Synthesis of Inhibitors Against Mycobacterium Tuberculosis

BY

Manjula Wijegunasinghe

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Chemistry

University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

Infections by *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), have reemerged as a public health threat in recent years. The resurgence in TB has sparked renewed interest in identifying new antibiotics that can be used to treat this disease, which claims nearly three million lives worldwide each year. This disease has been difficult to treat, which can be attributed in part to the unusual structure of the cell wall of the organism. In order to identify new anti-tuberculosis agents, our research goal is to develop new analogues that mimic the linker, α-L-Rha-(1$\rightarrow$3)-GlcNAc-1-phosphate of the cell wall. We carried out two studies. In our first study, we synthesized analogues that replace the *N*-acetylglucosamine fragment of the disaccharide linker with a D-threonine amino acid.

The second study focused on the synthesis of analogues formed by modifying the C-1´and C-4´positions of L-rhamnose. We attached a heterocycle (imidazole or thiazole) to the C-4´position of L-rhamnose and a octyl group to the C-1´position. Difficulties arose in the reduction of C-4´hydroxyl in these analogues. Biological investigation of these analogues were performed by our collaborators Dr. P. J. Brennan and Dr. D. C. Crick at Colorado State University, USA. Unfortunately, all the compounds showed no inhibitory activity. However, these results provided us the needed information and knowledge to design our future work.

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to my parents and teachers

Acknowledgements

I wish to express my sincere gratitude to my supervisor Dr. P. G. Hultin, for his never ending patience, invaluable advice and encouragement in completing my research. I consider myself privileged to have had the opportunity to pursue my masters studies under his guidance. I wish to express my deep appreciation to Dr. N. Hunter and Dr. Linda Donald for their encouragement and guidance. I am thankful to my friends, Jason Hein and Xi Wen for their support in numerous ways. The technical assistance of Dr. K. Marat of the NMR facility is greatly appreciated. I wish to acknowledge the financial support from the NSERC of Canada.

Finally, this work would not have been a reality without the love and support of my dear family. I extend my heart felt gratitude to my parents. I thank my brother, sister, and my husband for their continuous support, understanding, and encouragement given over the years.

List of Symbols and Acronyms

$[\alpha]$	specific rotation
Ac	acetyl
AG	arabinogalactan
AIBN	2,2´-azobisisobutyronitrile
Ar	aryl
Ara*f*	arabinofuranose
aq	aqueous
B	base
Bz	benzoyl
c	concentration in g/100 mL
δ	chemical shift in ppm
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
equiv	molar equivalent(s)
Et	ethyl
Gal*f*	galactofuranose
GlcNAc	*N*-acetyl glucosamine
h	hour(s)
Im	imidazole
J	coupling constant (in NMR)

LDA	lithium diisopropylamide
lit.	literature
Me	methyl
mp	melting point
Ms	methanesulfonyl (mesyl)
MS	mass spectrometry
m/z	mass to charge ratio
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
Nu	nucleophile
Ph	phenyl
Py	pyridine
Rha	rhamnose
s	singlet (in NMR)
rt	room temperature
THF	tetrahydrofuran
Ts	tosyl, *p*-toluenesulfonyl

Chapter 1

Introduction

Tuberculosis (TB) is caused by the microorganism *Mycobacterium tuberculosis*. It has infected a third of the world's population (approximately 2 billion people). Among those, there were about 8 million new cases of TB with 3 million deaths in 1995.[3,4]

This global epidemic is growing and becoming more threatening. The breakdown in health services, the spread of human immunodeficiency virus (HIV) infection, and the emergence of multidrug-resistant TB are contributing to the worsening impact of this disease. In 1993, WHO declared TB as a global emergency. It is estimated that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will get sick, and 35 million will die from TB, if no better control is found.[5]

Several factors have contributed to the increase in TB infection. HIV is accelerating the spread of TB.[6] HIV and TB form a lethal combination, each speeding the other's progress. HIV weakens the immune system. Someone who is HIV-positive and exposed to TB is many times more likely to become sick with TB than someone who is HIV negative. TB is a leading cause of death among people who are HIV-positive, accounting for about 15% of AIDS deaths worldwide.[5]

Furthermore, poorly managed TB treatment programmes are threatening to make

TB incurable. Drug-resistant TB is caused by inconsistent or partial treatment, when patients do not take all their drugs regularly for the required period because they start to feel better or when doctors and health workers prescribe the wrong treatment. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), defined as the disease due to TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs.[6–8]

1.1 Structure of the mycobacterial cell envelope

The cell wall of *Mycobacterium* spp. is required for growth and survival.[9] Its formation has become the focus of the search for essential targets in the development of new drugs against tuberculosis.[10]

The mycobacterial cell wall is composed of three major layers, the plasma membrane, a peptidoglycan and a mycolic acid bilayer (Fig.1.1).[1] The plasma membrane is the innermost layer of the cell wall and consists of a phospholipid bilayer containing diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and the phosphatidylmannoside family (PIMs) (Fig.1.1). The outer layer of the mycobacterial cell wall consists of mycolic acids, which are esters of long chain fatty acids. The mycolic acids are high molecular weight α-alkyl, β-hydroxy fatty acids, present mostly as covalently bound esters of a branched polysaccharide, the arabinogalactan (AG).

The peptidoglycan is the backbone of the mycobacterial cell wall and consists of a regular hetero-polymer that contains alternating molecules of N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) linked via a β-(1$\rightarrow$4) glycosidic bond.[11] These hetero-polymers lie side by side in the cell wall and are cross-linked by short chain peptides. This rigid peptidoglycan structure sheathes the entire plasma mem-

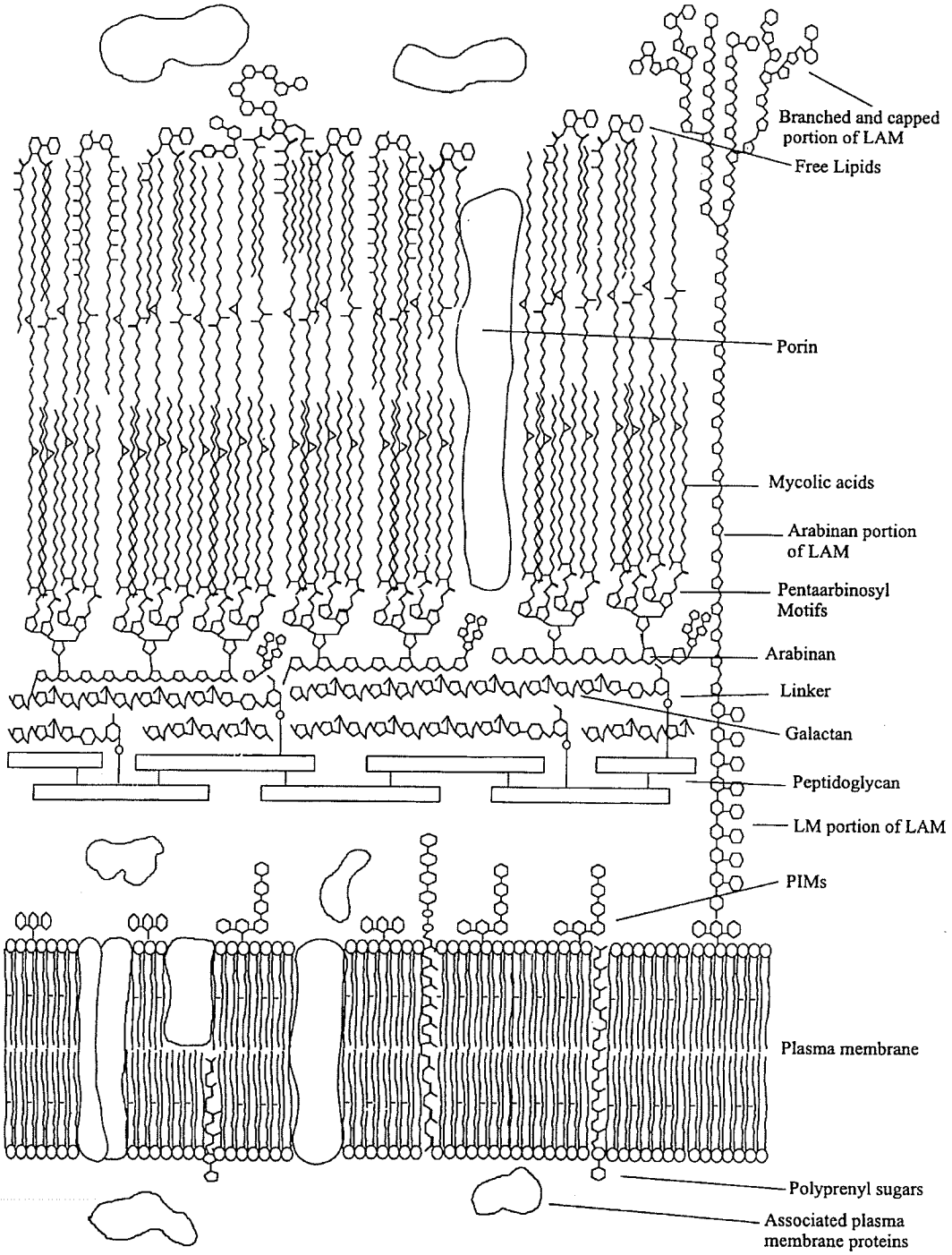


Figure 1.1: The Mycobacterium tuberculosis cell envelope[1]

brane and prevents the cell wall from rupturing due to the osmotic pressure of the interior. The arabinogalactan (AG) resides next to the peptidoglycan. It is made of arabinose and galactose. Both these monosaccharides are in their furanose form. The linear galactan chain that extends from the peptidoglycan linkage is composed of 12 to 15 repeating disaccharide units of alternating 5- and 6-linked β-D-Gal$f$ molecules (Fig.1.2). Arabinan chains are attached to the C-5 hydroxyl of various 5,6-linked Gal$f$ residues of the linear galactan. These arabinan chains are composed of linear 5-linked D-Ara$f$ residues with branches extending from 3,5-linked D-Ara$f$ residues. The non-reducing termini of the arabinan branches are composed of two identical disaccharide units (β-D-Ara$f$-(1$\rightarrow$2)-α-D-Ara$f$), one of which is attached to the 3-position of the branch point, the other attached to the 5-position. Thus the termini of the arabinan are non-reducing. Mycolic acid residues are attached to the 5-position of the terminal and 2-linked Ara residues at the end of the branches.[11]

The reducing end of the AG is linked to peptidoglycan through a unique disaccharide linker consisting of a α-L-Rha-(1$\rightarrow$3)-GlcNAc-1-phosphate. In the biosynthesis of the mycobacterial cell wall,[12] the disaccharide linker is formed first as a diphosphoprenol ester. The poly(galactofuranose) chain of the AG is then constructed from the C-4$'$ hydroxyl of the linker, after which the arabinofuranosyl and mycolate components are added. The linkage disaccharide is thus a key structure in the overall biosynthesis of the cell wall. Therefore it has become a good target for drug design. In our study, we design analogues that mimic portions of the linker.

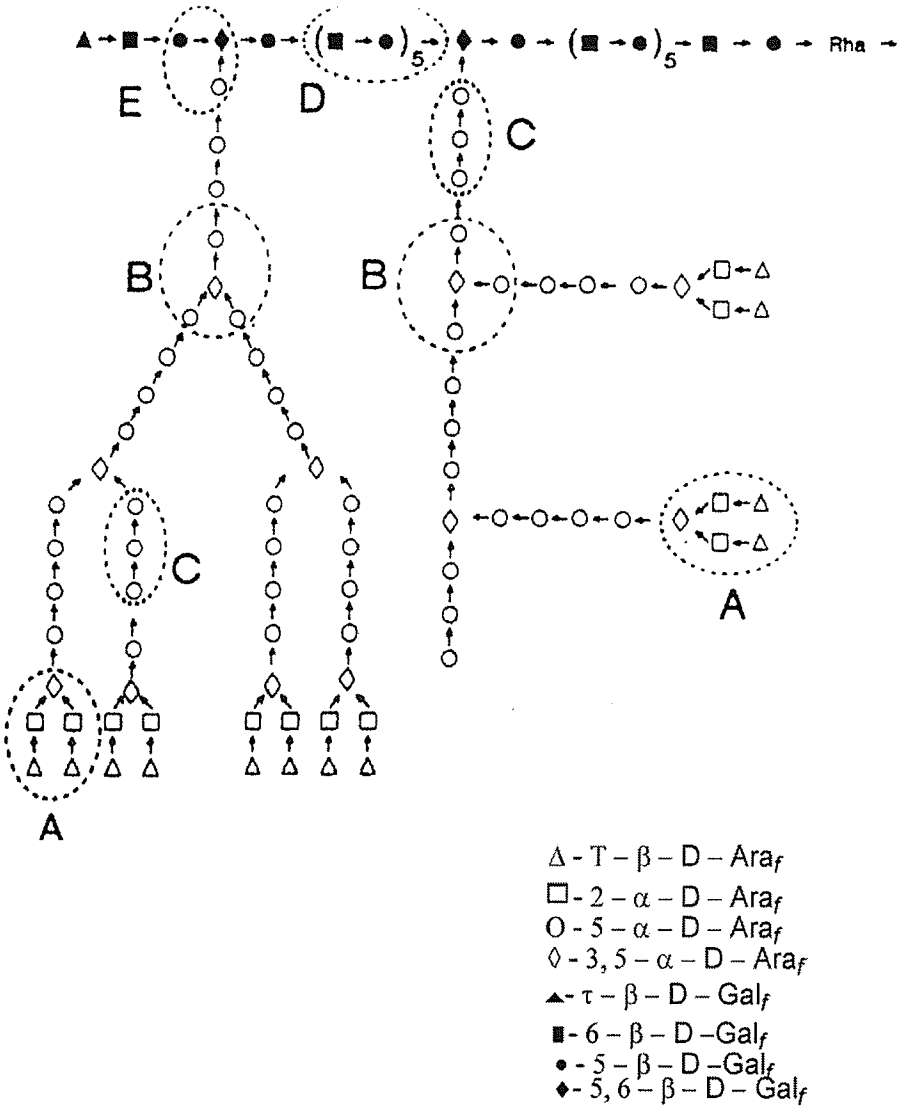


Figure 1.2: Structural motifs of the cell wall arabinogalactan

1.1.1 Structure of the linker region

In order to design compounds that mimic the linker, it is important to understand the detailed structure and the biosynthesis of this linker.

The linker was discovered by Brennan et al. in 1990[13] and has not been found in humans or in other bacteria.[14] It is a pyranosidic disaccharide α-L-Rha-(1→3)-GlcNAc-1-phosphate, linking arabinogalactan to the underlying peptidoglycan layer (Fig.1.3). The rhamnose moiety in the linker is attached to the galactan portion of the arabinogalactan via its C-4′ hydroxyl group. The rhamnose sugar is attached to the third position of *N*-acetyl glucosamine via its C-1′ hydroxyl group, and *N*-acetyl glucosamine is anchored to the peptidoglycan through a phosphate.

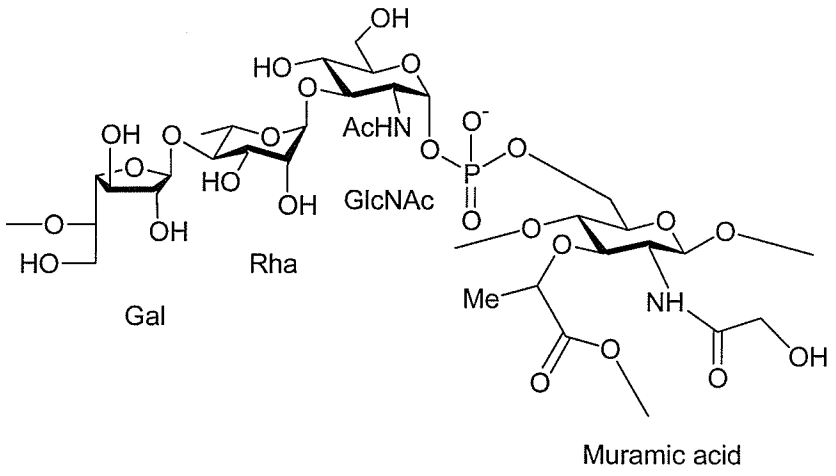


Figure 1.3: The α-L-Rha-(1→3)-GlcNAc-1-phosphate linker

1.2 Biological formation of oligosaccharides

The biosynthesis of oligosaccharides is controlled by glycosyltransferases. Glycosyltransferases are the enzymes that catalyze the transfer of sugars from specific glycosyl

donors to specific acceptors. Example is shown in Fig.1.4. The structures of glycosyl donors have been useful in the design of glycosyltransferase inhibitors.[15]

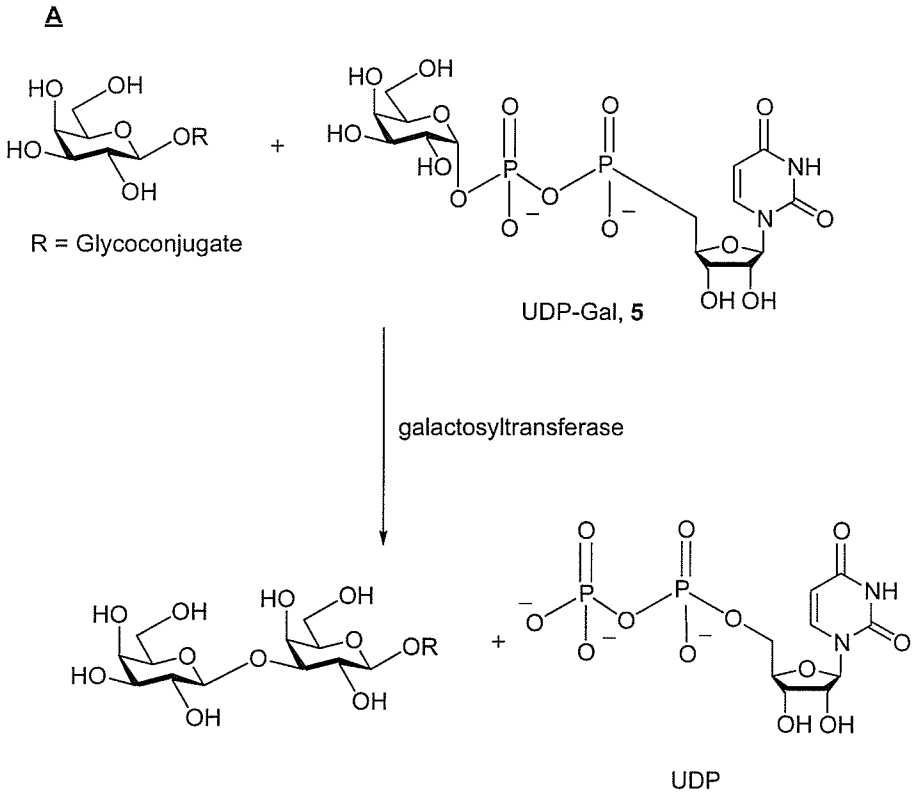


Figure 1.4: An example of the glycosyltransferase reaction

1.2.1 Glycosyl donors and glycosyltransferase reaction mechanism

There are two types of glycosyl donor substrates for glycosyltransferases (Fig.1.5).[16] One is the glycosyl nucleotide. Most glycosyl nucleotides consist of a glycose, diphosphate (also called pyrophosphate), a ribose and a base, except that the donor CMP-Neu5Ac has only one phosphate. The glycosyltransferases that use these types of donors are called Leloir glycosyltransferases. The Leloir pathway is responsible for the synthesis of most mammalian glycoconjugates and some aspects of bacterial polysac-

Table 1.1: Common sugar nucleotides used by mammalian glycosyltransferases

UDP	GDP	CMP
N-Acetylgalactosamine	Fucose	Sialic acid
N-Acetylglucosamine	Mannose	
N-Acetylmuramic acid		
Galactose		
Glucose		
Glucuronic acid		
Xylose		

charide synthesis. Table 1.1 gives the common sugar nucleotides used by mammalian glycosyltransferases.[17] Glycosyltransferases that utilize other types of glycosyl donors are called non-Leloir glycosyltransferases. A typical non-Leloir donor is composed of a glycose, diphosphate or monophosphate and a lipid moiety. The non-Leloir pathway is mostly involved in the biosynthesis of bacterial cell walls.[18]

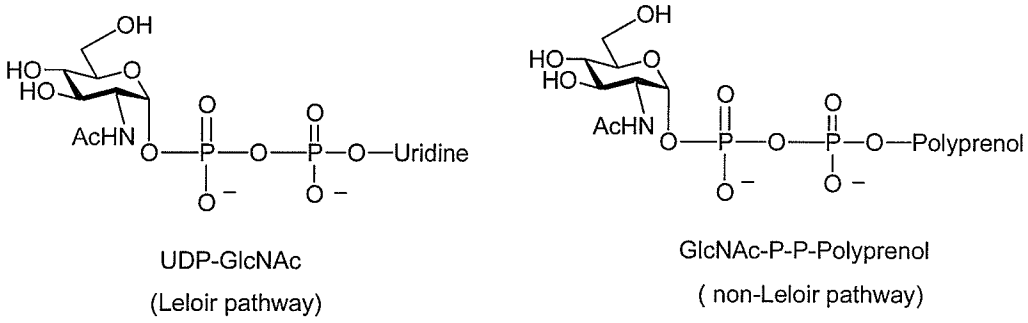


Figure 1.5: Two types of glycosyl donors

Glycosyltransferases are extremely difficult to isolate because they are membrane bound and secreted in very small amounts. Thus, there are only a few glycosyltransferases that have had their structures resolved so far.[19] It is commonly perceived that a glycosyltransferase has two domains in its structure. One domain harbors an acceptor and the other domain is occupied by a donor during the glycosylation reaction. In the donor domain, there is a divalent metal cation such as Mn^{2+} or Mg^{2+}, to bind

to the diphosphate moiety and assist the nucleotide in leaving.[20]

It is believed that glycosyltransferases carry out the glycosylation in such a way that the anomeric configuration of the donor sugars is inverted (Fig.1.6).[21] The carboxylate in the acceptor domain provides the base catalysis to activate the acceptor as a nucleophile. The acceptor nucleophile then attacks the donor anomeric carbon center in a S_N2 fashion. The divalent metal cation in the donor domain site provides Lewis acid assistance to the cleavage of the donor glycosidic bond. As a result, the anomeric configuration of the donor sugars is inverted when the new glycosyl bond is formed.[21]

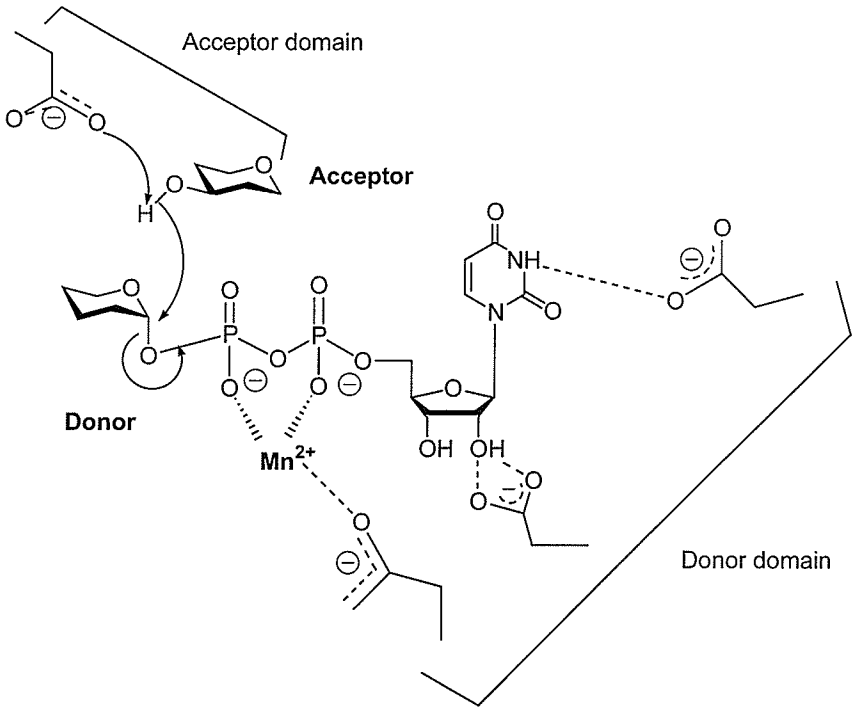


Figure 1.6: Interactions of a glycosyltransferase with a donor and an acceptor

1.3 Chemical glycosidic bond formation

Chemical synthesis of a glycosidic bond is much more complicated than the synthesis of other biopolymers such as peptides or nucleic acids. The stereoselective (α/β selectivity) introduction of the glycosidic linkage is a major problem. Glycosidic bond formation is generally achieved by condensing a fully protected glycosyl donor, which bears a potential leaving group at its anomeric centre, with a suitably protected acceptor that often contains only one free hydroxyl group.

Traditionally, the most widely used glycosylation methods have exploited anomeric halide derivatives of carbohydrates as glycosyl donors. These are often referred to as "Konigs-Knorr" reactions. However, these compounds often suffer from instability and require drastic conditions for their preparation. The introduction of the orthoester[22] and imidate[23] procedures provided the first practical alternatives to the glycosyl halide methodologies. Since these original disclosures, many other leaving groups for anomeric centres have been reported (Fig.1.7).[24]

However, of these glycosyl donors, the anomeric bromides, trichloroacetimidates and thioglycosides have been applied most widely. These compounds can be prepared under mild conditions and are sufficiently stable to be purified and stored for a considerable period of time. In addition, they undergo glycosylation under mild conditions. By selecting the appropriate reaction conditions, high yields and good α/β ratios can be obtained.

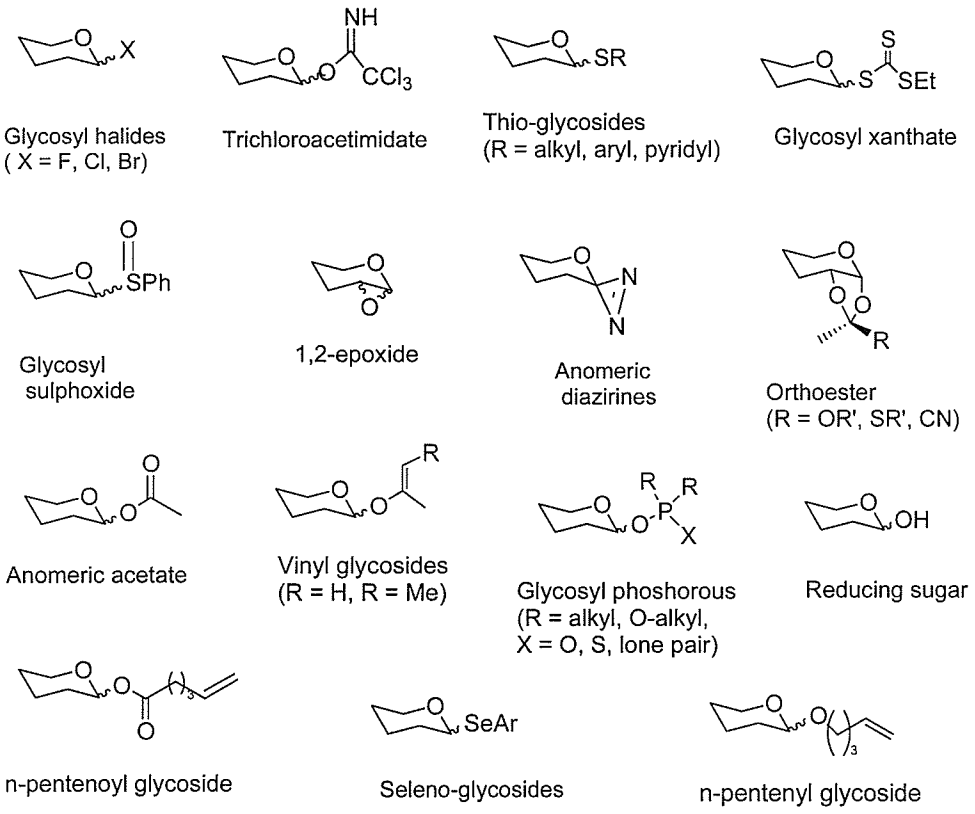
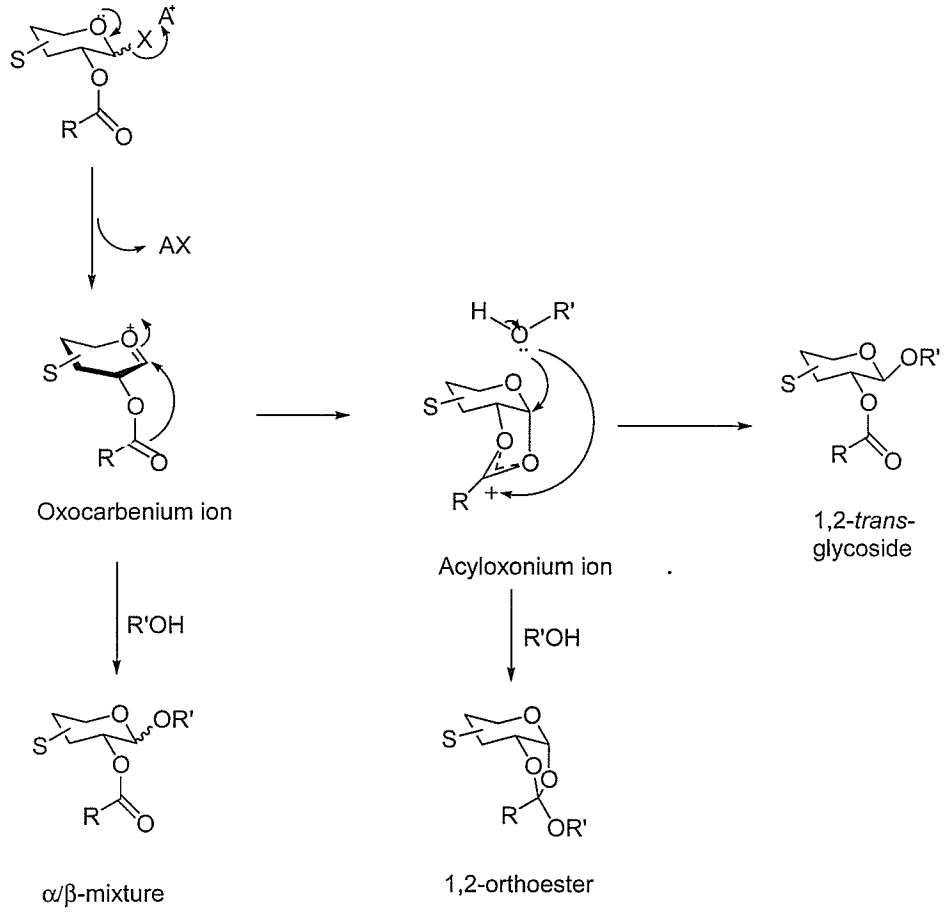


Figure 1.7: Glycosyl donors used for glycosidic bond synthesis

1.3.1 Neighbouring-group assisted procedures

The most reliable method for the construction of 1,2-*trans*-glycosidic linkages utilizes neighbouring-group participation from a 2-*O*-acyl functionality. The principle of this approach is schematically illustrated in Scheme 1.1 and Scheme 1.2.



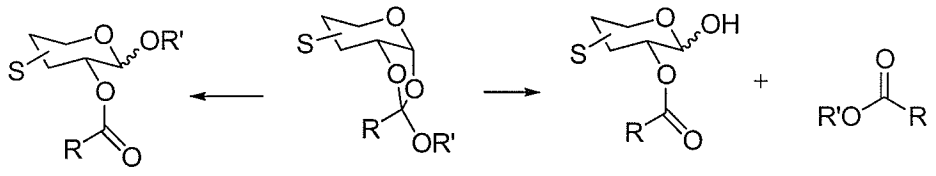
Scheme 1.1: Neighbouring-group participation

A promoter (A) activates an anomeric leaving group thus assisting in its departure. The result is the formation of an oxocarbenium ion. Subsequently, neighbouring-group participation of a 2-*O*-acyl protecting group leads to the formation of a more stable acyloxonium ion. In the latter intermediate, additional resonance stabilization

of the positive charge is provided by two oxygen atoms. In the case of the oxocarbenium ion, only the ring oxygen atom gives resonance stabilization and hence this is less stable than the acyloxonium ion. Attack of an alcohol at the anomeric centre of the acyloxonium results in the formation of a 1,2-*trans*-glycoside.

In some glycosylations, the alcohol will attack at the C(2) position of the dioxolane ring of the acyloxonium ion, resulting in the formation of an undesired orthoester. In some cases, the orthoester can be isolated as a moderately stable product, but in other reactions it may rearrange to the desired glycosidic or to an aldose and an acetylated sugar alcohol (Scheme 1.2).

Orthoester formation may be prevented by the use of a C(2) benzoyl or pivaloyl group. In these cases, orthoester formation is disfavoured by the presence of the bulky phenyl or *tert*-butyl group attached to the dioxolane ring. In some cases, the glycosylation may also proceed via the oxocarbenium ion to give mixtures of anomers.

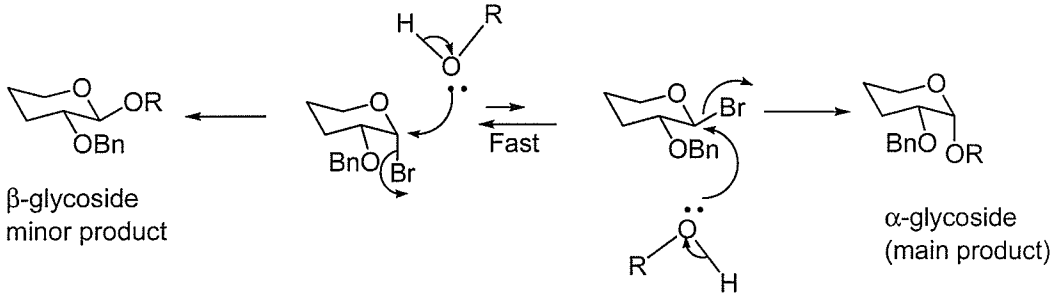


Scheme 1.2: Rearrangement of the orthoester

1.3.2 *In situ* anomerisation

In situ anomerisation is a kinetically-controlled process. This concept was first introduced by Lemieux in 1965[25] who observed that a rapid equilibrium can be established between α and β halides by the addition of tetra-*n*-butyl ammonium bromide (Scheme 1.3). The anomerisation is believed to proceed through several intermediates. At equilibrium, there is a shift towards the α bromide since this compound is stabilized by an endoanomeric effect. However, the β bromide is much more reactive

towards nucleophilic attack by an alcohol than is the more stable α-bromide. Thus, glycosylation takes place preferentially on the β bromide in an S_N2 fashion and gives mainly α glycoside. An important requirement for this reaction is that the rate of equilibration should be much faster than that of glycosylation.



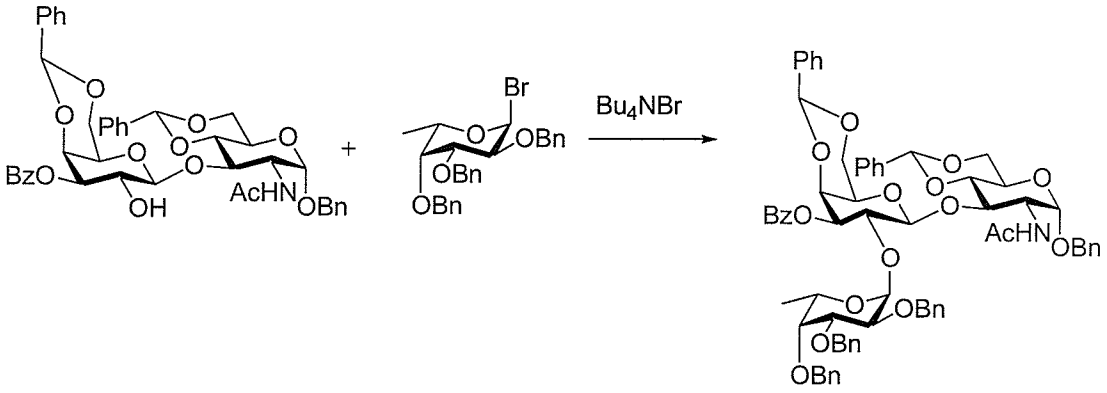
Scheme 1.3: α-Glycosidic bond synthesis by *in situ* anomerisation

The anomeric outcome of glycosylation can also be discussed in more general mechanistic terms. First, the product ratio is governed by competing rates of formation of the α and β glycoside and therefore glycosylation is kinetically controlled. Second, according to the Curtin-Hammett principle, if two reactants are in fast equilibrium the position of this equilibrium and therefore the reactant ratios will not determine the product ratio. The product ratio, however will depend on the relative activation energies of the reactions from each of the two reactants (α and β halide). In the case of the *in situ* anomerisation procedure, the activation energy for the glycosylation of the β anomer is significantly lower than for the α anomer and therefore the reaction proceeds mainly through the β anomer. The origin of the higher reactivity of β halides is a kinetic anomeric effect. Additionally, the α anomer is less reactive due to ground state stabilization by an endo anomeric effect.

The solvent used in the reaction medium plays an important role in the *in situ* anomerisation procedure. It is essential that *in situ* anomerisation is performed in a solvent of low polarity. In polar solvents, the reaction proceeds via an oxocarbenium

ion and the anomeric selectivity is reduced.

The efficacy of *in situ* anomerisation was demonstrated by the condensation of a fucosyl bromide with a glycosyl acceptor in the presence of tetra-*n*-butyl ammonium bromide to give a trisaccharide mainly as the α anomer (Scheme 1.4). It should be noted that tetra-*n*-alkyl ammonium halides react only with very reactive glycosyl halides. More reactive activators are required for more demanding glycosylations and nowadays a range of activators with different reactivities are available, including $Hg(CN)_2$, $HgBr_2$, $AgClO_4$ and AgOTf.[26] Glycosyl bromides, glycosyl imidates, thioglycosides and glycosyl fluorides also undergo *in situ* anomerisation.

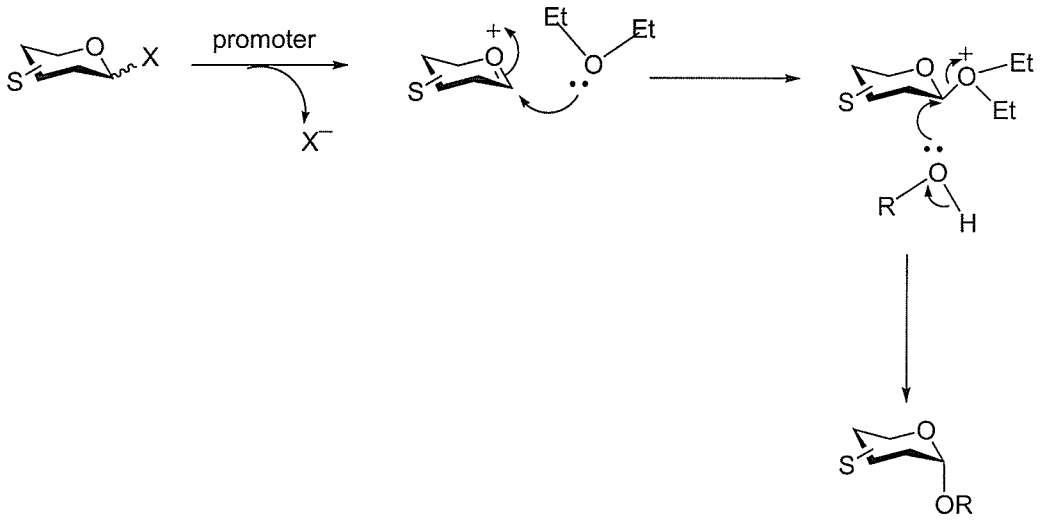


Scheme 1.4: An example of *in situ* anomerization

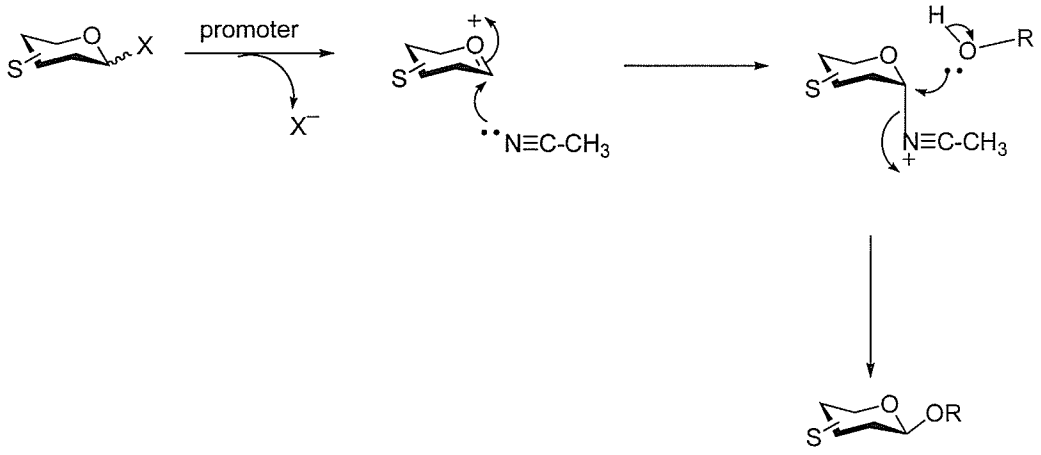
1.3.3 Solvent participation

Solvents also play an important role in glycosylation, especially in promoting stereoselectivity. The donors that are affected by solvents the most are those that do not have a participating group at C-2. Solvents of low polarity can dramatically increase their α-selectivity because *in situ* anomerisation is facilitated and the formation of oxocarbenium ions is suppressed.[27]

Some solvents also participate in glycosylation.[27] It is well known that diethyl ether or a mixture of toluene and dioxane promotes α anomeric selectivity, whereas acetonitrile increases β selectivity. The reaction mechanism for diethyl ether is shown in Scheme 1.5 and the reaction mechanism for acetonitrile is shown in Scheme 1.6. When diethyl ether complexes with the oxocarbenium ion, it forms an intermediate in which the diethyl oxonium ion adopts the equatorial position. Nucleophilic displacement of the β diethyl oxonium gives an α glycoside. In contrast, when acetonitrile complexes with the oxocarbenium ion, the nitrilium ion prefers an axial orientation. Nucleophilic substitution of the α-nitrilium intermediate leads to a β-glycoside. There has been no good explanation so far as to why the diethyl oxonium favours the equatorial position and the nitrilium prefers an axial orientation.



Scheme 1.5: Particiption of diethyl ether

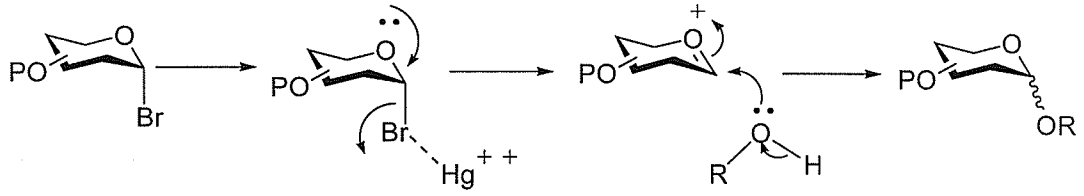


Scheme 1.6: Participation of acetonitrile

1.4 Glycosyl donors

1.4.1 Glycosyl halides

Koenigs and Knorr introduced the use of glycosyl bromides and chlorides as glycosyl donors in 1901.[28] This classical approach uses heavy metal salts mainly silver and mercury salts or alkyl ammonium halides to achieve the activation of the anomeric center.[29] Complexation of the anomeric bromide or chloride with a silver or mercury salt greatly improves their leaving group ability. Departure of the anomeric leaving group will give an oxocarbenium ion, which in turn will react with an alcohol to give a glycoside (Scheme 1.7).



Scheme 1.7: Activation of glycosyl halides

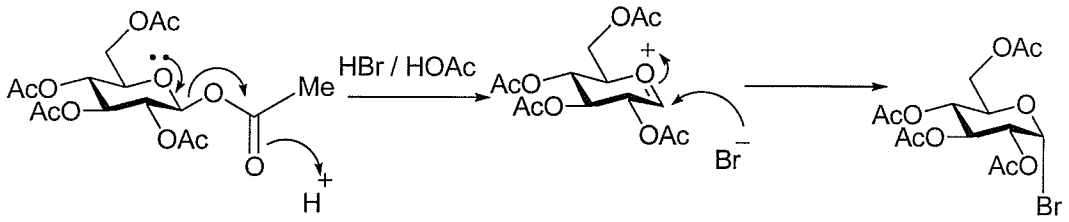
Alternatively, an activated anomeric halide may be substituted by an alcohol via

an S_N2 mechanism leading to a glycoside. The reactivity of glycosyl halides is determined by the protecting group pattern and in general ether protected derivatives are more reactive than analogous ester protected glycosyl donors. Furthermore, Fraser-Reid developed the "Armed-Disarmed concept.[30] That is, acyl protecting groups at C(2) depress the anomeric reactivity as compared with ether groups. This observation can be easily rationalized. Departure of the anomeric leaving group results in a positive charge at the anomeric centre. Esters are strongly electron-withdrawing and will destabilize the resulting positively charged intermediate and hence displacement of such halides is energetically less favourable. Protecting groups also determine the stability of glycosyl halides. For example, 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl bromide is a reasonably stable compound which can be stored for a considerable period of time. However, the analogous *O*-benzylated derivative will decompose within several hours after preparation.

Glycosyl bromides are more reactive than glycosyl chlorides but are also more labile. In general, glycosyl iodides are too labile to be used in glycosylations. It was believed that glycosyl fluorides were too stable to be used as glycosyl donors. However, in 1981 Mukaiyama and coworkers demonstrated that these compounds can be activated with $AgClO_4/SnCl_2$.[31] Subsequent reports expanded the knowledge about activators for glycosyl fluorides, and commonly used promoters are $BF_3{\cdot}OEt_2$,[32] $CpMCl_2$-$AgClO_4$ (M = Hf, Zr)[32] and Cp_2HfCl_2-AgOTf.[32] Glycosyl fluorides can be purified by silica gel column chromatography and have a good shelf-life. They can even undergo a limited number of protecting group manipulations.

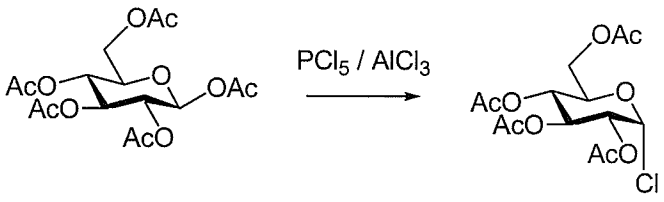
Glycosyl bromides are most commonly prepared by treatment of a per-*O*-acetylated sugar derivative with a solution of HBr in acetic acid (Scheme 1.8). The more stable α-anomer is usually obtained in high yield. In this reaction, the anomeric acetyl

moiety is converted into a good leaving group by protonation. Next, an oxocarbenium ion is formed by departure of acetic acid, which is substituted by bromide. In the first instance, a mixture of anomeric bromides may be formed and these quickly equilibrate to the thermodynamically more stable α anomer. The latter compound is stabilized by a strong endoanomeric effect. Other non-anomeric acetyl moieties in this compound may also be protonated. However, departure of these groups will result in the formation of highly unstable carbonium ions. Thus, normally this type of reaction will not occur.



Scheme 1.8: Preparation of glycosyl bromide

Glycosyl chlorides can be obtained by the treatment of aldosyl acetates with aluminium trichloride or phosphorus pentachloride (Scheme 1.9). This procedure is relatively harsh and many functionalities will not survive under these conditions.



Scheme 1.9

Several milder methods have been described with the Vilsmeier-Haack reagent,[33] one of the most useful reagents for the preparation of labile glycosyl chlorides or bromides. The Vilsmeier-Haack reagent ($Me_2N^+ =CHClCl^-$) is formed by the reaction of

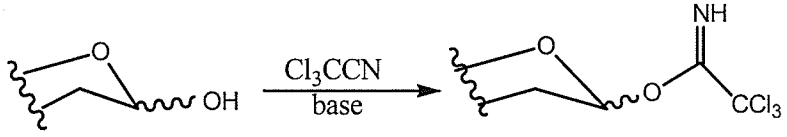
dimethylformamide (DMF) with oxalyl chloride [ClC(O)C(O)Cl]. An anomeric bromide will be formed when oxalyl bromide is used in the formation of Vilsmaier-Haack reagent (Me_2N^+ =CHBrBr$^-$).

Several methods have been reported for the preparation of glycosyl fluorides[34] but the most common procedure is treatment of a thioglycoside with NBS (*N*-bromosuccinimide) and (diethylamino)sulfur trifluoride (DAST)[35] or the reaction of lactol with DAST or 2-fluoro-1-methylpyridinium p-toluenesulfonate.[36] An alternative method is the treatment of a 1,2-anhydro-pyranoside with tetra-*n*-butylammonium fluoride (TBAF).

1.4.2 Trichloroacetimidates

Sinay was the first to use an imidate for the synthesis of a glycoside,[37] but it was to be Schmidt who would extend the method and make the use of trichloroacetimidates rival the well established Koenigs-Knorr procedure.[38] In fact, the trichloroacetimidate method is now often preferred for the synthesis of a 1,2-*trans*-glycoside, simply because it does not involve the use of heavy metal reagents in the promotion step.

Trichloroacetimidates are usually prepared from the corresponding hemiacetals (Scheme 1.10) by base catalyzed reaction with trichloroacetonitrile in dichloromethane as solvent. The choice of base is critical since it determines the anomeric configuration of the product. The strong bases such as sodium hydride,[39] cesium carbonate[40] and DBU[41] give the thermodynamically more stable α-trichloroacetimidate, while weaker bases such as potassium carbonate[42] give the β-anomers as kinetic products.



Scheme 1.10: Synthesis of trichloroacetimidates

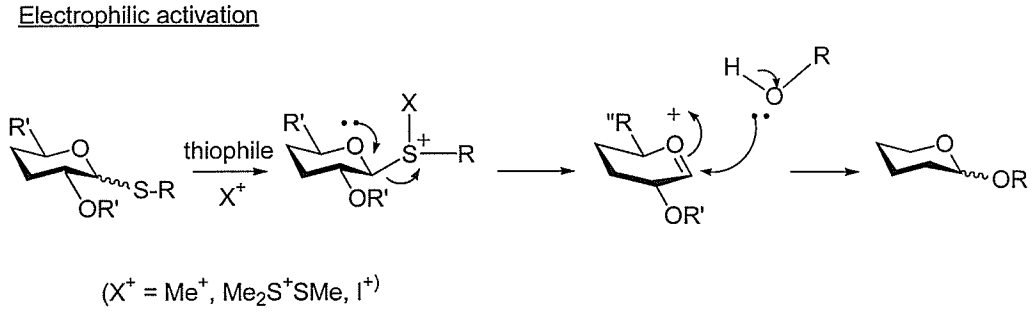
The coupling of trichloroacetimidates with glycosyl acceptors is usually mediated by Lewis acids, the choice of which influences both the rate and stereochemical outcome of the coupling. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) and boron trifluoride etherate[43] are usually the Lewis acids of choice. They allow coupling to occur between -78^{0}C and 0^{0}C over time periods ranging from minutes to hours, depending on the reactivity of the acceptors.[41-44] In addition weaker Lewis acids, such as silver triflate, sometimes work very efficiently.[45]

1.4.3 Thioglycosides

Thioglycosides were first introduced in glycosidation reactions by Ferrier in 1973,[46] who used the ethylthio group at the anomeric position. Subsequently, Hanessian[47] demonstrated the usefulness of the 2-pyridylthiol. Currently, the phenylthio and the ethylthio carbohydrate derivatives are the most commonly used glycosyl donors from this class of compounds. Thioglycosides are quite versatile and useful intermediates in oligosaccharide synthesis owing to their ease of preparation, stability, and rich chemistry. One of their distinct advantages is that their thio group can serve as a temporary protecting group stable to almost all glycosidation conditions currently in use.

Common usage of thioglycosides in glycosidation reactions is a relatively new development[48] compared with the Koenigs-Knorr method. The accepted mechanism of the thioglycoside based glycosidation reaction is similar to that of the Koenigs-Knorr reaction. Thus the thiophilic reagent used as an activator initiates displacement of the sulfur by the lone pair of electrons of the ring oxygen to form the oxonium species, which is then trapped by the glycosyl acceptor in the usual fashion as shown in Scheme 1.11. A variety of thiophiles "X" have been employed for the

activation of thioglycosides. The initial studies involved heavy-metal salts, such as those of mercury, copper, and lead.[48] These reagents, however, suffer from low reactivity and inconvenience and have not been extensively employed. Recently, several more effective activators have been introduced, including NBS,[49] methyl triflate (MeOTf),[50] dimethyl(methylthio)sulfonium triflate (DMTST),[51] *N*-iodosuccinimide-triflic acid (NIS-TfOH),[52] and trimethylsilyl triflate (TMSOTf).[53]

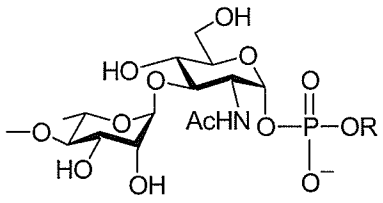


Scheme 1.11: Direct activation of thioglycosides

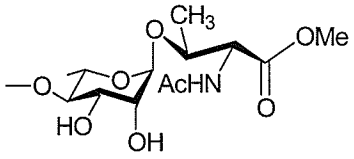
Chapter 2

Results and Discussion I

This chapter presents the synthesis of analogues in which the *N*-acetylglucosamine fragment of the disaccharide linker α-L-Rha-(1→3)-α-D-GlcNAc is replaced with a D-threonine amino acid (Fig.2.1). In this section, we will also describe both our successful and unsuccessful attempts toward the stereocontrolled synthesis of D-threonine-α-L-rhamnopyranoside (**23**). We attempted the glycosidic bond synthesis using rhamnosyl bromide, rhamnosyl imidate and rhamnosyl thioglycoside. We found out that the reaction utilizing the rhamnosyl thioglycoside produced the best yield.



α–L-Rha-(1-->3)-α–D-GlcNAc linker

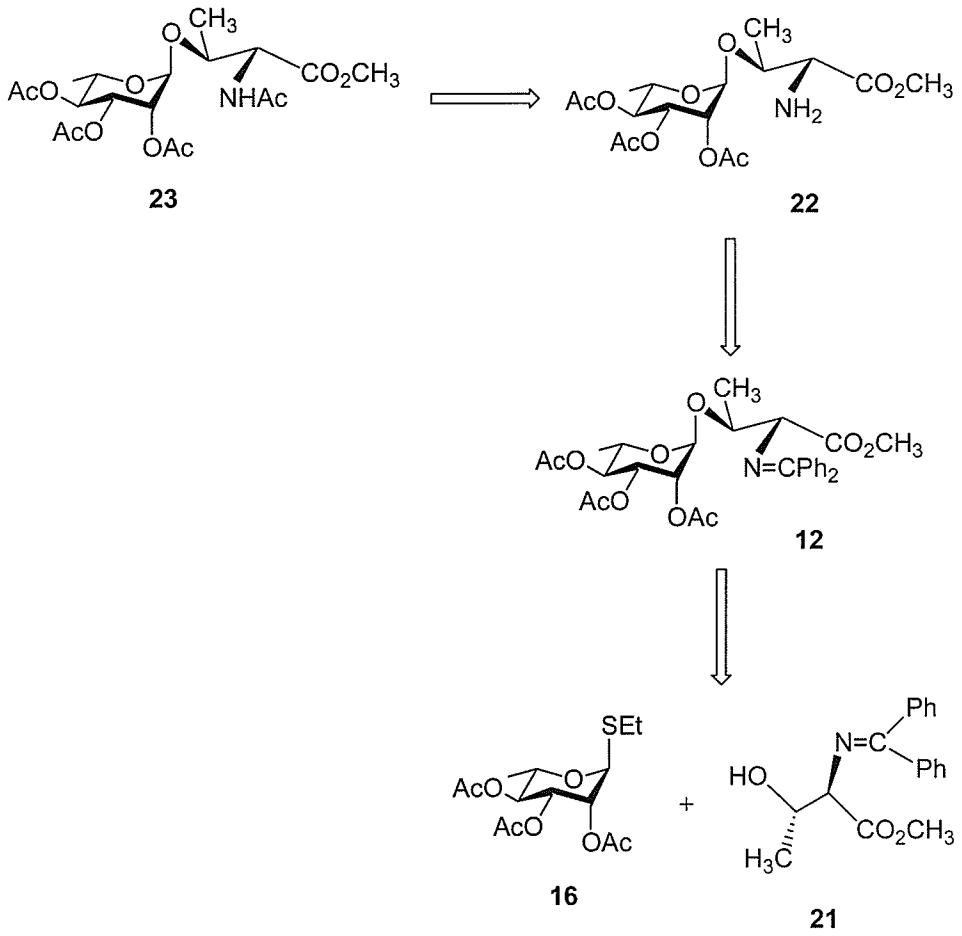


N-acetyl-*O*-(α-L-rhamnopyranosyl)-D-threonine methyl ester

Figure 2.1: A representation of how D-threonine fragment mimics the *N*-acetyl glucosamine fragment of the linker

2.1 Retro-synthetic analysis of 23

As reviewed in chapter 1, there are many approaches available to form glycosidic bonds. However, thioglycosides, glycosyl imidates and glycosyl halides are the most commonly used glycosyl donors. Therefore we envisioned that the compound **12** could be synthesized from building blocks **16** and **21** as shown in Scheme 2.1. Final target compound **23** could be prepared from **12** via functional group manipulations.



Scheme 2.1: Retrosynthetic analysis of compound **23**

2.2 Methods for making amino acid glycosides

The synthesis of *O*-glycosylated serine or threonine was a challenging problem due to the acid lability of the glycosidic bond and the base sensitivity (retro-Michael reaction) of the amino acid *O*-glycosides.[54–56] Therefore, a synthesis of *O*-glycosides demanded the development of versatile and selective protecting group techniques. Initially, the Boc group[57] was used for *N*-terminus protection. Consequently, the use of 9-fluorenylmethoxycarbonyl (Fmoc)[58–60] based coupling strategies and Cbz[61,62] based strategies appeared to be superior methods as they avoided acidic conditions for deprotection of the *N*-termini.

However, glycosylation of *N*-acylated *β*-amino alcohols, such as Fmoc-protected or Cbz-protected serine and threonine derivatives, as well as the structurally related ceramides and protected sphingosines,[63] is not efficient. Problems encountered by pioneers[64,65] in this area include low yields and poor α/β selectivity. Polt et al.[66] attributed these problems to the decreased nucleophilicity of the glycosyl acceptor due to an unfavourable hydrogen bonding pattern which arises from amide type protecting groups, or an adjacent peptide bond (Fig.2.2).

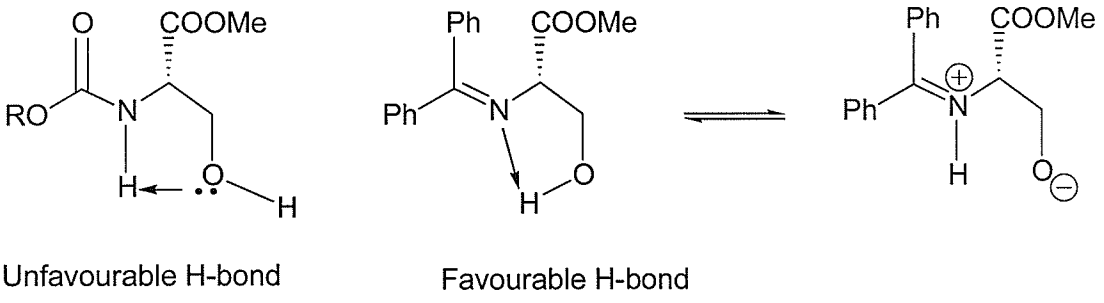
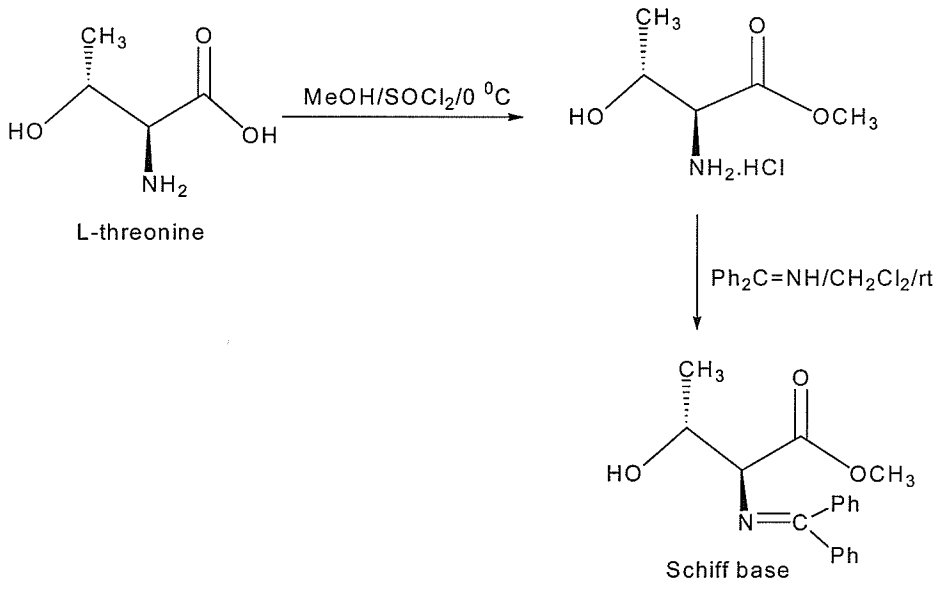


Figure 2.2: Reactivity of imine protected threonines/serines

Therefore, they suggested protection of the *N*-terminus with an imine group which would generate a favourable hydrogen bonding pattern. Furthermore, this would

increase the nucleophilicity of the neighbouring hydroxyl.

Polt et al. have developed a synthetic methodology for glycosidation of L-threonine. It has been shown that L-threonine Schiff base can be synthesized from commercially available L-threonine as shown in Scheme 2.2. In addition, they had successfully synthesized various glycosides by using this L-threonine Schiff base according to the Koenigs-Knorr procedure. Similarly, we synthesized D-threonine Schiff base **21** and tried to synthesize the required glycoside **12**.

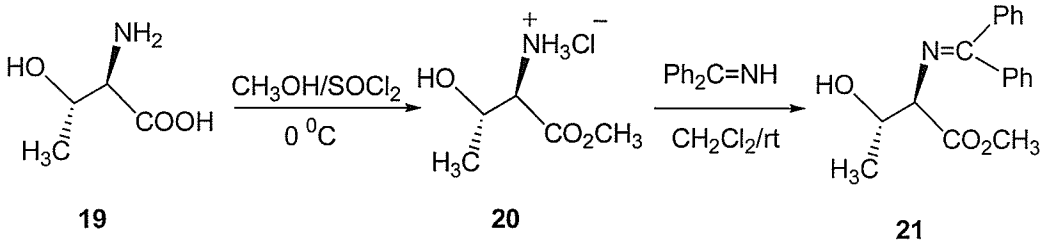


Scheme 2.2: Robin Polt's procedure

2.2.1 Synthesis of methyl *N*-(diphenyl methylene)-D-threoninate

According to our synthetic plan illustrated in Scheme 2.1, we also needed to synthesize the acceptor **21** (Schiff base). Commercially available D-threonine was boiled in methanol with thionyl chloride to synthesize the crude D-threonine methyl ester hydrochloride **20** in 98% yield. The crude compound **20** was then treated with benzophenone imine according to O'Donnell and Polt's procedure (Scheme 2.3).[67]

After 2 days reaction time, the crude product was purified by recrystallization (ethyl ether:hexane, 1:1). Schiff base **21** was obtained as needle shaped crystals in 85% yield.

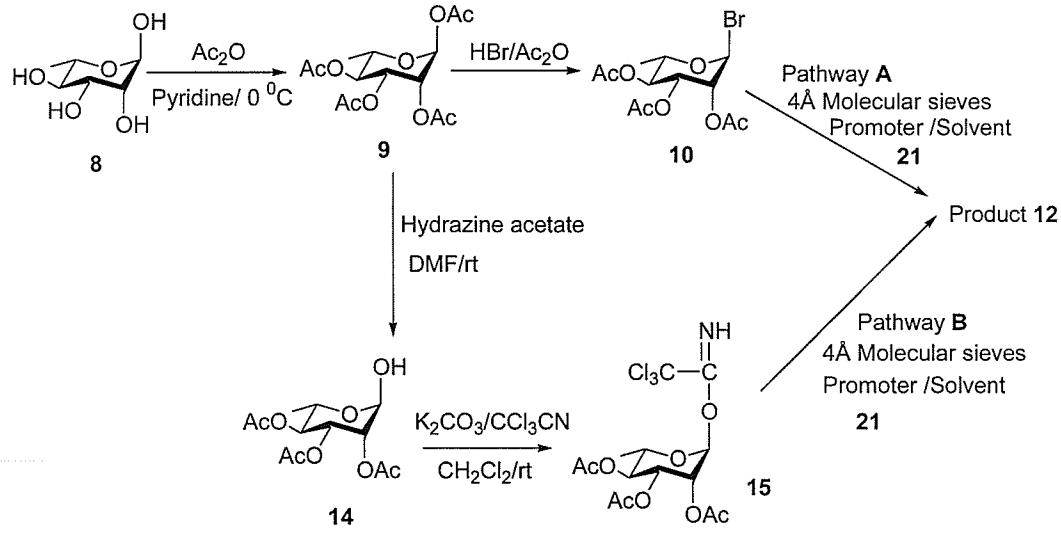


19 **20** **21**

Scheme 2.3: Formation of the Acceptor

2.2.2 Attempted glycosylation with bromide

Earlier reports on Koenigs-Knorr glycosylation demonstrated that glycosyl bromides with a participating group at C-2 were appropriate donors for the synthesis of 1,2-*trans*-glycosides.[68] This prompted us to investigate this approach for the synthesis of α-L-rhamnopyranoside **12** (Scheme 2.4, pathway **A**).



8 **9** **10** **14** **15**

Scheme 2.4: Attempted glycosylation using rhamnosyl bromide as a donor

Compound **8** was acetylated quantitatively with acetic anhydride in pyridine to give tetraacetate **9**.[69] Treatment of **9** with HBr provided the rhamnosyl bromide **10** which was purified by column chromatography. The compound **10** was reacted with the acceptor **11** with variation of the solvent, temperature and promoter to find the best conditions for this reaction.[70] Table 2:1, paths a-h present the results obtained from these experiments. In all of these reaction paths, compound **10** was stirred with **11** for approximately 24 hours but no product could be detected by TLC.

2.2.3 Attempted glycosylation with imidate

Because we required the coupling product **12** for the synthesis of target analogues, the coupling reaction was attempted again by changing the donor to rhamnosyl imidate **15** (Scheme 2.4, pathway **B**).[66,71] We tested the synthesis by varying the solvent, promoter and temperature. In Table 2:1, paths i-m present the results obtained from these experiments.

Compound **14** was treated with 6 equivalents of trichloroacetonitrile in the presence of potassium carbonate in dichloromethane[72] and we obtained α-rhamnosyl imidate **15** as the major product. In addition, we found a small amount of β-rhamnosyl imidate as the minor product.

We used TMSOTf as a promoter for this process (Table 2.1, paths j-m). Reaction attempted with $BF_3{\cdot}OEt_2$ was generally unsatisfactory (Path i in Table 2.1). In path j, we changed the promoter to TMSOTf and the solvent to CH_3NO_2. TLC showed no product formation.

We next attempted the same reaction by changing the solvent to diethyl ether (Path k in Table 2.1). After stirring for 16 hours, two new carbohydrate spots and spots for the two reactants appeared in the TLC. Purification was done by flash

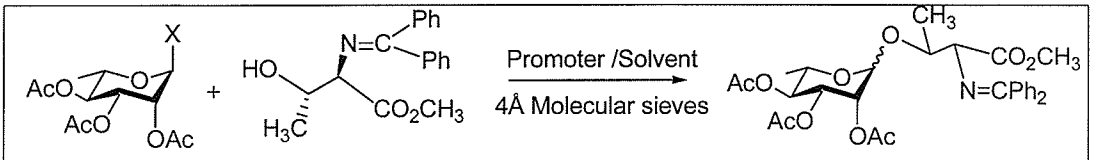
column chromatography (hexane:EtOAc, 2:1) and the products were identified by NMR. NMR confirmed that these two new spots on TLC were α and β anomers of the coupling product. Unfortunately, the yield of the required α anomer **12** was 7% and of the β anomer **13** was 38%. The amount of recovered starting material was substantial. However, in this attempt the reaction did work to a certain extent.

We then tried the same reaction in dichloromethane at room temperature (Path l in Table 2.1). We obtained a crude product that was purified by column chromatography. The yield of the α anomer **12** was 16%, while the β anomer **13** was obtained in 31% yield. In addition, we isolated a considerable amount of starting material. As we obtained a larger portion of β anomer in this reaction at room temperature, we next studied the same system at -78 ^{0}C (Path m in Table 2.1). This reaction was unsuccessful as we only obtained 2% of the expected anomer.

2.2.4 Glycosylation with a thioglycoside donor

We next studied glycosylation using a thioglycoside as the donor. As depicted in Scheme 2.5, the rhamnosyl thioglycoside donor **16** was prepared from L-rhamnosyl tetraacetate **9**. Acetylated rhamnose **9** was treated with ethanethiol in the presence of the Lewis acid $BF_3{\cdot}OEt_2$. This reaction was monitored by TLC. After 5 h reaction time, TLC showed that the reaction was complete and two products had formed. Products were isolated by recrystallization and identified by NMR. The α anomer **16** was isolated as the major product. It was obtained as a crystalline solid in 80% overall yield.[73] The minor β-thioglycoside was obtained in 15% yield.

The reaction mechanism for preparing rhamnosyl thioglycosides from peracetylated rhamnose **9** has been studied (Scheme 2.5).[74] An intermediate oxocarbenium ion **17** is first formed by cleaving the exocyclic acetyl group with the assistance of



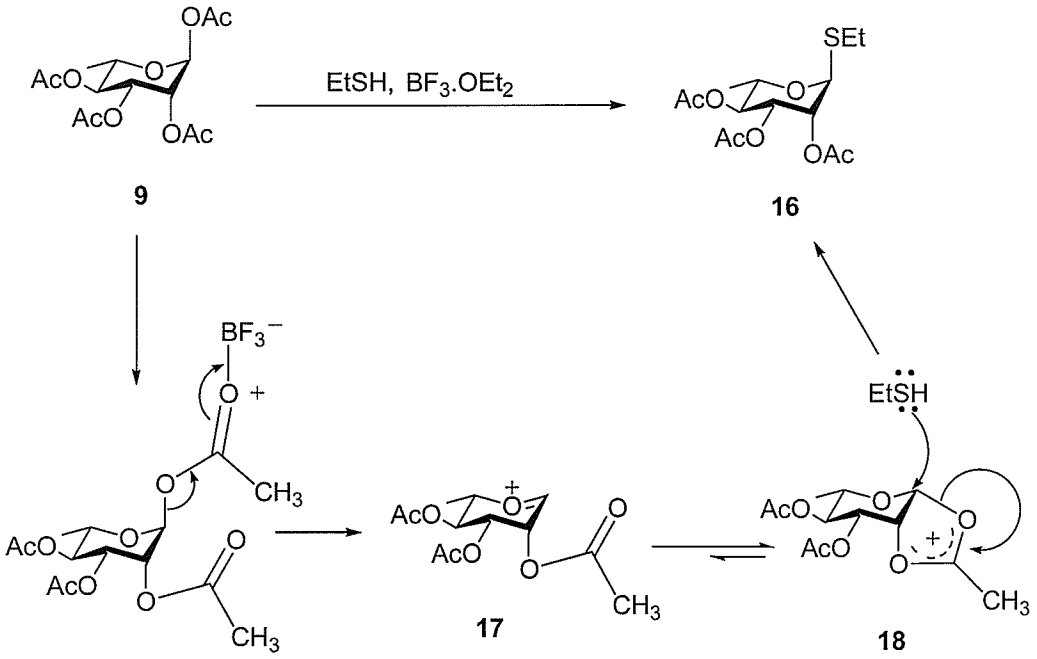
10 , X=Br
15 , X=OC=NHCCl$_3$
16 , X=SEt

α / β mixture

Path	Donor	Temp. (^{0}C)	Promotor	Solvent	Yield(%) =α:β
a	Bromide **10**	rt	$HgCN_2$:$HgBr_2$	CH_2Cl_2	0
b	Bromide **10**	rt	$HgCN_2$	CH_3NO_2:Benzene	0
c	Bromide **10**	rt	$HgCN_2$	CH_2Cl_2	0
d	Bromide **10**	rt	$HgCN_2$	CH_3NO_2	0
e	Bromide **10**	rt	$HgCN_2$	Acetonitrile	0
f	Bromide **10**	rt	AgOTf	Benzene	0
g	Bromide **10**	-25	AgOTf	CH_2Cl_2	0
h	Bromide **10**	-25	AgOTf	CH_3NO_2:Benzene	0
i	Imidate **15**	rt	$BF_3.OEt_2$	Hexane	0
j	Imidate **15**	-40	TMSOTf	CH_3NO_2	0
k	Imidate **15**	rt	TMSOTf	Et_2O	7:38
l	Imidate **15**	rt	TMSOTf	CH_2Cl_2	16:31
m	Imidate **15**	-78	TMSOTf	CH_2Cl_2	2:0
n	Thioglycoside **16**	-78	TfOH/NIS	CH_2Cl_2	6:0
o	Thioglycoside **16**	rt	TfOH/NIS	CH_2Cl_2	35:25

Table 2.1: Different conditions used for glycosidic bond formation

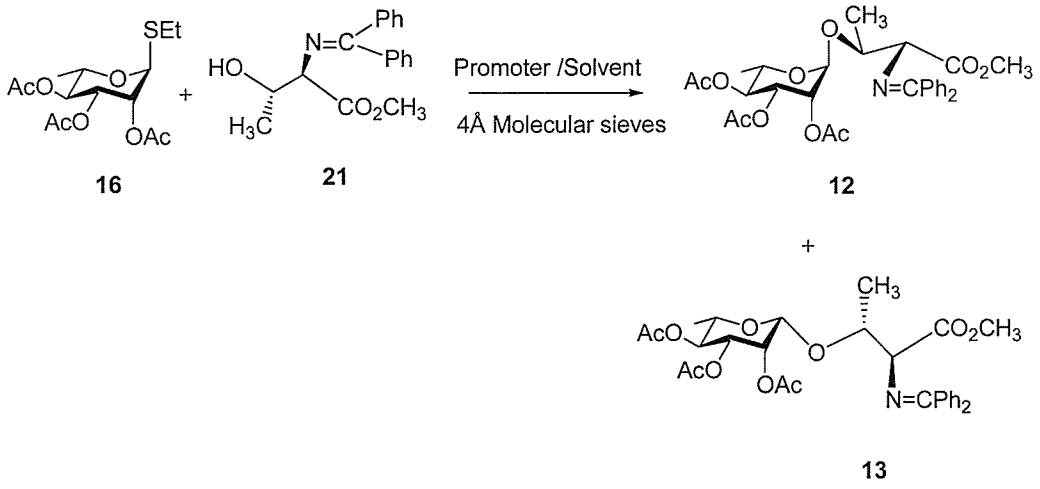
$BF_3{\cdot}OEt_2$. The intermediate **17** equilibrates to the more stable intermediate, dioxonium ion **18,** by neighbouring group assistance of the C-2 acetyl group. Nucleophilic attack by ethanethiol on the intermediate **18** affords the α rhamnosyl thioglycoside **16** via an S_N2 mechanism.



Scheme 2.5: Mechanism of rhamnosyl thioglycoside

2.2.5 Synthesis of coupling product **12**

Rhamnosyl thioglycoside **16** was coupled with the acceptor **21** (Scheme 2.6). This reaction is very sensitive even to a trace amount of moisture and it is important to use extremely dry conditions. The promoters were *N*-iodosuccinimide and triflic acid.



Scheme 2.6: Glycosylation using rhamnosyl thioglycoside as donor

First, the reaction was performed at -78^{0}C in dichloromethane (Path n in Table 2.1). Completion of the reaction was monitored by TLC. After 2 hours reaction time, TLC confirmed that two products had formed. However, after work up and purification, a substantial amount of starting material was recovered. In addition, α anomer **12** was isolated in 6% yield, and structure was identified by NMR. We presumed that the β anomer **13** was formed by comparing the R_f values ($R_f = 0.28$) of the TLC, even though we could not isolate it.

We also conducted the same coupling reaction at room temperature (Path o in Table 2.1). This reaction produced two new products which were purified by column chromatography. They were identified by using NMR as **12** (Fig.2.3) and **13** (Fig.2.4). The two anomers can be distinguished by nuclear Overhauser effect (nOe) analysis (Fig.2.5). In compound **12,** average nOe enhancements were found between the H-5 and the CH_3 and H-3 positions of the sugar ring (7% and 2% enhancements respectively). No nOe was observed between H-5 and the anomeric proton. This indicated that the compound **12** was the α anomer. Compared to the compound **12**, compound **13** showed nOe between H-5 and the anomeric proton (4.4% enhance-

ment). This confirmed compound **13** was the β anomer. In addition, compound **13** showed nOe between H-5 and H-3 and CH_3 positions of the sugar ring (5.5% and 4% enhancements respectively).

Based on the results in Table 2.1, we concluded that this reaction at room temperature (Path o) was a better approach than the others. This gave 35% yield of the α-isomer and 25% of the β anomer. Since all our efforts to synthesize α anomer using different donors under different conditions proved to give very low yields, we decided to continue our study with Path o for which the results are shown in Table 2.1.

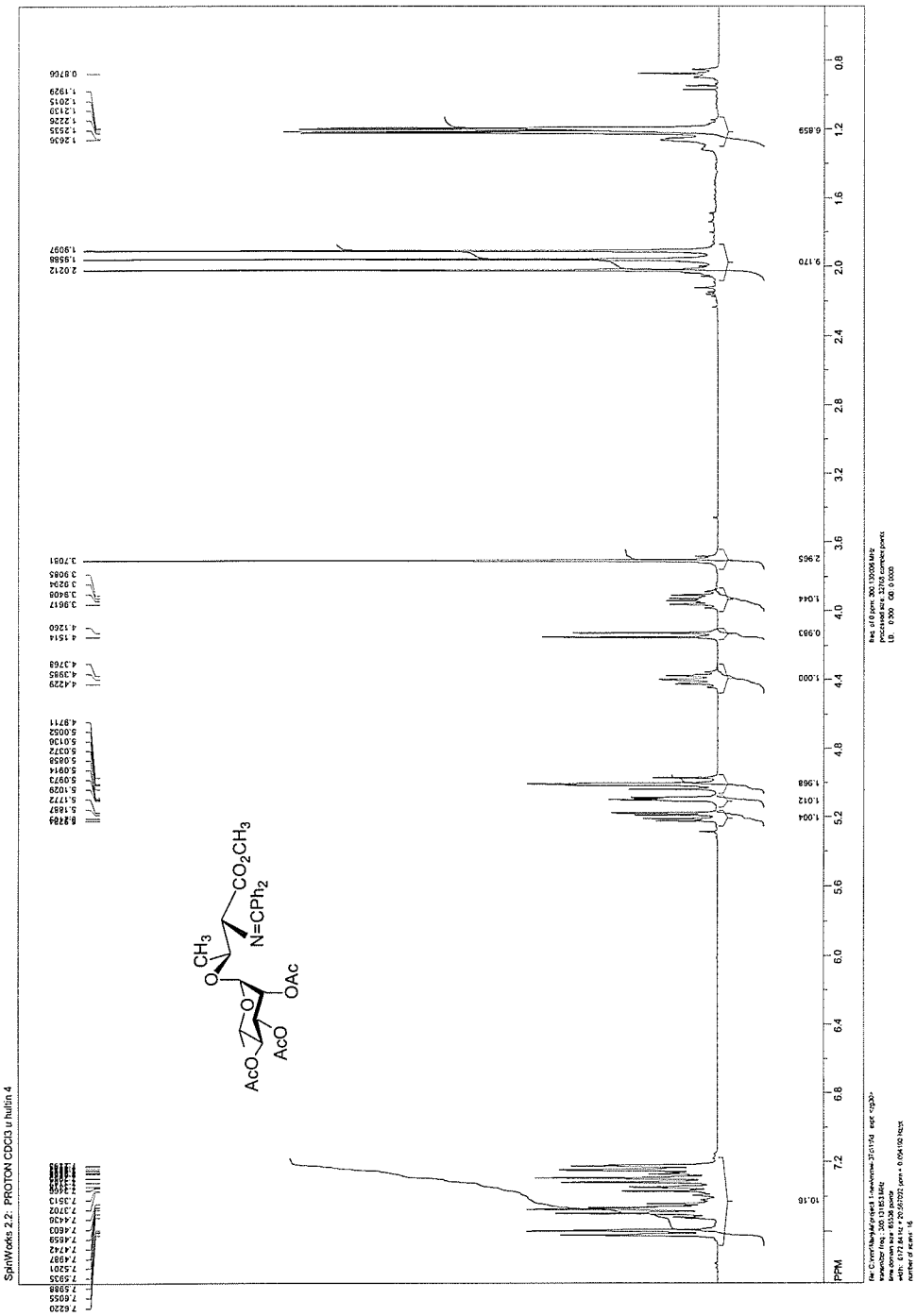


Figure 2.3: The ^{1}H NMR spectrum of **12**

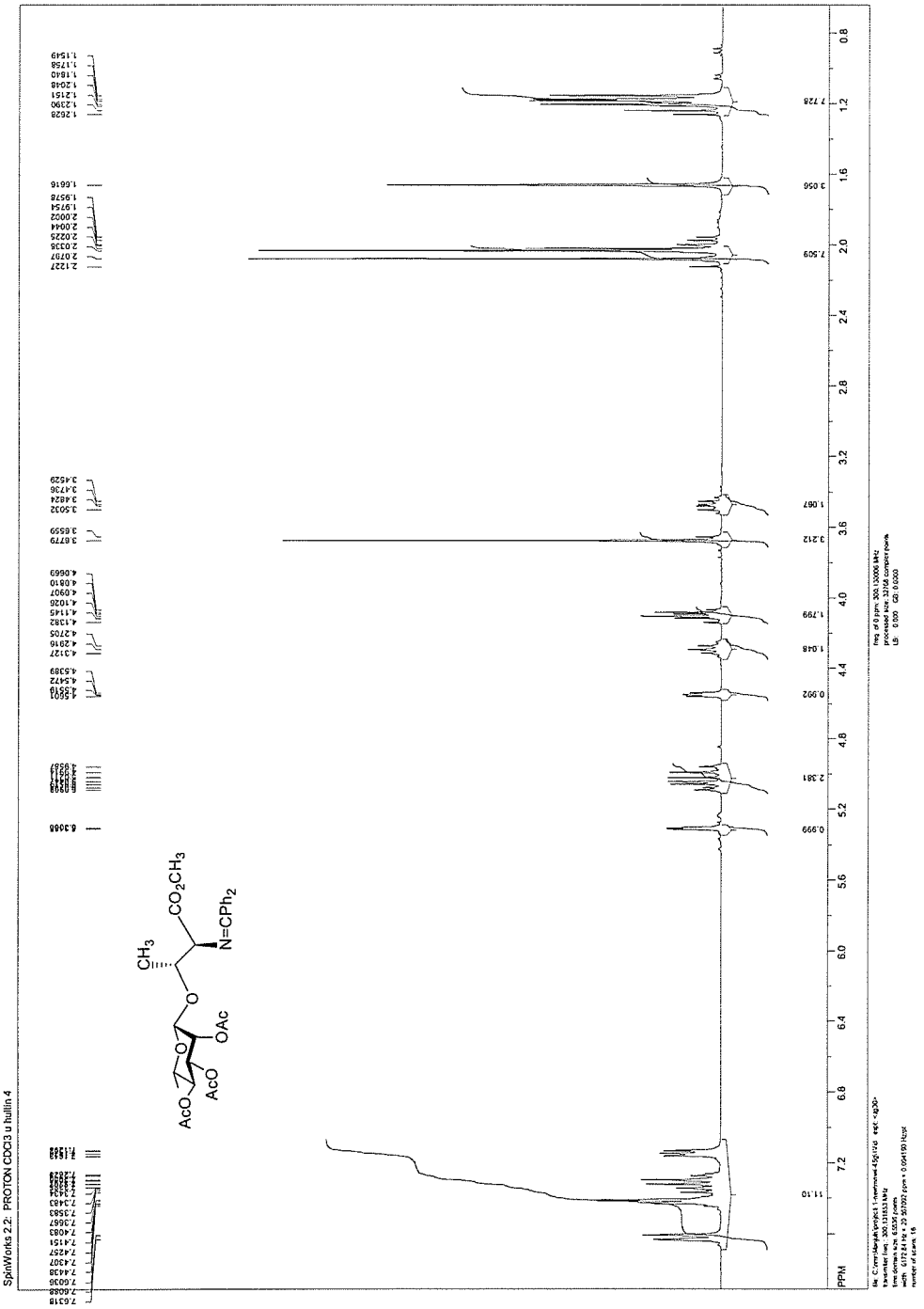


Figure 2.4: The ^{1}H NMR spectrum of **13**

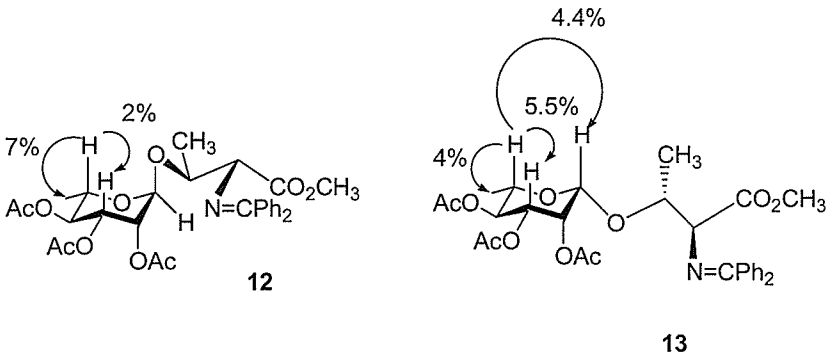
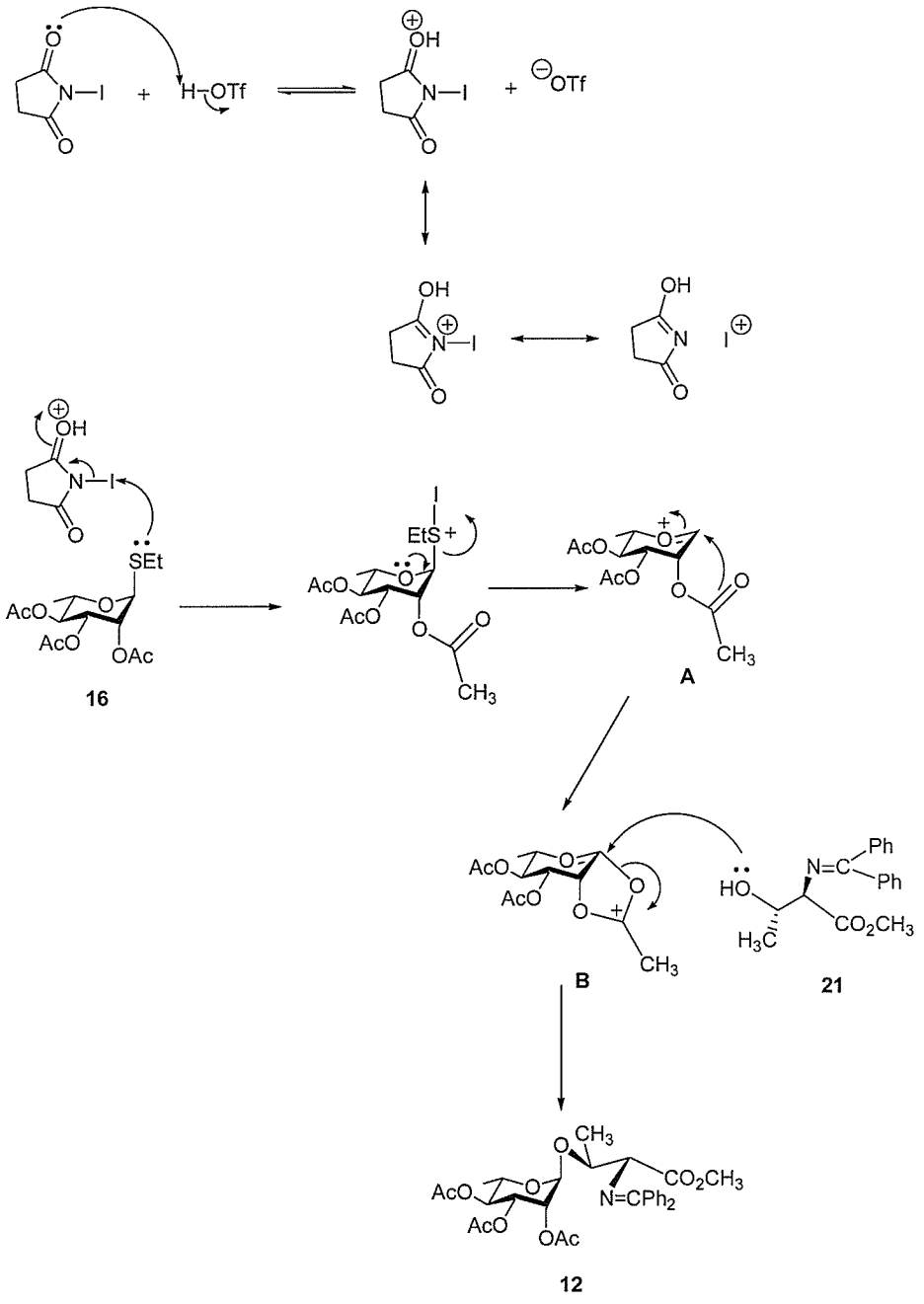


Figure 2.5: The nOe results of compound **12** and **13**

The mechanism of the coupling process can be sketched as shown in Scheme 2.7. The electrophile in this reaction is a combination of two promoters NIS/TfOH. First, sulfur from the thioglycoside donor adds to the electrophile and then the sulfur-carbon bond breaks with the assistance of a kinetic anomeric effect. This forms the oxocarbenium ion **A**, which would generate the more stable cyclic dioxonium ion **B** by neighbouring group participation. Nucleophilic attack by acceptor on intermediate **B** would produce the α coupling product.

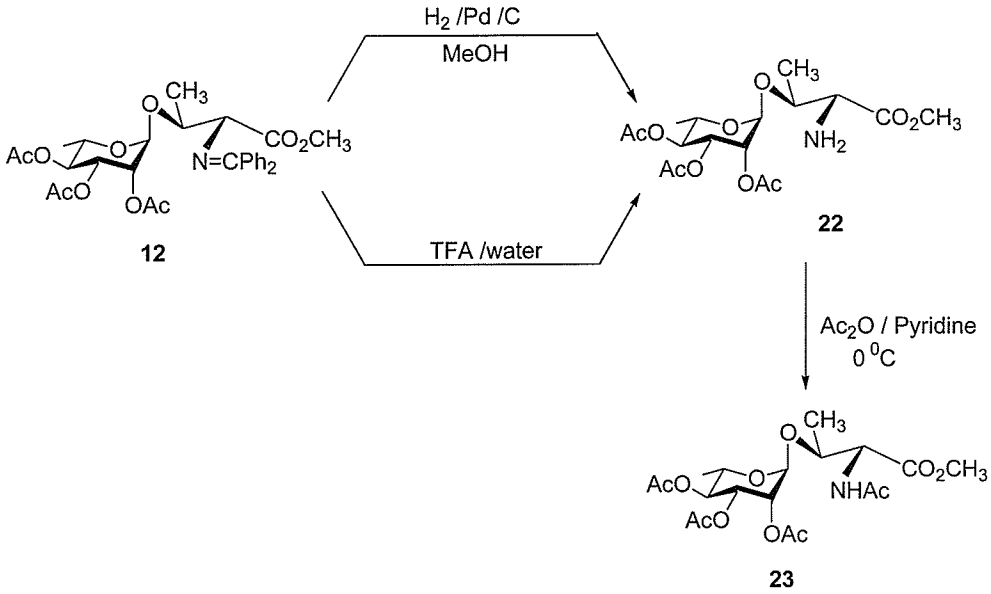


Scheme 2.7: Mechanism of the coupling reaction

We next focused our attention on the deprotection of the α anomer **12**.[75] This was first tried by using Pd/C in methanol, stirring at room temperature under H_2 (Scheme

2.8). After 10 minutes, the reaction was complete, as determined by TLC. The crude product was diluted with dichloromethane and filtered through Celite. The product was purified by column chromatography to afford **22** in 67% yield. The NMR spectra were used to identify the compound. The ^{1}H NMR spectrum confirmed the structure of this compound through the disappearance of the proton peaks corresponding to the two phenyl groups (Fig.2.6).

It is worthy of note that we tried the same reaction using trifluoroacetic acid and water in THF. We obtained the deprotected product **22** within 5 minutes in 80% yield.



Scheme 2.8: Synthesis of compound **22** and **23**

In the next step, the compound **22** was acylated and we obtained the desired analogue **23** in 96% yield. The product **23** was purified and fully characterized by NMR. An additional singlet appeared at 2.14 ppm in the proton spectrum, as well as a broad doublet at 6.3 ppm corresponding to the NHAc group (Fig.2.7).

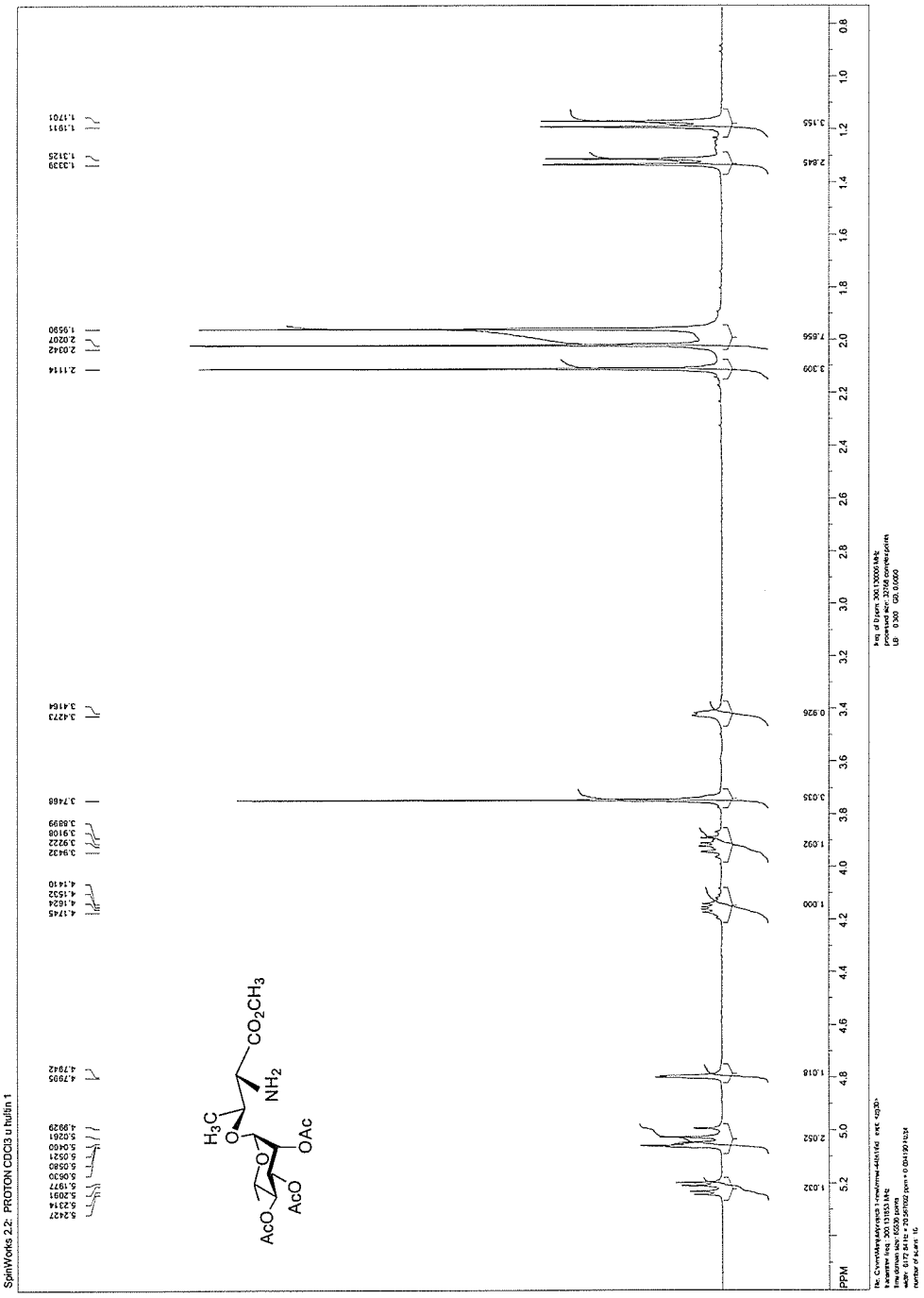


Figure 2.6: The ^{1}H NMR spectrum of **22**

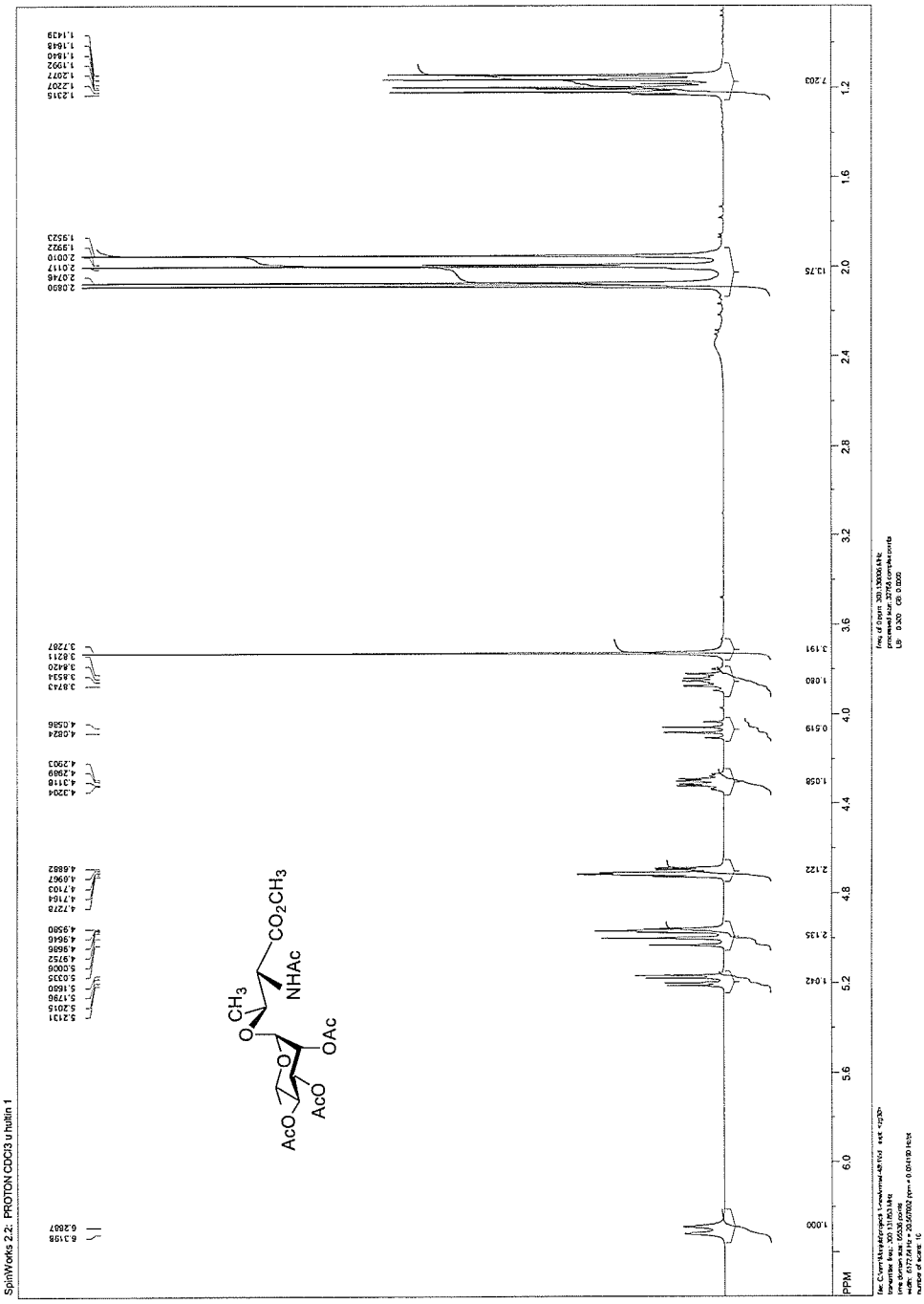


Figure 2.7: The ^{1}H NMR spectrum of **23**

Chapter 3

Results and Discussion II

This chapter outlines the synthesis of analogues formed by modifying the C-1´ and C-4´ positions of L-rhamnose. Our plan was to attach an octyl group to the anomeric carbon of the sugar and a heterocycle to the C-4´ position (Fig.3.1).

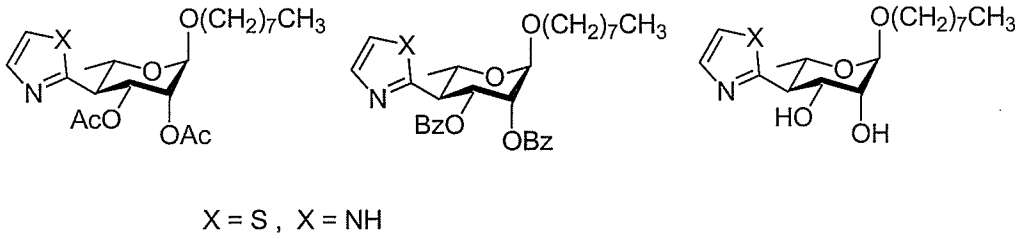


Figure 3.1: Analogues synthesized by modifying C-1´and C-4´position of L-rhamnose

These analogues were expected to disrupt the conversion of **GL-2** to **GL-3** (Fig.3.2) in arabinogalactan biosynthesis. Therefore, it is important to understand the detailed description of AG biosynthesis.

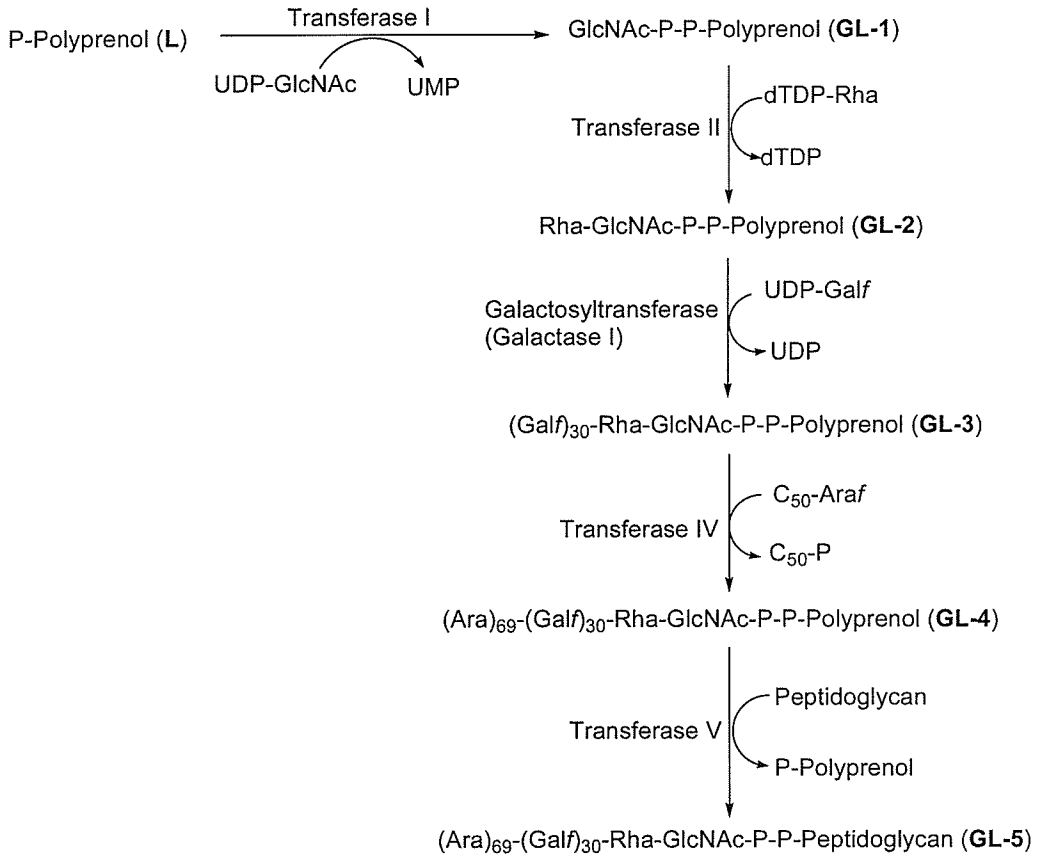


Figure 3.2: Arabinogalactan biosynthesis

3.1 Arabinogalactan Biosynthesis

AG biosynthesis begins with a lipid polyprenol phosphate (**L**) (Fig.3.2). The P-polyprenol is abundant in the mycobacterial cell wall, and serves as an acceptor for glycosyltransferase I. The glycosyltransferase I catalyzes the transfer of *N*-acetyl D-glucosamine 1-phosphate (GlcNAc-1-P) from the nucleotide donor (UDP-GlcNAc) to the polyprenol phosphate (Pol-P), yielding glycolipid GlcNAc-P-P-polyprenol (**GL-1**). In turn the **GL-1** serves as an acceptor for the next step of glycosylation. Glycosyltransferase II transfers L-rhamnopyranose from the sugar nucleotide donor (dTDP-Rha) to **GL-1**, forming the disaccharide lipid Rha-GlcNAc-P-P-polyprenol (**GL-2**).

At this stage, the disaccharide linker is synthesized. The galactofuranose and arabinofuranose are sequentially introduced from sugar donors UDP-Gal*f* and C_{50}-P-Ara*f* to the disaccharide linker through the rhamnose end, forming AG-Rha-GlcNAc-P-P-polyprenol glycolipid **GL-4**. At the last step of the biosynthesis, **GL-4** serves as a glycosyl donor and is transferred to a peptidoglycan.

3.2 Synthesis of imidazole analogues

Having established the procedure for synthesis of the D-threonine analogues, we focused our attention on the next aspects of the project. The area of inhibitors of mycobacterial glycosyltransferases has developed rapidly over the past few years. A survey of the progress of this field helped us to design our project. Mainly there are 2 types of mycobacterial glycosyltransferase inhibitors. They are galactosyltransferase inhibitors and arabinosyltransferase inhibitors.

3.2.1 Galactosyltransferase inhibitors

The polysaccharide AG in the mycobacterial cell wall (Fig.1.1) has many galactofuranose residues. These galactose linkages are the ideal targets for designing drugs against mycobacterial galactosyltransferases. Reynolds and coworkers have reported several galactofuranose disaccharides with $\beta1\rightarrow5$ and $\beta1\rightarrow6$ glycosidic linkages similar to the natural galactan (Fig.3.3).[76] They were designed with the aim of disrupting the arabinogalactan biosynthesis (**GL-2** to **GL-3**) at a late stage, when the galactan portion is already under construction. All these compounds have their reducing end, C-1 blocked by an *n*-octyl group. The *n*-octyl group is considered to be a suitable mimic of the lipophilic polyprenol moiety of the natural sugar donors.

The analogues **25** and **30** were found to be cytotoxic in an *in vitro* whole cell

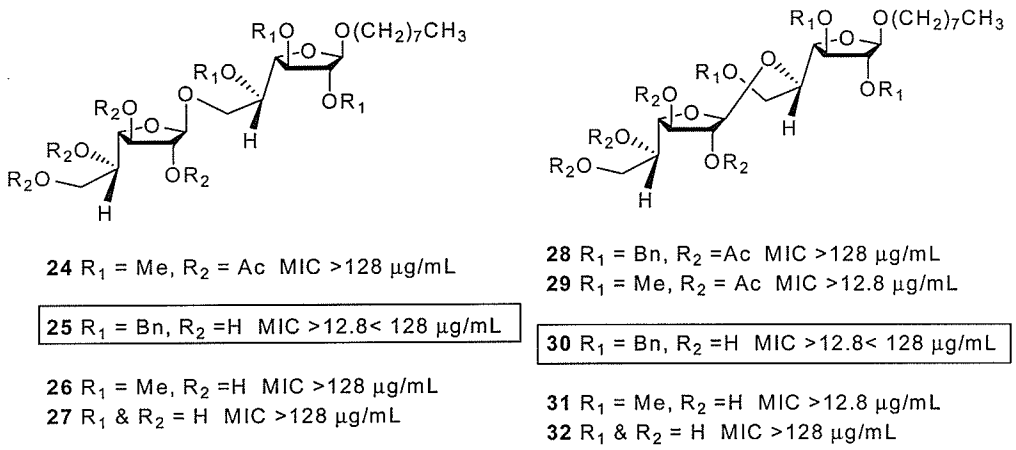
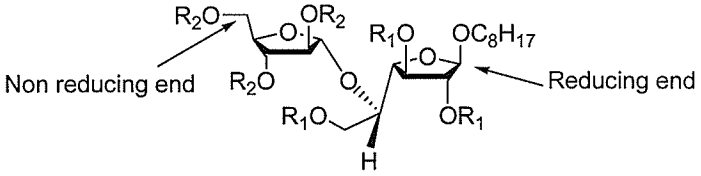


Figure 3.3: The galactofuranose disaccharides with $\beta 1 \rightarrow 5$ and $\beta 1 \rightarrow 6$ glycosidic linkages

assay, but they were not active as either acceptors or inhibitors in a cell free glycosyltransferase assay. Clearly, the benzyl groups in the reducing sugar are too large to allow enzyme binding and inhibition. In addition, the fully de-protected analogues **27** and **32** were found to be the only active galactosyltransferase acceptors in the mycobacterial galactosyltransferase assay.

As a continuation of their earlier work, Reynolds and coworkers[77] have synthesized a group of analogues that target the formation of an Ara*f* $\alpha(1\rightarrow5)$-Gal*f* linkage (Fig.3.4). They were aimed to disrupt the arabinogalactan biosynthesis (**GL-3** to **GL-4**) in the assembly of the arabinan. All these compounds have their reducing end, C-1 blocked by an *n*-octyl group. In addition, analogues **33** and **34** have their non reducing end blocked while **35** and **36** have their non reducing end open. All of these analogues have been tested against the cell growth of *M. tuberculosis*. The test results showed that the partially deprotected disaccharide analogue **35** was the best inhibitor. That means the Ara*f* $\alpha(1\rightarrow5)$-Gal*f* octyl linkage in **35** is similar to the natural substrate. The fully de-blocked disaccharide **37** was the only arabinosyltransferase

acceptor found in the acceptor assay. The other compounds were not recognized as substrates for arabinosyltransferase enzymes, presumably due to the presence of the methyl and benzyl ether protecting groups on the reducing sugar.



33 R_1 = Bn, R_2 = Bz MIC >128 µg/mL
34 R_1 = Me, R_2 =Bz MIC >128 µg/mL
35 R_1 = Bn, R_2 = H MIC =32 µg/mL
36 R_1 = Me, R_2 =H MIC >128 µg/mL
37 R_1 & R_2 = H MIC >128 µg/mL

Figure 3.4: Analogues that target Ara*f* $\alpha(1\rightarrow5)$ gal*f* linkage

Analogues **38**, **39** and **40** (Fig.3.5) have also been synthesized and tested.[2] They were aimed to disrupt at the first steps beyond the linker in arabinogalactan biosynthesis (**GL-2** to **GL-3**). The cell growth assay showed that the partially protected analogue **38** and fully protected analogue **39** were better inhibitors than the fully unprotected analogue **40**. The acceptor assay indicated that the fully deprotected disaccharide analogue **40** and the partially deprotected analogue **38** were acceptors in the galactosyltransferase catalyzed reaction. Both of them have their non-reducing end C-6´open. Disaccharide **39** was the only analogue that showed inhibition activity but was not an acceptor. This is probably due to the blockage of both its C-1 and C-6´ ends. The blockage prevented it from being used as a glycosyl acceptor by the enzyme.

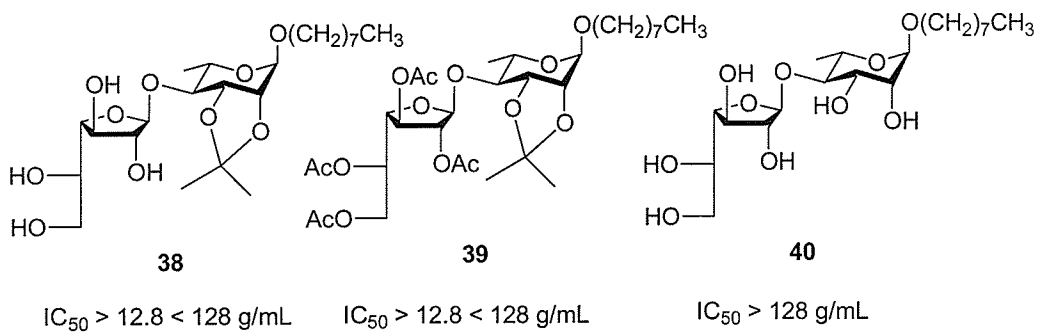


Figure 3.5: The results of Reynolds and coworkers[2]

3.2.2 Arabinosyltransferase inhibitors

Reynolds et al.[78] studied the effect of $\alpha(1\rightarrow5)$ linked octyl arabinofuranosyl disaccharides (Fig.3.6) for mycobacterial arabinosyltransferase activity. All of these compounds have their reducing end, C-1, blocked by an *n*-octyl group. Compounds **41** and **42** are fully protected disaccharide analogues, **43** and **44** are partially protected disaccharide analogues and **45** is a fully unprotected disaccharide analogue. Analogues **44** and **45** have their reducing end closed, and **43-45** have their non-reducing end C-5´ open.

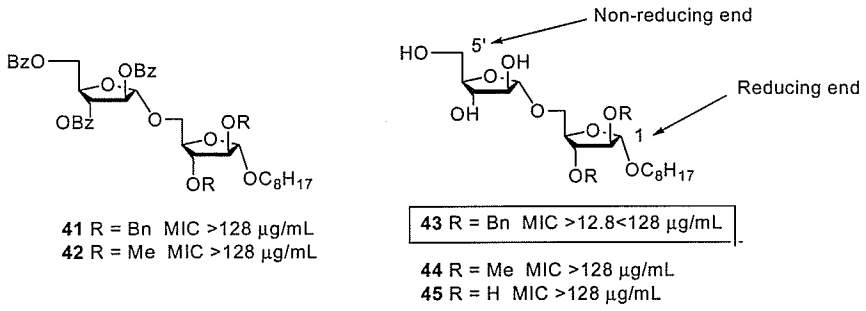


Figure 3.6: The $\alpha(1\rightarrow5)$ linked octyl arabinofuranosyl disaccharide

In-vitro assays of these analogues were conducted with a multi-drug resistant

strain of *M. tuberculosis*. The tests indicated that **43** was the best inhibitor (IC_{50}>12.8<128 μg/ml). Acceptor activity assays showed that the fully unprotected disaccharide analogue **45** was a good acceptor of the arabinosyltransferase, but the partially protected analogue **43** was not. Enzyme assays of these analogues indicated that **43** was an inhibitor of arabinosyltransferase with an IC_{50} value of 1.12 mM. The partially deprotected disaccharide **44** was also an inhibitor, but with much weaker activity. All of these results implied that **43** retained the recognition sites of the natural substrate, but prevented the enzyme from using it as an acceptor.

All the literature on mycobacterial glycosyltransferase inhibitors suggested to us that it is logical to synthesize analogues with an *n*-octyl group at the reducing end. Therefore, we decided to incorporate an octyl group on the C-1´ position of rhamnose sugar and synthesized our second set of compounds. In addition, we decided to modify the C-4´ position of rhamnose sugar.

The C-4´position was modified by incorporating an imidazole and also a thiazole group. At physiological pH imidazole may protonate so that the resulting structure, with a positively charged imidazole, mimics the partial positive charge of the galactofuranose residue (Fig.3.7). If the imidazole could be attached via a spacer (O or C), the structure would be a better representative of the natural linker with galfuranose than one without a spacer. However, we faced problems of finding a synthetic strategy to incorporate a spacer. Therefore, we decided to synthesize our compounds without a spacer.

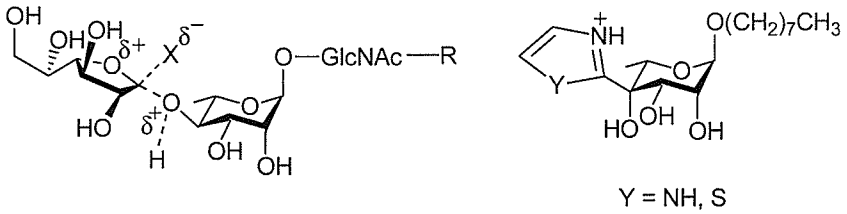
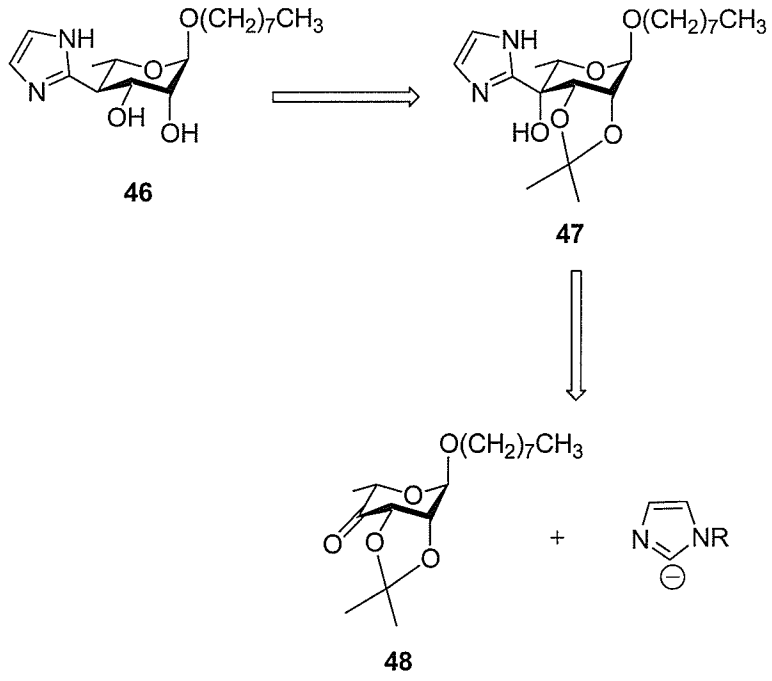


Figure 3.7: A representation of how positively charged heterocycle mimics the partial positive charge of the galactofuranose residue

3.2.3 Retrosynthetic analysis

For the preparation of **46**, we chose ketone **48** and imidazole as the building blocks as shown in Scheme 3.1. Compound **47** was the main intermediate in this synthesis.



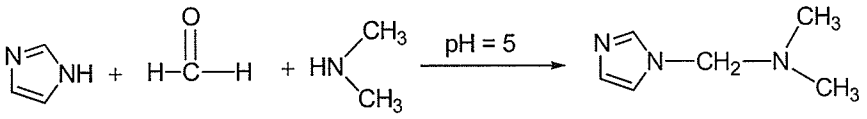
Scheme 3.1: Retrosynthetic analysis of **46**

3.2.4 Synthesis of the imidazole anion

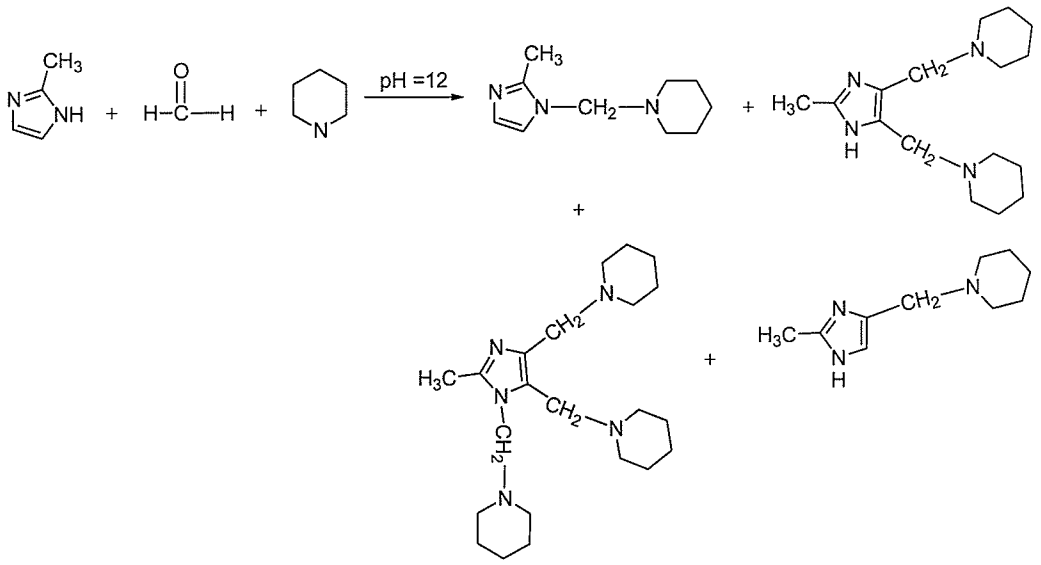
According to the retrosynthetic pathway in Scheme 3.1, we are required to synthesize the imidazole anion at position 2. This can be done by synthesizing the *N*-substituted imidazole. This *N*-protection can be achieved by using different groups (methyl, tert-butyl).[79] However, many of them suffer from drawbacks. In some cases, lithiated derivatives fail to undergo reaction with certain electrophiles.

Katritzky et al., have described an excellent *N*-protecting method [*N*-(dialkylamino) methyl protection] which avoids all the above problems. In addition, this method gave us an additional advantage as it does not need a deprotection step. That is because the *N*-(dialkylamino)methyl group hydrolyzes at room temperature on treatment with a mild acid during the work up.

The synthesis of *N*-(dialkylamino)methyl derivatives of imidazole can be done under Mannich reaction conditions. In the Mannich reaction of imidazole, the ring is nucleophilic at four possible sites, the 1, 2, 4 and 5 positions.[79] The relative nucleophilicity of these four positions is determined by the pH of the medium. Under acidic conditions, substitution occurs on the nitrogen (position 1) as shown in Scheme 3.2.[80] However, under basic conditions, substitution is possible in all four possible sites. For an example, the Mannich reaction between 2-methyl imidazole, piperidine and formaldehyde gave four Mannich bases (Scheme 3.3).[80]



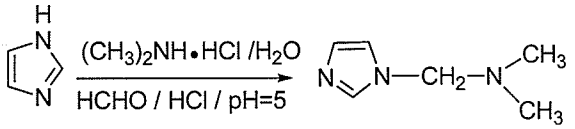
Scheme 3.2: The nucleophilicity of position 1 in imidazole



Scheme 3.3: Nucleophilicity of imidazole at 1,4 and 5 positions

According to the above literature, we mixed imidazole in water with dimethylamine hydrochloride and 37% aqueous formaldehyde solution. The pH was maintained at 5 by adding concentrated HCl (Scheme 3.4). After 48 hours, the solution was made strongly alkaline by adding 20% KOH and then the crude product was extracted with chloroform.

However, this gave a very low yield. The extraction was inefficient. That is because the product **49** tends to be more soluble in water than in $CHCl_3$. Therefore, we employed a continuous extractor to extract the product into dichloromethane. After 12 hours, we managed to extract the crude product in 92% yield. The crude product was identified by NMR and found to be clean enough to use directly in the next step.

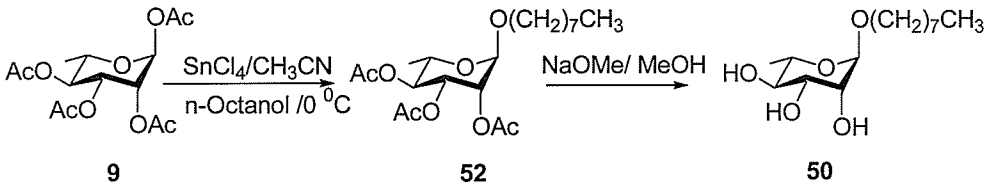


49

Scheme 3.4: Synthesis of compound **49**

3.2.5 Synthesis of L-rhamnosyl ketone **48**

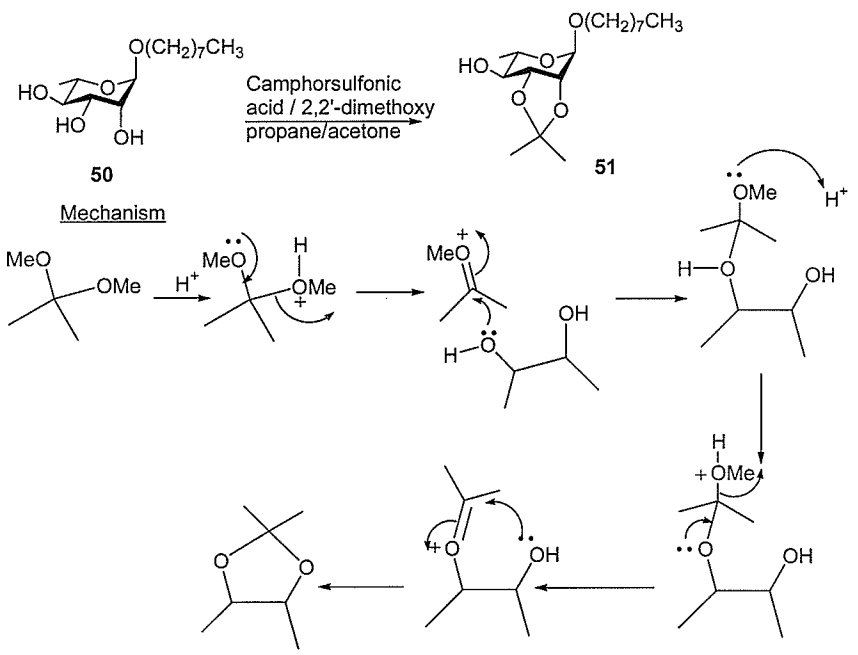
The ketone **48** was efficiently synthesized from L-rhamnosyl tetraacetate **9** in four steps. First, rhamnosyl tetraacetate **9** was converted to octyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside **52** in 80% yield.[81] The compound **52** was then deprotected with sodium methoxide in methanol and we obtained compound **50** (Scheme 3.5) as a white solid in 88% yield.[81]



9 **52** **50**

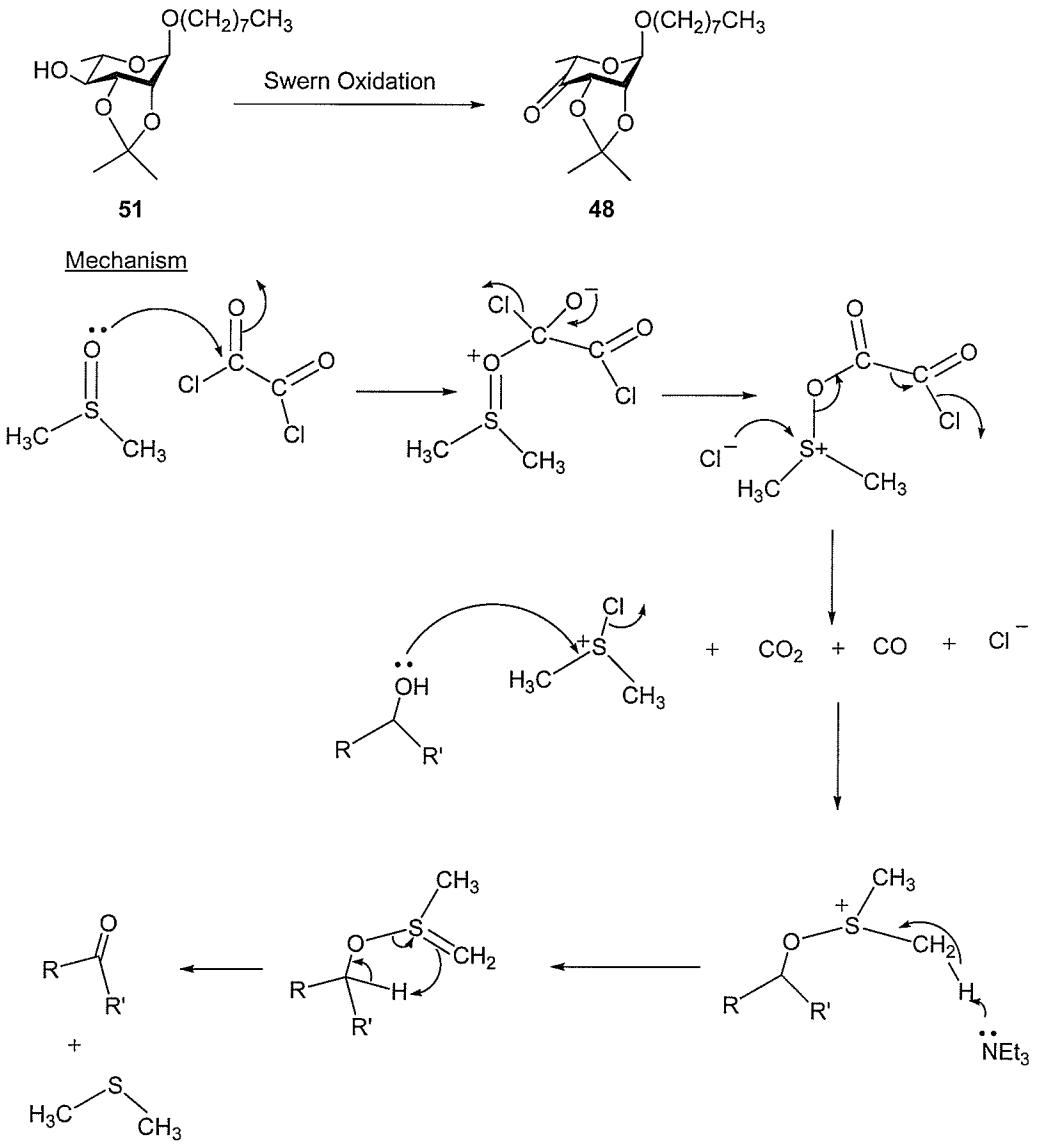
Scheme 3.5: Synthesis of **50**

The deprotected product **50** was then treated with 2,2´-dimethoxypropane and camphorsulfonic acid in acetone.[81] After 5 hrs, the 2,3-diol **50** was selectively protected with an isopropylidene group. The 2,3-isopropylidene ketal is formed preferentially over the 3,4-isopropylidene ketal because the cis-fused ketal is more thermodynamically stable than the trans-fused ketal.[82] A reaction mechanism is proposed in Scheme 3.6.



Scheme 3.6: Synthesis of **51**

In the next step, the free hydroxyl at the C-4 position was oxidized by the Swern method to obtain the required ketone **48** as a colourless oil in 92% yield.[83] A mechanism for oxidation of secondary alcohols is shown in Scheme 3.7. The key step in the mechanism is the formation of a sulfoxonium species, a strong oxidizing agent.

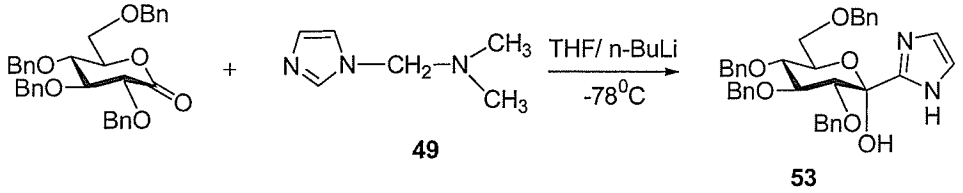


Scheme 3.7: Synthesis of **48**

3.2.6 Coupling of **49** with the rhamnosyl ketone **48**

Katritzky *et al.*[79] have shown that lithiation occurs regiospecifically at the 2 position of imidazole derivative **49**. Moreover, Andrea Vasella and coworkers[84] have reacted 1-(Me_2NCH_2)Im with tetra-*O*-benzylgluconolactone in the presence of n-BuLi and

have obtained the crystalline α-D-glucopyranose **53** in 68% yield (Scheme 3.8).

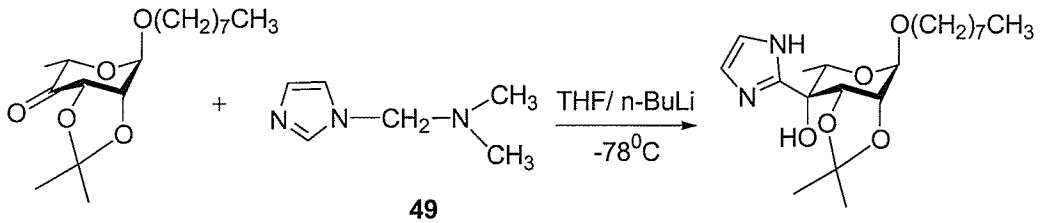


49 **53**

Scheme 3.8: The results of Andrea Vasella coworkers

As depicted in Scheme 3.8, the resulting heterocyclic carbanion of **49** can be reacted with ketone type electrophiles. Therefore, we reacted ketone **48** with the anion derived from **49** (Scheme 3.9). The reaction yielded the coupling product **47** in 67% yield. The appearance of new peaks at δ 6.94 (1H), 7.07 (1H) for imidazole hydrogen and 9.49 (brs, 1H) for the NH group in the ^{1}H NMR spectrum confirmed the successful formation of the coupling product (Fig.3.8).

In this reaction we chose n-BuLi as a base, which degrades even with a trace amount of moisture. Therefore, it is very important to handle n-BuLi under an argon atmosphere under extremely dry conditions.



48 **49** **47**

Scheme 3.9: Coupling reaction of ketone **48** and the imidazole anion **49**

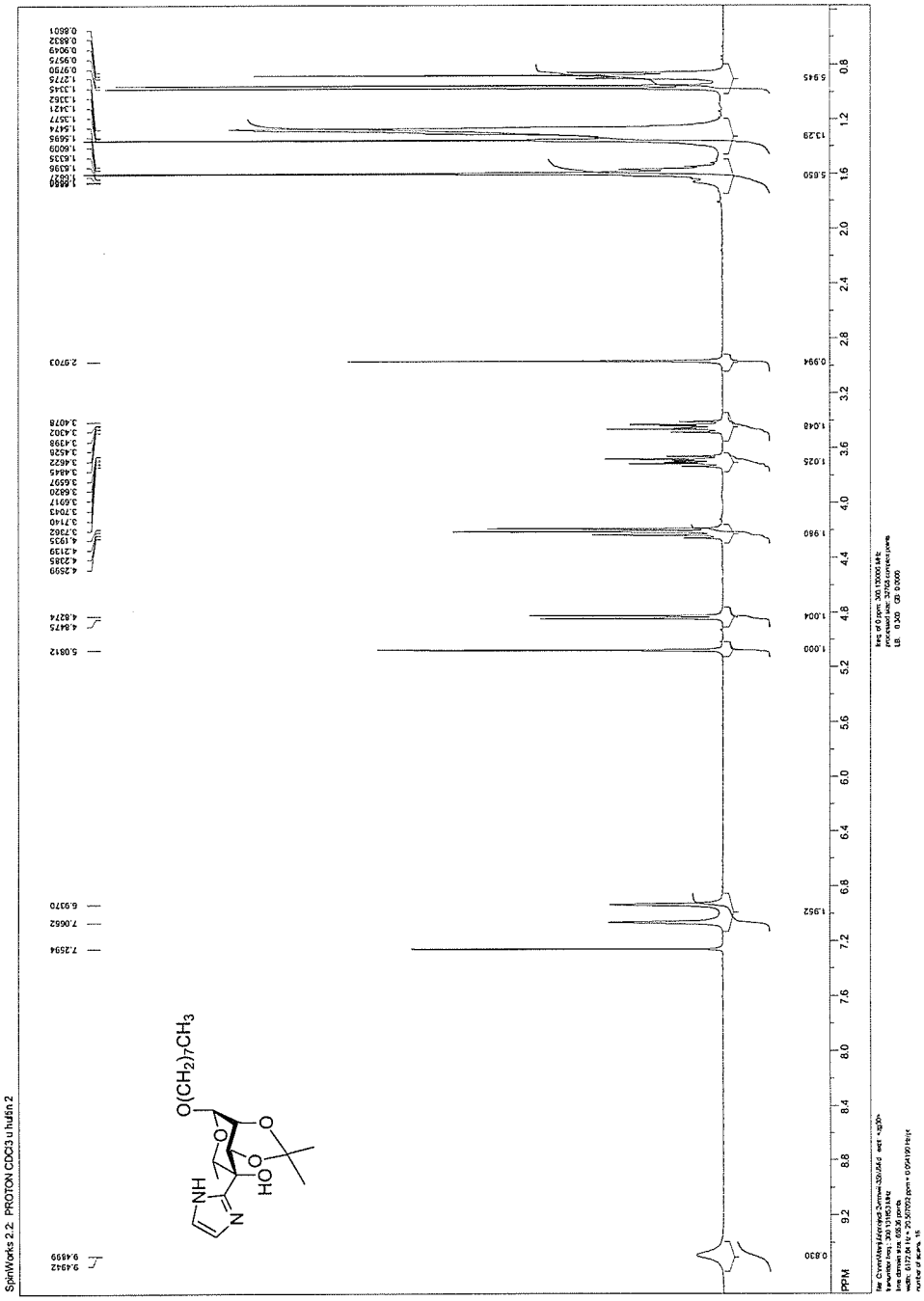
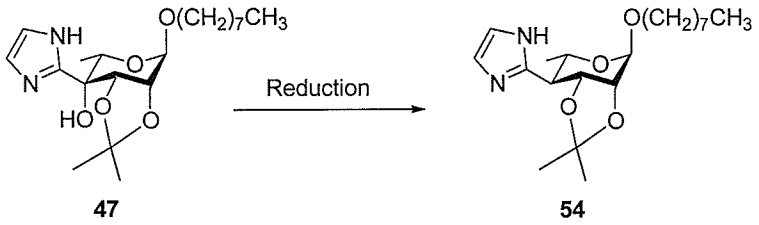
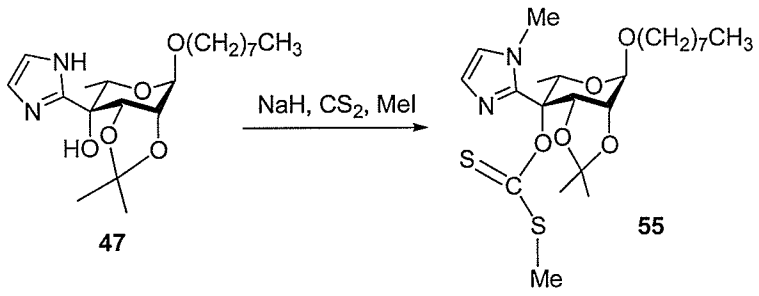


Figure 3.8: The ^{1}H NMR spectrum of **47**

In accordance with our initial synthetic plan (Fig.3.7), we next attempted the reduction of the axial C-4´hydroxyl group in **47** as portrayed in Scheme 3.10. First, we attempted the reduction by employing $NaH/CS_2/MeI$ and Bu_3SnH.[85] In the first step, we stirred the **47** in THF with $NaH/CS_2/MeI$. Consequently, we obtained intermediate **55** with a NMe group (Scheme 3.11). Disappointingly, this reaction was not successful as it incorporates a methyl group to the imidazole nitrogen.



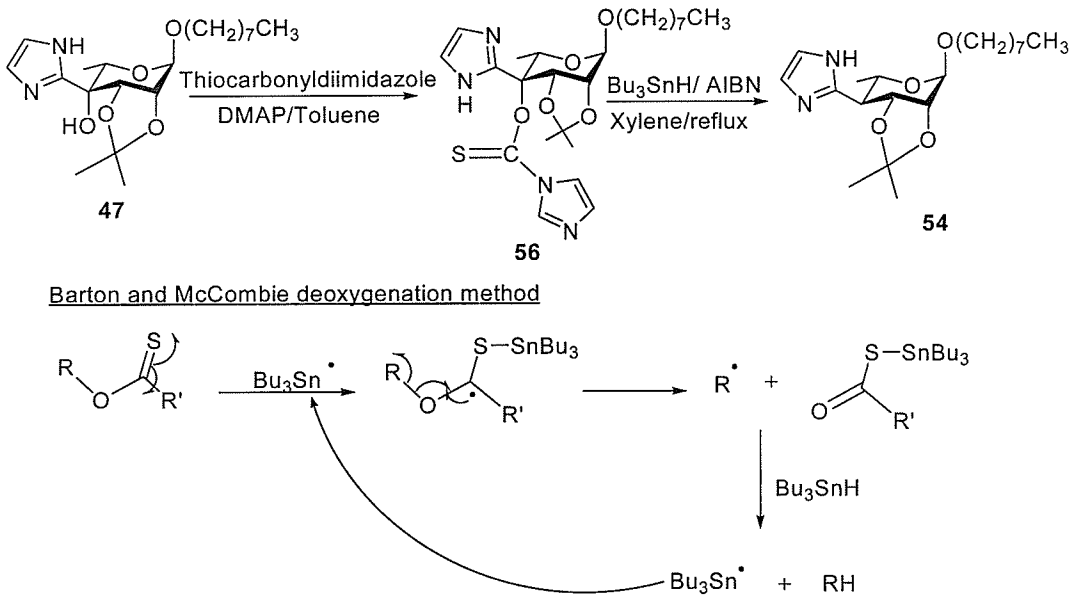
Scheme 3.10: Proposed reduction of compound **54**



Scheme 3.11: Attempted reduction of **47**

As the base NaH deprotonates both OH and NH in **47**, we next tried the Barton and McCombie method. Barton and McCombie first employed thionobenzoate, S-methyl xanthate and thiocarbonylimidazolide derivatives for deoxygenation of carbohydrate secondary alcohols in 1975.[86] Since then, this method has been extensively applied to the preparation of deoxy sugars. The mechanism is shown in Scheme 3.12.[86] An α-stabilized thiocarbon radical is formed when thiocarbonyl derivatives are attacked by the tributyltin radical. The phenyl, SMe or imidazole groups stabilize the radical. The β scission of the radical followed by hydrogen transfer from the

stannane affords the desired deoxygenated product.



Scheme 3.12: Barton and McCombie method

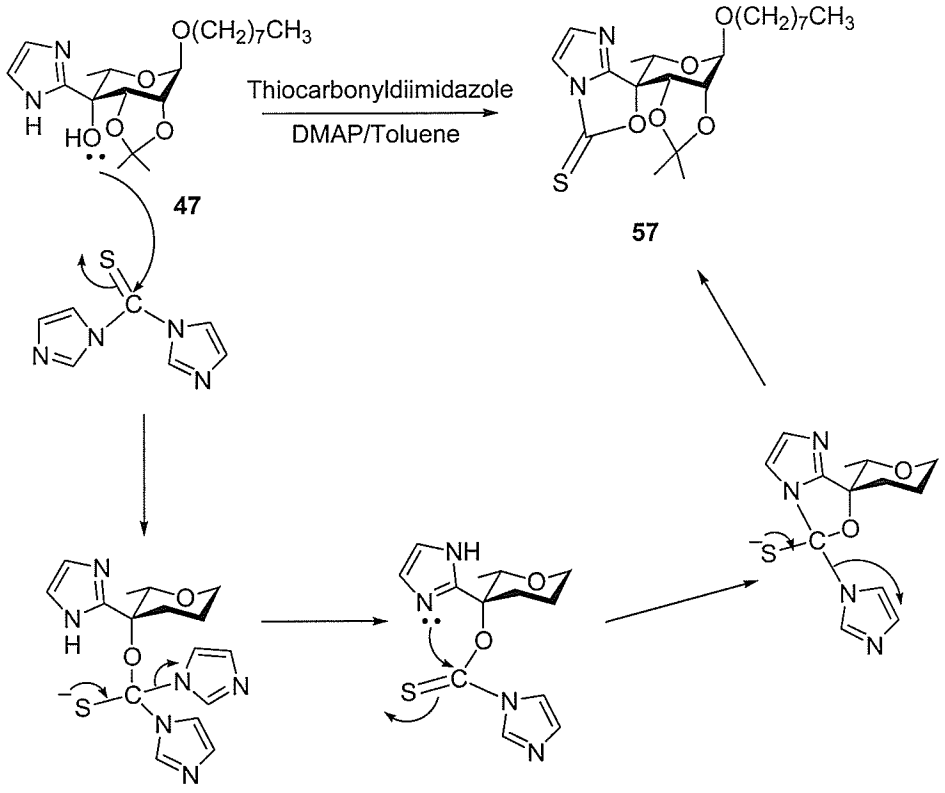
According to the Barton and McCombie method, we heated **47** in toluene with thiocarbonyldiimidazole and DMAP. As depicted in Scheme 3.12 we expected to have the intermediate **56**. However, we were unsuccessful in preparing the required thiocarbonylimidazole derivative **56** probably due to the steric hindrance at this C-4 centre. Instead of **56**, we obtained **57** as shown in Scheme 3.13. We thought perhaps this is quite similar to the expected thiocarbonylimidazolide derivative **56** and we refluxed it with AIBN/Bu_3SnH in xylene. Surprisingly, we obtained the product **58**, which was expected to be a high energy structure. Even though this seems to be a very high energy structure, we found that it can be isolated at room temperature. The identity of the structure was confirmed by NMR (Fig.3.9) and mass spectrometry analysis.

Two mechanisms were proposed for the formation of this interesting product **58** as shown in Scheme 3.14. Mechanism **A** is a radical process. The reaction medium has a radical initiator AIBN. Therefore, it produces the $Bu_3Sn^{\cdot}$ radical. Then a

homolytic bond cleavage occurs in **57** as shown in Scheme 3.14 and produces the intermediate A. The CO bond in intermediate A breaks and produces intermediate B. Both the intermediate A and B are stable tertiary radicals which are logically possible. Therefore, this mechanism is also acceptable.

Mechanism **B** is a concerted electrocyclic reaction. According to the Woodward and Hoffman theory,[87] thermal reactions with 4n π electron systems will undergo electrocyclic reactions by conrotatory motion whereas systems with 4n + 2 π electrons will react by the disrotatory mode. The compound **57** has a 8 π electron system. Therefore, it is a 4n system which must undergo conrotatory mode if this reaction happens via the mechanism **B**.

Both these mechanisms are logically acceptable according to the experimental results. However, we can determine the mechanism by doing further experiments. If we carry out the same reaction without a radical initiator and Bu_3SnH and if it still gives the product, then it is a pericyclic process. Otherwise it a radical process.



Scheme 3.13: Proposed mechanism for the formation of **57**

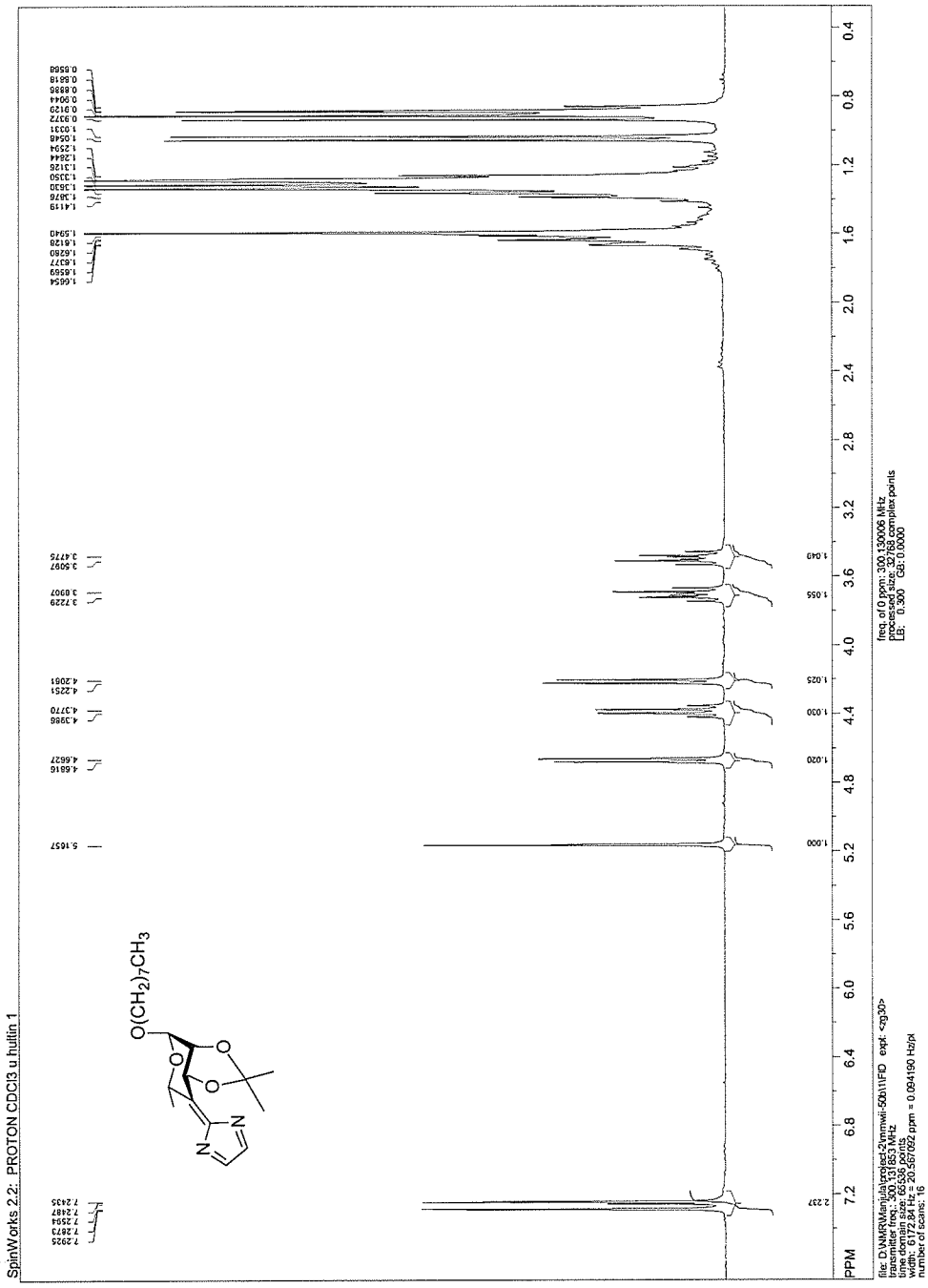
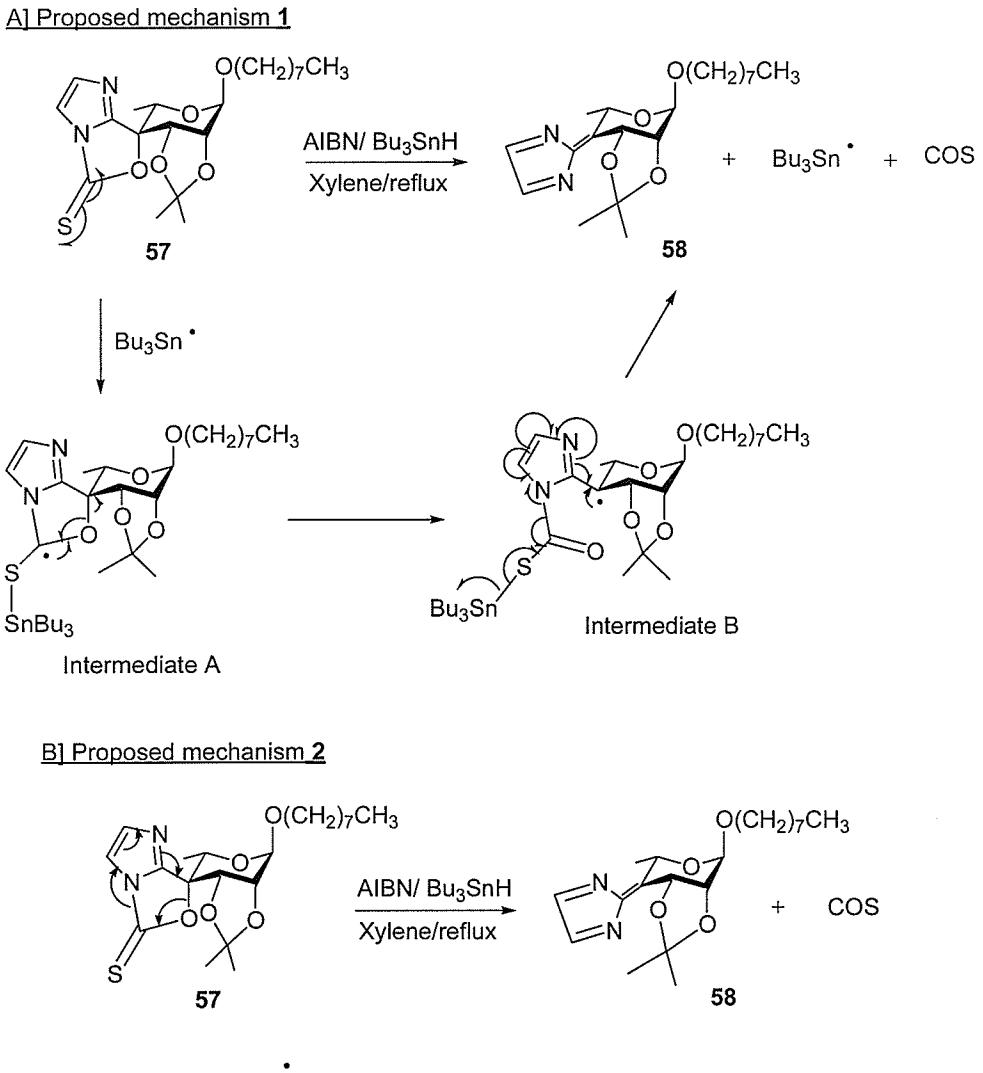
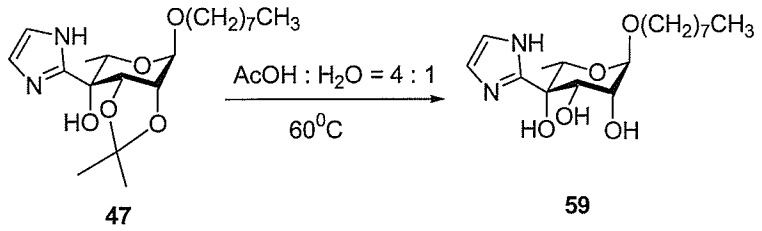


Figure 3.9: The ^{1}H NMR spectrum of **58**



Scheme 3.14: Proposed mechanism for **58**

We also tried reduction of **47** with H_2/Pd/C, monitoring the reaction by TLC. Only starting material was found after 12 h reaction time. All our efforts to reduce **47** to **54** proved to be impossible. This may be due to the steric hindrance at the C-4 position. Therefore, we decided to synthesize analogues with the C-4´hydroxyl group. As shown in Scheme 3.15, the next step involved the removal of the isopropylidene group. We heated **47** with aqueous glacial acetic acid and obtained **59** in 90% yield.

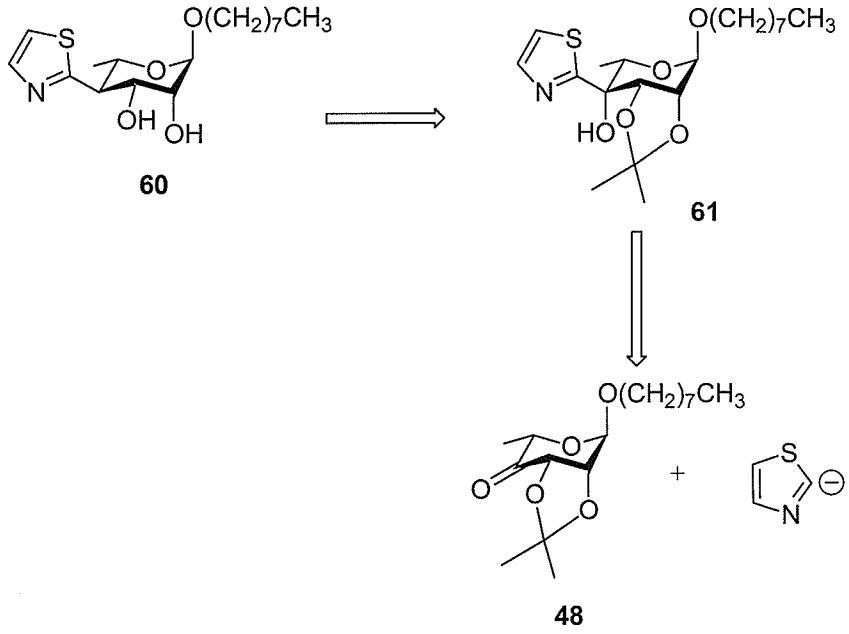


47 59

Scheme 3.15: Deprotection of **47**

3.2.7 Retrosynthetic analysis

Compound **61** was the coupling product of **48** and thiazole anion. Compound **60** was expected to be obtained from **61** by functional group manipulation (Scheme 3.16). In this synthesis, we used a synthetic strategy similar to that used for imidazole analogues.

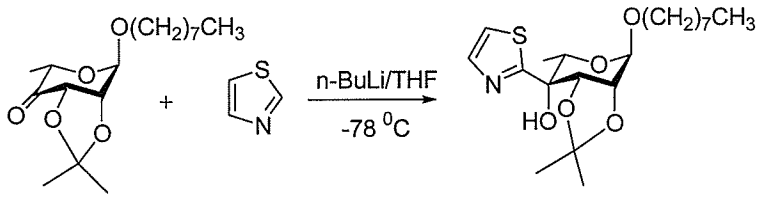


60 61 48

Scheme 3.16: Retrosynthetic analysis of analogue **60**

3.2.8 Synthesis of analogues **61-65**

We attached the thiazole to C-4 position of ketone **48** by stirring at -78 ^{0}C with n-BuLi (Scheme 3.17).[88] The reaction was monitored by TLC and after 4 h reaction time there was one new carbohydrate spot visible both under UV and after spraying with 5% sulfuric acid.



Scheme 3.17: Coupling reaction of the ketone **48** and thiazole

Since we chose optimum conditions, the reagents were completely converted to product and the isolation of the desired product was accomplished without any difficulty. The final product was extracted with extra Et_2O and purified by column chromatography. This provided a colourless oil **61** in 73% yield. The ^{1}H NMR of the isolated product **61,** provided the evidence for the formation of this compound. Two doublets appeared at 7.25 and 7.76 ppm indicating that the thiazole group had been attached to the sugar (Fig.3.10). These results were also confirmed by ^{13}C NMR analysis.

The mechanism of the coupling process can be sketched as shown in Scheme 3.18. The nucleophile is produced by deprotonating thiazole by n-BuLi at -78 ^{0}C. In the next step, the thiazole anion attacks the electrophile (ketone) and produces the coupling product **61**. Special notice should be given to the importance of the bulky isopropylidene group which protects the hydroxyl groups at the 2 and 3 positions. This provides the steric hindrance for the axial side of the C-4 position and leaves the equatorial side open for nucleophilic attack.

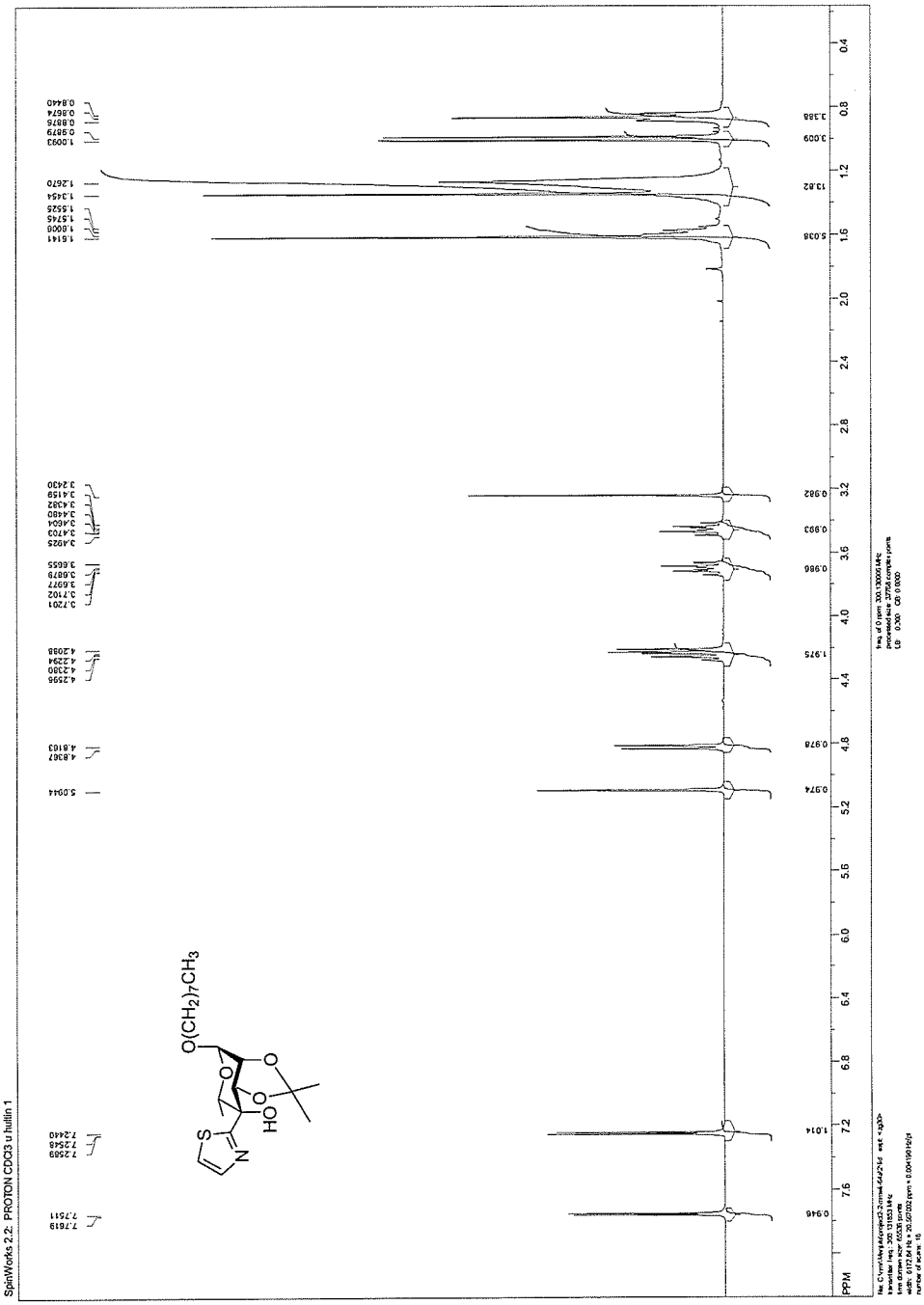
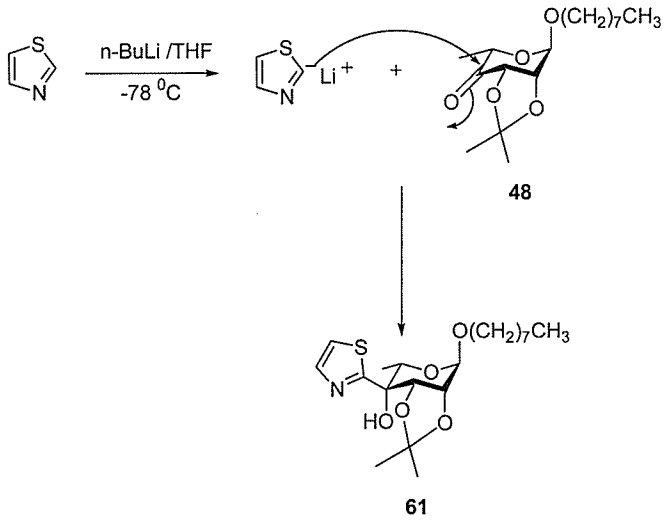


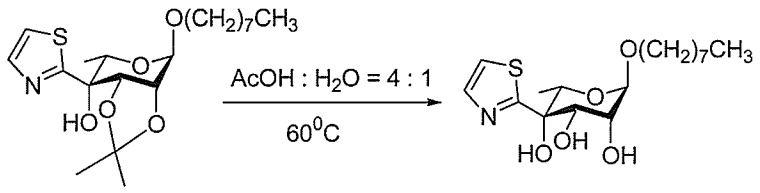
Figure 3.10: The ^{1}H NMR spectrum of **61**



Scheme 3.18: Mechanism of the coupling reaction

We next studied the Barton deoxygenation method for our coupling product **61**. The compound **61** in toluene was refluxed 2 days with thiocarbonyldiimidazole. The reaction was monitored by TLC. Disappointingly, there was no product formed.

Then we decided to continue our study with the C-4 hydroxyl group in compound **61**. In the next step, we removed the isopropylidene group without affecting other functional groups (Scheme 3.19). Initially we stirred the compound **61** for 12 h with a 4:1 acetic acid and water mixture, at room temperature. TLC showed that there was no product formation. Then, we increased the temperature to 40^0C and left the reaction overnight. Again, we observed only the starting material. After that, we increased the temperature to 60 ^{0}C. As we anticipated, the deprotection went to completion within 2 hours. The product **60** was isolated by column chromatography and obtained in 98% yield. The ^{1}H NMR spectrum of compound **60** was compared with the ^{1}H NMR spectrum of **61**. The deprotected structure was confirmed by the disappearance of the isopropylidene peaks at 1.35 and 1.61 ppm (singlets), which correspond to the two methyl groups (Fig.3.11).



61 **62**

Scheme 3.19: Deprotection of **61**

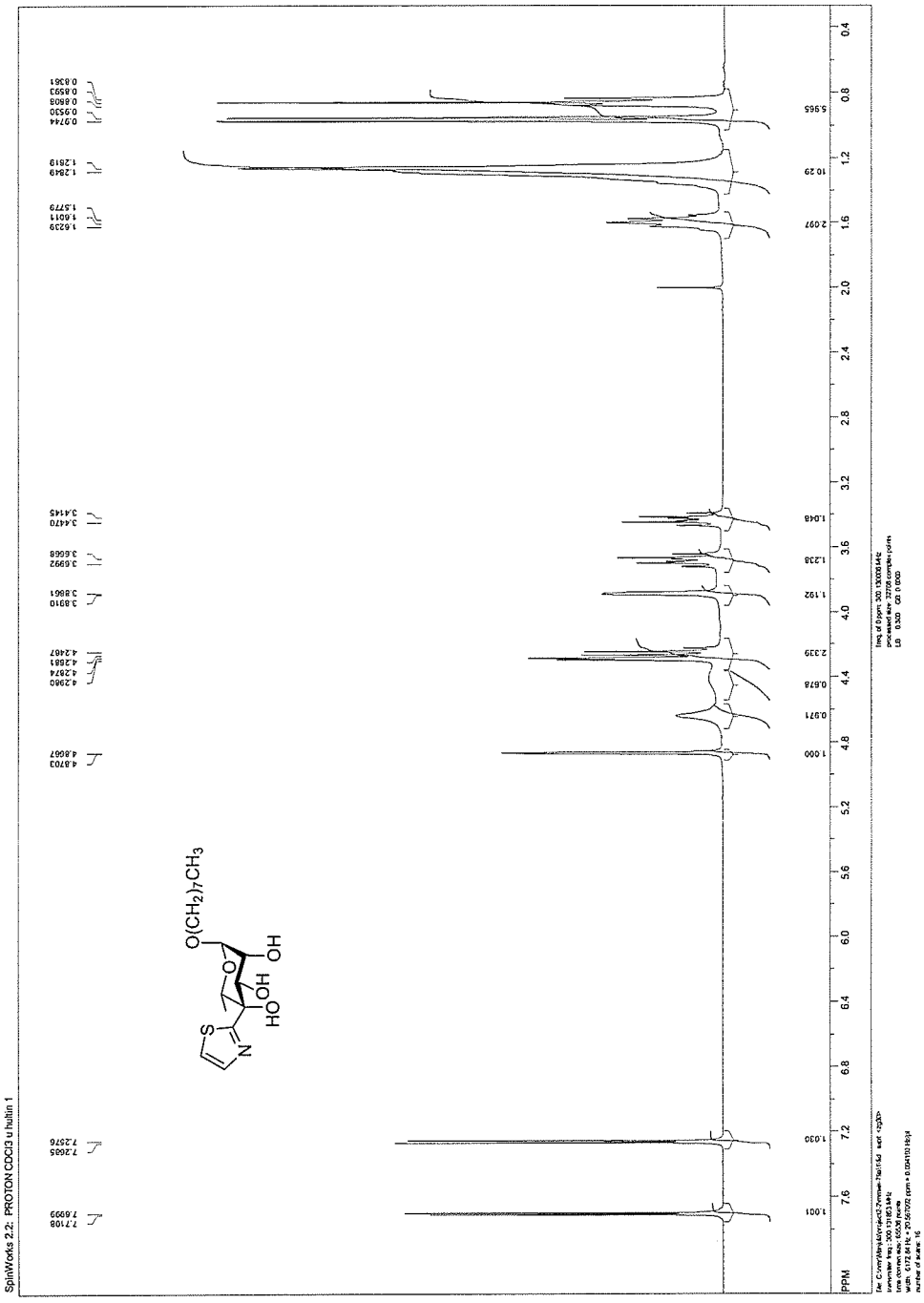
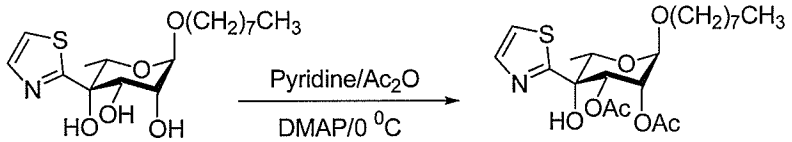


Figure 3.11: The ^{1}H NMR spectrum of **62**

We noticed that compound **62** has a tertiary hydroxyl at C-4. All other hydroxyls are secondary groups. Because $Et_3SiH/BF_3{\cdot}Et_2O$ at -10 ^{0}C reportedly reduces only tertiary hydroxyls,[89] we tried this reaction to reduce **62** and monitored the reaction by TLC. Disappointingly, we found that the reaction was unsuccessful and the starting material was not consumed. We next attempted the reduction by treating **62** with Et_3SiH/CF_3COOH.[90] The reaction was done at -10 ^{0}C. This reaction was also unsuccessful and starting material was recovered .

Since all our efforts to reduce **62** proved to be unsuccessful, we decided to continue our study with the C-4 hydroxyl group. Compound **62** was simply protected with Ac_2O at 0 ^{0}C to obtain **63** in 73% yield (Scheme 3.20). Appearance of peaks at δ 1.82 and 2.17 ppm as singlets for two OAc groups in the ^{1}H NMR spectrum confirmed the structure (Fig.3.12).



62 **63**

Scheme 3.20: Synthesis of analogue **63**

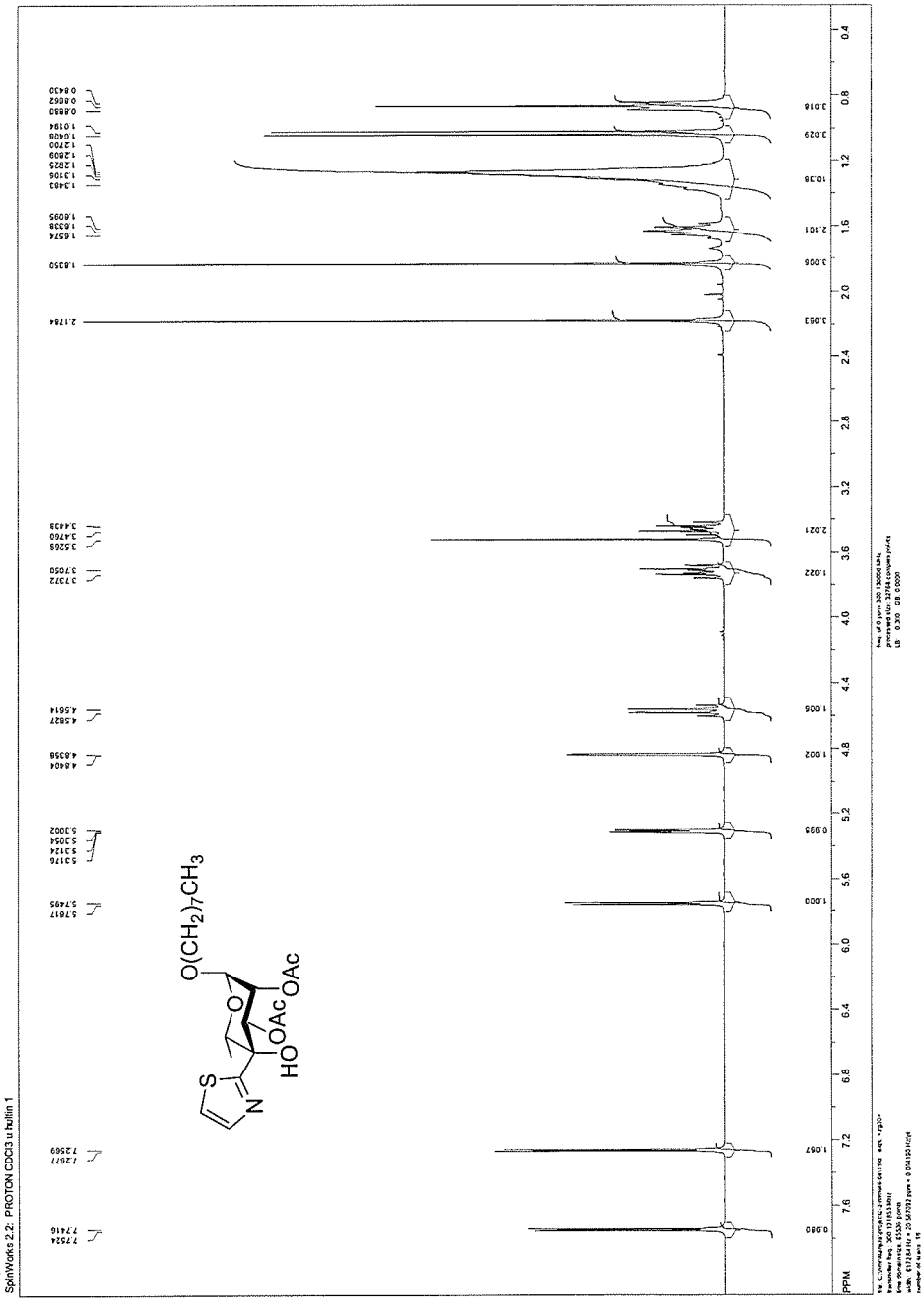
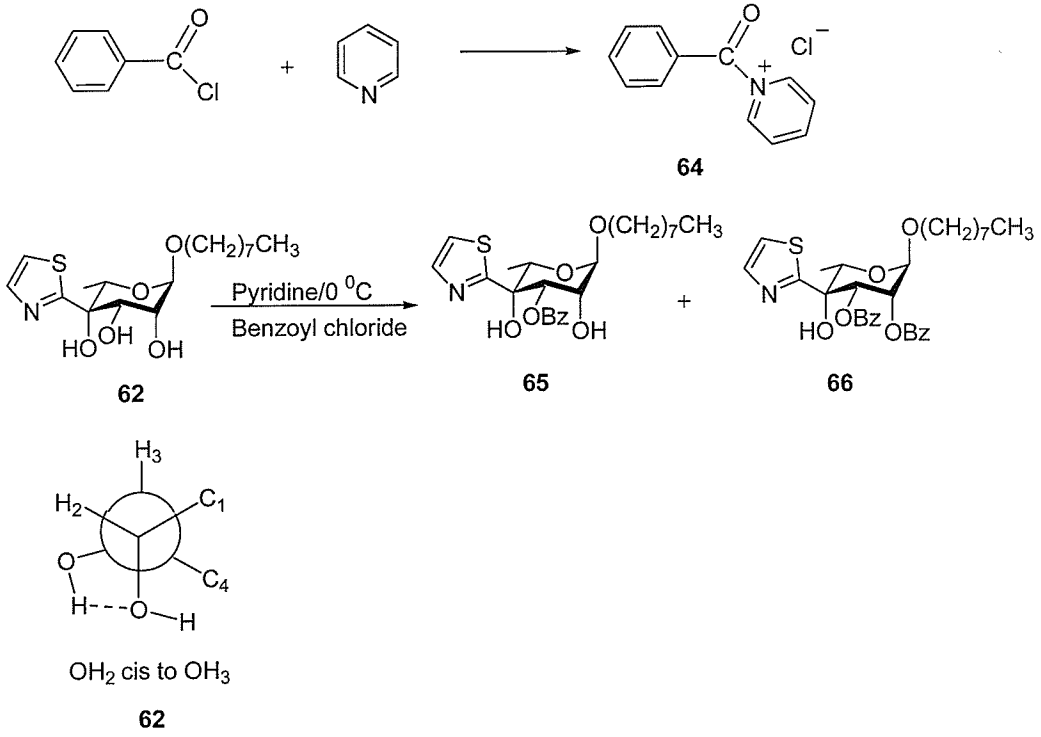


Figure 3.12: The ^{1}H NMR spectrum of **63**

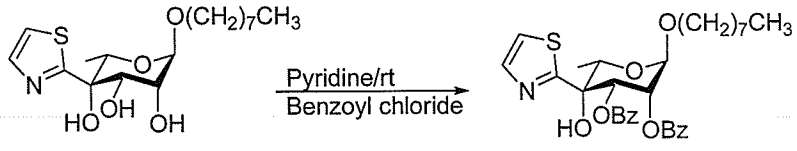
Regioselective acylation of monosaccharides was extensively studied two decades ago. The acyl pyridinium salt **64** is believed to form first in this reaction (Scheme 3.21), and then it reacts with the hydroxyl that has intramolecular hydrogen bonding.[91] Equatorial hydroxyls are generally more reactive than axial hydroxyls as they are more acidic. There is only one equatorial hydroxyl in **62**. In addition, this C-3 hydroxyl is cis to the C-2 hydroxyl and forms a hydrogen bond with it.[91] Therefore, the C-3 hydroxyl would be benzoylated first, giving monobenzoylated product **65** as the major product in 73% yield in low temperature (Scheme 3.21). In this reaction, **66** was obtained in 10% yield. The structures were confirmed by NMR. Regioselectivity of the benzoylated products were confirmed by comparing the H-2 and H-3 proton shifts of both **65** and **66** with **62**. In **65**, the chemical shift of H-3 has shifted downfield 0.67 ppm compared to the chemical shift of H-3 in **62**. But chemical shift of H-2 has only shifted 0.34 ppm. In **66**, both chemical shifts of H-2 and H-3 have shifted downfield in 0.78 ppm and 1.80 ppm respectively compared to chemical shifts of H-2 and H-3 in **62**.



64 62 65 66 62

Scheme 3.21: Synthesis of analogues **65** and **66**

As we needed to synthesize **66** as the main product for our study, we tried the same reaction by increasing the temperature (Scheme 3.22). We were successful in synthesizing **66** in 77% yield as a single product as identified by NMR. Appearance of peaks between δ 7.20-8.03 ppm in the ^{1}H NMR spectrum indicated the attachment of two phenyl groups (Fig.3.13). The C-4 hydroxyl in **62** was a tertiary hydroxyl group and also sterically hindered compared to the two secondary hydroxyl group at C-3 and C-2 position. Therefore, the C-4 was not benzoylated.



62 66

Scheme 3.22: Synthesis of **66**

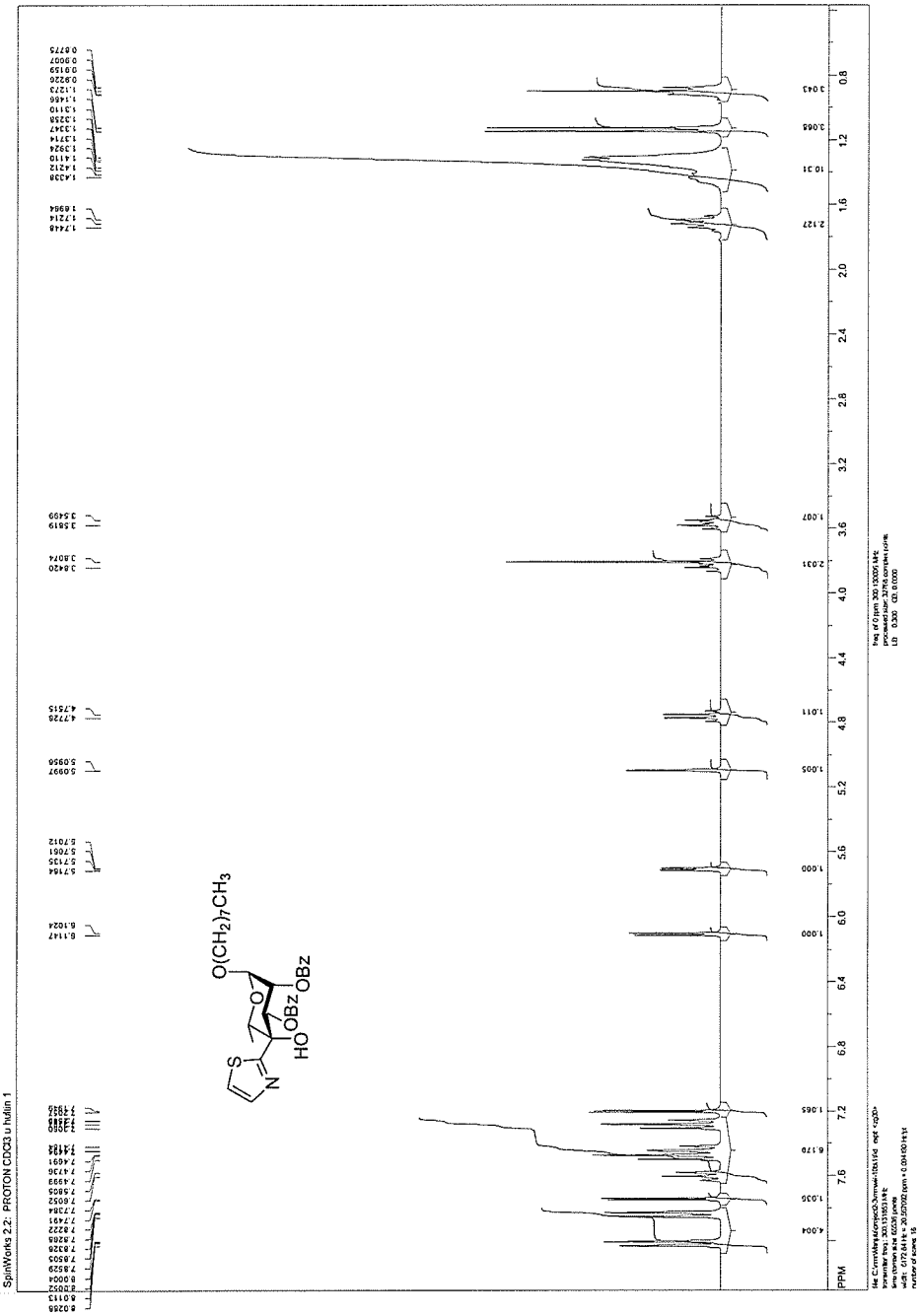


Figure 3.13: The ^{1}H NMR spectrum of **66**

3.3 Biological investigations

Biological investigations were carried out by our collaborators Dr. P.J. Brennan and Dr. D.C. Crick at Colorado State University, USA. Nine analogues (Fig.3.14) were sent for biological testing and the effectiveness of the compounds as inhibitors of mycobacterial AG biosynthesis was assayed[92] at up to 300 μg compound/mL. We found that the compounds had no inhibitory effect on the formation of **GL-3**. Therefore they are inactive.

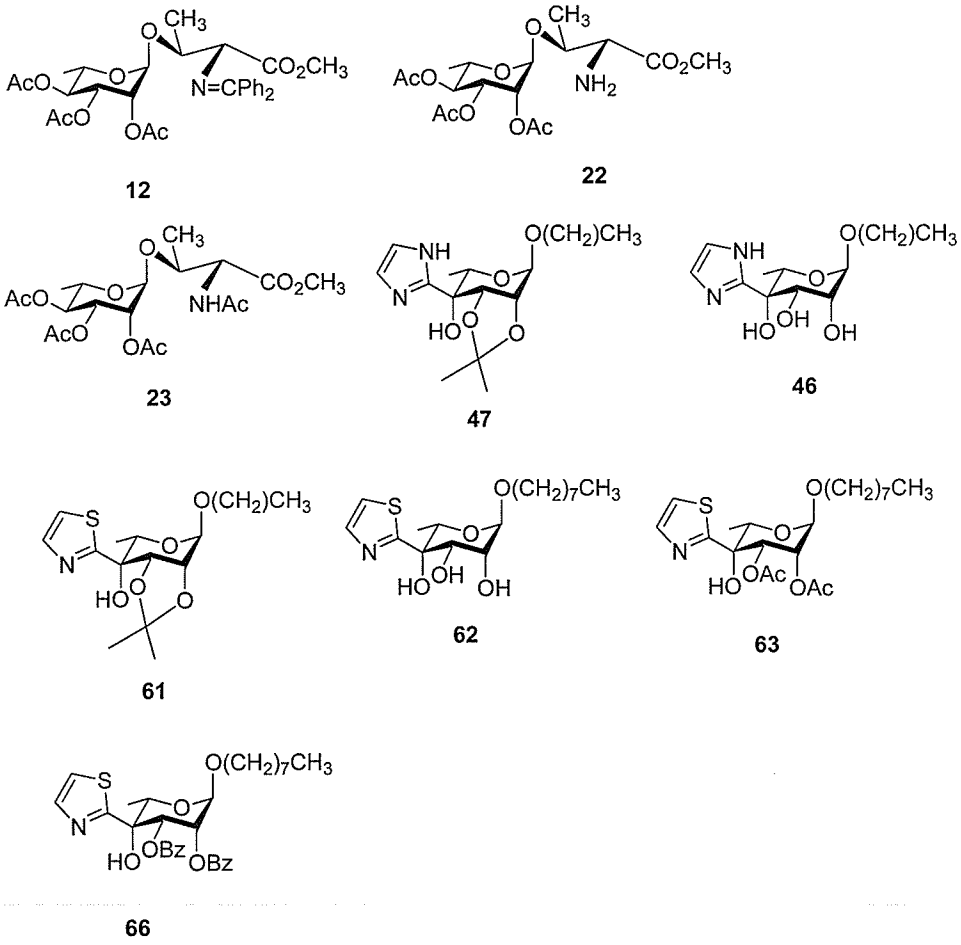


Figure 3.14: Tested compounds

Chapter 4

Conclusions

1. We constructed a route to synthesize the analogue **23**. In order to synthesize this analogue **23**, we coupled an acceptor with a donor. The procedure using TfOH/NIS in CH_2Cl_2 at room temperature to couple the acceptor, Schiff base **21** with the donor, thioglycoside **16** was the best of other methods attempted. We achieved the deprotection of coupling product **12** in good yield (80%) within 5 minutes. The method incorporating $CF_3COOH/THF/H_2O$ at room temperature worked well for this purpose.

2. We successfully constructed imidazole analogues **47** and **46**. In order to improve the efficacy of the coupling reaction, we synthesized imidazole derivative **49**. The coupling reaction of imidazole derivative **49** with L-rhamnose ketone **48** was successfully achieved with nBuLi/THF at -78^{0}C in 67% yield. The deprotection of the 2 and 3 hydroxyl was done successfully in the presence of AcOH:H_2O (4:1) at 60^{0}C. The reduction of the C-4´hydroxyl of compound **47** was attempted. However, reduction was unsuccessful under the conditions we attempted. For efficient reduction, the use of other methods for a hindered tertiary hydroxyl is recommended.

3. We successfully prepared thiazole analogues **60**, **63** and **66**. The attachment of

the thiazole to L-rhamnose ketone **48** was carried out in the presence of nBuLi/THF at -78^{0}C. The coupling reaction was accomplished in high yield (73%). The deprotection was performed in the same manner as imidazole compounds. The protected analogues **63** and **66** were synthesized successfully in high yield (73% and 77% respectively).

4.1 Suggestions for future work

As shown in the results, this thesis project has accomplished its initial goal to synthesize the analogues for both projects. However, we found out that none of the compounds had any significant galactosyltransferase inhibitory activity. Therefore, I would like to point out a few suggestions to improve the synthesis and to design the future work.

1. The glycosylation reaction in the first project only has 35% yield. This can be improved by finding the optimum conditions. Thioglycoside donors may be tried in different solvent systems and under different temperatures.

2. We have only tried a few methods for the reduction of C-4´ hydroxyl group in imidazole compound **47** and in thiazole compound **61**. This can be tried under different methods. For example, alcohol can be converted to a thiol and then reduced[93] or it may be converted to a sulfonate and then reduced[94] (Fig.4.1).

This will give analogues without C-4´hydroxyl and they look like better mimetics of the linker α-L-Rha-(1$\longrightarrow$3)-α-D-GlcNAc. Therefore, these compounds could have an inhibitory activity against mycobacterial glycosyltransferases.

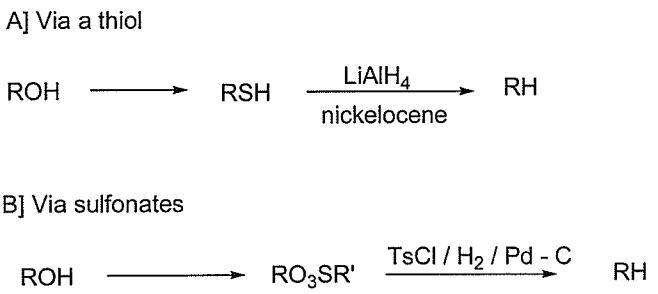


Figure 4.1: Other possibilities to reduce **47** and **61**

3. The analogues could be synthesized by attaching the thiazole or imidazole to the C-4´ position of rhamnose via a carbon or oxygen (spacer). For example, Fig.4.2

shows two possibilities. We could assume that these analogues would be a better representation of the natural linker with galfuranose than ones without a spacer.

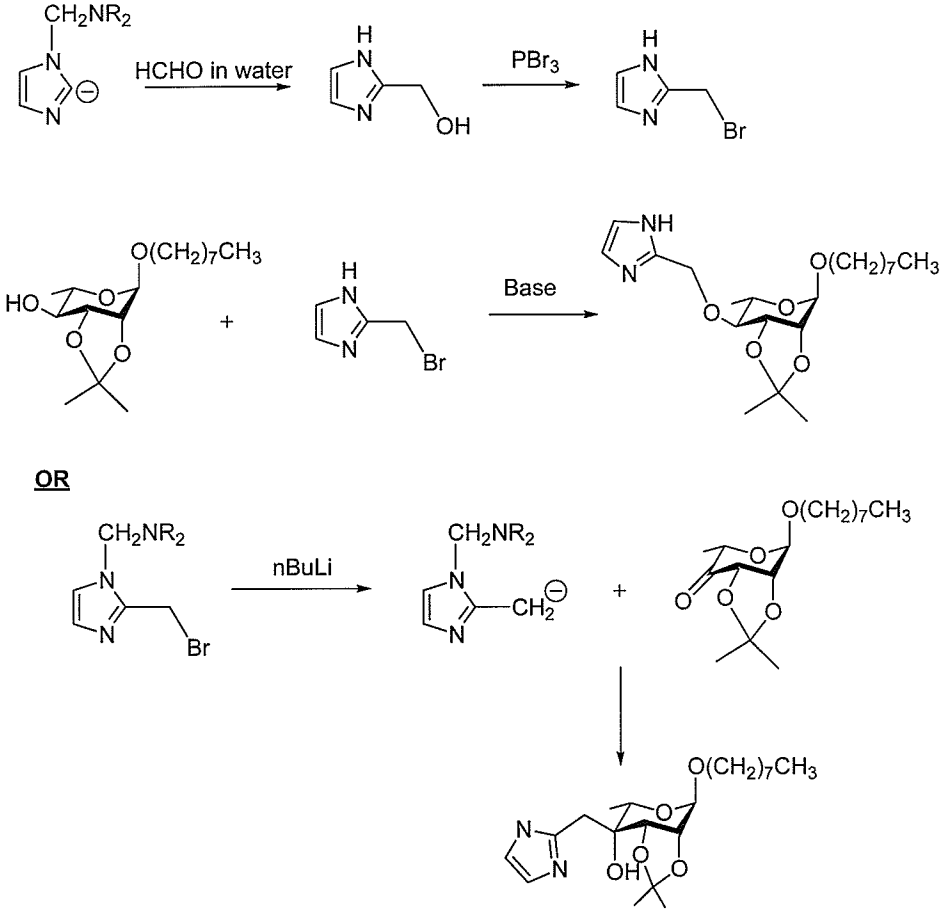


Figure 4.2: Attachment of the heterocycle to L-rhamnose via a spacer

4. The compound **58** was synthesized unexpectedly via Barton and McCombie method. This compound can be used in other areas in organic synthesis.

Chapter 5

Experimental

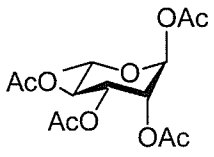
5.1 General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 FT instrument using Xwinnmr software. Residual $CHCl_3$ in $CDCl_3$ was used as the chemical shift standard for ^{1}H spectra (7.26 ppm) and the carbon resonance of the solvent was used as the standard for the ^{13}C spectra (77.2 ppm). Compounds were visualized on analytical thin layer chromatograms (TLC) by UV light or by 5% H_2SO_4 in ethanol. Flash Chromatography was performed on silica gel 60, eluting with the solvent mixtures indicated. Melting points were determined in open capillaries and are uncorrected. Optical rotations were recorded at room temperature in a microcell, 1 dm path length. Microanalyses were obtained on pure compounds by Guelph Laboratories, Guelph, ON.

Solvents and reagents were dried and purified using standard procedures.[95] Reactions requiring an inert atmosphere were conducted under a positive pressure of argon or nitrogen in glassware oven dried overnight at 120 - 140 ^{0}C. Reaction temperatures recorded are bath temperatures. "Drying" of organic extracts refers to the use of

anhydrous $MgSO_4$.

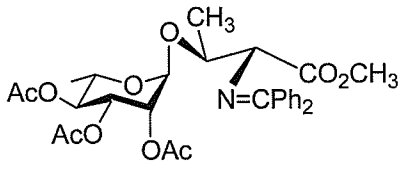
1,2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranoside (9)



9

A solution of L-rhamnose (5.04 g, 27.6 mmol) in pyridine (50 mL) was cooled to 0 ^{0}C. Acetic anhydride (20 mL, 211.5 mmol) was added to this cooled solution and the mixture was stirred overnight at 0 ^{0}C. The resulting mixture was poured into cooled ice water (600 mL) and the product was extracted with diethyl ether (3 $\times$ 100 mL). The combined organic layers were washed with 10% HCl (3 $\times$ 50 mL) and saturated $NaHCO_3$ (3 $\times$ 100 mL), before being dried and concentrated. The crude product (8.82g, 96%) was essentially one material by ^{1}H NMR. The crude product was used within several days. The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[69]

***N*-Diphenylmethylene-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-D-threonine methyl ester (12)**



12

The acceptor **21** (0.37 g, 1.3 mmol, 1 equiv.) and freshly activated powdered 4 Å molecular sieves (0.2 g) were added to a solution of thioglycoside donor **16** (0.49 g, 1.5 mmol, 1.2 equiv.) in CH_2Cl_2 (3 mL). The mixture was vigorously stirred for 1 h under argon. *N*-Iodosuccinimide (0.38 g, 1.7 mmol, 1.4 equiv.) and TfOH (18.4

μl) were added, and the suspension was stirred at room temperature for 5 h. The reaction mixture was diluted with CH_2Cl_2 (3 mL) and filtered through Celite. The filtrate was washed with saturated $Na_2S_2O_3$ and saturated $NaHCO_3$. The organic solution was then dried and concentrated. The crude product was purified by flash column chromatography (4:1 hexane:EtOAc) and **12** was obtained as a colourless oil (255 mg, 35%), along with **13** (175 mg, 25%).

TLC (2:1 hexane:EtOAc) R_f 0.48.

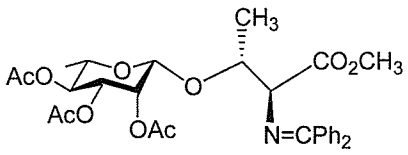
$[\alpha]_D$=+172.0 (c 1.0, CH_2Cl_2)

^{1}H NMR ($CDCl_3$): δ 1.19 (d, 3H, J=2.0 Hz, CH_3), 1.21 (d, 3H, J=1.9 Hz, H-6'), 1.91, 1.96, 2.02 (s, 3 $\times$ OAc) 3.70 (s, 3H, CO_2CH_3), 3.93 (dq, 1H, J=1.9 Hz, J=9.9 Hz, H-5), 4.13 (d, 1H, J=7.5 Hz, NCH), 4.38 (dq, 1H, J=7.5 Hz, J=2.0 Hz, CH_3CH), 5.00 (dd, 1H, J=9.9 Hz, J=8.4 Hz, H-4), 5.02 (d, 1H, J=1.7 Hz, H-1), 5.08 (dd, 1H, J=1.7 Hz, J=3.5 Hz, H-2), 5.19 (dd, 1H, J=3.5 Hz, J=8.4 Hz, H-3), 7.20 - 7.60 (m, 10H, Ar-H).

^{13}C NMR ($CDCl_3$): δ 17.3 (CH_3), 18.6 (C-6'), 20.6, 20.7, 20.8 (3 $\times$ OAc), 52.2 (OCH_3), 66.6 (CH_3CH), 69.1 (C-3), 69.7 (C-2), 71.1 (C-4), 71.5 (C-5), 76.6 (CH_3CH), 98.76 (C-1).

Anal. Calcd for $C_{30}H_{35}NO_{10}$: C, 63.26; H, 6.19; N, 2.46. Found: C, 63.00; H, 6.20; N, 2.42.

N-Diphenylmethylene-O-(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)-D-threonine methyl ester (13)



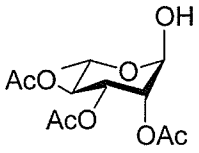
13

TLC (2:1 hexane:EtOAc) R_f 0.28.

^{1}H NMR ($CDCl_3$): δ 1.17 (d, 3H, J=6.3 Hz, CH_3), 1.20 (d, 3H, J=6.2 Hz, H-6′), 1.67, 2.05, 2.09 (s, 3 × OAc) 3.49 (m, 1H, H-5), 3.69 (s, 3H, CO_2CH_3), 4.10 (d, 1H, J=6.5 Hz, NCH), 4.29 (dq, 1H, J=6.5 Hz, J=6.3 Hz, CH_3CH), 5.04 (m, 2H, *H-3*, H-4), 5.31 (d, 1H, J=2.7 Hz, H-1), 7.14 - 7.65 (m, 10H, Ar-H).

^{13}C NMR ($CDCl_3$): δ 17.5 (C-6′), 18.6 (CH_3), 20.7, 23.8, 25.6 (3 × OAc), 52.0 (OCH_3), 69.2 (C-5), 70.4 (C-3), 70.5 (C-4), 70.6 (CH_3C), 76.2 (C-2), 97.1 (C-1), 124.4 (N=C), 127.9, 128.1, 128.4, 128.6, 128.9 (C-Ar), 169.7, 170.4, 170.9, 171.6 (4 × C=O).

2,3,4-tri-O-acetyl-α-L-rhamnopyranoside (14)

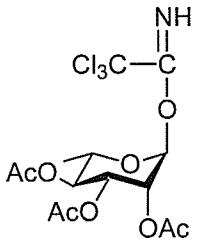


14

Compound **9** (7.30 g, 21.9 mmol) was dissolved in dry DMF (50 mL) and hydrazine acetate (2.02 g, 21.9 mmol) was added. After 12 hours, the solution was poured into brine (50 mL) and extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with water (3 × 100 mL), dried and concentrated. Recrystallization

(3:2, ethyl ether:hexane) yielded **14** (5.64g, 88%). The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[72]

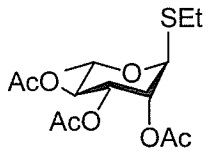
***O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl) trichloroacetimidate (15)**



15

A solution of **14** (3.38 g, 11.8 mmol) in CH_2Cl_2 (25 mL) was mixed with potassium carbonate (5.78 g, 59.0 mmol, 5 equiv.) and trichloroacetonitrile (7.1 mL, 70.0 mmol, 6 equiv.). The resulting suspension was stirred for 12 h at room temperature. The mixture was diluted with CH_2Cl_2 (25 mL), filtered through Celite and dried. The organic solution was concentrated and the residue was purified by column chromatography (2:1, petroleum ether:ethyl ether). This yielded product **15** (4.91 g, 96%). The ^{1}H and ^{13}C NMR data of the product were consistent with the literature.[71]

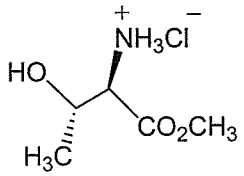
Ethyl 2,3,4-tri-*O*-acetyl-1-thio-α–L-rhamnopyranoside (16)



16

Tetra-*O*-acetyl-α-L-rhamnopyranose **9** (1.40 g, 4.20 mmol) was added to dry CH_2Cl_2 (6 mL) and freshly activated powdered 4 A^0 molecular sieves (0.5 g). The solution was cooled to 0 ^{0}C. $BF_3.Et_2O$ (1.60 mL, 12.6 mmol, 3 equiv.) was added, followed by ethanethiol (0.62 mL, 8.4 mmol, 2 equiv.). The resulting mixture was stirred for 0.5 h at 0 ^{0}C. The mixture was allowed to come to room temperature and was stirred for an additional 5 h. The mixture was diluted with CH_2Cl_2 (15 mL) and filtered through Celite. The organic solution was washed with $NaHCO_3$ (3 $\times$ 20 mL), brine (3 $\times$ 10 mL), dried and the solvent was evaporated. The crude product was purified by crystallisation (diethyl ether) to yield (1.13 g, 80%) of the pure α-L anomer **16**. The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[73]

D-threonine methyl ester hydrochloride (20)



20

The D-threonine (7.00 g, 58.8 mmol) was dissolved in methanol (120 mL). The solution was cooled to 0 ^{0}C. Thionyl chloride (20 mL, 277.0 mmol) was added dropwise at 0 ^{0}C, during a period of 1.5 h. The mixture was refluxed over a period of 12 h. The solution was concentrated and the residue was re-evaporated from methanol

4 times. The crude product (9.95 g, 98%) was used for the next step without any purification.

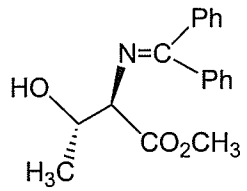
TLC (1:1:0.4 EtOAc:CH_2Cl_2:CH_3OH) R_f 0.44.

$[\alpha]_D$=+7.8 (c 1.0, CH_3OH)

^{1}H NMR ($CDCl_3$): δ 1.46 (d, 3H, J=6.6 Hz, CH_3), 3.83 (s, 3H, CO_2CH_3), 4.20 (d, 1H, J=4.1 Hz, H-2), 4.35 (dq, 1H, J=4.1 Hz and J=6.6 Hz, H-3).

^{13}C NMR ($CDCl_3$): δ 20.4 (CH_3), 53.3 (OCH_3), 59.7 (C-2), 66.4 (C-3), 206.8 (C=O).

Methyl *N*-(Diphenylmethylene)-D-threoninate (21)



21

Compound **20** (9.76 g, 57.5 mmol) was dissolved in CH_2Cl_2 (150 mL) and benzophenone imine (9.7 mL, 57.5 mmol) was added. After two days, the mixture was diluted with CH_2Cl_2 (100 mL), filtered, dried and concentrated. The white solid residue was recrystallized from 1:1 hexane:ethyl ether to afford white needlelike crystals of **21** (14.54 g, 85%).

TLC (2:1 hexane:EtOAc) R_f 0.42.

mp 73 ^{0}C.

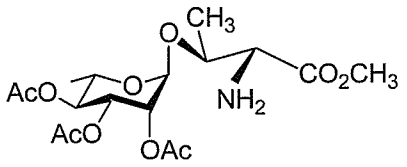
$[\alpha]_D$=+172.0 (c 1.0, CH_2Cl_2)

^{1}H NMR ($CDCl_3$): δ 1.32 (d, 3H, J=6.1 Hz, CH_3), 3.75 (s, 3H, CO_2CH_3), 3.56 (d, 1H, J=8.0 Hz, H-2), 4.10 (dq, 1H, J=8.0 Hz and J=6.1 Hz, H-3), 7.15-7.83 (m, 10H, Ar-H).

^{13}C NMR ($CDCl_3$): δ 20.1 (CH_3), 52.4 (OCH_3), 60.0 (C-2), 66.9 (C-3), 99.9 (N=C), 125.6, 126.3, 127.4, 127.5, 127.7, 128.1, 128.2, 128.3, 128.7, 128.9, 144.9 (C_{Ar}), 171.5 (C=O).

Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 73.14; H, 6.88; N, 4.84.

_O_-(2,3,4-tri-_O_-acetyl-α-L-rhamnopyranosyl)-D-threonine methyl ester (22)



22

Compound **12** (111 mg, 0.2 mmol) was dissolved in MeOH (20 mL). 10% Pd/C (100 mg) was added and the solution was vigorously stirred under an atmosphere of H_2. After 10 min. the solution was filtered through Celite and diluted with CH_2Cl_2 (10 mL). The organic solution was then dried and concentrated. The crude product was purified by flash column chromatography (1:1:1 hexane:EtOAc:MeOH) to afford **22** as a colourless oil (53 mg, 67%).

$[\alpha]_D$= (c 1.0, $CHCl_3$)

TLC (1:1:2 hexane:EtOAc:MeOH) R_f 0.28.

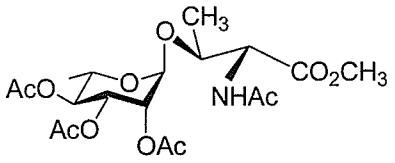
^{1}H NMR ($CDCl_3$): δ 1.18 (d, 3H, J=3.0 Hz, H-6´), 1.32 (d, 3H, J=6.0 Hz, CH_3), 1.96, 2.02, 2.11 (s, 3 × OAc), 3.42 (d, 1H, J=3.0 Hz, $CHNH_2$), 3.75 (s, 3H, CO_2CH_3), 3.92 (dq, 1H, J=3.0 Hz, J=9.0 Hz, H-5), 4.16 (dq, 1H, J=3.0 Hz, J=6.0 Hz, CH_3CH), 4.80 (d, 1H, J=2.7 Hz, H-1), 5.00 (dd, 1H, J=12.0 Hz, 9.0 Hz, H-4), 5.05 (dd, 1H, J=3.0 Hz, J=2.7 Hz, H-2), 5.22 (dd, 1H, J=9.0 Hz, J=3.0 Hz, H-3).

^{13}C NMR ($CDCl_3$): δ 17.2 (C-6´), 18.1 (CH_3), 20.7, 20.8, 20.9 (3 × CH_3C=O),

52.3 (OCH_3), 59.1 ($CHNH_2$), 66.7 (C-5), 68.9 (C-3), 70.0 (C-2), 71.1 (C-4), 77.6 (CH_3CH), 98.5 (C-1), 169.8, 169.9, 170.0 (3 × C=O).

Anal. Calcd for $C_{17}H_{27}NO_{10}$: C, 50.37; H, 6.71; N, 3.46. Found: C, 50.38; H, 6.90; N, 3.52.

_N_-acetyl-_O_-(2,3,4-tri-_O_-acetyl-α-L-rhamnopyranosyl)-D-threonine methyl ester (23)



23

Compound **22** (378 mg, 0.9 mmol) was dissolved in pyridine (670 μl). Ac_2O (88 μl, 0.9 mmol) was added and the solution was stirred 1h at 0 ^{0}C. The reaction was neutralized with 10% HCl and diluted with CH_2Cl_2 (5 mL). The organic solution was then dried and concentrated. The crude product was purified by flash column chromatography (4:1 hexane:EtOAc) to afford **23** as a colourless oil (401 mg, 96%).

$[\alpha]_D$=-73.4 (c 1.0, $CHCl_3$)

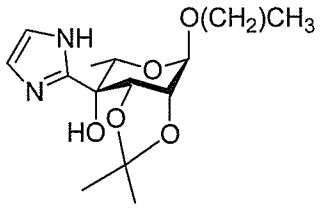
TLC (1:1 hexane:EtOAc) R_f 0.38.

^{1}H NMR ($CDCl_3$): δ 1.15 (d, 3H, J=6.4 Hz, H-6′), 1.21 (d, 3H, J=6.5 Hz, CH_3), 1.95, 2.00, 2.09 (s, 4 × OAc), 3.73 (d, 1H, J=3.0 Hz), 3.73 (s, 3H, CO_2CH_3), 3.85 (dq, 1H, J=6.4 Hz, J=3.5 Hz, H-5), 4.31 (dq, 1H, J=6.5 Hz, J=2.5 Hz, CH_3CH), 4.71 (m, 2H, CHNHAc, H-1), 4.96 (dd, 1H, J=3.5 Hz, H-4), 5.00 (dd, 1H, J=10.1 Hz, H-2), 5.18 (dd, 1H, J=3.5 Hz, J=10.1 Hz, H-3), 6.30 (d, 1H, J=9.23 Hz, NH)

^{13}C NMR ($CDCl_3$): δ 17.2 (C-6′), 18.2 (CH_3), 20.7, 20.8, 20.9, 20.1 (4 × CH_3C=O), 52.7 (OCH_3), 56.3 (CHNHAc), 68.8 (C-3), 69.9 (C-4), 70.9 (C-2), 77.3 (CH_3CH), 98.6 (C-1), 169.8, 169.9, 170.1, 170.5, 170.6 (5 × C=O).

Anal. Calcd for $C_{19}H_{29}NO_{11}$: C, 51.00; H, 6.53; N, 3.13. Found: C, 50.78; H, 6.50; N, 3.12.

Octyl 2,3-isopropylidene-4-(1H-imidazol-2yl)-α-L-rhamnopyranoside (47)



47

A solution of **49** (0.6 g, 4.8 mmol) in THF (28 mL) was cooled to -78 ^{0}C. *tert*-butyllithium (2.5 M in hexane; 1.91 mL) was added and the resulting yellow solution was stiired 1 h at -78 ^{0}C. A solution of ketone **48** (1.0 g, 3.2 mmol) in THF (15 mL) at -78 ^{0}C was added to the above mixture and stirred 8 h while maintaining the temperature. The reaction was quenched with saturated NH_4Cl and extracted to Et_2O (3 $\times$ 30 mL). The combined organic layers were successively washed with water (3 $\times$ 30 mL), dried and evaporated to afford crude **47**. Purification was done using flash chromatography (4:1 hexane:EtOAc) and provided **47** (0.8 g, 67%).

TLC (2:1 hexane:EtOAc) R_f 0.20.

$[\alpha]_D$= +17.2 (c 1.0, $CHCl_3$)

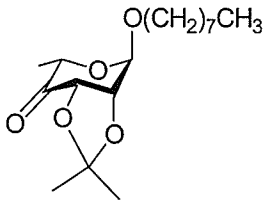
^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J=6.8 Hz, CH_3), 0.96 (d, 3H, J=6.8 Hz, H-6), 1.27 (m, 8H, 4 $\times$ CH_2), 1.36 (s, 3H, CH_3), 1.60 (m, 5H, CH_2,CH_3), 2.97 (s, 1H, OH), 3.44 (m, 2H, CH_2), 3.69 (m, 2H, CH_2), 4.22 (m, 2H, J=6.1 Hz, J=6.8 Hz, H-5, H-2), 4.83 (d, 1H, J=6.1 Hz, H-*3*), 5.08 (s, 1H, H-1), 6.94 (s, 1H, imidazol H), 7.07 (s, 1H, imidazol H), 9.49 (brs, 1H, NH).

^{13}C NMR ($CDCl_3$): δ 13.8 (CH_3), 14.1 (C-6), 22.6 (CH_2), 25.2 (CH_3), 25.9 (CH_3), 26.1, 29.2, 29.4, 31.8 (4 $\times$ CH_2), 67.1 (C-5), 68.0 (OCH_2), 74.4 (C-2), 76.7 (C-3), 71.9

(C-4), 97.0 (C-1), 109.3 $(CH_3)_2C$, 114.9 (Imidazole C), 129.3 (Imidazole C), 148.6 (N=C-NH).

Anal. Calcd for $C_{20}H_{34}N_2O_5$: C, 62.80; H, 8.96; N, 7.32. Found: C, 63.12; H, 9.16; N, 7.37.

Octyl 2,3-isopropylidene-4-keto-α-L-rhamnopyranoside (48)



48

A solution of oxalyl chloride (2.4 mL, 27.5 mmol) in CH_2Cl_2 (68 mL) at -78 ^{0}C was added to a solution of dimethyl sulfoxide (4.1 mL, 57.8 mmol) in CH_2Cl_2 (18 mL). The resulting mixture was stirred 1 h at -78 ^{0}C. A solution of alcohol (5.5 g, 17.4 mmol) in CH_2Cl_2 (68 mL) at -78 ^{0}C was added to it and stirred another 1 h at the same temperature. The Et_3N (10.5 mL) was added and allowed the reaction to warm to room temperature. The reaction was again stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with brine (3 $\times$ 30 mL), water (3 $\times$ 30 mL). The organic layer was dried, concentrated and purified by flash chromatography (2:1 hexane:EtOAc) to obtain **48** (5.0 g, 92%).

$[\alpha]_D$=-86.7 (c 1.0, $CHCl_3$)

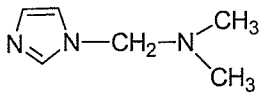
TLC (15:1 hexane:EtOAc) R_f 0.41.

^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J=6.3 Hz, CH_3), 1.27 (m, 10H, 5 $\times$ CH_2), 1.36 (s, 3H, CH_3), 1.39 (d, 3H, J=6.8 Hz, H-6), 1.49 (s, 3H, CH_3), 1.59 (m, 2H, CH_2), 3.50 (m, 1H, OCH), 3.73 (m, 1H, OCH), 4.24 (q, 1H, J=6.9 Hz, H-5), 4.43 (m, 2H, H-*3*, H-2), 4.92 (s, 1H, H-1), 9.75 (s, 1H, CHO).

^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3), 16.0 (C-6), 26.0 (CH_3), 26.7 (CH_3), 22.1, 22.7, 25.4, 29.0, 29.2, 31.6 (5 $\times$ CH_2), 68.7 (C-5), 69.9 (OCH_2), 75.9 (C-3), 76.6 (C-4), 78.8 (C-2), 96.9 (C-1), 111.3 ($C(CH_3)_2$), 204.8 (C=O).

Anal. Calcd for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62. Found: C, 65.23; H, 10.01.

1-[(Dimethylamino)methyl]imidazole (49)



49

Imidazole (5.00 g, 73.0 mmol) and dimethylamine hydrochloride (6.03 g, 73 mmol) were dissolved in water (10 mL) and concentrated hydrochloride acid was added untill the pH was just less than 5. Aqueous formaldehyde solution (37%, 7.02 g, 87.6 mmol) was added, and the mixture was allowed to stand at room temperature for 48 h. The solution was made strongly alkaline with saturated KOH solution, and extracted with CH_2Cl_2 by continuous extraction method. The organic layer were dried and concentrated to give an oil **49** (8.4 g, 92%)

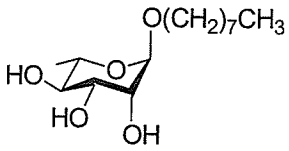
TLC (1:1:2 hexane:EtOAc:ether) R_f 0.21.

$[\alpha]_D$= +1.0 (c 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 2.24 (s, 6H, CH_3), 4.62 (s, 2H, CH_2), 6.92 (s, 1H, H-5), 7.03 (s, 1H, H-4), 7.46 (s, 1H, H-2).

^{13}C NMR ($CDCl_3$): δ 41.9 (CH_3), 69.3 (CH_2), 119.8 (C-5), 128.9 (C-4), 137.6 (C-2).

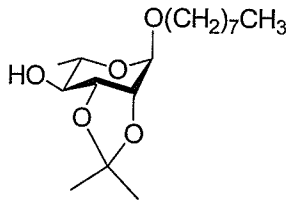
Octyl α-L-rhamnopyranoside (50)



50

A solution of **49** (5.00 g, 12.4 mmol) in methanol (14 mL) was reacted with NaOMe (3.75 g, 69.0 mmol) in methanol (15 mL). The resulting solution was stirred for 12 h. The reaction was diluted with methanol and filtered through Celite. The organic solution was concentrated and purified by flash chromatography (6:1 $CHCl_3$:CH_3OH) to obtain **50** (3.0 g, 88%). The ^1H and ^{13}C NMR spectra of the product were consistent with the literature.[81]

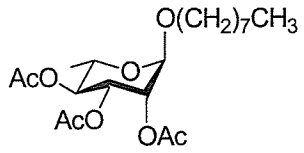
Octyl 2,3-isopropylidene-α-L-rhamnopyranoside (51)



51

Octyl α-L-rhamnopyranoside **50** (1.44 g, 5.2 mmol) and (+)-10-camphorsulfonic acid (120.8 mg, 0.52 mmol) in dry acetone (10 mL) was treated with 2,2´-dimethoxy propane (1.6 mL, 13.2 mmol). After stirring for 5 h, the mixture was neutrallized to pH 7.0 by adding triethyl amine. The reaction was filtered through Celite, concentrated and purified by flash chromatography (9:1 hexane:EtOAc) to obtain **51** (1.0 g, 62%). The ^1H and ^{13}C NMR spectra of the product were consistent with the literature.[81]

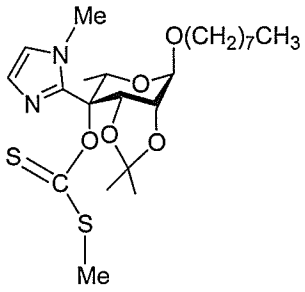
Octyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (52)



52

Compound **9** (4.80 g, 14.5 mmol) was taken up into dry CH_3CN (57 mL) and the flask was purged with N_2 and cooled to 0 ^{0}C. Stannic chloride (2 mL, 17.3 mmol) was added and the resulting mixture was stirred 0.5 h at 0 ^{0}C. *n*-Octanol (2.3 mL, 14.5 mmol) was added dropwise to the cooled reaction mixture over a period of 30 min. The reaction was allowed to warm up to the room temperature and stirred 4 h. The reaction was quenched with water (25 mL) and extracted with $CHCl_3$(3 $\times$ 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 $\times$ 20 mL), brine (3 $\times$ 20 mL), dried and purified by flash chromatography (6:1 hexane:EtOAc) and obtained a colourless oil **52** (4.6 mg, 80%). The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[81]

Octyl 2,3-isopropylidene-4-C-(imidazol-2-yl)-4-*O*-
(S-methyl dithiocarbonate)-α-L-rhamnopyranoside (55)



55

Glycoside **47** (200 mg, 0.53 mmol) and catalytic amount of imidazole were dissolved in THF (2 mL). A 60% sodium hydride dispersion (42 mg, 1.05 mmol) was

added at 0 ^{0}C. After the reaction mixture was stirred for 30 min, carbon disulfide (0.24 mL, 3.95 mmol) was added and stirred for another 30 min. Iodomethane (35.8 μl, 0.58 mmol) was then added and stirred at room temperature for 12 h. The suspension was mixed with water (10 mL) and extracted with Et_2O (3 $\times$ 10 mL). The combined organic layers were washed with saturated NH_4Cl (2 $\times$ 10 mL), brine (3 $\times$ 10 mL), dried and evaporated. The crude mixture was purified by using flash chromatography (6:1 hexane:EtOAc) and provided **55** (108 mg, 42%).

TLC (2:1 hexane:EtOAc) R_f 0.58.

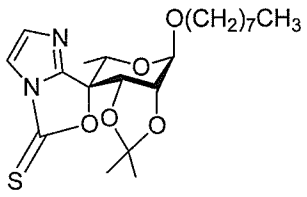
$\alpha]_D$= -63.0 (c 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J=6.7 Hz, CH_3), 1.12 (d, 3H, J=6.2 Hz, H-6), 1.28 (m, 8H, 4 $\times$ CH_2), 1.36 (s, 3H, CH_3), 1.58 (m, 2H, CH_2), 1.65 (s, 3H, CH_3), 2.80 (d, 3H, J=5.6 Hz, SCH_3), 3.44 (m, 2H, CH_2), 3.71 (m, 2H, OCH_2), 3.90 (s, 3H, NCH_3), 4.14 (d, 1H, J=6.2 Hz, H-2), 4.39 (q, 1H, J=6.2 Hz, H-*5*), 4.64 (d, 1H, J=6.2 Hz, H-*3*), 5.07 (s, 1H, H-1), 6.69 (d, 1H, J=1.2 Hz, imidazol H), 6.92 (s, 1H, J=1.2 Hz, imidazol H).

^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3), 14.2 (C-6), 19.7 (SMe), 25.5, 25.8 (2 $\times$ CH_3), 22.6 (CH_2), 26.1, 29.2, 29.3, 29.4, 31.8 (5 $\times$ CH_2), 35.6 (NMe), 67.6 (C-5), 68.0 (OCH_2), 74.0 (C-4), 74.6 (C-2), 78.3 (C-3), 96.8 (C-1), 109.3 $(CH_3)_2C$, 122.5 (Imidazole C), 126.8 (Imidazole C), 146.6 (N=C-NMe), 199.9 (OC=S).

Anal. Calcd for $C_{23}H_{40}N_2O_5S_2$: C, 56.53; H, 8.25; N, 5.73. Found: C, 57.00; H, 8.30; N, 5.75.

Octyl 2,3-isopropylidene-4-C-(imidazol-2-yl)-4-*O*-(dithiocarbonate)-α-L-rhamnopyranoside (57)



57

Glycoside **47** (200 mg, 0.53 mmol) and thiocarbodiimidazole (0.15 g, 0.84 mmol) were refluxed in toluene (6 mL) for overnight. The resulting mixture was concentrated and residue was purified by using flash chromatography (2:1 hexane:EtOAc) and provided **57** (150 mg, 67%).

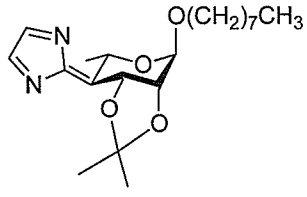
TLC (2:1 hexane:EtOAc) R_f 0.53.

$\alpha]_D$= -73.0 (c 1.0, $CHCl_3$)

^{13}C NMR ($CDCl_3$): δ 13.8 (CH_3), 14.1 (C-6), 25.5, 25.6 (2 $\times$ CH_3), 22.6, 26.1, 29.2, 29.3, 29.4, 31.8 (6 $\times$ CH_2), 64.7 (C-5), 68.5 (OCH_2), 73.0 (C-4), 73.6 (C-2), 88.2 (C-3), 96.9 (C-1), 111.3 $(CH_3)_2C$, 113.4 (Imidazole C), 137.0 (Imidazole C), 153.5 (N=C-N), 178.9 (OC=S).

Anal. Calcd for $C_{21}H_{32}N_2O_5S$: C, 59.41; H, 7.60; N, 6.60. Found: C, 59.40; H, 7.90; N, 6.62.

Octyl 2,3-isopropylidene-4-C-(imidazol-2-yl)-α-L-rhamnopyranoside (58)



58

Glycoside **57** (150 mg, 0.36 mmol) in xylene (4.5 mL) was refluxed with tributyl-stannane (0.2 mL, 0.57 mmol) for 6 h. The mixture was washed with dilute HCl (3

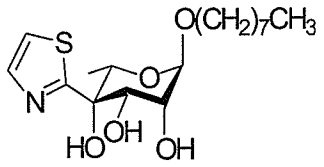
× 5 mL) and water (3 × 5 mL), dried and concentrated. The resulting residue was purified by flash chromatography (4:1 hexane:EtOAc) and afford **58** (53 mg, 41%).

TLC (4:1 hexane:EtOAc) R_f 0.28.

^{1}H NMR ($CDCl_3$): δ 0.92 (m, 3H, CH_3), 1.05 (d, 3H, J=6.5 Hz, H-6), 1.32 (m, 10H, 5 × CH_2), 1.34 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 3.50 (m, 1H, OCH_2), 3.71 (m, 1H, OCH_2), 4.22 (d, 1H, J=5.6 Hz, H_3), 4.39 (q, 1H, J=6.6 Hz, H-5), 4.67 (d, 1H, J=5.6 Hz, H-*2*), 5.17 (s, 1H, H-1), 7.25 (d, 1H, imidazol H), 7.29 (d, 1H, imidazol H)

Anal. Calcd for $C_{20}H_{32}N_2O_4$: C, 65.91; H, 8.85; N, 7.69. Found: C, 65.90; H, 8.75; N, 7.60.

Octyl-4-(thiazol-2yl)-α-L-rhamnopyranoside (60)



60

Compound **61** (2.7 g, 6.6 mmol) dissolved in 4:1 acetic acid:water solution (30 mL). The resulting mixture was stirred at 60 ^{0}C for 2 h. A solution was concentrated, purified by flash chromatography (1:1 hexane:EtOAc) and obtained **60** (2.3 g, 98%).

TLC (1:1 hexane:EtOAc) R_f 0.39.

$[\alpha]_D$= -38.8 (c 1.0, $CHCl_3$)

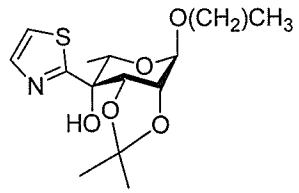
^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J=6.5 Hz, CH_3), 1.01 (d, 3H, J=6.4 Hz, H-6), 1.29 (m, 10H, 5 × CH_2), 1.63 (m, 2H, CH_2), 3.47 (m, 1H, OCH), 3.74 (m, 1H, OCH), 3.93 (m, 1H, H-2), 4.31 (m, 3H, H-3, H-4, H-5), 4.94 (d, 1H, J=1.3 Hz, H-1), 7.34 (d, 1H, J=3.2 Hz,Thiazoyl H), 7.77 (d, 1H, J=3.2 Hz,Thiazoyl H)

^{13}C NMR ($CDCl_3$): δ 13.6 (C-6), 14.07 (CH_3), 22.6, 26.1, 29.2, 29.3, 29.4, 31.8

(6 × CH_2), 68.2 (OCH_2), 69.8 (C-5), 70.2 (C-3), 70.9 (C-2), 79.6 (C-4), 99.9 (C-1), 119.8 (Thiazoyl C), 142.5 (Thiazoyl C), 172.1 (N=C-S).

Anal. Calcd for $C_{17}H_{29}NO_5S$: C, 56.80; H, 8.13; N, 3.90. Found: C, 57.01; H, 8.47; N, 3.91.

Octyl 2,3-isopropylidene-4-(thiazol-2yl)-α-L-rhamnopyranoside (61)



61

A solution of thiazole (1.2 g, 14.0 mmol) in THF (30 mL) was cooled to -78 ^{0}C. *tert*-Butyllithium (2.5 M in hexane; 6.76 mL, 16.0 mmol, 1.2 equiv.) was added and the resulting yellow solution was stirred 1 h at -78 ^{0}C. A solution of ketone **48** (4.4 g, 14 mmol) in THF (24 mL) at -78 ^{0}C was added to the above mixture and stirred 4h while maintaining the temperature. The reaction was quenched with large excess of water and extracted to Et_2O (3 × 100 mL). The combined organic layers were successively washed with water (3 × 30 mL), dried and evaporated to afford a crude oil. Purification of the crude oil using flash chromatography (10:1 hexane:EtOAc) provided **61** (4.1 g, 73%) as a colourless oil.

TLC (10:1 hexane:EtOAc) R_f 0.33.

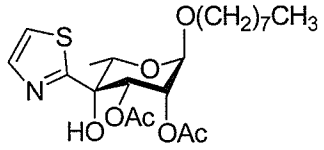
$[\alpha]_D$= +14.2 (c 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, J=6.9 Hz, CH_3), 0.99 (d, 3H, J=6.5 Hz, H-6), 1.27 (m, 8H, 4 × CH_2), 1.35 (s, 3H, CH_3), 1.57 (m, 2H, CH_2), 1.61 (s, 3H, CH_3), 3.24 (s, 1H, OH), 3.45 (m, 2H, CH_2), 3.70 (m, OCH_2), 4.24 (m, 2H, H-5, H-2), 4.82 (d, 1H, J=5.6 Hz, H-*3*), 5.09 (s, 1H, H-1), 7.25 (d, 1H, J=3.2 Hz,Thiazoyl H), 7.76 (d, 1H, J=3.2 Hz,Thiazoyl H)

^{13}C NMR ($CDCl_3$): δ 13.7 (C-6), 14.1 (CH_3), 22.6 (CH_2), 25.1 (CH_3), 25.9 (CH_3), 26.1, 29.2, 29.3, 29.4, 31.8 (5 $\times$ CH_2), 67.5 (C-5), 68.0 (OCH_2), 74.6 (C-2), 74.7 (C-4), 77.4 (C-3), 97.0 (C-1), 109.5 $(CH_3)_2C$, 119.2 (Thiazoyl C), 143.0 (Thiazoyl C), 174.4 (N=C-S).

Anal. Calcd for $C_{20}H_{33}NO_5S$: C, 60.12; H, 8.32; N, 3.51. Found: C, 60.45; H, 8.42; N, 3.62.

Octyl 2,3-acetyl-4-(thiazol-2-yl)-α-L-rhamnopyranoside (63)



63

The purified product **62** (281 mg, 0.78 mmol) was suspended in dry pyridine (3 mL) containing a catalytic amount of DMAP. Ac_2O (0.3 mL, 3.06 mmol) was added at 0 ^{0}C and stirred for 6 h at 0 ^{0}C. The resulting solution was quenched with concentrated HCl and then diluted with EtOAc (20 mL). The mixture was poured into cooled water (20 mL) and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 $\times$ 10 mL), brine (3 $\times$ 10 mL), dried and concentrated. The crude product was purified by flash chromatography (2:1 hexane:EtOAc) and obtained a colourless oil **63** (252 mg, 73%).

TLC (2:1 hexane:EtOAc) R_f 0.43.

$[\alpha]_D$= -46.8 (c 1.0, $CHCl_3$)

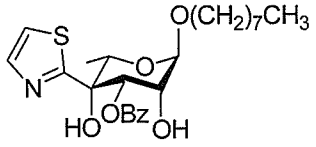
^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, J=6.9 Hz, CH_3), 1.03 (d, 3H, J=6.4 Hz, H-6), 1.30 (m, 8H, 4 $\times$ CH_2), 1.63 (m, 2H, CH_2), 1.82, 2.17 (s, 2 $\times$ OAc), 3.45 (m, CH_2), 3.53 (s, 1H, OH), 3.71 (m, OCH_2), 4.57 (q, 1H, J=6.4 Hz, H-5), 4.84 (d, 1H, J=1.4 Hz, H-1), 5.31 (dd, 1H, J=1.4 Hz, J=3.7 Hz, H-2), 5.75 (d, 1H, J=3.7 Hz, H-3),

7.26 (d, 1H, J=3.2 Hz,Thiazoyl H), 7.75 (d, 1H, J=3.2 Hz,Thiazoyl H)

^{13}C NMR ($CDCl_3$): δ 13.5 (CH_3), 14.1 (C-6), 20.3, 20.9(2 $\times$ OAc), 22.6, 26.0, 29.1, 29.2, 29.3, 31.8 (6 $\times$ CH_2), 68.6 (OCH_2), 70.1 (C-2), 70.2 (C-3), 70.3 (C-5), 77.9 (C-4), 97.8 (C-1), 119.4 (Thiazoyl C), 143.0 (Thiazoyl C), 168.9, 169.1 (2 $\times$ C=O), 171.0 (N=C-S).

Anal. Calcd for $C_{21}H_{33}NO_7S$: C, 56.86; H, 7.50; N, 3.16. Found: C, 57.36; H, 7.81; N, 3.13.

Octyl 3-benzoyl-4-(thiazol-2-yl)-α-L-rhamnopyranoside (65)



65

The purified product **62** (243 mg, 0.68 mmol) was suspended in dry pyridine (2 mL) at 0 ^{0}C. Benzoyl chloride (0.16 mL, 1.35 mmol) was added and stirred for 2 h at 0 ^{0}C. The resulting solution was diluted with CH_2Cl_2 (10 mL) and washed with 10% HCl (2 $\times$ 5 mL), saturated $NaHCO_3$ (3 $\times$ 5 mL), dried, concentrated and purified by flash chromatography (6:1 hexane:EtOAc). This afford **65** (230 mg, 73%) and **66** (33 mg, 10%).

TLC (6:1 hexane:EtOAc) R_f 0.48.

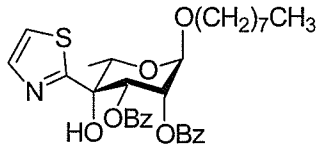
$[\alpha]_D$= +100.6 (c 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, J=6.8 Hz, CH_3), 1.08 (d, 3H, J=6.3 Hz, H-6), 1.30 (m, 10H, 5 $\times$ CH_2), 1.66 (m, 2H, CH_2), 3.20 (s, 1H, OH), 3.52 (m, 1H, OCH), 3.78 (m, 1H, OCH), 4.27 (m, 1H, H-2), 4.56 (q, 1H, J=6.5 Hz, H-5), 4.67 (s, 1H, OH), 4.98 (d, 1H, J=1.5 Hz, H-3), 5.75 (d, 1H, J=3.2 Hz, H-1), 7.26-7.94 (m, OBz), 7.22 (d, 1H, J=3.2 Hz,Thiazoyl H), 7.69 (d, 1H, J=3.2 Hz,Thiazoyl H)

^{13}C NMR ($CDCl_3$): δ 13.6 (C-6), 14.1 (CH_3), 22.7, 26.1, 29.3, 29.4, 29.4, 31.8 (6 $\times$ CH_2), 68.4 (OCH_2), 69.7 (C-5), 70.4 (C-2), 73.0 (C-3), 78.6 (C-4), 100.1 (C-1), 119.8 (Thiazoyl C), 128.2, 128.4, 129.4, 129.8, 133.2, 133.2, (C-Ar), 142.6 (Thiazoyl C), 164.9 (N=C-S), 171.3 (C=O).

Anal. Calcd for $C_{24}H_{33}NO_6S$: C, 62.18; H, 7.17; N, 3.02. Found: C, 65.03; H, 6.97; N, 2.56.

Octyl 2,3-benzoyl-4-(thiazol-2yl)-α-L-rhamnopyranoside (66)



66

The purified product **62** (561 mg, 1.56 mmol) was suspended in dry pyridine (2 mL) at room temperature. Benzoyl chloride (0.55 mL, 4.68 mmol) was added and stirred for 2 h. The resulting solution was diluted with CH_2Cl_2 (10 mL) and washed with 10% HCl (2 $\times$ 5 mL), saturated $NaHCO_3$ (3 $\times$ 5 mL), dried and concentrated. The crude product was purified by flash chromatography (10:1 hexane:EtOAc) and obtained a colourless oil **66** (685 mg, 77%).

TLC (4:1 hexane:EtOAc) R_f 0.47.

$[\alpha]_D$= +107.6 (c 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H, J=6.9 Hz, CH_3), 1.13 (d, 3H, J=6.4 Hz, H-6), 1.33 (m, 8H, 4 $\times$ CH_2), 1.43 (m, 2H, CH_2), 1.72 (m, 2H,CH_2), 3.60 (m, 1H, OCH), 3.82 (m, 2H, OCH, OH), 4.76 (q, 1H, J=6.4 Hz, H-5), 5.09 (d, 1H, J=1.3 Hz, H-1), 5.71 (dd, 1H, J=1.5 Hz, J=3.7 Hz, H-2), 6.11 (d, 1H, J=3.7 Hz, H-3), 7.19-8.01 (m, 2 $\times$ OBz), 7.20 (d, 1H, J=3.1 Hz,Thiazoyl H), 7.74 (d, 1H, J=3.1 Hz,Thiazoyl H)

^{13}C NMR ($CDCl_3$): δ 13.6 (C-6), 14.1 (CH_3), 22.7, 26.1, 29.2, 29.3, 29.4, 31.8

(6 × CH_2), 68.7 (OCH_2), 70.3 (C-5), 70.8 (C-2), 71.3 (C-3), 73.0 (C-4), 97.9 (C-1), 119.5 (Thiazoyl C), 119.7, 128.2, 128.3, 128.7, 129.2, 129.5, 129.6, 129.7, 129.8, 133.0, 133.2, 133.6 (C-Ar), 143.1 (Thiazoyl C), 164.9, 171.3 (2 × C=O), 171.0 (N=C-S).

Anal. Calcd for $C_{31}H_{37}NO_7S$: C, 65.59; H, 6.57; N, 2.47. Found: C, 65.20; H, 6.93; N, 2.44.

References

1. Shinnick, T. M. *Tuberculosis;* Springer-Verlag Berlin Heideberg: 1996.

2. Pathak, A. K.; Besra, G. S.; Crick, D.; Maddry, J. A.; Morehouse, C. B.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **1999,** *7,* 2407-2413.

3. Snider, D. S.; Raviglione, M.; Kochi, A. *In Tuberculosis: Pathogenesis, Protection and Control;* American Society of Microbiology: Washington, D. C., 1994.

4. Enarson, D. A.; Murray, J. F. *In Tuberculosis;* Little, Brown and Co.: Boston, 1996.

5. *World Health Organization, "world wide website http://www.who.int/en/"* .

6. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Tuberculosis;* Springer: Berlin, 1996.

7. Reichman, L. B. *J. Am. Med. Assoc.* **1997,** *278,* 865.

8. Pablos-Mendez, A.; Raviglion, M. C.; Laszlo, A.; Binkin, N.; Rieder, H. L.; Bustreo, F.; Cohn, D. L.; Weezenbeek, C. S. B. L.-V.; Kim, S. J.; Chaulet, P.; Nunn, P. N. *Engl. J. Med.* **1998,** *338,* 1641.

9. Daffe, M.; Draper, P. *Adv. Microbiol. Physiol.* **1998,** *39,* 131-203.

10. Crick, D. C.; Brennan, P. J. *Curr. Opin. Anti-Inf. Invest. Drugs* **2000,** *2,* 154-163.

11. Brennan, P. J. *Annu. Rev. Biochem.* **1995,** *64,* 29-63.

12. Mikusova, K.; Mikus, M.; Bersa, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.* **1996,** *271,* 7820.

13. McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1990,** *265,* 18200.

14. Brennan, P. J.; Besra, G. S. *Biochem. Soc. Trans.* **1997,** *25,* 188-194.

15. Qian, X.; Palcic, M. M. *Carbohydrates in Chemistry and Biology* **2000,** *3,* 293-312.

16. Leloir, L. F. *Science* **1971,** *172,* 1299-1303.

17. Zechel, D.; Withers, S. G. *Comprehensive Natural Product Chemistry;* C. D. Poulter: 1999.

18. Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001,** *9,* 3077-3092.

19. Bourne, Y.; Henrissat, B. *Cur. Opin. Struct. Biol.* **2001,** *11,* 593-600.

20. Charnock, S. J.; Davies, G. J. *Biochem.* **1999,** *38,* 6380-6385.

21. Davies, G. J. *Nat Struct. Biol.* **2001,** *8,* 98-100.

22. Kochetkov, N. K.; Bochkov, A. F.; Sokolovskaja, T. A. *Carbohydr. Res.* **1971,** *16,* 17.

23. Sinay, P. *Pure Appl. Chem.* **1978,** *50,* 1437.

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

25. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975,** *97,* 4056.

26. Wolf, G.; Rohle, G. *Angew. Chem., Int. Ed. Engl.* **1974,** 13.

27. Boons, G.-J.; Hale, K. J. *Organic Synthesis with Carbohydrates;* Sheffield Academic Press Ltd, England: 2000.

28. Igarashi, K. *Adv. Carbohydr. Chem. Biochem.* **1977,** *34,* 243.

29. Paulsen, H. *ibid* **1982,** *21,* 155.

30. Mootoo, D. R.; Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988,** *110,* 5583-5584.

31. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981,** 431.

32. Shimizu, M. S.; Togo, H.; Yokoyama, M. *Synthesis* **1998,** 799.

33. Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988,** *174,* 211.

34. Wolfrom, M. L.; Groebke, W. *J. Org. Chem.* **1963,** *28,* 2986.

35. Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984,** *106,* 4189.

36. Posner, G. H.; Haines, S. R. *Terahedron Lett.* **1985,** *26,* 5.

37. Sinay, P. *Pure Appl. Chem.* **1978,** *50,* 1437-1452.

38. Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980,** *19,* 731.

39. Grundler, G.; Schmidt, R. R. *Carbohydr. Res.* **1985,** *135,* 203-218.

40. Urban, F. J.; Moore, B. S.; Breitenbach, R. *Tetrahedron Lett.* **1990,** *31,* 4421-4424.

41. Marino-Albernas, J. R.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1993,** *245,* 245-257.

42. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984,** 1826-1847.

43. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986,** *25,* 212-235.

44. Kobayashi, M.; Yamazaki, F.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990,** *201,* 51-67.

45. Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Carbohydr. Chem.* **1993,** *12,* 131-136.

46. Ferrier, R. J.; Hay, R. W.; Vethaviyasar, N. *Carbohydr. Res.* **1973,** *27,* 55.

47. Hanessian, S.; Bacquet, C.; Lehong, N. *Carbohydr. Res.* **1980,** *80,* C17.

48. Fugedi, P.; Garegg, P. J.; Lonn, H.; Norberg, T. *Glycocongugate J.* **1987,** *4,* 97.

49. Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1983,** *105,* 2430.

50. Lonn, H. *Carbohydr. Res.* **1985,** *139,* 105.

51. Fugedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986,** *149,* C9.

52. Veeneman, G. H.; Leeuwen, S. H. V.; Boon, J. H. V. *Tetrahedron Lett.* **1990,** *31,* 1331.

53. Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1987,** *28,* 4701.

54. Wakabayashi, K.; Pigman, W. *Carbohydr. Res.* **1974,** *35,* 3-14.

55. Garg, H. G.; Jeanloz, R. W. *Carbohyr. Res.* **1976,** *49,* 482-488.

56. Lacombe, J. M.; Pavia, A. A. *J. Org. Chem.* **1983,** *48,* 2557-2563.

57. Lavielle, S.; Ling, N. C.; Saltman, R.; Guillemin, R. C. *Carbohydr. Res.* **1981,** *89,* 229-236.

58. Nakahara, Y.; Iijima, H.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1991,** *216,* 211-225.

59. Luning, B.; Norberg, T.; Tejgrant, J. *J. Chem. Soc., Chem. Commun.* **1989,** 1267-8.

60. Jansson, A. M.; Medal, M.; Bock, K. *Terahedron Lett.* **1990,** *31,* 6991-4.

61. Filira, F.; Biondi, L.; Cavaggion, F.; Scolaro, B.; Rocchi, R. *Int. J. Pept. Protein Res.* **1990,** *36,* 86-96.

62. Bardaji, E.; Torres, J. L.; Clapes, P.; Albericio, F.; Barany, G.; Rodriguez, R. E.; Sacristan, J. L.; Valencia, G. *J. Chem. Soc., Perkin Trans. 1* **1991,** 1755-9.

63. Schmidt, R. R.; Zimmermann, P. *Tetrahedron Lett.* **1986,** *27,* 481-4.

64. Wakabayashi, K.; Pigman, W. *Carbohyr. Res.* **1974,** *35,* 3.

65. Kerevitskaya, V.; Vafina, M. G.; Kochetkov, N. K. *Carbohydr. Res.* **1967,** *3,* 377.

66. Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992,** *114,* 10249-10258.

67. Martin, J.; O'Donnell,; Polt, R. L. *J. Org. Chem.* **1982,** *47,* 2663-2668.

68. Garegg, P. J.; Konradsson, P.; Kvarnstrom, I.; Norberg, T.; Svensson, S. C. T.; Wigilius, B. *Acta. Chem. Scand. B* **1985,** *39,* 569-577.

69. Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C. *Bioorganic and Medicinal Chemistry* **1997,** *5,* 661-672.

70. Chatterjee, D.; Cho, S.; Brennan, P. J.; Aspinall, G. O. *Carbohydr. Res.* **1986,** *156,* 39-56.

71. Bing,; Schmidt, R. R. *Liebigs Ann. Chem.* **1992,** 817-823.

72. Schmidt, R. R.; Wegmann, B.; Jung, K. *Liebigs Ann. Chem* **1991,** 121-124.

73. Azuzanneau, F.-I.; Bundle, D. R. *Carbohydr. Res.* **1991,** *212,* 13-24.

74. Lemieux, R. U.; Brice, C. *Can. J. Chem.* **1954,** *33,* 109-119.

75. Szabo, L.; Ramza, J.; Langdon, C.; Polt, R. *Carbohydr. Res.* **1995,** *274,* 11-28.

76. Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. *Bioorganic and Medicinal Chemistry* **2001,** *9,* 3129-3143.

77. Pathak, A. K.; Pathak, V.; Suling, W. J.; Gurcha, S. S.; Morehouse, C. B.; Besra, G. S.; Maddry, J. A.; Reynolds, R. C. *Bioorganic and Medicinal Chemistry* **2002,** *10,* 923-928.

78. Pathak, A. K.; Pathak, V.; Maddry, J. A.; Suling, W. J.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Bioorganic and Medicinal Chemistry* **2001,** *9,* 3145-3151.

79. Katritzky, A. R.; Reweastle, G. W.; Fan, W. *Am. Chem. Soc.* **1988,** *53,* 5685-5689.

80. Stocker, F. B.; Kurtz, J. L.; Gilman, B. L.; Forsyth, D. A. *J. Org. Chem* **1970,** *35,* 883-887.

81. Pathak, A. K.; Besra, G. S.; Morehouse, C. B.; Suling, W. J.; Reynolds, R. C. *Bioorganic and Medicinal Chemistry* **1999,** *7,* 2407-2413.

82. Boons, G. J.; Hale, K. J. *Organic Synthesis with Carbohydrates;* Sheffield Academic Press Ltd: England: 2000.

83. Michael, J. E.; Ganem, B. *Carbohydr. Res.* **1988,** *176,* 316-323.

84. Granier, T.; Vasella, A. *Helvetica Chimica Acta* **1995,** *78,* 1738-1746.

85. Enders, D.; Hundertmark, T.; Lampe, C.; Jegelka, U.; Scharfbillig, I. *Eur. J. Org. Chem.* **1998,** 2839-2849.

86. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc. Perkin Trans. I* **1975,** 1574-1585.

87. Woodward, R. B.; Hoffmann, R. *J. Am. Chem. Soc.* **1965,** *87,* 395.

88. Chikashita, H.; Itoh, K. *Heterocycles* **1985,** *23,* 295-300.

89. Golubovskaya, L. E.; Rzheznikov, V. M. *Russian Journal of Organic Chemistry* **2001,** *37,* 881-882.

90. Draper, R. W.; Hou, D.; Iyer, R.; Lee, G. M.; Liang, J. T.; Mas, J. L.; Vater, E. J. *Organic Process Research Development* **1998,** *2,* 186-193.

91. Haines, A. H. *Adv. Carbohydr. Chem.;* Biochem. Academic press: 1976.

92. Mikusova, K.; Mikus, M.; Bersa, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.* **1996,** *271,* 7820.

93. Chan, M.; Cheng, K.; Ho, K. M.; Ng, C. T.; Yam, T. M. *J. Org. Chem.* **1988,** *53,* 4466-4471.

94. Kirmse, W.; Konrad, W.; Schnitzler, D. *J. Org. Chem.* **1994,** *59,* 3821-3829.

95. Perrin, D. D.; F., W. L. *Purification of Laboratory Chemicals;* Pergamon Press: New York: 3 ed. ed.; 1988.