78%

Synthesis of protected methyl mannopyranoside, **13**, followed by its reaction to form the azide (as in scheme 3.5.1) gave a relatively low yield, 48% for **18**. As a result, we attempted the synthesis of azide **11** first, followed by its protection with tetramethoxycyclohexane to yield **18**. This sequence, however, failed to proceed and the cyclohexane reagent was recovered in 85% yield.

IR spectroscopy was the most useful technique for confirming the formation of azide **18**. The IR spectrum shows the characteristic N_3 stretch at 2099 cm^{-1} , indicating the formation of the azido-derivative. The presence of the azide substituent on C6 had no great effect on the NMR spectra of **13**, and hence both the azide derivative **18** and the

precursor **13** had almost identical NMR spectra.. The ^1H NMR spectrum of **18** shows the two diagnostic methoxy singlets (from the protecting unit) seen in **13** at almost the same chemical shifts (δ 3.19, and 3.16 ppm compared to δ 3.21 and 3.20 ppm, respectively). The chemical shift of the anomeric proton is identical in both **18** and **13** (δ 4.71 ppm). The anomeric methoxy of **18** resonates at almost the same chemical shift as that of **13** (δ 3.38 and 3.35 ppm, respectively). The ^1H NMR spectrum of **18** is less resolved, however, where the remaining sugar protons give rise to overlapping signals resulting in multiplets. The ^{13}C NMR spectra of both compounds are identical. key chemical shift include that of C1 which gives rise to a signal δ 101.1 ppm for both **13** and **18**, the two diagnostic methoxy group of the protecting moiety which resonate at δ 46.7 and 46.9 ppm for both **13** and **18**. The fragmentation pattern in the FAB-mass spectrum of **18** resembles that of **13**. The base peak for both results from the loss of an -OMe fragment (M-31, 100%).

Alkylation of **18** was conducted in DMF using sodium hydride and the appropriate alkylating agent to furnish **19** and **22** in good yields (74% and 81%, respectively). Deprotection of **19** was achieved by its treatment with TFA-water (20:1) mixture over a period of 1 hr to yield **20** in a decent yield (49%). 40% TFA solution was used to deprotect **22** and yield **23** in 45%. The NMR spectra of the deprotected products, **15** and **17**, lack the diagnostic, two methoxy signals of the protecting unit indicating its complete removal. The IR spectra retained the N_3 stretch, as expected, and showed the appearance of an OH stretch around 3000 cm^{-1} .

Finally, hydrogenation of **20** and **23** over palladium catalyst yielded the target methyl 6-amino-6-deoxy-2-*O*-alkyl- α -D-mannopyranosides in excellent yields (98% and 78% for R= Et and Bn, respectively). IR spectra of these compounds showed the disappearance of the N₃ stretch and the appearance of a stretch in the 3400 cm⁻¹ region along with a bend around 1600 cm⁻¹ indicating the reduction of the azide functionality to primary amine functionality.

3.6 Inhibitory Effect of methyl 6-amino-6-deoxy- and 2-*O*-alkyl-6-amino-6-deoxy- α -D-mannopyranosides

Three compounds were tested for their inhibitory effect on the binding of Con A to yeast mannan: methyl 6-amino-6-deoxy- α -D-mannopyranoside (**12**), methyl 2-*O*-ethyl-6-amino-6-deoxy- α -D-mannopyranoside (**21**), methyl 2-*O*-benzyl-6-amino-6-deoxy- α -D-mannopyranoside (**24**). Methyl α -D-mannopyranoside was used as a standard for comparison purposes. Stock solutions were prepared by dissolving 1-3 mg of sample in 1mL PBS solution. Each sample underwent a 2-fold serial dilution and each dilution was tested in duplicate. The preliminary inhibition results obtained by ELLA are shown in table 3.6.1 and Figure 3.6.1. Preliminary results indicate that the modified methyl mannopyranosides are poorer inhibitors than the standard. None of the tested compounds even reached 50% inhibition. However, in order to quantify the inhibitory effect of these compounds, the IC₅₀ values were extrapolated from the inhibition curves. Methyl α -D-mannopyranoside was found to be 25-95 times better inhibitor than the three tested compounds (IC₅₀ 2.78 μ M compared to 42.9-179 μ M, respectively).

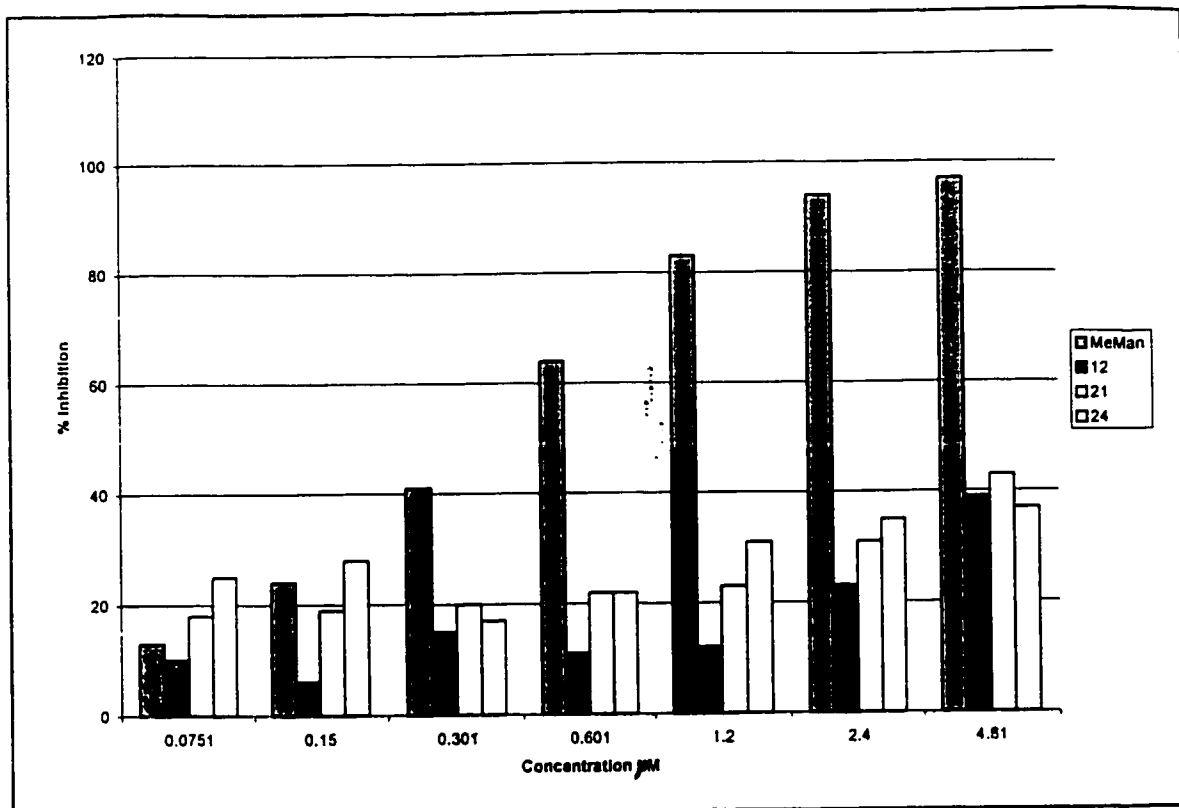


Figure 3.6.1 ELLA preliminary inhibition results for modified methyl mannopyranosides

Table 3.6.1 Preliminary inhibition results for the binding of Con A to yeast mannan by 6- and 2-,6- modified methyl- α -D-mannopyranosides

Compound	IC₅₀[†]	Relative Potency
Methyl Man	2.78	1
12	70.1	0.04
21	42.9	0.065
24	179	0.016

[†]- Values extrapolated from inhibition curves.

3.7 Conclusions

Selective manipulation of the secondary hydroxyl groups of methyl α -D-mannoside requires the use of protecting groups. Hydroxyl-6 is primary and hence can be efficiently manipulated without the use of protecting groups. This chapter demonstrated the synthesis of 2-, 6-, and 2-,6- modified methyl α -D-mannopyranoside. Most of the results are preliminary and further effort is required to refine the results.

Preliminary results of ELLA for the inhibition of the binding of Con A to yeast mannan by compounds **12**, **21**, and **24** showed that they are poorer inhibitors than the standard methyl α -D-mannopyranoside. Further testing is required to obtain actual IC₅₀ values for the above mentioned compounds.

3.8 Experimental

Synthesis of Methyl 6-azido-6-deoxy- α -D-mannopyranoside (11)

Methyl α -D-mannopyranoside (0.55g, 2.83 mmol) and triphenyl phosphine (1.36g, 5.18mmol) were dissolved in anhydrous DMF (20 mL). The solution was cooled in an ice bath and NIS (1.26g, 5.56mmol) was added. The solution turned deep red and emitted heat upon addition of NIS. The reaction flask was equipped with a condenser and heated to 55 °C until TLC indicated the consumption of all starting material (2-3.5 hrs, 10% MeOH in CH₂Cl₂). The solution was then cooled and MeOH was added to destroy excess NIS. NaN₃ was added (1.76g, 27.1mmol) and the reaction mixture heated to 88 °C until tlc indicated completion of reaction (2.5 hrs, 10% MeOH in CH₂Cl₂). DMF was evaporated and co-evaporated with toluene. The brown oily residue was dissolved in water and washed with chloroform/hexane (1:1) solution to remove triphenylphosphine oxide. The aqueous phase was evaporated to dryness and the resulting solid suspended in acetone then filtered. The filtrate was evaporated and the resulting syrup chromatographed on a silica column using 10% MeOH in CH₂Cl₂ to yield 11 in 88% yield (547.5 mg, 2.50mmol); oil;; ¹H NMR (200MHz, D₂O): δ = 4.68 (s, 1H, H1), 4.56 (br s, OH, 3H), 3.82-3.64 (m, 3H), 3.50 (s, 2H), 3.36 (s, 3H, OMe); IR (neat, cm⁻¹): 2101.9; FAB-MS Calcd for C₇H₁₃N₃O₅ 220.09(M+1), found 220.11 (M+1 6.6%), 192.1105 (M-N₂+1 18.0%)

Synthesis of the Methyl 6-amino-6-deoxy- α -D-mannopyranoside (12)

11 (48.2mg, 0.222mmol) was dissolved in ethanol (3mL) and hydrogenated over Pd catalyst (10% on charcoal, 5 mg) under ambient conditions until TLC indicated the completion of the reaction (1 hr, 10% MeOH in CH₂Cl₂). The mixture was filtered over Celite and the filtrate evaporated to yield 12 as the sole product in 87% (37.1 mg, 0.192mmol). No further purification was necessary. m.p. 107 °C (decomp.); [α]_D +66.1 (c. 0.5, MeOH); ¹H NMR (200MHz, D₂O): δ = 4.53 (d, 1H, H1), 3.68 (dd, 1H), 3.55 (dd, 1H), 3.35 (t, 1H, H4), 3.33 (m, 1H, H5), 3.28 (s, 3H, O-CH₃), 2.90 (dd, 1H), 2.7 (dd, 1H); IR (neat, cm⁻¹): 3400.3, 1600.1; FAB-MS Calcd for C₇H₁₅NO₅ 193.09, found 193.14 (0.2 %)

Synthesis of (1'S,2'S)-methyl 3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside (13)

CSA (370.7mg, 1.60 mmol) was added to a solution of methyl α -D-mannopyranoside (2.04g, 10.5 mmol), 1,1,2,2-tetramethoxycyclohexane (3.42g, 16.7mmol) and dry trimethyl orthoformate (0.1 ml/ mmol monosaccharide) in dry MeOH (15mL). The reaction mixture was refluxed for 16 hrs after which it was neutralized with saturated NaHCO₃ and evaporated under reduced pressure. The crude product was purified by column chromatography (gradient elution ether to 4% EtOH in ether) to yield the title compound as an off-white solid in 55% yield (1.91g, 5.73mmol). m.p. 166-168 °C, [α]_D +192 (c 1.0, CHCl₃) [lit. m.p. 168 °C, [α]_D +191 (c 0.94, CHCl₃)]; ¹H NMR (500 MHz, CDCl₃): δ = 4.71 (d, 1H, H1), 4.24 (t, 1H, H4), 4.14 (dd, 1H, H3), 3.92-3.91 (m, 1H, H2), 3.83-3.75 (m, 3H, H5, H6a and H6b), 3.35 (s, 3H, 1-OMe), 3.21 (s, 3H, 1'-OMe), 3.20 (s,

3H, 2'-OMe), 1.97 (br s, 2H, OH-2 and OH-6), 1.77-1.70 (m, 4H, 3'-H and 4'-H), 1.52-1.49 and 1.38-1.34 (2Xm, 2X 2H, 5'-H and 6'-H).

Synthesis of (1'S,2'S)-methyl 2-O-benzyl-3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside (14)

To a solution of 13 (537.2mg, 1.61 mmol) in DMF (8ml) was added sodium hydride (96.1 mg, 2.41 mmol, 60% suspension in mineral oil) and the mixture stirred under N₂ atmosphere at room temperature for 2hrs. Benzyl bromide (0.20ml, 1.77 mmol) was injected into the reaction flask using a syringe and the mixture was left to stir for another 16hrs. Methanol was added to destroy excess NaH and the solution evaporated under reduced pressure. The crude product was chromatographed on a silica column (eluent EtOAc/ Hex 1:1) to yield 14 in 46% yield (314.1 mg, 0.74 mmol) along with (1'S,2'S)-methyl 2,6-O-dibenzyl-3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside in 24% yield.), oil, $[\alpha]_D +37$ (c 0.85, CHCl₃), [lit. $[\alpha]_D +33$ (c. 0.96, CHCl₃)], IR (neat, cm⁻¹): 2099.2; ¹H NMR (200MHz, CDCl₃): δ = 7.42-7.22 (m, H arom, 5H), 4.94 (d, CH_AH_BPh, *J*=11.8, 1H), 4.62 (d, H1, 1H), 4.56(d, CH_AH_BPh, *J*=11.8, 1H), 4.34-4.06 (m, H6a-b, 2H), 3.76- 3.67(m, H2, H3, H4, H5, 4H), 3.26(s, 1-OMe, 3H), 3.20(s, 1'-OMe, 3H), 3.21(s, 2'-OMe, 3H), 2.31 (br s, OH-6, 1H), 1.91-1.71 (m, 3H), 1.67-1.37 (m, 3H)

Synthesis of methyl 2-O-benzyl- α -D-mannopyranoside (15)

To protected compound 14 (314.1mg, 0.74 mmol) was added 2ml of 40% aqueous TFA. The reaction mixture was left to stir for 24hrs after which the solvent was removed under

vacuum and the residue neutralized with 5 drops of triethylamine. The crude was dissolved in minimal amount of CH₂Cl₂ and chromatographed on a silica column (eluent 5%MeOH in CH₂Cl₂) to yield the title compound as oil in 65% yield (136.6 mg, 0.48 mmol). ¹H NMR (200MHz, D₂O): δ= 7.34-7.26 (m, 5H, CH arom.), 5.20 (d, 1H, CH_AH_BPh), 5.03 (d, 1H, CH_AH_BPh), 4.66 (d, 1H, H1), 4.46-4.25 (m, 3H, H3, H6a, H6b), 4.06 (m, 1H, H4), 3.82-3.78 (m, 2H, H2, H5), 3.47 (s, 3H, OCH₃); ¹³C NMR (200MHz, D₂O): δ= 37.9, 128.4, 128.2, 128.9, 98.5 (C1), 80.6, 75.8, 73.7, 70.7, 62.8 (C6), 55.3 (OCH₃); FAB-MS calc'd for C₁₄H₂₀O₆ 285.1338 (M+1) found 285.1352 (M+1)

Synthesis of (1'S,2'S)-methyl 2-O-methyl-3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)-α-D-mannopyranoside (16)

To a solution of 13 (296.6mg, 0.88 mmol) in DMF (4ml) was added sodium hydride (36.9 mg, 0.92 mmol, 60% suspension in mineral oil) and the mixture stirred under N₂ atmosphere at room temperature for 2hrs. Methyl iodide (0.06ml, 0.976 mmol) was injected into the reaction flask using a syringe and the mixture was left to stir for another 16hrs. Methanol was added to destroy excess NaH and the solution evaporated under reduced pressure. The crude product was chromatographed on a silica column (eluent EtOAc/ hex 2:1) to yield 16 in 47% yield (145.2 mg, 0.42 mmol). m.p. (oil); [α]_D +119.8 (c 0.49, MeOH); ¹H NMR (500MHz, CDCl₃): δ= 4.67 (d, 1H, H1), 4.15-4.10 (m, 2H, H3, H4), 3.73 (m, 1H, H6a), 3.69-3.65 (m, 2H, H5, H6b), 3.42 (s, 3H, 1-OCH₃), 3.89 (s, 1H, H2), 3.29 (s, 3H, 2-O-CH₃), 3.14 (s, 3H, 1'-OCH₃), 3.12 (s, 3H, 2-OCH₃), 2.28 (br s, OH-6), 1.75-1.60 (m, 4H), 1.56-1.42 (m, 2H), 1.31-1.27 (m, 2H); ¹³C NMR (125MHz, CDCl₃): δ= 101.3 (C1), 99.2, 98.8, 78.7 (C2), 71.0 (C5), 68.8, 64.3, 61.5 (C6), 58.9 (1-

OCH₃), 54.7 (2-OCH₃), 46.7 (1'-OCH₃), 46.5 (2'-OCH₃), 26.9, 26.8, 21.3, 21.2; FAB-MS calc'd for C₁₆H₂₈O₈ 348.1784 found 348.1471 (2.1%), 317.1560 (M-OMe, 100%)

Synthesis of methyl 2-*O*-methyl- α -D-mannopyranoside (17)

Protected **16** (139.9mg, 0.402 mmol) was dissolved in 1.5ml of 20:1 TFA/H₂O solution. The reaction mixture was stirred for 30 min after which the solvent was removed under vacuum and the residue was neutralized with triethylamine. The crude product was dissolved in minimal amount of MeOH and chromatographed on a silica column to furnish the title compound in 60.3% yield (50.4 mg, 0.242 mmol). ¹H NMR (200MHz, CDCl₃): δ = 4.59 (d, 1H, H1), 3.95-4.10 (br s, 3H, OH), 3.78-3.53 (m, 3H, H5, H6a, H6b), 3.38 (s, 3H, -OCH₃), 3.40 (s, 1H, H2), 3.29 (s, 3H, OCH₃); ¹³C NMR (125MHz, CDCl₃): δ = 98.2 (C1), 77.8, 72.4, 67.5, 61.4 (C6), 58.9, 54.6; FAB-MS calc'd for C₈H₁₆O₆ 208.09 found 209.10 (M+1, 15.2%)

Synthesis of (1'S,2'S)-methyl 6-azido-6-deoxy-3,4-*O*-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside (18)

Protected methyl α -D-mannopyranoside, **13**, (0.72 mg, 2.14 mmol) and triphenyl phosphine (1.18g, 5.18mmol) were dissolved in anhydrous DMF (15 ml). The solution was cooled in an ice bath and NIS (1.02g, 4.49mmol) was added. The reaction flask was equipped with a condenser and heated to 55 °C until TLC indicated the consumption of all starting material (1.5 hrs, EtOAc/ Hex 1:1). The solution was then cooled and MeOH was added to destroy excess NIS. NaN₃ was added (1.38g, 21.4mmol) and the reaction mixture heated to 88 °C until TLC indicated completion of reaction (2.5 hrs, EtOAc/ Hex

1:1). The solution was evaporated and co-evaporated with toluene and the resulting syrup chromatographed on a silica column (eluent EtOAc/ Hex 1:1) to yield **18** in 46% yield (350 mg, 0.97 mmol). m.p. 141-142 °C, $[\alpha]_D +136.8$ (c. 0.83, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ = 4.71 (d, H1, 1H), 4.14-4.08 (m, H3 & H4, 2H), 3.92-3.87 (m, H2 & H5, 2H), 3.49-3.39 (m, H6a & H6b, 2H), 3.38 (s, 1-OMe, 3H), 3.19 (s, 1'-OMe, 3H), 3.16 (s, 2'-OMe, 3H), 2.41 (br s, OH-2, 1H), 1.74-1.65 (m, H3' & H4', 4H), 1.63-1.49 and 1.36-1.34 (2X m, H5' and H6', 2H, 2H); ¹³C NMR (125MHz, CDCl₃): δ = 101.1(C1), 99.1 and 98.(C1', C2'), 70.1 and 69.9 (C2, C5), 68.7(C3), 64.8(C4), 54.9(1-OMe), 50.6(C6), 46.9 and 46.7 (OMe-1', OMe-2'), 26.9, 26.8, 21.3, 21.4 (C1', C2', C3', C4'); FAB-MS for C₁₅H₂₅N₃O₇ Calc'd 359.20, found 328.1675 (M-OMe, 100%), 332.2196 (M-OMe-N₂, 2.7%); IR (neat, cm⁻¹): 2099.5

Synthesis of (1'S, 2'S)-methyl 6-azido-6-deoxy-2-O-ethyl-3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside (19)

To a solution of **18** (329.3 mg, 0.917 mmol) in DMF (8ml) was added sodium hydride (150.5 mg, 3.76 mmol, 60% suspension in mineral oil) and the mixture stirred under N₂ atmosphere at room temperature for 6hrs. Ethyl iodide (0.11 ml, 1.38 mmol) was injected into the reaction flask using a syringe and the mixture was left to stir for another 14hrs. Methanol was added to destroy excess NaH and the solution evaporated under reduced pressure. The residue was suspended in dichloromethane, washed with water (2X), dried over Na₂SO₄ and concentrated. Column chromatography (eluent EtOAc/ Hex 1:2) furnished the title compound in 74% yield (262 mg, 0.677 mmol). $[\alpha]_D +120.0$ (c 6.2); ¹H NMR (200MHz, CDCl₃): δ = 4.59 (s, 1H, H1), 3.99-4.02 (m, 2H, H2, H3), 3.78-3.3.60

(m, 2H, H5 H6a), 3.57-3.45 (m, 2H, H6b, CH_AH_BCH₃), 3.33-3.29 (m, 2H, H4, CH_AH_BCH₃), 3.28 (s, 3H, 1-OMe), 3.09 (s, 3H, 1'-OMe), 3.06 (s, 3H, 2'-OMe), 1.72-1.50 (m, 4H), 1.46-1.19 (m, 4H), 1.09 (t, 3H, -CH₂CH₃); ¹³C NMR (200MHz, CDCl₃): δ= 100.9 (C1), 99.2, 98.9, 78.4, 71.3, 69.6, 67.5 (CH₂CH₃), 65.8, 55.3, 51.1(1-OMe), 47.3 (1'-OMe), 47.1 (2'-OMe), 27.5, 21.9, 21.8, 16.1 (CH₂CH₃); IR (neat, cm⁻¹): 2099.5; FAB-MS calc'd for C₁₇H₂₉N₃O₇ 387.2007 found 387.2341 (1.3%), 356.2164 (M-OMe 25.1%)

Synthesis of methyl 6-azido-6-deoxy-2-O-ethyl-α-D-mannopyranoside (20)

19 (255.8 mg, 0.661 mmol) was stirred in 5% aqueous TFA (2ml) for 1 hr. The solvent was removed under reduced pressure and the residue dissolved in minimal amount of the dichloromethane, neutralized with Et₃N and chromatographed on a silica column (eluent 10% MeOH in CH₂Cl₂) to yield **20** in 49% yield (80 mg, 0.324 mmol). [α]_D +67.7 (c. 1.09, MeOH); ¹H NMR (200MHz, CDCl₃): δ= 4.74 (s, 1H, H1), 3.73-3.43 (m, 8H), 3.36 (s, 3H, 1-OMe), 1.19 (t, 3H, CH₂CH₃); ¹³C NMR (200MHz, CDCl₃): δ= 99.4(C1), 80.0, 75.7, 72.2, 71.1, 63.9 (CH₂CH₃), 55.1(1-OMe), 48.2 (C6), 15.0 (CH₂CH₃); IR (neat, cm⁻¹): 2100.5; FAB-MS calc'd for C₉H₁₇N₃O₅ 248.1247 (M+1) found 248.1340 (48.3%)

Synthesis of methyl 6-amino-6-deoxy-2-O-ethyl-α-D-mannopyranoside (21)

20 (49.7 mg, 0.201 mmol) was dissolved in ethanol (2mL) and hydrogenated over Pd catalyst (10% on charcoal, 7.7 mg) under ambient conditions until TLC indicated the completion of the reaction (24 hrs, 10% MeOH in CH₂Cl₂). The mixture was filtered over Celite and the filtrate evaporated to yield **21** as the sole product in 98% (43.8 mg, 0.198

mmol). No further purification was necessary. m.p. oil, $[\alpha]_D +15.6$ (c.1.07, MeOH); ^1H NMR (200MHz, CDCl_3): $\delta= 4.46$ (s, 1H, H1), 3.72-3.47 (m, 8H), 3.31 (s, 3H, 1-OMe), 1.18 (t, 3H, CH_2CH_3); ^{13}C NMR (200MHz, CDCl_3): $\delta= 99.7$ (C1), 81.0, 75.3, 71.6, 70.7, 64.3 (CH_2CH_3), 55.1(1-OMe), 42.6 (C6), 15.0 (CH_2CH_3); IR (neat, cm^{-1}): 3402..3, 1615.1; FAB-MS calc'd for $\text{C}_9\text{H}_{19}\text{NO}_5$ 222.1342 (M+1) found 222.1286 (95.1%)

Synthesis of (1'S,2'S)-methyl 6-azido-2-O-benzyl-6-deoxy-3,4-O-(1',2'-dimethoxycyclohexane-1',2'-diyl)- α -D-mannopyranoside (22)

To a solution of **18** (300 mg, 0.835 mmol) in DMF (8ml) was added sodium hydride (113.7 mg, 2.84 mmol, 60% suspension in mineral oil) and the mixture stirred under N_2 atmosphere at room temperature for 6hrs. Benzyl bromide (0.15 ml, 1.25 mmol) was injected into the reaction flask using a syringe and the mixture was left to stir for another 14hrs. Methanol was added to destroy excess NaH and the solution evaporated under reduced pressure. The residue was suspended in dichloromethane, washed with water (2X), dried over Na_2SO_4 and concentrated. Column chromatography (eluent EtOAc/ Hex 1:2) furnished the title compound in 81% yield (294.7 mg, 0.677 mmol). $[\alpha]_D +106.9$ (c. 0.51, CHCl_3); ^1H NMR (200MHz, D_2O): $\delta= 7.44$ -7.24 (m, 5H, CH arom.), 4.96 (d, 1H, $J= 11.9$, $\text{CH}_A\text{H}_B\text{Ph}$), 4.66 (s, 1H, H1), 4.60 (d, 1H, $J= 11.9$, $\text{CH}_A\text{H}_B\text{Ph}$), 4.24-4.18 (m, 2H, H6a, H6b), 3.70 (m, 1H, H5), 3.69 (m, 1H, H3), 3.46-3.43 (m, 2H, H2, H4), 3.33 (s, 3H, 1-OCH₃), 3.20 (s, 3H, 1'-OCH₃), 3.19 (s, 3H, 2'-OCH₃); ^{13}C NMR (200MHz, D_2O): $\delta= 101.1$ (C1), 99.3, 99.1, 76.6, 73.8, 71.3, 70.1, 66.0, 55.4(C6), 54.39(1-OCH₃), 47.5(1'-OCH₃), 47.4(2'-OCH₃), 27.7, 27.6, 22.0, 21.9; IR (neat, cm^{-1}): 2099.2; FAB-MS calc'd for $\text{C}_{21}\text{H}_{29}\text{N}_3\text{O}_7$ 435.2007 found 436.2013 (M+1, 8.5%)

Synthesis of methyl 6-azido-2-*O*-benzyl-6-deoxy- α -D-mannopyranoside (23)

22 (294.2 mg, 0.676 mmol) was stirred in 40% TFA (1.5ml) for 24 hrs. Saturated NaHCO₃ was added drop-wise until no more CO₂ evolved and the mixture extracted with ether (2 X 10 ml). The combined organic layers were washed with saturated NaHCO₃ (10ml), dried over Na₂SO₄ and concentrated. The crude product was chromatographed on a silica column (eluent 10% MeOH in CH₂Cl₂) to yield the title compound in 45% yield (103.2 mg, 0.300 mmol). $[\alpha]_D +20.4$ (c. 0.71, CHCl₃); ¹H NMR (200MHz, CDCl₃): δ = 7.34-7.31 (m, 5H, CH arom.), 4.71 (s, 1H, H1), 4.67 (d, 1H, J = 11.8, CH_AH_BPh), 4.53 (d, 1H, J = 11.8, CH_AH_BPh), 3.71-3.61 (m, 4H), 3.47-3.41 (m, 2H), 3.34 (s, 3H, 1-OCH₃), 3.06(br s, 2H, OH); ¹³C NMR (200MHz, D₂O): δ = 137.9, 128.4, 127.9, 99.9(C1), 84.9, 76.2, 73.6(CH₂-Ph), 72.2, 71.1, 55.1 (1-OCH₃), 48.2(C6); IR (neat, cm⁻¹): 3675.4, 2098.6; FAB-MS calc'd for C₁₇H₁₇N₃O₅ 343.1169 found 236.1723 (M⁺-OBn)

Synthesis of methyl 6-amino-2-*O*-benzyl-6-deoxy- α -D-mannopyranoside (24)

23 (72.5 mg, 0.211 mmol) was dissolved in methanol (4mL) and hydrogenated over Pd catalyst (10% on charcoal, 9.7 mg) under ambient conditions until TLC indicated the completion of the reaction (2 hrs, 10% MeOH in CH₂Cl₂). The mixture was filtered over Celite and the filtrate evaporated to yield **24** as the sole product in 78% (52.5 mg, 0.166 mmol). No further purification was necessary. m.p. 91-93 °C, $[\alpha]_D +37.2$ (c. 0.25, MeOH); ¹H NMR (200MHz, CDCl₃): δ = 7.34-7.26 (m, 5H, CH arom.), 4.66 (s, 1H, H1), 4.63 (d, 1H, J = 11.8, CH_AH_BPh), 4.52 (d, 1H, J = 11.8, CH_AH_BPh), 3.72-3.59 (m, 4H), 3.48-3.41 (m, 2H), 3.33 (s, 3H, 1-OCH₃), 3.06(br s, 4H, OH); ¹³C NMR (200MHz, CDCl₃): δ = 137.9, 128.4, 127.9, 98.9(C1), 84.2, 76.3, 73.5(CH₂-Ph), 72.5, 70.7, 55.1 (1-

OCH₃), 42.6(C6); IR (neat, cm⁻¹): 3526.3, 3405.2, 1621.3; FAB-HRMS calc'd for C₁₄H₂₁NO₅ 284.1498 (M+1) found 284.1457 (M+1, 48.4%)

Appendix 1

Enzyme Linked Lectin Assay (ELLA)

Nunc microtitration plates were coated with yeast mannan (100 μL / well) diluted from a stock solution of 10 $\mu\text{g}/\text{mL}$ in 0.01M phosphate buffer saline (PBS, pH 7.3 containing 0.1 mM Ca^{2+} and 0.1 mM M^{2+}) at 37 °C for 2 hours. The wells were then washed three times with 300 $\mu\text{L}/\text{well}$ of washing buffer (PBS containing 0.05% (v/v) Tween 20) (PBST). This washing procedure was repeated after each incubation period throughout the assay. The wells were then blocked with 1% BSA/PBS for 1 hour at 37 °C (150 $\mu\text{L}/\text{well}$), washed with PBST, and filled with 100 $\mu\text{L}/\text{well}$ of serial dilutions of Conacavalin A-peroxidase labeled (Con A-HRP) from 10^{-1} to 10^{-5} mg/mL in PBS and incubated for 1hr at 37 °C. After washing, 50 $\mu\text{L}/\text{well}$ of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1mg/ 4mL) in citrate-phosphate buffer (0.2M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 $\mu\text{L}/\text{well}$ of 1M H_2SO_4 and the optical density (O.D.) was measured at 410 nm relative to 570 nm. Blank wells contained citrate-phosphate buffer. The concentration of each lectin-enzyme conjugate that read on O.D. between 0.8-1.0 was used for inhibition experiments.

Inhibition Experiments

The microtiter plates were coated for 2hrs at 37 C with yeast mannan (100 μL of 10 $\mu\text{g}/\text{mL}$ solution). The wells were washed with PBST and blocked with BSA, as described previously. Stock solutions were prepared varying from 1-3 mg/mL in PBS.

Each stock solution underwent a serial 2-fold dilutions in PBS. Each dilution (60 $\mu\text{L}/\text{well}$) along with Con A (60 $\mu\text{L}/\text{well}$) were incubated in a Nunclon (Delta) microtiter plate for 1 hr at 37 °C. The above solutions (100 μL) were then transferred to the antigen-coated plates and incubated at 37 °C for another 1 hr. The wells were then washed as described and ABTS was added (50 $\mu\text{L}/\text{well}$). Color development was stopped after 20 min and the O.D. was measured at 410 nm relative to 570 nm. The percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A_{\text{no-inhibitor}} - A_{\text{with-inhibitor}}) / A_{\text{no-inhibitor}} \times 100$$

IC₅₀ values were reported as the concentration required to cause 50% inhibition of the binding of Con-A to the coating antigen.

CLAIMS TO ORIGINAL FINDINGS

1. Glycosylation of mannose can be easily achieved from mannose pentaacetate in the presence of a Lewis acid.
2. The relative inhibitory effect on the binding of Con A to yeast mannan of aryl *O*- and *S*-mannopyranosides and corresponding sulfoxides and sulfones can be measured using ELLA.
3. The order of inhibitory effect is: Aryl *S*-mannopyranosides are better inhibitors than arylsulfonyl mannopyranosides > arylsulfinyl mannopyranosides > aryl *O*-mannopyranosides.
4. *p*-methoxyphenylsulfonyl mannopyranoside is 33 times more potent than methyl mannopyranoside.
5. Modification of mannose at the 2- and 6-positions results in poorer inhibitors to the binding of Con A to yeast mannan than methyl mannopyranoside.

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