Synthesis of Carbohydrate-based Glycosyltransferase Inhibitors

By

Xianghui Wen

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Department of Chemistry
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

Synthesis of Carbohydrate-based Glycosyltransferase Inhibitors

BY

Xianghui Wen

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

Of

Doctor of Philosophy

Acknowledgments

First of all, I would like to express my sincere thanks to my supervisor Dr. Philip G. Hultin for his encouragement and advice during this Ph. D. research. His expertise and enthusiasm are appreciated.

I also would like to thank our collaborators Dr. Dean C. Crick and Dr. Patrick J. Brennan at Colorado State University for testing our samples. Also thanks to Dr. Kirk Marat and Dr. Hélène Perreault for their assistance in obtaining NMR and MS spectra.

I also wish to thank the members of my advisory committee, Dr. James L. Charlton, Dr. Norman R. Hunter and Dr. Brian Hasinoff for their comments and advice regarding my Ph.D. research.

I am also grateful to the staff at the chemistry department, Barbara, Cheryl, Tricia and Wayne for their help whenever it was needed. And also thanks to the past and current group members Marion, Jason, Manjula, Tim and Pedro for making the lab a pleasant place to work. I especially thank Pedro and Richard for proofreading the manuscript.

Finally, I would like to thank my wife Wenlian, my son Eway and my daughter Melissa for their love, support and encouragement.

The financial support from the University of Manitoba and the Natural Sciences and Engineering Research Council of Canada for this research is also acknowledged.

Abstract

This thesis describes the synthesis of three proposed carbohydrate-based glycosyltransferase inhibitors. They were β *N*-acetyl *C*-mannosamine and *C*-glucosamine 1-phosphonates, α *N*-acetyl D-glucosamine 2-hydroxy and 2-keto phosphonates, and α-L-Rha-(1→3)-α-D-GlcNAc disaccharide analogues.

N-acetyl D-mannosamine and *N*-acetyl D-glucosamine 1-phosphates are two glycosyl donors used by glycosyltransferases to synthesize a variety of important biological components such as the bacterial cell wall. However, β *C*-glycosyl analogues of these glycosyl donors have not been explored as potential inhibitors yet. In order to prepare these analogues, several synthetic routes have been proposed and evaluated in this thesis. It has been found that the Horner-Emmons/Michael reaction approach to the *C*-glycosyl analogue of mannosaminyl 1-phosphate and the gluconolactone approach to the analogue of glucosaminyl 1-phosphate were the most efficient synthetic routes.

One of the key features in the glycosyltransferase-catalyzed glycosylation is the binding of the donor diphosphate and the divalent metal ion at the enzyme active site, in which the divalent metal ion activates the diphosphate moiety as a leaving group. However, the currently reported analogues of glycosyl pyrophosphate haven't taken full account of this binding nature. Therefore, both glycosyl 2-hydroxy and 2-keto phosphonates were proposed as close mimics to the glycosyl pyrophosphate in this research. Both glycosyl 2-hydroxy and 2-keto phosphonates were prepared through an epoxide approach. Nucleophilic addition of a phosphonate nucleophile to the glycosyl epoxide gave a glycosyl 2-hydroxyl phosphonate in good yield. This glycosyl 2-hydroxy phosphonate was then converted to a glycosyl 2-keto phosphonate with a good yield by oxidation with acetic anhydride/DMSO.

The synthesis of mycobacterial glycosyltransferase inhibitors was based on the structure of a specific mycobacterial cell wall Rha-(1→3)-GlcNAc disaccharide linker. This linker is a key component in the biosynthesis of the mycobacterial cell wall, but has not been targeted for drug design so far. It provides a novel structure for modifications as potential inhibitors of mycobacterial glycosyltransferases. These disaccharide analogues were prepared using a thioglycoside approach. For this purpose, a series of glycosyl donors and acceptors was synthesized. Glycosylation of these donors and acceptors by the thioglycoside approach resulted in disaccharides in nearly quantitative yields and with excellent stereoselectivity. These disaccharides were then further derivatized. Seventeen disaccharide analogues of this particular linker have been prepared so far. Three of them exhibited inhibitory activity against mycobacterial glycosyltransferases. The biological test results have provided guidance for the future drug design of mycobacterial glycosyltransferase inhibitors.

List of Abbreviations

[α]	specific rotation
Ac	acetyl
AG	arabinogalactan
AIBN	2,2'-azobisisobutyronitrile
Ar	aryl
Ara*f*	arabinofuranose
B	base
Bn	benzyl
Bu	butyl
*t*Bu	*tert*-butyl
Bz	benzoyl
c	concentration in g/100 ml
δ	chemical shift in parts per million (ppm)
d	doublet
de	diastereomeric excess
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEPT	distortionless enhancement by polarization transfer
DIBALH	diisobutylaluminum hydride
DMAP	4-(dimethylamino) pyridine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
equiv	equivalent
ESI	electrospray ionization
Et	ethyl
g	gram (s)
Gal*f*	galactofuranose
GlcNAc	*N*-acetyl glucosamine
h	hour (s)
Hz	hertz
IDCP	iodonium dicollidine perchlorate
Im	imidazole
J	coupling constant (in NMR)
L	liter (s)
LAM	lipoarabinomannan
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl) amide
LiNaphth	lithium naphthalenide
lit.	literature
μ	micro

m	multiplet
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
mp	melting point
Ms	methanesulfonyl (mesyl)
MS	mass spectrometry
m/z	mass to charge ratio
NIS	*N*-iodosuccinimide
NOE	nuclear Overhauser effect
NMR	nuclear magnetic resonance
Nu	nucleophile
Ph	phenyl
Py	pyridine
Rha	rhamnose
rt	room temperature
s	singlet
t	triplet
TBDMS	*t*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl (triflyl)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Ts	*p*-toluenesulfonyl (tosyl)

Table of Contents

Part II: Ph.D. Research

Part I: Introduction

Chapter 1

The Mycobacterial Cell Wall and Mycobacterial Glycosyltransferase Inhibitors

1.1 Introduction

Tuberculosis caused by the microorganism *Mycobacterium tuberculosis* is responsible for over 2 million deaths worldwide annually, largely in developing countries due to the fact that people living there have little access to appropriate medical treatment. In industrialized countries, tuberculosis is becoming a growing concern due to the appearance of multidrug resistant mycobacterial strains and the increasing number of infections in HIV patients.[1]

This chapter will discuss the structure of the mycobacterial cell wall, the problems associated with the current anti-tuberculosis drugs, and a proposed solution to the problems.

1.2 The mycobacterial cell wall

The mycobacterial cell wall is composed of three major layers, the plasma membrane, the peptidoglycan and the mycolic acid bilayer (Figure 1.1).[2] The plasma membrane is located in the inner layer of the cell wall and consists of a phospholipid bilayer. Peptidoglycan is the backbone of the mycobacterial cell wall. It contains a linear polysaccharide of alternating β (1→4) linked *N*-acetyl glucosamine and *N*-acetyl muramic acid residues. The linear polymers lie side by side in the cell wall, cross-linked by short chain peptides. The rigid peptidoglycan structure sheathes the entire plasma membrane and prevents the cell wall from rupturing due to the osmotic pressure of the interior. The outer layer of the mycobacterial cell wall consists of mycolic acid, a long chain fatty acid. The mycolic acid bilayer is packed so tightly that it forms a waxy barrier to control the traffic of the chemicals that go in and out of the cell wall.

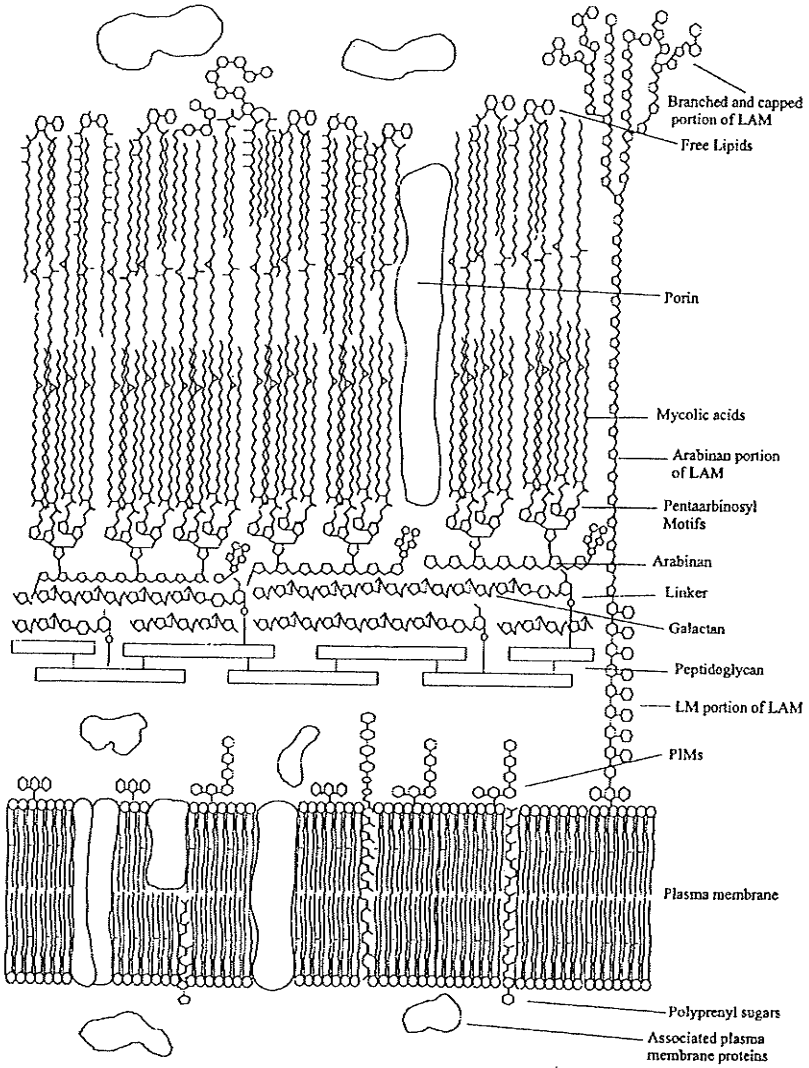


Figure 1.1 Mycobacterial cell wall (Adapted from Shinnick, T. M. 'Tuberculosis' published by Springer-Verlag, Berlin, 1996, p23)

Between the peptidoglycan and mycolic acid layer resides a polysaccharide called the arabinogalactan (AG) (Figure 1.2).[3] Both arabinose and galactose residues in the AG are in their furanose forms. The AG consists of linear galactan cores that have alternating β (1→5) and β (1→6) Gal*f* linkages. The galactan cores are branched at C-5 of a β (1→6) galactofuranose residue by α (1→5) Ara*f* linked arabinan. At the reducing end of the AG,

linked to the peptidoglycan, is a unique disaccharide linker. It consists of a Rha-(1→3)-GlcNAc-1-phosphate that connects the AG and the peptidoglycan together.

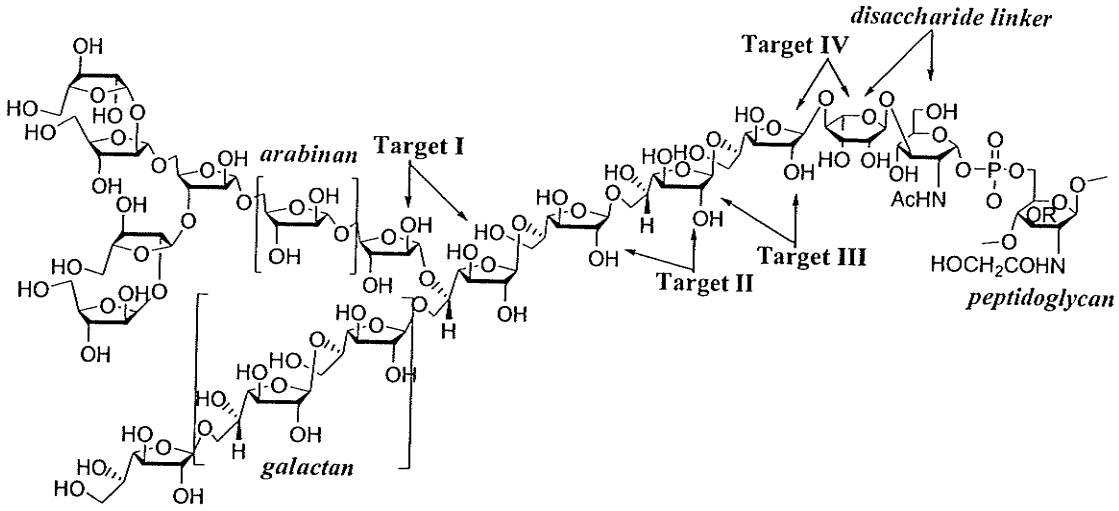


Figure 1.2 Mycobacterial arabinogalactan (AG) and targets for drug design

Lipoarabinomannan (LAM) is another important polysaccharide stretching across the peptidoglycan and the mycolic acid bilayer. The LAM is a very complex polymer, consisting of α (1→6) linked mannan and α (1→5) linked arabinan cores. The arabinan core has branches of various arabinan segments.[4] Although the LAM plays an important role in the mycobacterial cell wall, it will not be discussed further in this thesis.

1.3 Problems for the anti-tuberculosis drugs

The bacterial cell wall has been an effective target for many conventional antibacterial drugs. Two of the most important antimycobacterial agents, isoniazid and ethambutol, specifically target cell wall biosynthesis, inhibiting the biosynthesis of the AG and mycolic acids, respectively.[5,6] However, most of the commonly-used antibacterial drugs are ineffective against mycobacteria due to the impermeable mycobacterial cell wall. So far, only a few drugs have been found to be able to penetrate the cell wall barrier.[7]

Until fairly recently, most people believed that tuberculosis no longer posed a threat to public health in industrialized countries. However, the emergence of mycobacterial strains that are resistant to some or all of the front-line anti-tuberculosis drugs has caused alarm.[8] There are several general ways that a bacterium can produce resistance to antibiotics, such as (1) prevention of the drug from reaching its target either by active efflux or reduced uptake, (2) deactivation of the antibiotic by enzymatic modification, (3) modification of the drug's target, (4) metabolic bypass of the inhibited reaction, and (5) overproduction of the antibiotic target.[9]

The resistant mycobacterial strains also follow these mechanisms. For example, catalase-peroxidase is an enzyme that converts the anti-tuberculosis drug isoniazid into a toxic derivative that inhibits the synthesis of mycolic acids. Mycolic acid synthesis is a step in the assembly of the mycobacterial cell wall. However, the highly isoniazid-resistant strains have lost their catalase-peroxidase activity completely, thus they do not activate isoniazid and are unaffected by it.[10]

1.4 Glycosyltransferase inhibitors as a solution

A solution to the aforementioned problems is the driving force for new therapeutic strategies to develop drugs based on rational design. In recent years, a lot of attention has been focused on the development of mycobacterial glycosyltransferase inhibitors as potential antimycobacterial agents. There are two reasons for this. One is that the inhibitors will prevent biosynthesis of the mycobacterial cell wall by inhibiting mycobacterial glycosyltransferase activity. The other is that drug design can start from the enzyme reaction products – the mycobacterial cell wall structure. With advances in knowledge of the mycobacterial cell wall structure, the key points potentially susceptible to therapeutic effects will be identified.[7]

Strategies for drug design can begin from several vantage points. Firstly, the design can be started from the unusual monosaccharides found in the mycobacterial cell wall. Several unusual monosaccharides such as L-rhamnopyranose, D-arabinofuranose and D-galactofuranose have been identified as critical components of cell wall

polysaccharides. None of these sugar forms are found in humans.[11-13] Thus, the inhibitors that prevent the glycosyltransferases from incorporating these monosaccharides into mycobacterial cell wall biosynthesis will be potentially selective and effective.[14]

Secondly, the design can target a particular type of glycosyltransferase. It has been found that some of the monosaccharides in the mycobacterial cell wall have very diverse linkages. For instance, arabinose in the arabinogalactan polysaccharide forms a wide range of linkages such as Ara*f*-(α-1,5)-Ara*f*, Ara*f*-(β-1,2)-Ara*f* and Ara*f*-(α-1,5)-Gal*f* (Figure 1.2). The synthesis of each linkage is catalyzed by a specific glycosyltransferase. However, these glycosyltransferases may use the common arabinose donor as the building block. A drug that inhibits one of these glycosyltransferases may inhibit all other glycosyltransferases that utilize the same sugar donor. This feature makes the drug design very desirable.[7] Many glycosyl linkages in the AG and the LAM have been targeted for drug design in recent years.[11]

Finally, the design can aim at the unique structures that only mycobacteria possess. For instance, mycolic acids are a unique feature of the mycobacterial cell wall. Several currently used antituberculosis drugs have targeted the biosynthesis of mycolic acids. This feature makes the drug very specific and active.[14]

Overall, there are several advantages for drug design based on carbohydrate substrates for the mycobacterial glycosyltransferase inhibitors:

(1) The analogues based on the substrate structure can potentially bind to the glycosyltransferases.

(2) These mimics not only deceive the glycosyltransferases but also block the biosynthesis process because they lack the properties of the natural substrates.

(3) The natural substrates would provide many opportunities for drug design. For example, the sugar donor, the acceptor and the bisubstrate complex are all potential drug targets.

1.5 Target for drug design in this Ph.D. research

This Ph.D. research uses the disaccharide linker between the AG and the peptidoglycan as a target for the design of glycosyltransferase inhibitors that may be effective against mycobacteria (Figure 1.2). There are several reasons why this particular linker was chosen as the target.

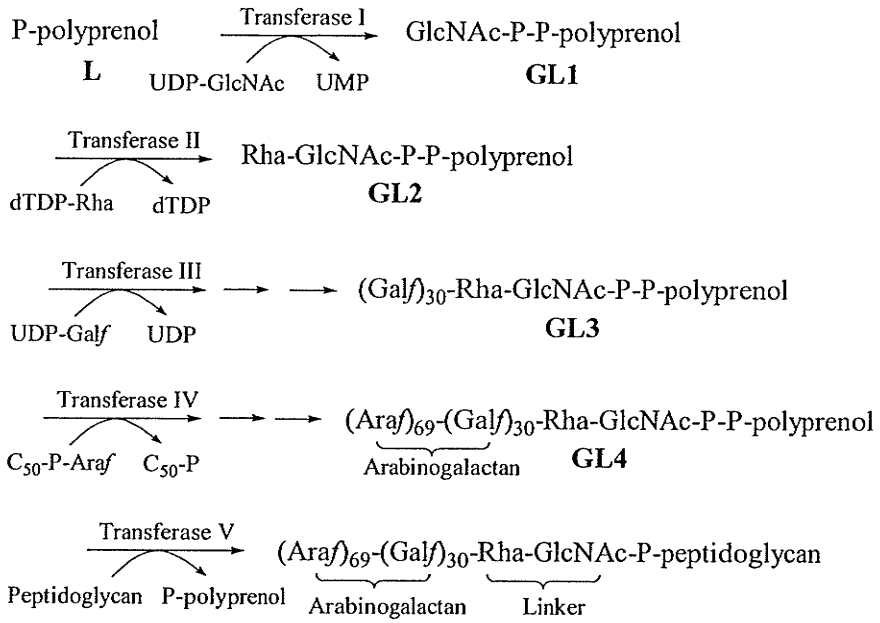
(1) This linker is unique to mycobacteria. Such a disaccharide unit has not been found in humans or other bacteria.[15] Drugs that target the biosynthetic processing of this linker will probably only be directed at mycobacteria and have little side effect on humans.

(2) This linker is pivotal in bringing the AG and the peptidoglycan together. Any drugs that inhibit the biosynthetic processing of this linker will seriously disrupt the biosynthesis of the mycobacterial cell wall.[15]

(3) This linker was discovered by Brennan in 1990.[16] Despite its importance, no one has targeted it for drug design so far.

(4) The biosynthetic pathway of the AG involving this linker has been proposed (Scheme 1.1).[15] The synthesis starts with a lipid polyprenol phosphate (**L**). The P-polyprenol is abundant in the mycobacterial cell wall, and serves as an acceptor to glycosyltransferase I. Glycosyltransferase I transfers GlcNAc-P from the sugar nucleotide UDP-GlcNAc to the polyprenol phosphate (L), generating the glycolipid GlcNAc-P-polyprenol (**GL1**). **GL1** in turn serves as an acceptor for the next step of glycosylation. Glycosyltransferase II transfers rhamnopyranose from the sugar nucleotide dTDP-Rha to **GL1**, forming the disaccharide lipid Rha-GlcNAc-P-Polyprenol (**GL2**). At this stage, the disaccharide linker is synthesized. In the following events, galactofuranose and

arabinofuranose are sequentially introduced from sugar donors UDP-Gal*f* and C_{30}-P-Ara*f* to the disaccharide linker through the rhamnose end, forming AG-Rha-GlcNAc-P-Polyprenol glycolipid **GL4**. At the last step of the biosynthesis of this particular mycobacterial cell wall region, **GL4** serves as a glycosyl donor and is transferred to the peptidoglycan. As a result, the linker serves as a bridge between the AG and the peptidoglycan. In order to inhibit the biosynthesis of the mycobacterial cell wall, this linkage is a logical target for the design of glycosyltransferase inhibitors against mycobacteria.



Scheme 1.1 Biosynthesis of AG-peptidoglycan domain of the mycobacterial cell wall

In the following chapters, I will discuss *C*-glycosides as glycosyltransferase inhibitors (chapter 2), the latest progress in mycobacterial glycosyltransferase inhibitor research (chapter 3) and how to prepare *C*-glycoside and disaccharide compounds as potential glycosyltransferase inhibitors (chapters 4 and 5).

Chapter 2

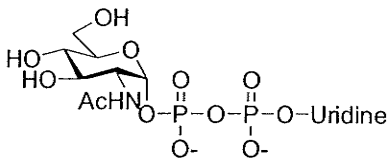
C-glycosides as Glycosyltransferase Inhibitors

2.1 Introduction

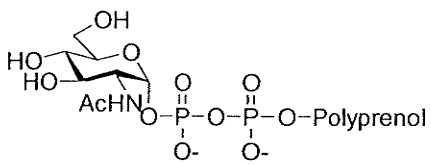
Glycotransferases catalyze the transfer of sugars from specific glycosyl donors to specific acceptors. The structures of glycosyl donors have been useful in the design of glycosyltransferase inhibitors.[17] This chapter will survey the literature on glycosyltransferase inhibitors that use *C*-glycosyl analogues to mimic a glycosyl donor, both in its ground state and transition state. This chapter also discusses how these *C*-glycosyl analogues are designed and how the modifications of their structures affect their biological activity. This review will provide us with information for the design of novel structures of mycobacterial glycosyltransferase inhibitors.

2.2 Glycosyl donors and glycosyltransferase reaction mechanism

There are two types of glycosyl donor substrates for glycosyltransferases (Figure 2.1).[18] 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 polysaccharide synthesis. Table 2.1 gives the common sugar nucleotides used by mammalian glycosyltransferases.[19] 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.[20] Figure 2.2 lists some mycobacterial glycosylphosphopolyprenols. They are the sugar donors of the mannose and arabinose units in the AG and the LAM biosynthesis.[2]



UDP-GlcNAc
(Leloir pathway)

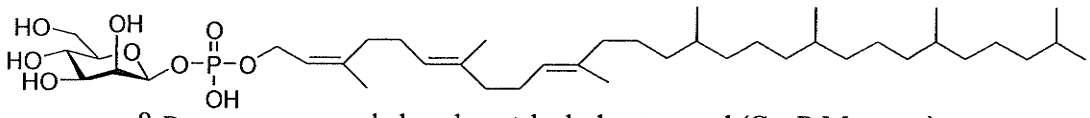


GlcNAc-P-P-Polyprenol
(non-Leloir pathway)

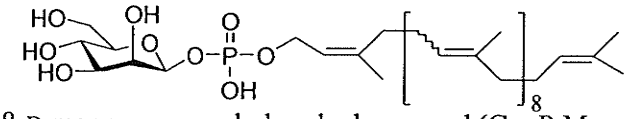
Figure 2.1 Two types of glycosyl donors

Table 2.1 Common glycosyl nucleotides used by mammalian glycosyltransferases

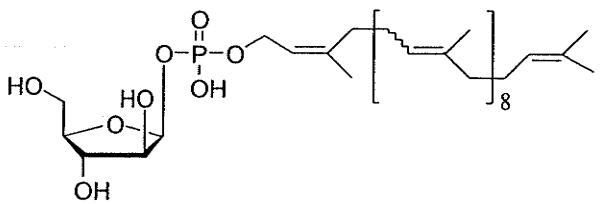
UDP	GDP	CMP
N-Acetylgalactosamine	Fucose	Sialic acid
N-Acetylglucosamine	Mannose	
N-Acetylmuramic acid		
Galactose		
Glucose		
Glucuronic acid		
Xylose		



β-D-mannopyranosyl phosphooctahydroheptaprenol (C_{35}-P-Mannose)



β-D-mannopyranosyl phosphodecaprenol (C_{50}-P-Mannose)



β-D-arabinofuranosyl phosphodecaprenol

Figure 2.2 Mycobacterial glycosylphosphopolyprenols

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.[21] 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 (Figure 2.2).[22] 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.

It is believed that glycosyltransferases carry out the glycosylation in such a way that the anomeric configuration of the donor sugars is inverted (Figure 2.3).[23] 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.[23]

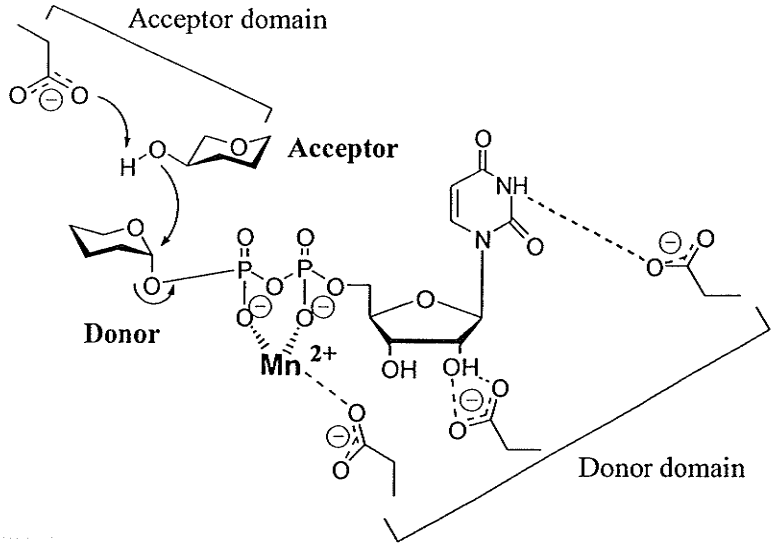


Figure 2.3 Interactions of a glycosyltransferase with a donor and an acceptor

2.3 *C*-glycosides as analogues to glycosyl phosphates and nucleotides

One of the strategies for the design of glycosyltransferase inhibitors is to use *C*-glycosides to mimic *O*-glycosyl 1-phosphates and nucleotides.[20] There are several reasons why *C*-glycosides are good mimics of *O*-glycosides.

Firstly, *C*-glycosides have similar conformations to the *O*-glycosides, thus they confer the structural elements recognized by glycosyltransferases.[24] Secondly, because *O*-aglycons serve the purpose of a leaving group to transfer the glycosyl residue, *C*-glycosides would inactivate the glycosyl residue toward the glycosyl transfer, therefore inhibiting the glycosyltransferases by failing to fulfill the duty as a glycosyl donor.[25] Finally, *C*-glycosides are more stable than the *O*-glycosides. The glycosidic C-C bonds in *C*-glycosides are not susceptible to hydrolysis by acids, bases, or glycosidases. Therefore, *C*-glycosides make plausible drug candidates.[26] So far, several types of *C*-glycosyl analogues of glycosyl phosphates and nucleotides have been designed and prepared.

2.3.1 Analogues of glycosyl 1-phosphates

The first type of *C*-glycoside analogues is the mimic of glycosyl 1-phosphates. Both Leloir donors and non-Leloir donors have glycose and 1-phosphate fragments. Figure 2.4 lists the analogues that have been reported so far.[25,27-30]

Compounds **1a-b** and **4-9** are close mimics to glycosyl 1-phosphates. They use a CH_2 to replace glycosidic oxygen atom, and keep the rest of the donor structure intact. Compounds **2** and **3** are modified further than **1a-b** and **4-9**. They use one more carbon to link the glycosyl residue with the phosphonate or phosphate moieties. The purpose of using one more carbon atom is to resemble the transition state formed during the enzymatic reaction. It is postulated that the anomeric C-O bond gradually dissociates in the transition state, therefore, the C-O bond length would be expected to be longer than the regular C-O bond length.[31] Unlike compounds **1-6**, **7-9** feature a nitrogen atom as the replacement for the ring oxygen in the monosaccharide unit.

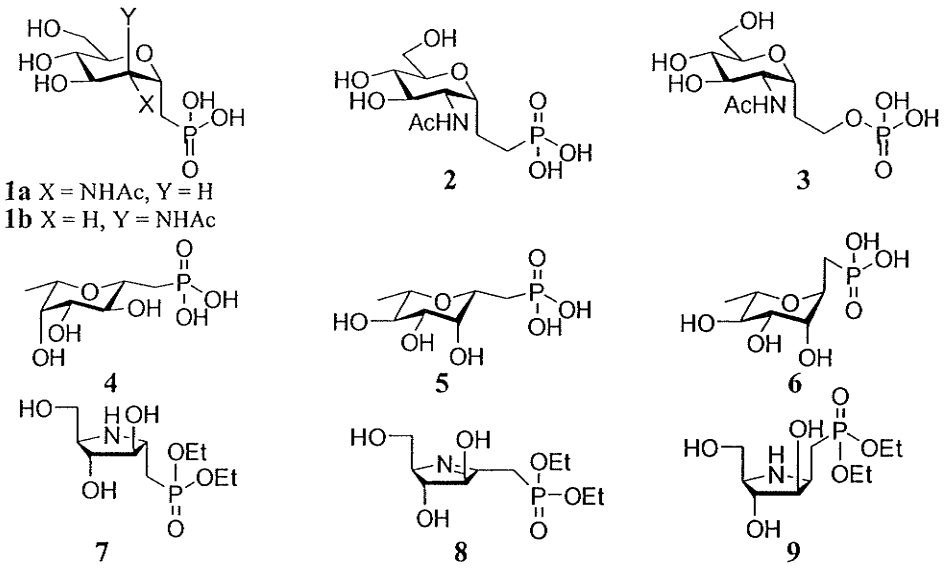


Figure 2.4 Analogues of the glycosyl 1-phosphates

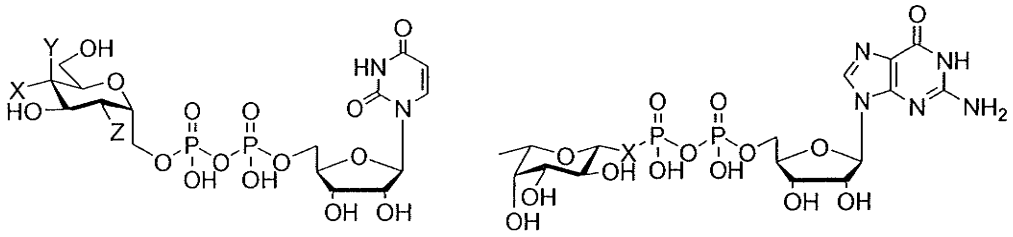
In addition, all of these compounds, except **3**, replace the phosphate moiety with a phosphonate fragment. Unlike the phosphate group, the phosphonate group is not a leaving group, therefore, the phosphonate replacement will deactivate the glycosyl analogues as glycosyl donors.

However, none of these drug design ideas has been tested. The inhibition activities of these mimics towards glycosyltransferases are currently unknown.

2.3.2 Analogues of sugar nucleotides

The second type of *C*-glycosides comprises analogues of sugar nucleotides. Some mimics are illustrated in Figure 2.5.[26,33] Analogues **10a-c** and **11b** are close mimics of sugar nucleotides. The glycosidic oxygen atom is replaced by a carbon atom while the rest of the nucleotide fragment is kept intact. Analogue **11c** is similar to **10a-c** and **11b**, but has one more carbon between the glycosyl unit and the nucleotide fragment. The purpose of adding one more carbon is to mimic more closely the transition state of glycosylation. The distance between the glycosyl residue and the phosphorus moiety in analogues **11a** to **11c** is different. The purpose of preparing such a series of analogues is to test whether or not the distance between the glycosyl unit and the phosphorus moiety is

important to the activity. Unfortunately, none of these drug design ideas have been tested. The activities of these sugar nucleotide mimics toward glycosyltransferases have not been reported.



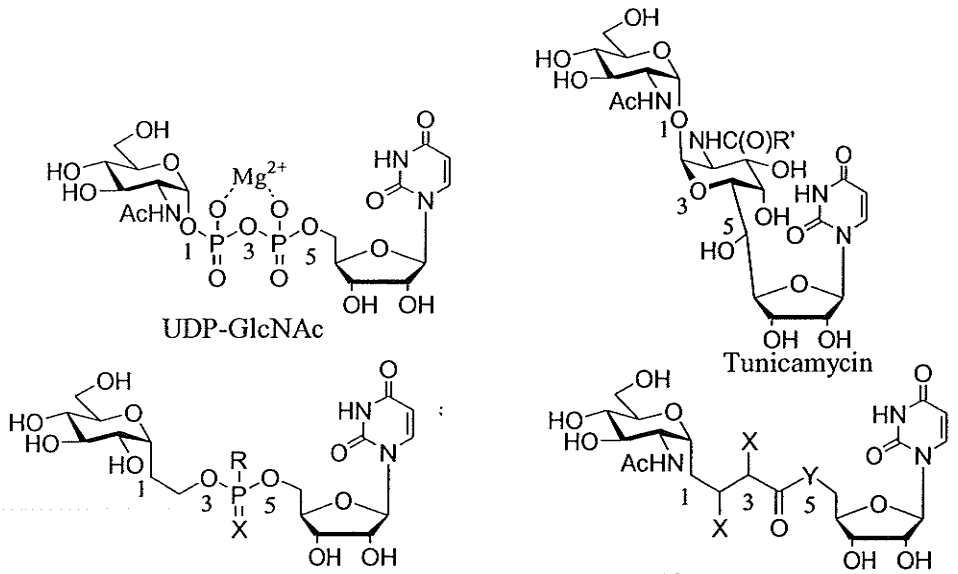
10a X = H, Y = OH, Z = OH
10b X = OH, Y = H, Z = NHAc
10c X = H, Y = OH, Z = NHAc

11a X = CH_2
11b X = CH_2O
11c X = CH_2CH_2O (α and β)

Figure 2.5 Analogues of the sugar nucleotides

2.3.3 Analogues of sugar pyrophosphates

The third type of *C*-glycoside analogues also mimics sugar nucleotides, but focuses on the pyrophosphate fragment. Figure 2.6 list some examples of these analogues.[34,35]



UDP-GlcNAc

Tunicamycin

12a R = OH, X = O
12b R = CH_3, X = O
12c R = CH_3, X = S
12d R = Ph, X = O

13a X = H, Y = O
13b X = H, Y = NH
13c X = OH, Y = O
13d X = OH, Y = NH

Figure 2.6 Analogues of sugar nucleotides based on pyrophosphate mimics

Compounds **12a-d** and **13a-d** mimic the five-atom diphosphate bridge of UDP-GlcNAc. UDP-GlcNAc is a glycosyl donor of *N*-acetyl glucosaminyltransferase, and tunicamycin is known to inhibit this enzyme, preventing the transfer of UDP-GlcNAc to dolichyl-pyrophosphoryl-GlcNAc.[34-36] Analogues **12a-d** are designed to test two hypotheses. The first hypothesis is the effect of electric charge. Compound **12a** is a charged mimic and **12b-d** are neutral. The second hypothesis is to use a five-atom monophosphate to replace the diphosphate moiety. Molecular modeling studies indicate that the five-atom distance in analogues **12a-d** is in good agreement with the spatial arrangements of UDP-GlcNAc and tunicamycin. Preliminary biological tests of these compounds show that all of them have a small inhibiting effect on the biosynthesis of glycolipids.[36]

Analogues **13a-d** use a different design concept.[34] They feature a modification that uses a nonhydrolyzable carbon chain to replace the five-atom pyrophosphate. The purpose of doing this is also to prevent the analogues from being used as glycosyl donors. Analogues **13c-d** imitate the diphosphonate moiety by adding two hydroxyls at C-2 and C-3. This design aims to increase the binding to glycosyltransferases. However, this group of analogues has not been tested for activity yet.

2.4 *C*-glycosides as transition state inhibitors

The *C*-glycosyl analogues discussed in the above sections all mimic the ground state of the sugar donors. *C*-glycosyl analogues that mimic the transition state of the reaction have also been developed.[20]

There are two approaches to the design of glycosyltransferase transition state inhibitors. One is to mimic only the donor portion of the transition state. These inhibitors are called mono-substrate mimics. In this case, the analogues are made of unsaturated glycosyl residues that have a half-chair conformation. The other is to mimic both donor and acceptor in the reaction in which they are covalently connected. These inhibitors are called bi-substrate mimics.

2.4.1 Mono-substrate transition state analogues

Figure 2.7 lists some mono-substrate transition state inhibitors. Analogue **14** is a mimic of UDP-Gal*p*.[37] It uses a glycal to mimic the half chair conformation of the galactose in the transition state, with the rest of the donor structure unaltered. It has been shown to be a good inhibitor of β-galactosyltransferase from bovine milk.

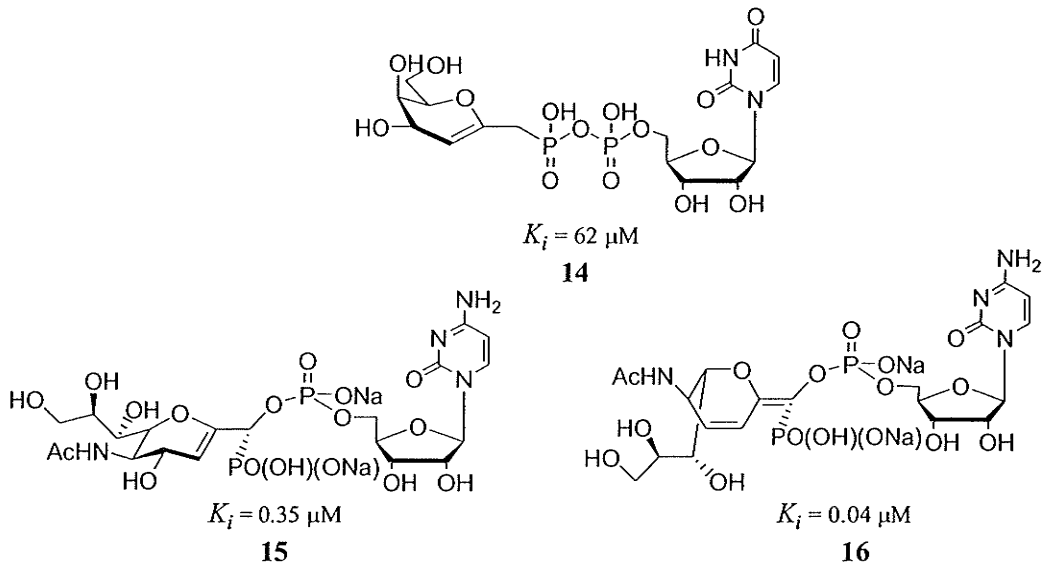


Figure 2.7 Some mono-substrate transition state inhibitors

Analogues **15** and **16** are two of a series of transition state inhibitors that target the glycosyl donor CMP-Neu5Ac prepared by Schmidt and coworkers.[38,39] These compounds also use a glycal unit to mimic the half chair conformation of the sialic acid. Structure-activity studies indicated that both the pyrophosphate moiety and the flat glycal structure of **15** and **16** were essential to the inhibitory activity.[39] The two negative charges located on the pyrophosphate moiety are perfectly separated by five bonds. This arrangement makes it possible that these two charges strongly bind to the divalent metal cation present at the active site of the glycosyltransferases. Any attempt to change this arrangement decreased the activity significantly. For example, when the phosphate unit close to the sialic acid was replaced with a carboxylate group, the binding affinity decreased by one order of magnitude. In the glycals of **15** and **16**, the anomeric center is

planar due to the double bond structure. This configuration is similar to the geometry of the transition state. Schmidt and coworkers showed that any analogues that did not have this flat glycal feature had lower binding affinity.[39] Analogues **15** and **16** are the best currently known inhibitors of α (2,6)-sialyltransferase from rat liver.

2.4.2 Bi-substrate transition state analogues

Some bi-substrate transition state inhibitors are listed in Figure 2.8.[40-42] Both **17** and **18** are designed to inhibit glucuronosyltransferases that transfer a glucuronosyl residue from UDP-GlcA to xenobiotics and endogenous compounds in the liver. Analogue **17** mimics the transition state for steroid metabolization by glucuronosyltransferases. At the transition state, the steroid is covalently connected to the GlcA donor. Thus, **17** covalently links the glycosyl donor and the steroid acceptor together. This analogue has exhibited some inhibition toward these enzymes. Analogue **18** is a simplified version of **17** in which the steroid fragment and uridine nucleotide have been removed. It also shows a small inhibitory effect.[40]

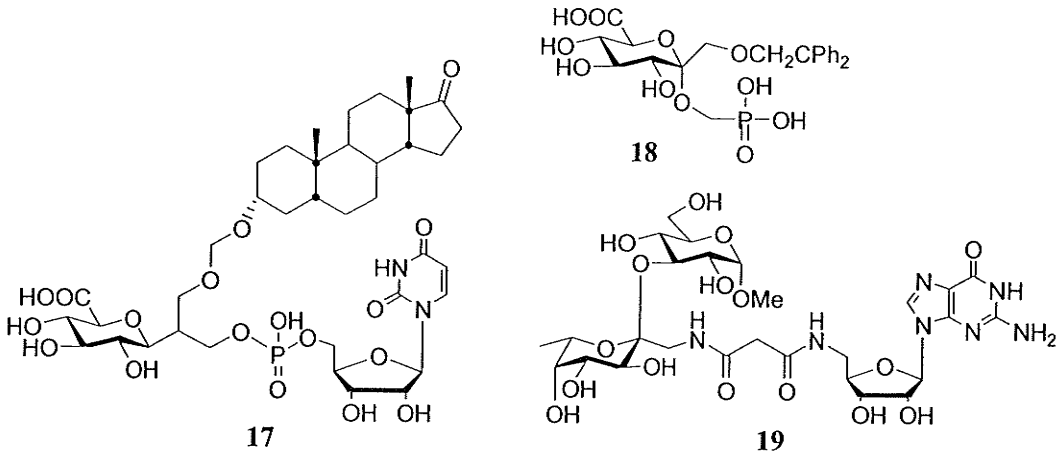


Figure 2.8 Some bisubstrate transition state inhibitors

Analogue **19** is designed to inhibit fucosyltransferases that transfer a fucopyranose residue from GDP-Fuc to glycoconjugate acceptors. It uses the amide chain to replace the pyrophosphate moiety in anticipation that the non-charged amides will

facilitate membrane transport. Unfortunately, this design concept has not been proven. Analogue **19** does not exhibit any inhibitory effect in biological tests.[42]

2.5 Concluding remarks

The last 10 years have witnessed the rapid growth of glycosyltransferase inhibitors based on glycosyltransferase substrate mimics. *C*-Glycosides have a variety of advantages over their *O*-glycoside counterparts. They deactivate glycosyl transfer from glycosyl donors by preventing the aglycone moiety from cleaving. They retain the enzymatic recognition features by resembling the conformation of the natural substrates. *C*-Glycosides have been applied to design a wide range of carbohydrate-based mimics, such as mimics to glycosyl 1-phosphates, glycosyl pyrophosphates, glycosyl nucleotides, glycosyl transition state mono-substrates and bi-substrates.

Chapter 3

Inhibitors of Mycobacterial Glycosyltransferases

3.1 Introduction

Inhibitors of mycobacterial glycosyltransferase have developed rapidly in the last two to three years. They target biosynthesis of the polysaccharides in the mycobacterial cell wall. This chapter will survey the progress of this field and discuss how these glycosyltransferase inhibitors are designed and what information the biological tests of these analogues provide.

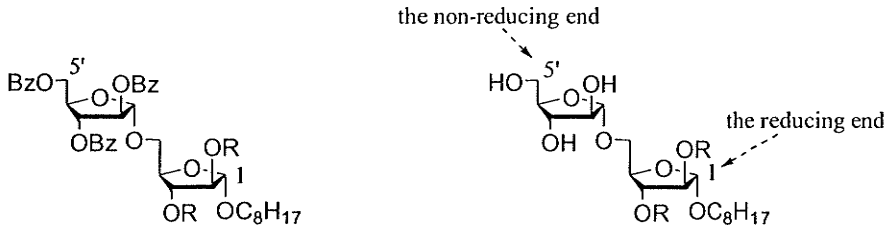
3.2 Inhibitors of mycobacterial glycosyltransferases

3.2.1 Arabinosyltransferase inhibitors

A group of α (1→5) arabinofuranosyl disaccharide analogues that target the mycobacterial arabinosyltransferase have been synthesized (Figure 3.1).[11] All of these compounds have their reducing end C-1 blocked by an *n*-octyl group. In previous studies, the *n*-octyl group has been found to be a suitable mimic of the lipophilic polyprenol moiety of the natural sugar donors.[12] Compounds **20a-b** are fully protected disaccharide analogues, **21b-c** are partially protected disaccharide analogues and **21a** is a fully unprotected disaccharide analogue. Analogues **20a-b** have their non-reducing end C-5′ closed, but **21a-c** have their non-reducing end C-5′ open.

In vitro assays of these analogues were conducted with the multi-drug resistant strain of *Mycobacterium tuberculosis*. The tests indicated that **21b** was the best inhibitor (IC_{50} >12.8<128 μg/ml). Acceptor activity assays showed that the fully de-blocked disaccharide analogue **21a** was a good acceptor of the arabinosyltransferase, but the partially protected analogue **21b** was not. Enzyme assays of these analogues indicated that **21b** was an inhibitor of arabinosyltransferase with an IC_{50} value of 1.12 mM.

Another partially deprotected disaccharide **21c** was also an inhibitor, but with much weaker activity. All of these results implied that **21b** retained the recognition sites of the natural substrate, but prevented the enzyme from using it as an acceptor.



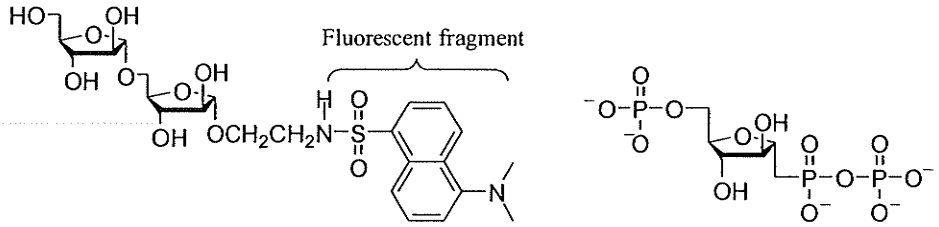
20a R = Bn IC_{50} > 128 μg/ml
20b R = Me IC_{50} > 128μg/ml

21a R = H IC_{50} > 128 μg/ml
21b R = Bn IC_{50} > 12.8 < 128 μg/ml
21c R = Me IC_{50} > 128μg/ml

IC_{50}: concentration at 50% inhibition of the cell growth of *Mycobactrium tuberculosis*

Figure 3.1 Analogues of α (1→5) arabinofuranosyl linkage

Arabinofuranosyl disaccharide analogue **22** was prepared with a fluorescent fragment (Figure 3.2).[43] It was used to monitor the arabinofuranosyltransferase activity. An assay using radiolabeling is the traditional technique for the measurement of inhibitor activities. Radiolabeled sugar donor and synthetic inhibitors are incubated with a mycobacterial cell wall enriched fraction. This cell wall enriched fraction contains the necessary glycosyltransferases and other components. The reaction is monitored by thin-layer chromatography and autoradiography. There are several shortcomings to this technique:[43] (1) low throughput; (2) requirement of radiolabeled chemicals; (3) special handling and waste disposal. Compound **22** was designed to solve this problem. It facilitates high throughput drug screening. However, the test result has not been reported yet.



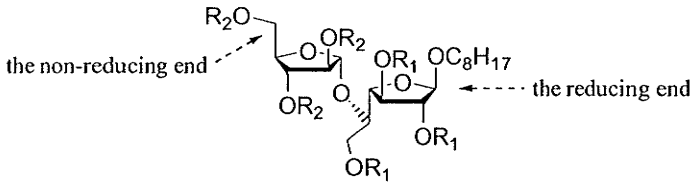
22 **23**

Figure 3.2 Mimics of arabinofuranosides

Mimic **23**, shown in Figure 3.2, is an analogue of the arabinose donor 5-phospho-D-arabinosyl pyrophosphate (pApp).[44] It uses a *C*-glycosyl phosphonate to replace the *O*-glycosyl 1-phosphate. pApp is an intermediate in the biosynthesis of arabinofuranosyl phospho-polyprenol (Ara*f*-P-Polyprenol). Ara*f*-P-Polyrenol is the sugar donor for the biosynthesis of mycobacterial cell wall polysaccharides LAM and AG. Analogue **23** has shown less than 20% inhibition of the transformation of pApp to Ara*f*-P-Polyprenol at 5 mM concentration.[44]

3.2.2 Galactosyltransferase inhibitors

The polysaccharide AG in the mycobacterial cell wall has many galactofuranose residues. These galactose linkages are ideal targets for designing drugs against mycobacterial galactosyltransferases.[3] Figure 1.2 depicts the targeted linkages that have been studied so far.



24a R_1 = Bn, R_2 = Bz *MIC* > 128 μg/ml
24b R_1 = Me, R_2 = Bz *MIC* > 128 μg/ml
24c R_1 = Bn, R_2 = H *MIC* = 32 μg/ml
24d R_1 = Me, R_2 = H *MIC* > 128 μg/ml
24e R_1 & R_2 = H *MIC* > 128μg/ml

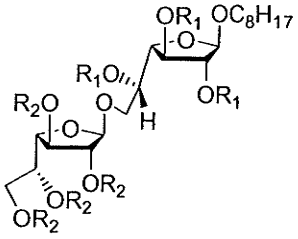
MIC: minimiun inhibition concentration against the cell growth of *Mycobactrium tuberculosis*

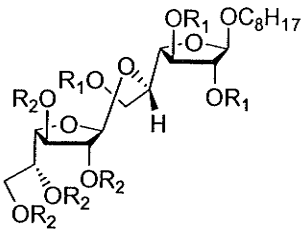
Figure 3.3 Analogues that target Ara*f* α (1→5) Gal*f* linkage

Figure 3.3 lists a group of analogues that target the formation of the Ara*f* α (1→5)-Gal*f* linkage (Target **I** in Figure 1.2, page 4).[45] These compounds all also have their reducing end, C-1, blocked by an *n*-octyl group. Analogues **24a-b** have their non-reducing end blocked while **24c-d** have their non-reducing end open. All of these analogues have been tested against the cell growth of *Mycobacterium tuberculosis*. The test results showed that the partially deprotected disaccharide analogue **24c** was the best inhibitor. The fully de-blocked disaccharide **24e** was the only arabinosyltransferase acceptor found in the acceptor assay. The other analogue compounds were not acceptors,

probably due to the presence of the protecting groups on those molecules. Partially protected disaccharide analogues **24c** and **24d** inhibited galactosyltransferases in the enzyme assay, with IC_{50} values of 1.16 and 3.20 mM respectively, indicating that they bound to the enzymes better than the fully protected sugars.

Analogues **27a-d** and **28a-e**, shown in Figure 3.4, are mimics of β (1→6) and β (1→5) Gal*f* disaccharides, which target the formation of the linkages **II** and **III** illustrated in Figure 1.2 (page 4).[13] The reducing ends of these analogues are all blocked. The cell-free enzyme assay indicated that the fully deprotected disaccharide analogues **27d** and **28e** were not inhibitors of the mycobacterial galactosyltransferases, but acted as glycosyl acceptors instead. Both the partially protected disaccharide analogues **27c** and **28d** were effective enzyme inhibitors, but **27c** did not show any inhibition effect in the cell growth assay against *Mycobacterium tuberculosis*. The reason is unknown. Compounds **27b** and **28c** are partially protected disaccharide analogues and have demonstrated cell growth inhibition against *Mycobacterium tuberculosis*. However, they did not show any inhibiting effect against the mycobacterial glycosyltransferases in cell-free enzyme assay. It was envisaged that these disaccharide analogues might bind to and lyse cells as nonspecific surfactants, but that they were too large to bind to and inhibit the glycosyltransferases due to the presence of large benzyl protecting groups.[13]





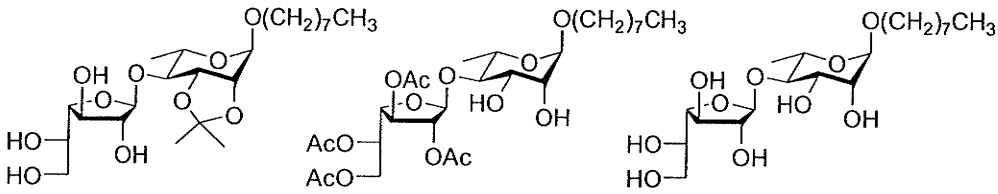
27a R_1 = Me, R_2 = Ac *MIC* > 128 μg/ml
27b R_1 = Bn, R_2 = Ac *MIC* > 12.8 < 128 μg/ml
27c R_1 = Me, R_2 = H *MIC* > 128 μg/ml
27d R_1 & R_2 = H *MIC* > 128 μg/ml

28a R_1 = Bn, R_2 = Ac *MIC* > 128 μg/ml
28b R_1 = Me, R_2 = Ac *MIC* > 12.8 μg/ml
28c R_1 = Bn, R_2 = H *MIC* > 12.8 < 128 μg/ml
28d R_1 = Me, R_2 = H *MIC* > 12.8 μg/ml
28e R_1 & R_2 = H *MIC* > 128μg/ml

MIC: minimiun inhibition concentration against the cell growth of *Mycobactrium tuberculosis*

Figure 3.4 Analogues that target β (1→6) and β (1→5) Gal*f* linkages

Disaccharide analogues **29a-c** targeted the formation of the β-D-Gal*f*- (1→4)-α-L-Rha*p* linkage (target **IV** in Figure 1.2, page 4) (Figure 3.5).[12] These analogues also have their reducing ends blocked by an *n*-octyl group. The cell growth assay indicated that the partially protected disaccharide analogues **29a-b** were better inhibitors than the fully unprotected saccharide analogue **29c**. The acceptor assay indicated that the fully deprotected disaccharide analogue **29c** and the partially deprotected disaccharide analogue **29a** were acceptors in the galactosyltransferase-catalyzed reaction. Both of them have their non-reducing end C-6′ open. Disaccharide **29b** 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.



29a IC_{50} > 12.8 < 128 μg/ml **29b** IC_{50} > 12.8 < 128 μg/ml **29c** IC_{50} > 128 μg/ml

IC_{50}: concentration at 50% inhibition against the cell growth of *Mycobactrium tuberculosis*

Figure 3.5 Analogues that target β-D-Gal*f*- (1→4)-α-L-Rha*p* linkage

3.2.3 Other glycosyltransferase inhibitors

Mannosyltransferases have been suggested to be responsible for the biosynthesis of the cell wall polysaccharide LAM.[46] In order to establish an assay to measure mannosyltransferase activity and probe the acceptor/donor specificities, a series of mannosides was prepared (Figure 3.6).[47] These analogues are divided into two groups. The first group is disaccharide analogues **30a-c** and trisaccharide analogue **31**. They are the analogues of the α (1→6) mannosyl linkage found in LAM. The second group comprises disaccharides **32** and **33.** They are not mimics of any of the known mannosyl linkages found in the LAM. Three mannosyl donors, GDP-mannose **34**, C_{35}-P-mannose **35** and C_{50}-P-mannose **36** were used for the test as well (Figure 3.7).

The results indicated that the mimic compounds **30a-c** and **31** were acceptors in the mannosyltransferase-catalyzed reaction, but the non-mimic compounds **32** and **33** were not substrates. Furthermore, the glycolipids **35** and **36** were glycosyl donors in the mannosyltransferase-catalyzed reaction, but the sugar nucleotide **34** was not. The results also showed that each acceptor was able to take up one or two mannose residues. The synthetic acceptors **30a-c** and **31** could be used as lead compounds for the design of mycobacterial mannosyltransferase inhibitors.

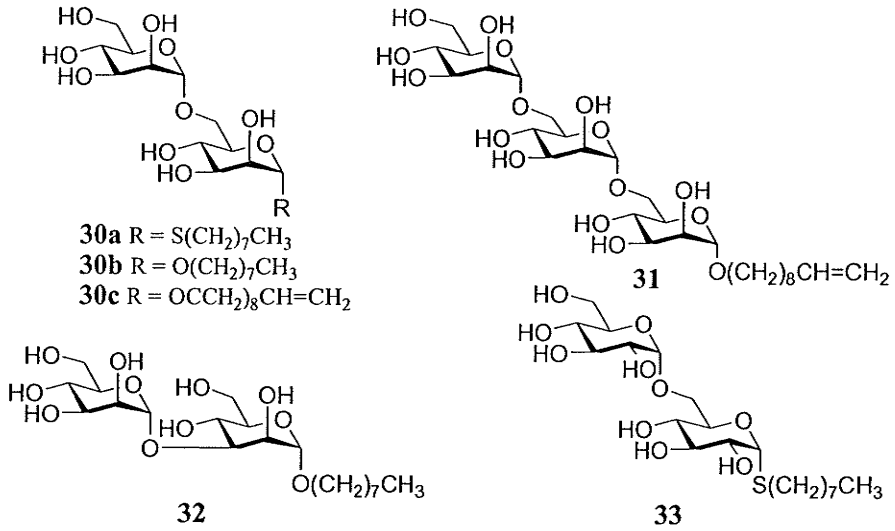


Figure 3.6 Potential acceptors for mycobacterial mannosyltransferases

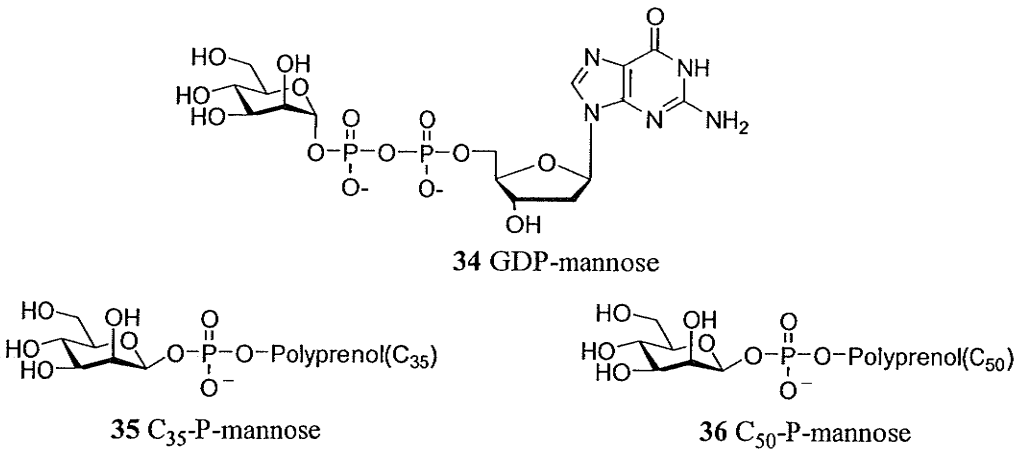
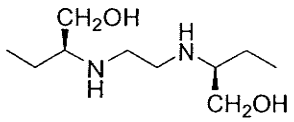
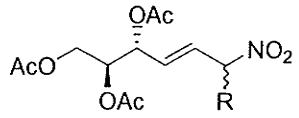


Figure 3.7 Potential donors for mycobacterial mannosyltransferases

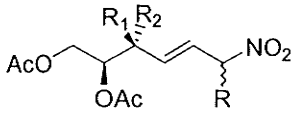
Last year, several acyclic deoxy monosaccharide derivatives were reported to possess active anti-mycobacterial activity.[48,49] They were prepared as mimics of the anti-tuberculosis drug ethambutol **37**. However, their biological targets and modes of action were not clear. Figure 3.8 depicts some of these compounds.



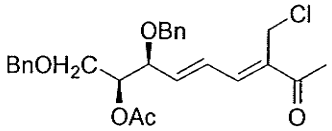
37 Ethambutol



38a R = H, *MIC* = 6.25 μg/ml
38b R = CH_3, *MIC* = 6.25 μg/ml



39a R_1 = H, R_2 = OAc, *MIC* = 6.25 μg/ml
39b R_1 = OAc, R_2 = H, *MIC* = 6.25 μg/ml



40 *MIC* = 3.12 μg/ml

MIC: minimiun inhibition concentration against the cell growth of *Mycobactrium tuberculosis*

Figure 3.8 Some anti-mycobacterial agents

3.3 Concluding remarks

This chapter has surveyed the literature on inhibitors of mycobacterial glycosyltransferases. In most cases, the inhibitors have been designed by mimicking the enzymatic reaction products – mycobacterial cell wall polysaccharides. Most of them are disaccharides that closely mimic the cell wall polysaccharide linkages. All these inhibitors have the common structural feature: one or both their linking ends have been blocked. This blockage prevents them from being used as glycosyl acceptors by the mycobacterial glycosyltransferases.

Chapter 4

Synthesis of *C*-glycosides

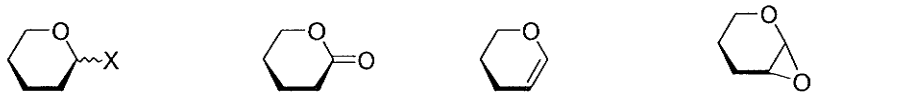
4.1 Introduction

The aim of this chapter is to survey the methodologies available for the preparation of *C*-glycosides. Mechanisms, stereochemistry, structures and relevant examples will be given for each *C*-glycosylation approach. Challenges in the preparation of amino *C*-glycosides and the conformation of *C*-glycosides in comparison with their *O*-glycoside counterparts will be discussed as well.

4.2 Approaches to *C*-glycosides

4.2.1 Electrophilic *C*-glycosylation

Electrophilic reactions are widely used for the preparation of *C*-glycosides, and there are several glycosyl electrophiles commonly used in electrophilic *C*-glycosylation. They are glycosyl halides, esters, imidates, lactols, glycosides, lactones, glycals and 1,2-anhydrides (Figure 4.1).



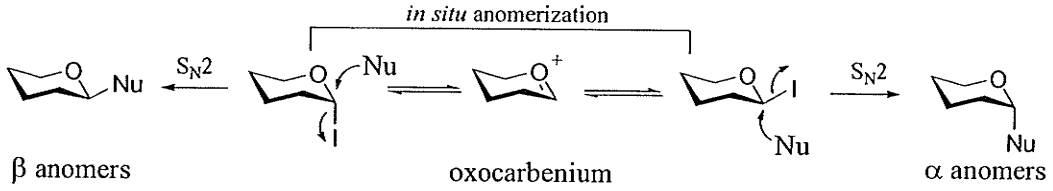
Glycosyl halides, esters, imidates, lactols and glycosides | Lactones | Glycals | 1,2-anhydroglycosides

Figure 4.1 Common glycosyl electrophiles for *C*-glycosylation

4.2.1.1 Glycosyl halides

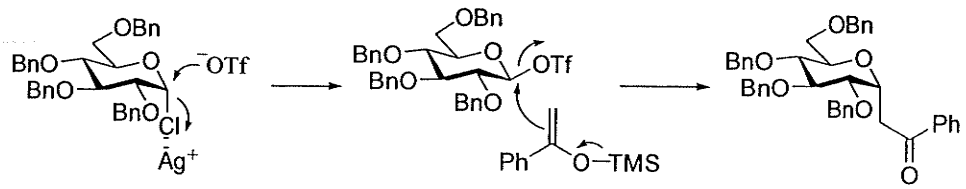
Glycosyl bromides and chlorides are commonly seen in the preparation of *C*-glycosides.[50] Nucleophiles can be lithium, copper, zinc, tin and magnesium complexes of organometallic compounds.[51,52] The selectivity between α and β anomeric products mainly depends on the glycosyl halides used and the reaction conditions employed.

For example, 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl iodide reacted with malonate anion to give α and β anomeric products in a 5:1 ratio, whereas the corresponding galactosyl iodide afforded a 1:10 ratio of α and β anomeric products under the same conditions. The β isomeric products in both cases came from direct S_N2 substitution, whereas the α isomeric products were generated through *in situ* anomerization followed by S_N2 reaction (Scheme 4.1).[53] The *in situ* anomerization occured because the equilibrium of α and β anomeric iodides occured faster than the nucleophilic substitution, and the β anomeric iodide was more reactive in the substitution than the α anomeric iodide. *In situ* anomerization in *C*-glycosylation is similar to the *in situ* anomerization mechanism in *O*-glycosylation discussed in section 5.3.4. In the case of the glucosyl iodide, the *in situ* anomerization went faster than direct S_N2 substitution, but for galactosyl iodide, direct displacement was faster.



Scheme 4.1 Reaction paths for α and β isomeric products

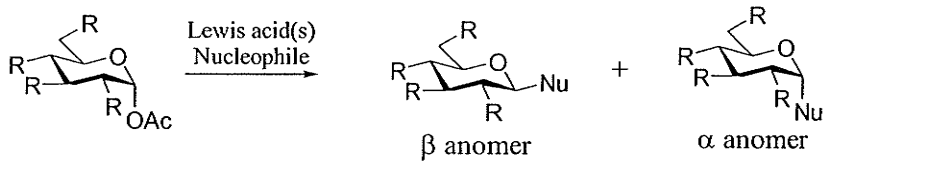
One of the approaches employed to increase the product stereoselectivity is to use AgOTf as a catalyst. Silver metal can activate the halide to leave and generate a β triflate intermediate in an S_N2 displacement. Substitution of this intermediate by a carbon nucleophile in an S_N2 fashion stereoselectively gives the α anomeric product. Scheme 4.2 illustrates the reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl chloride with a silyl enol ether in the presence of AgOTf. The α anomeric product was isolated in 88% yield.[54]



Scheme 4.2 Substitution of a glycosyl halide with AgOTf as catalyst

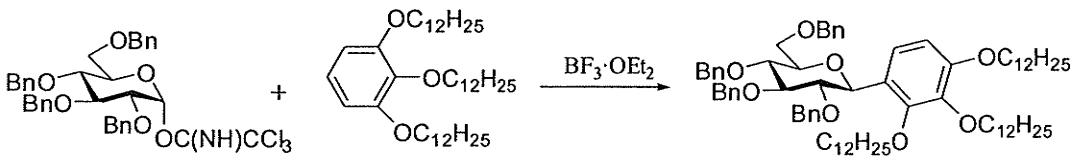
4.2.1.2 Glycosyl esters

Anomeric esters can be directly substituted to generate *C*-glycosides by allyl or acetylenic silanes, or by silyl ketene acetals catalyzed by Lewis acids TMSOTf or $BF_3{\cdot}Et_2O$.[55-59] The electronic and steric properties of the nucleophiles control the stereoselectivity of these reactions. A neighboring participating group, acetate or benzolate, or a highly sterically demanding nucleophile, will enhance β-selectivity.[60] Scheme 4.3 shows a *C*-glycosylation of glucopyranosyl acetates with a series of silyl nucleophiles. The results showed that silyl ketene acetals preferentially formed β *C*-glycosides. Allylsilane mainly produced α *C*-glycosides due to its relatively lower nucleophilicity. An increase in the steric hindrance of the nucleophiles resulted in enhanced β selectivity. The presence of a neighboring participating group (acetate) at C-2 of the glucopyranosyl acetate also greatly increased β selectivity.



	Lewis acid(s)	TMS	OTMS / OTMS	Me / Me OTMS / OMe	OTMS / O
R = OBn (β:α)	TMSOTf	<1:10	1.4:1	3:1	3:1
R = OAc (β:α)	$TMSOTf/BF_3{\cdot}OEt_2$	<1:10	>10:1	>10:1	NR

Scheme 4.3 Electrophilic *C*-glycosylation with glycosyl esters



Scheme 4.4 Electrophilic *C*-glycosylation with a glycosyl imidate

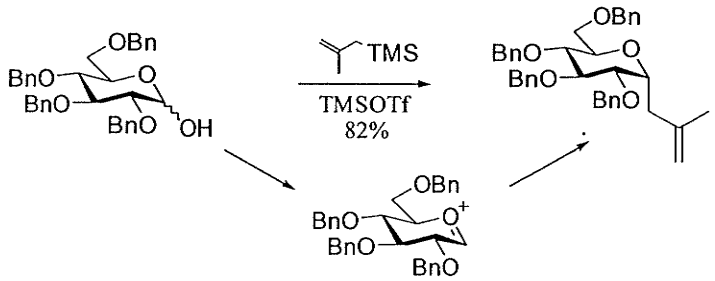
4.2.1.3 Glycosyl imidates

Trichloroacetimidate is a good leaving group when it is activated by a Lewis acid such as TMSOTf or $BF_3{\cdot}Et_2O$.[116] Glycosyl trichloroacetimidates, which are widely used in the synthesis of *O*-glycosides, are also useful in the preparation of *C*-glycosides. They

tend to couple particularly well with electron-rich aromatic compounds in the presence of $BF_3\,OEt_2$ (Scheme 4.4).[61]

4.2.1.4 Lactols

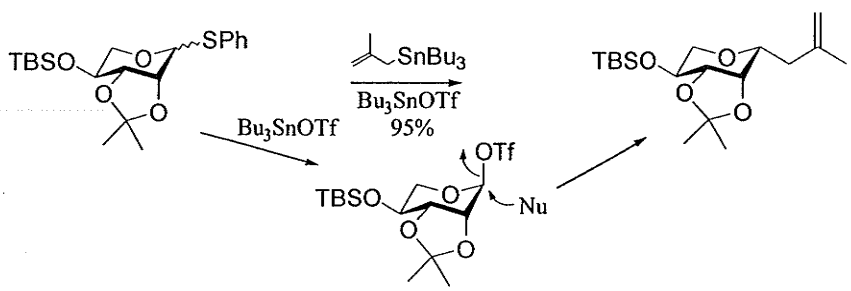
Glycoses can react directly with nucleophiles such as allyltrimethylsilanes and silyl enol ethers in the presence of Lewis acid catalysts such as TMSOTf or $BF_3\,Et_2O$ to afford *C*-glycosides. The reactions mostly yield α anomeric products through the oxocarbenium intermediate. This facial preference is due to the axial attack on the oxocarbenium species. Scheme 4.5 shows an example of this reaction.[62,63]



Scheme 4.5 Electrophilic *C*-glycosylation with a lactol

4.2.1.5 Glycosides

Like glycoses, methyl glycosides can also react directly with allyltrimethylsilanes and silyl enol ethers to generate *C*-glycosides in the presence of Lewis acid catalysts TMSOTf or $BF_3\,Et_2O$.[64,65] Besides methyl glycosides, thiol and sulfonyl glycosides give *C*-glycoside products as well. The reaction shown in Scheme 4.6 afforded exclusively the β anomeric product due to the formation of the pyranosyl triflate intermediate.[66-68]

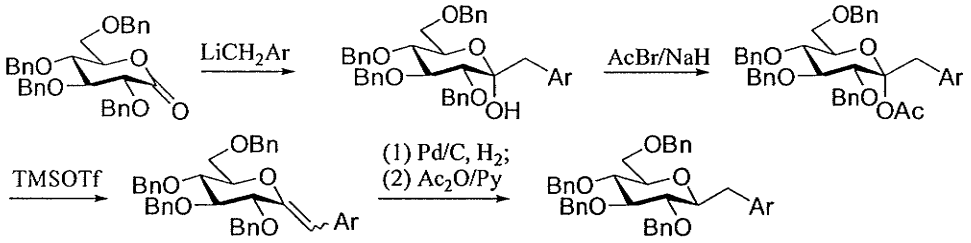


Scheme 4.6 Electrophilic *C*-glycosylation with a thio-glycoside

4.2.1.6 Lactones

The preparation of *C*-glycosides from glyconolactones involves a two-step process. The first step is the nucleophilic addition to the glycopyranolactone to give a ketopyranose with an α anomeric hydroxyl. The second step is to deoxygenate the anomeric hydroxyl to afford the *C*-glycoside. The deoxygenation reaction usually employs ionic reduction conditions such as $Et_3SiH/BF_3{\cdot}Et_2O$, stereoselectively generating β anomeric products.[69,70]

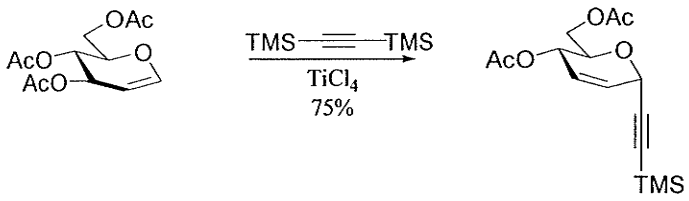
However, direct reduction of the ketopyranoses is sometimes not the best choice. In such cases, an alternative route has to be chosen. The ketopyranose shown in the example of Scheme 4.7 was converted to an olefin by elimination, and then the olefin was hydrogenated to afford the desired *C*-glycoside.[71]



Scheme 4.7 Electrophilic *C*-glycosylation with a lactone

4.2.1.7 Glycals

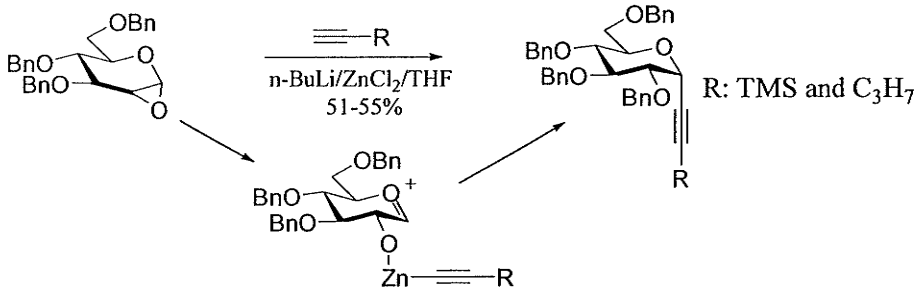
Glycals having a leaving group at C-3 react with nucleophiles such as silyl ketene acetals, allylsilanes, vinyloxysilanes and silyl acetylene to generate *C*-glycosides. This reaction requires a Lewis acid catalyst to activate the silyl nucleophiles. The reaction involves an allylic S_N2' mechanism, in which the allylic substituent at C-3 of the glycal (usually acetate) serves as a leaving group.[72,73] Scheme 4.8 shows an example of this reaction.



Scheme 4.8 Electrophilic *C*-glycosylation with a glycal

4.2.1.8 1,2-Anhydroglycosides

1,2-Anhydroglycosides, which are extensively used in *O*-glycosylation reactions,[74,75] are also useful in the preparation of *C*-glycosides. The reaction takes place similar to a normal S_N2 substitution of an epoxide, leading to 1,2-*trans* *C*-glycosides. The nucleophiles can be organocuprates or alkynyl lithiums.[76,77] However, when $ZnCl_2$ is used as catalyst in the reaction, 1,2-*cis* *C*-glycosides are produced. As depicted in Scheme 4.9, the reaction generates a zinc-alkyne complex in which zinc coordinates the alkyne nucleophile. This zinc-alkyne complex leads to the addition of the alkyne nucleophile from the bottom face of the oxocarbenium. Thus, the 1,2-*cis* diastereomer is predominantly found in the products.[78]



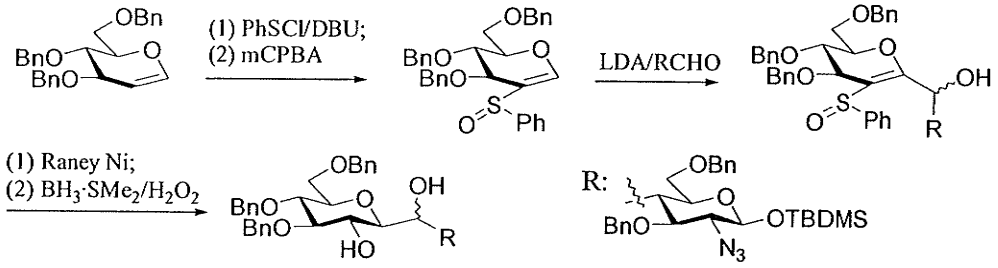
Scheme 4.9 Electrophilic *C*-glycosylation with a 1,2-anhydroglycoside

4.2.2 Nucleophilic *C*-glycosylation

4.2.2.1 Glycals

Nucleophilic *C*-glycosidation is complementary to the electrophilic strategy. The direct way to prepare an anomeric nucleophile is to deprotonate the glycals when there is a sulfoxide neighboring group to stabilize the anomeric negative charge or a neighboring phenylthiol to direct the anomeric lithiation.[79,80] The sulfoxide and thiol groups can be

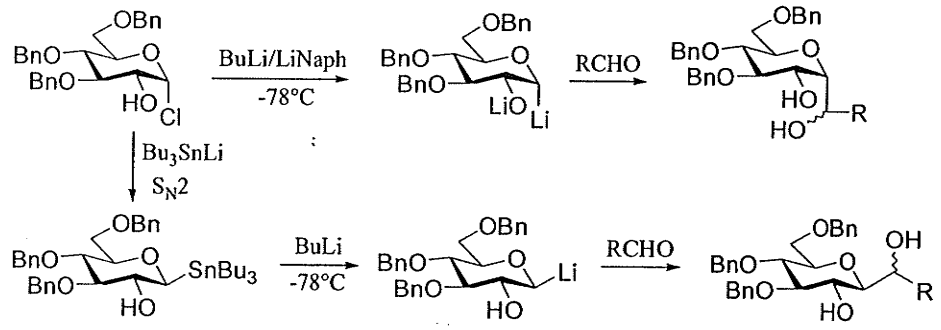
removed by Raney nickel in later stages of the synthesis. Scheme 4.10 depicts a synthetic route to a β *C*-glycoside from a glycal. The glycal was first converted to a vinyl sulfoxide by reacting with PhSCl/DBU followed by oxidation with mCPBA. Deprotonation of the vinylsulfoxide by LDA followed by addition to the aldehyde gave a *C*-glycosyl sulfoxide. Reduction with Raney Ni removed the sulfoxide group. Hydroboration/oxidation of the resulting glycal then produced the desired β *C*-glycoside.



Scheme 4.10 Nucleophilic *C*-glycosylation with a glycal

4.2.2.2 Glycosyl halides and stannanes

Another way to prepare an anomeric nucleophile is by tin-lithium transmetallation or metal exchange of glycosyl halides with BuLi. These reactions usually take place *in situ*.[81-85] Since the anomeric carbanions are sp^3 hybridized (tetrahedral) and inversion of the anomeric carbanions is slow at low temperature (–78°), the reaction products generally retain the configuration of the glycosyl halides or stannanes.[78,87,88] As depicted in Scheme 4.11, both α and β *C*-glycosyl epimers can be stereoselectively prepared by manipulation of the anomeric nucleophiles.

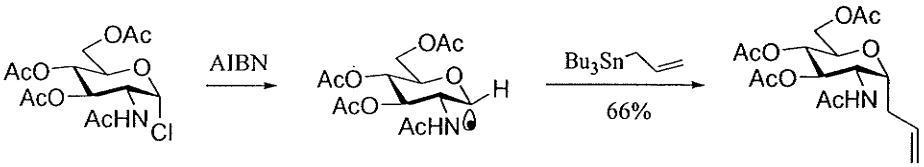


Scheme 4.11 Nucleophilic *C*-glycosylation with a glycosyl halide and a glycosyl stannane

4.2.3 Radical *C*-glycosylation

4.2.3.1 Glycosyl halides

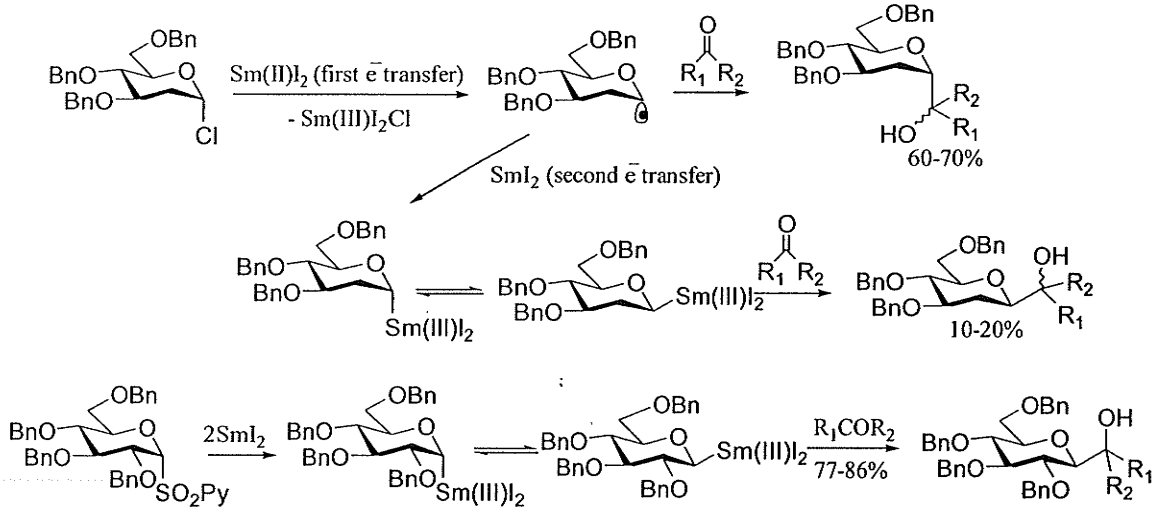
Radical coupling reactions are a very useful approach for preparing *C*-glycosides. The glycosyl radicals are generated through treatment of glycosyl halides with Bu_3SnH or $Bu_3SnSnBu_3$. These radicals can couple with a variety of reagents such as allyl stannanes, nitriles, sulfides and sulfones.[89-91] Scheme 4.12 depicts an example of such a reaction.



Scheme 4.12 Radical *C*-glycosylation with a glycosyl halide

4.2.3.2 *C*-Glycosylation with Samarium iodide

Samarium iodide (SmI_2) is an important reagent in organic synthesis. It promotes a wide range of important organic reactions such as reduction, cyclization and ketyl-olefin coupling. Reactions with SmI_2 involve either one electron or two electron transfer processes.[92] SmI_2 was introduced into *C*-glycosylation only a decade ago.[93]



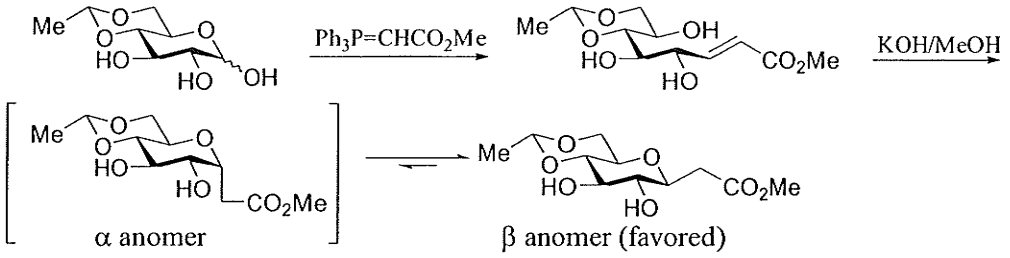
Scheme 4.13 Samarium iodide catalyzed *C*-glycosylation

Glycosyl halides, sulfones and phosphates are the glycosyl sources for *C*-glycosylation with SmI_2.[94-96] In general, *C*-glycosylation with SmI_2 can proceed through

single electron transfer and two electron transfer reactions. The first path involves the radical coupling. SmI_2 transfers one electron to the anomeric carbon to form a radical. This anomeric radical then couples with a ketone or aldehyde to afford a *C*-glycoside product. The second path involves the nucleophilic addition. After transferring one electron from the first SmI_2 to the anomeric carbon, the second SmI_2 transfer another electron to the anomeric carbon to produce an anion. The anomeric anion forms a complex with samarium. The newly formed glycosyl samarium tends to adopt a β configuration because it is thermodynamically more stable. This is because the electronegativity of samarium is smaller than that of carbon and no anomeric effect is occurring in the glycosyl samariums. As a consequence, nucleophilic addtion of the anion to an aldehyde or ketone generates the β *C*-glycosides. The overall stereoselectivity of the reaction depends on the glycoside and reaction conditions. Scheme 4.13 depicts two examples of samarium iodide catalyzed *C*-glycosylation. In the first case, the reaction with a glycosyl chloride produced predominantly the α product via the radical pathway, whereas the glycosyl sulfone in the second case gave mainly the β product via the anionic mechanism.

4.2.4 Intramolecular cyclization

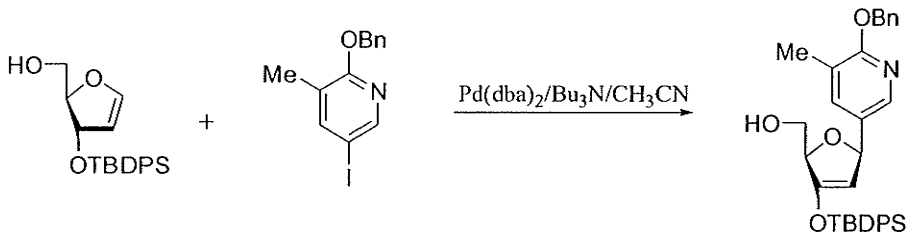
The Horner-Emmons/Michael reaction is the most popular intramolecular cyclization method for the preparation of *C*-glycosides. The reaction consists of a two-step event. The first step is the reaction of a glycose with an ylide to generate an α,β-unsaturated ester. The hydroxyl on the other position of the chain then attacks the α,β-unsaturated ester under the basic conditions of the reaction. The reaction first generates kinetic products, α *C*-glycosides. The reaction can sometimes be stopped at this stage and only α anomeric products are isolated. But the thermodynamically more stable products, β anomers, are often predominant in the reaction. An example of this reaction is given in Scheme 4.14.[97]



Scheme 4.14 *C*-glycosylation by intramolecular cyclization

4.2.5 *C*-Glycosylation catalyzed by palladium

The last approach to the preparation of *C*-glycosides is the coupling reaction of glycals catalyzed by palladium. The palladium method is especially useful in the synthesis of *C*-nucleoside analogues (Scheme 4.15).[98]



Scheme 4.15 *C*-Glycosylation catalyzed by palladium

4.3 Challenges in the preparation of amino *C*-glycosides

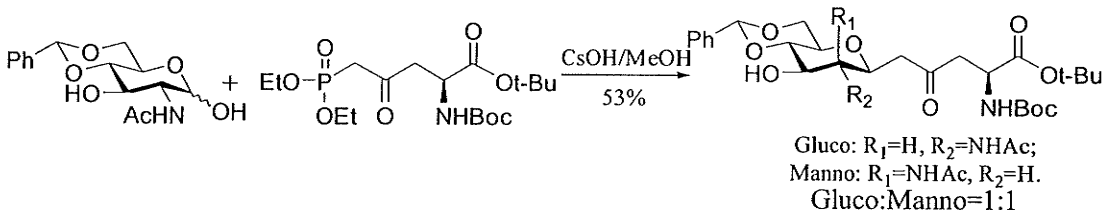
As seen from the above literature survey, a wide range of approaches is available for the preparation of *C*-glycosides. However, this is true only for non-amino sugars. As for amino sugars, the choice is very limited. Radical allylation, nucleophilic addition to lactones and intramolecular cyclization are a few methods that have been successfully applied to the synthesis of amino *C*-glycosides.[99] Here is a comment from Bertozzi regarding the challenges facing amino sugar *C*-glycoside synthesis:[91]

"*Unfortunately, C-glycosyl derivatives of 2-amino sugars are among the most difficult to prepare due to the incompatibility of neighboring nitrogen-based functional*

groups with common C-glycosylation strategies. Accordingly, few methods for their synthesis have been reported."

This comment was made in 1996, and little progress on the *C*-glycosidation of amino glycosides has been made since then. There are two reasons. First, the amino group at C-2 of the sugar ring strongly interferes with the manipulation of the *C*-glycosidic appendage due to its nucleophilicity. Any attempt to convert the *C*-glycosidic carbon of an *N*-acetyl glycoside or its derivatives into an electrophile has failed.[25]

Second, the *N*-acetyl group of 2-amino glycoses is extremely prone to epimerization. In the synthesis of a glycoamino acid derivative illustrated in Scheme 4.15, the Horner-Emmons/Michael reaction of *N*-acetyl D-glucosamine consistently gave a mixture of *gluco* and *manno* diastereomer products. Although various bases were used, the stereoselectivity of the reaction was not improved.[100] It is also reported that a prolonged reaction of 2-acetamido-3,4,5-tri-*O*-benzyl-2-deoxy-D-glucopyranose with TPAP/NMO (more than 2 hours) produced 50% of the *manno* derivative.[101]

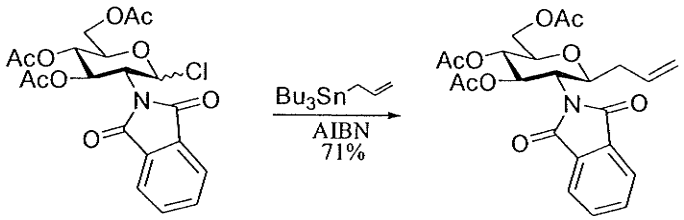


Scheme 4.15 *N*-acetyl group of GlcNAc is prone to epimerization

These features of amino sugars have hampered the choice of *C*-glycosylation methods for amino *C*-glycosides. To solve this problem, several alternatives have been developed over the years.

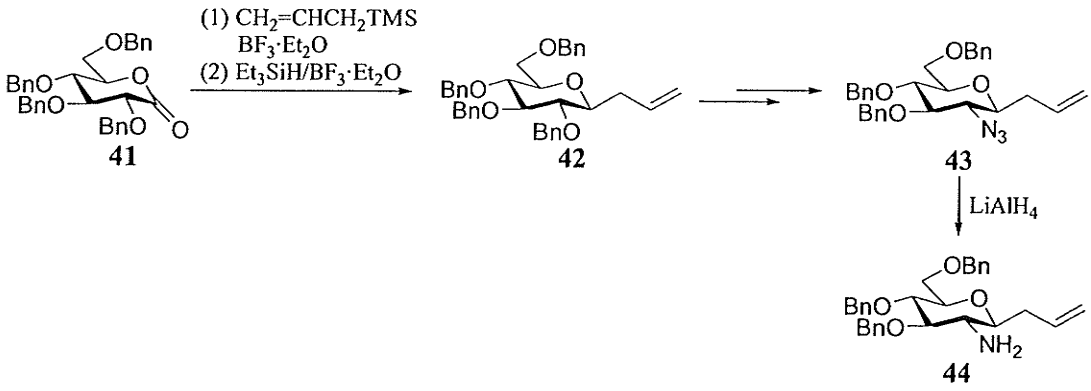
The first alternative is to use a protecting group for the amino group. The most commonly used *N*-protecting group apart from *N*-acetyl is the phthalimido group.[102] Scheme 4.16 depicts a radical allylation of a glucosamine compound. Due to the steric bulk of the phthalimido group, the reaction produced exclusively the β *C*-glycoside

product. The drawback of this strategy is that the carbonyls of the phthalimido protecting group are just as nucleophilic as that of the acetamido group.[91,103]



Scheme 4.16 *C*-glycosylation with protection group strategy

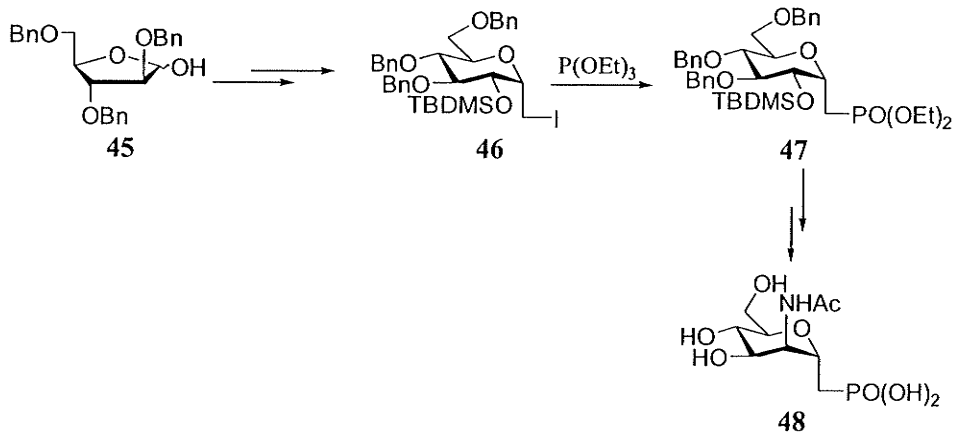
The second alternative is to use the azido group as a masking functionality, which is a common practice in preparations of amino *C*-glycosides. This is because the azido group is a nonparticipating group and is compatible with other functionalities. The azide is easily reduced to an amino group in the last step of the synthesis.[102] Scheme 4.17 depicts an example of this strategy. The synthesis started from a non-amino sugar glucosyl lactone **41**. After manipulation of the functionalities around the sugar ring, the molecule was converted to the azido sugar **43**. Reduction of **43** with lithium aluminumhydride afforded the desired β allyl *C*-glycoside **44**.[105]



Scheme 4.17 Azide as a masking group in the preparation of an allyl *C*-glycoside

The third alternative is to install the amino group at the last stage of the synthesis. This approach avoids the interference of the amino group with other functionalities.[105] The amino group can be introduced by many methods, including nucleophilic displacement, epoxide opening, addition to glycals, reduction of oximes and

intramolecular substitution.[104] Scheme 4.18 depicts an example of this approach.[106] The preliminary experiments indicated that the amino group would interfere with the introduction of a phosphonate moiety. Thus, the synthesis began with a non-amino sugar arabinose **45**. The phosphonate moiety was introduced to the molecule in the early stage of the synthesis, producing the glycosyl phosphonate **47** from **45** in a few steps. After all the manipulations were done, the amino group was installed at the last step of the synthesis by reduction of an oxime, generating the desired amino *C*-glycoside **48** in a few steps from **47**.



Scheme 4.18 Synthesis of a *C*-glycosyl analogue of *N*-acetyl D-mannosamine 1-phosphate

The drawback of both the second and third approaches is that they start with non-amino sugars instead of cheap and readily available amino sugars, thus making the procedures lengthy and less efficient.

4.4 The conformation of *C*-glycosides

A question arises when a *C*-glycoside is used as a glycosyl analogue to replace an *O*-glycoside: does the conformation of the *C*-glycoside resemble that of its related *O*-glycoside?[24,107]

In order to answer this question, the *O*-glycoside conformation is examined first. Modern carbohydrate conformational analysis started with the discovery of the anomeric effect and the *exo*-anomeric effect of *O*-glycosides by Lemieux.[108,110] Like cyclohexane derivatives, sugar pyranosides prefer to adopt a chair conformation and most substituents prefer an equatorial orientation due to steric effects. However, the difference between the pyranosides and the cyclohexane derivatives is that the axial pyranosides with a strongly electronegative substituent at the anomeric carbon are stabilized by the anomeric effect. The anomeric effect can be explained by molecular orbital theory. Figure 4.2 shows the Newman projections of the two possible configurations for an *O*-glycoside, the axial and equatorial anomers. In the axial anomer, the nonbonding *n* orbital of the ring oxygen overlaps with the σ* orbital of the anomeric C-O_{exo} bond, and the lone pair of the ring oxygen is delocalized into the σ* orbital of the anomeric C-O_{exo} bond. This interaction stabilizes the axial anomer, shortening the endocyclic C-O_{ring} bond and giving it some double bond character, meanwhile lengthening and weakening the exocyclic C-O_{exo} bond. This stabilization is not present in the equatorial anomer. The anomeric effect is also affected by solvents, with the axial anomers favored by solvents of low polarity.[32] Besides *O*-glycosides, the anomeric effect occurs in molecules that have a general feature C-X-C-Y where X has lone pair electrons such as N, O and S, and with Y as a more electronegative atom than carbon like Br, Cl, F, N, O and S.[109]

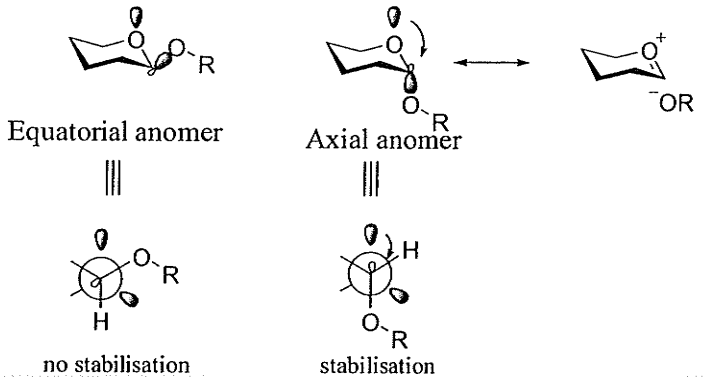


Figure 4.2 The anomeric effect

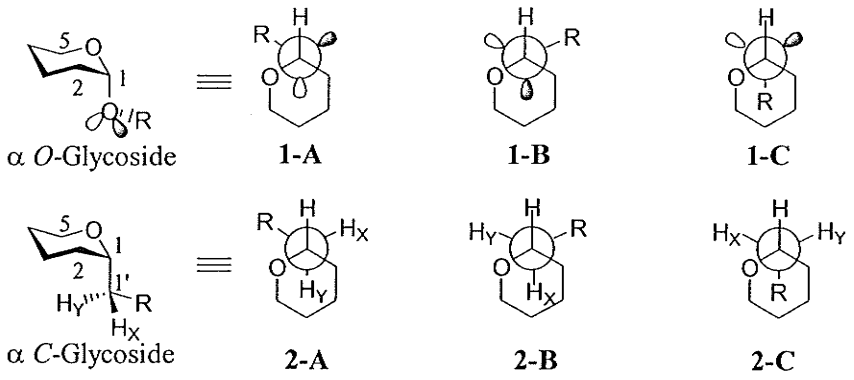


Figure 4.3 Comparison of the conformation of an α *O*-glycoside and an α *C*-glycoside

The term "*exo*-anomeric effect" is introduced to describe the orientation of the aglycon part of *O*-glycosides.[110] Figure 4.3 gives three possible rotamers of an α *O*-glycoside anomer. The conformation **1-A** of the α *O*-glycoside is preferred over **1-B** and **1-C** due to combination of the following preferences: (1) electronic stabilization of **I-A** and **I-C** is greater than that of **I-B**; (2) steric destabilization in **1-C** is more than that in **1-A** and **1-B**. Because the R substituent is *gauche* to the ring oxygen in the preferred conformation **1-A**, this preference is called the *exo*-anomeric effect.

In the case of an α *C*-glycoside, although its anomeric C-C bond (1.54Å) is longer than the anomeric C-O bond (1.43Å) of an *O*-glycoside, the steric effect of the anomeric substituent in the *C*-glycoside still has a profound influence on its conformations.[111,112] Among the three rotamers of the α *C*-glycoside, **2-A** and **2-B** are favored over **2-C** due to steric effects (Figure 4.3). Rotamer **2-A** is preferred over **2-B** due to a *1,3-diaxial-like* steric effect occurring in **2-B**. This 1,3-diaxial-like steric interaction occurs between the C-2 substituent and the C-1′ substituent R. Thus, a *C*-glycoside has the same preferred conformation as its related *O*-glycoside. This conclusion is supported both by molecular modeling and NMR experiments. The dihedral angle of O_{ring}-C_1-$C_{1'}$-R (also called the ϕ *angle*) in an α *C*-glucoside is found to be the same as the corresponding torsional angle of a methyl α glucopyranoside (both of them are 55°).[111] Solvents and temperature are found to have little effect on the α *C*-glycoside conformation.[111]

Although α *O*-glycopyranosides are theoretically more stable, β *O*-glycopyranosides exist abundantly in nature, and they can be prepared by *O*-glycosylation methods. Thus, comparing the conformation of a β *O*-glycoside and a β *C*-glycoside is also meaningful.[111] Figure 4.4 depicts the Newman projections of both a β *O*-glycoside and a β *C*-glycoside. Of the three rotamers of the β *O*-glycoside, **3-A** and **3-B** are favored over **3-C** due to steric effects. **3-A** is preferred over **3-B** due to a *1,3-diaxial-like* steric effect. Similar to the α *C*-glycoside shown in Figure 4.3, the **4-A** rotamer of the β *C*-glycoside, in which the substituent R is *gauche* to the ring oxygen atom, is also the most favored conformer. As well, this conclusion is supported both by molecular modeling and NMR experiments. The ϕ angle of a β *C*-glucoside (–80°) is found to be close to that of the corresponding methyl β glucopyranoside (–70°). Both solvents and temperature have little effect on the β *C*-glycoside conformation.[111]

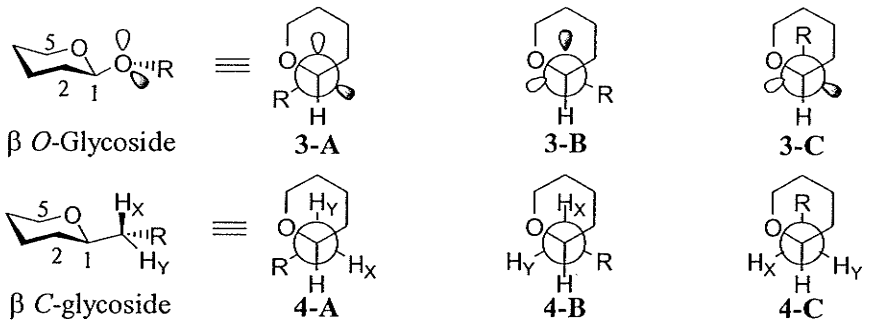


Figure 4.4 Comparison of the conformation of a β *O*-glycoside and a β *C*-glycoside

In conclusion, a simple *C*-glycoside has the same or similar conformation as its related *O*-glycoside.

4.5 Concluding remarks

This chapter has surveyed the methodologies available for the preparation of *C*-glycosides and discussed the challenges to be faced in preparing amino *C*-glycosides. Although many methods can be used for the preparation of non-amino sugars, they are not feasible for amino sugars due to the nature of amino sugars. So far there have only been a few approaches described for the preparation of amino *C*-glycosides, and they suffer from poor yields.

The conformation of simple *C*-glycosides has also been discussed in this chapter. Molecular modeling and NMR experiments both indicate that the *C*-glycosides have the same or similar conformations as *O*-glycosides.

Chapter 5

Synthesis of Disaccharides

5.1 Introduction

The synthesis of disaccharides and oligosaccharides is much more complicated than the synthesis of other biopolymers such as peptides and nucleic acids. There are two difficulties frequently encountered in the preparation of complex oligosaccharides:

(1) There are a great number of possibilities for combination of monosaccharides to form disaccharides and oligosaccharides;

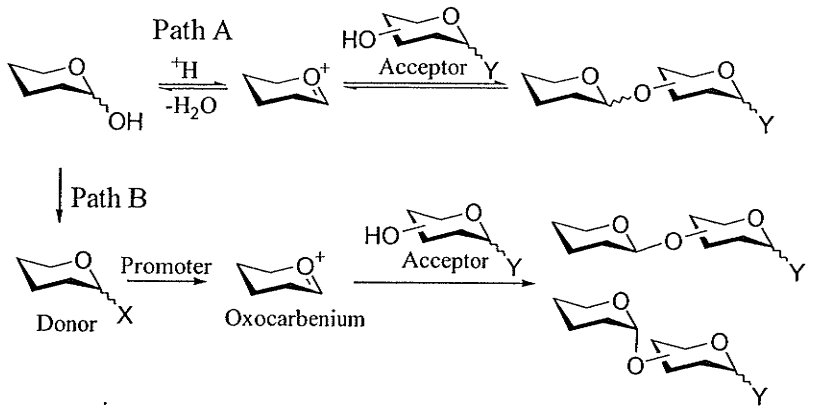
(2) The glycosidic linkages have to be introduced stereoselectively and sequentially.

Because each monosaccharide is a unique polyfunctional compound, there are no generally applicable methods for the assembly of complex oligosaccharides. Instead, contemporary carbohydrate synthesis often use the protecting-group strategy.[116] In recent years, some advanced approaches such as glycosyl transfer without protection and solid-phase glycosylation have made great progress, but they have not yet been broadly applied. The purpose of this chapter is to survey the literature about glycosylation strategies and mechanisms for the preparation of disaccharides. The focus will be on the mechanisms, stereochemistry and structures. Relevant examples will be given for each glycosylation approach as well.

5.2 Glycosylation and glycosyl donors

The synthesis of disaccharides involves the coupling of two monosaccharide units in a process where one monosaccharide functions as a glycosyl donor and the other as a glycosyl acceptor. This process is called "glycosylation". Because glycosylation always occurs at the anomeric carbon, it has been recognized that "half of sugar chemistry

resides at the anomeric carbon atom".[122] As depicted in Scheme 5.1, the glycosyl donor either possesses a good leaving group or becomes an oxocarbenium ion during the reaction. The glycosyl acceptor has to have at least one free hydroxy group to allow the glycosylation to occur.



Scheme 5.1 Two glycosylation paths

The relative reactivities of the acceptor hydroxyls are in the order: primary > secondary > tertiary, and equatorial > axial. However, some exceptions exist. In addition, the adjacent protecting groups usually play a role in the hydroxyl's reactivity. For instance, an ester group will deactivate the adjacent hydroxyl, whereas an ether group activates the adjacent hydroxy group. This concept has been developed into "remote-controlled glycosylation" or "selective activation glycosylation".[122]

The glycosyl donors are far more complex than the acceptors. This can be explained by the glycosylation paths illustrated in Scheme 5.1. Path A starts from a glycose, whereas path B begins with a glycoside derivative. In path A, the proton-induced dehydration makes the anomeric carbon become an oxocarbenium ion and the subsequent attack of an acceptor on this oxocarbenium would give a disaccharide. However, this process is not reliable. This is because this process is reversible, and in most cases, favors cleavage of the glycosyl bond (leading to hydrolysis). In order to control the glycosylation outcome, synthesis of disaccharides has to go through path B. In this path, the glycose is first converted to a glycoside derivative. The glycoside derivative is then transformed into the desired oxocarbenium ion in a controlled fashion (usually by a

promoter). Subsequent addition to the oxocarbenium by an acceptor would stereoselectively produce the desired disaccharide. Development of a novel glycosyl donor is a main part of modern carbohydrate synthesis.[122]

Figure 5.1 lists the glycosyl donors and the activation methods that have been developed in the last two decades.[116,122] Although the number of glycosyl donors and activation methods is continuing to grow, the glycosyl halides, glycosyl imidates and thioglycosides are considered to be the most commonly used glycosyl donors in modern carbohydrate synthesis.

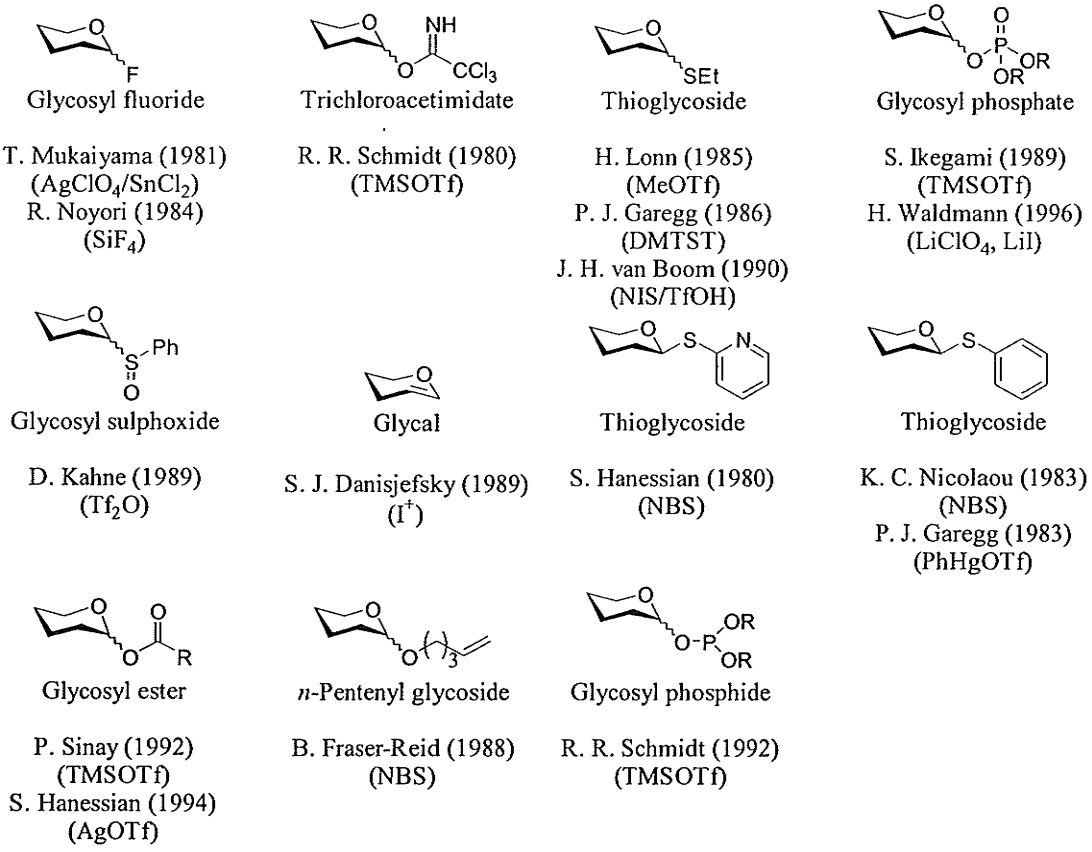


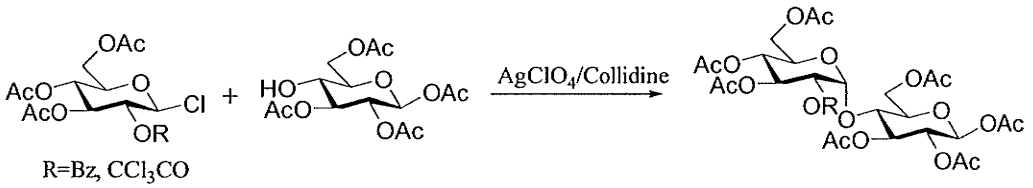
Figure 5.1 The development of major glycosyl donors in the last two decades

5.2.1 Glycosyl halides

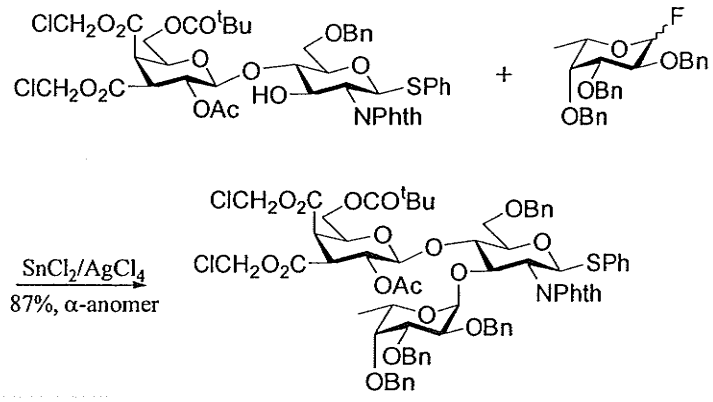
Glycosyl halides include glycosyl fluorides, chlorides, bromides and iodides. Their reactivities are in the order: iodides > bromides > chlorides > fluorides. Glycosyl

iodides are so unstable that they decompose very quickly at room temperature. Thus, their isolation and handling are very problematic. For this reason, glycosyl iodides are rarely used for glycosylation.[116]

Glycosyl bromides and chlorides were first introduced by Koenigs and Knorr in 1901.[127] Glycosylation with glycosyl bromides and chlorides is also referred to as the Koenigs-Knorr reaction. Glycosyl bromides are more reactive than the chlorides but also more labile. Although some of the glycosyl bromides and chlorides are stable enough to survive chromatographic purification, they are often prepared *in situ* and used directly. A variety of heavy metal salts such as AgOTf, Ag_2O, Ag_2CO_3, $AgClO_4$, $AgNO_3$, Ag-silicate, $Hg(CN)_2$, $HgBr_2$, $HgCl_2$, and HgI_2 are employed as promoters. Lewis acids like $SnCl_4$, $BF_3{\cdot}Et_2O$ and $Sn(OTf)_2$-collidine also act as promoters. The example shown in Scheme 5.2 uses $AgClO_4$ as the promoter.[114]



Scheme 5.2 Glycosylation with a glycosyl chloride



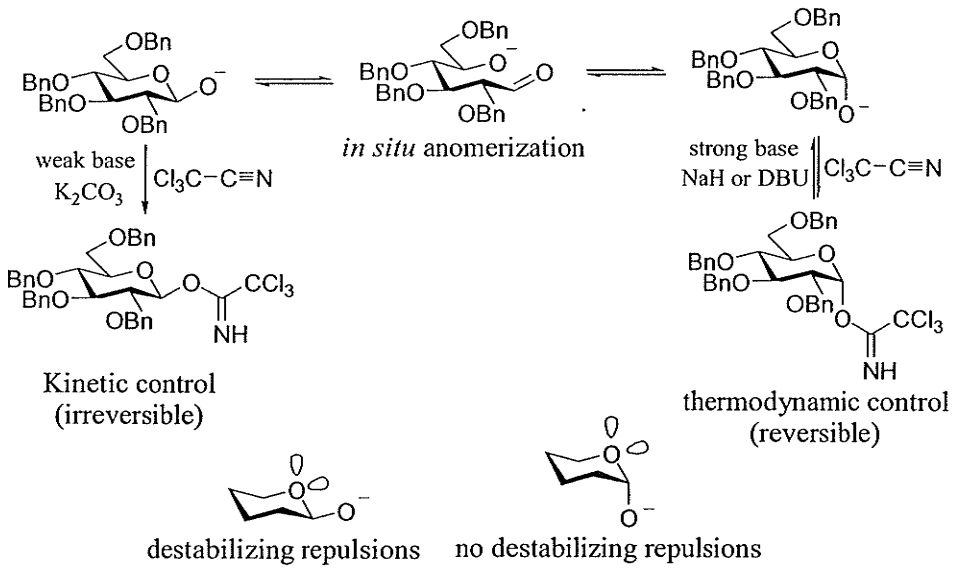
Scheme 5.3 Glycosylation with a glycosyl fluoride

Glycosyl fluorides are so stable that they cannot only be purified by column chromatography, but also stored at room temperature over a long period of time. Thus,

glycosyl fluorides are also frequently used in the preparation of disaccharides. Glycosyl fluorides can be activated by a wide range of promoters such as $SnCl_2$-$AgClO_4$, $SnCl_2$-$TrClO_4$, TMSOTf, SiF_4, SnF_4 and Tf_2O.[118] Scheme 5.3 depicts an example of glycosyl fluoride glycosylation.

5.2.2 Glycosyl imidates

Glycosyl imidates were only introduced as glycosyl donors in 1978. However, they have become one of the most widely used glycosyl donors in modern carbohydrate synthesis. There are several reasons contributing to their popularity.



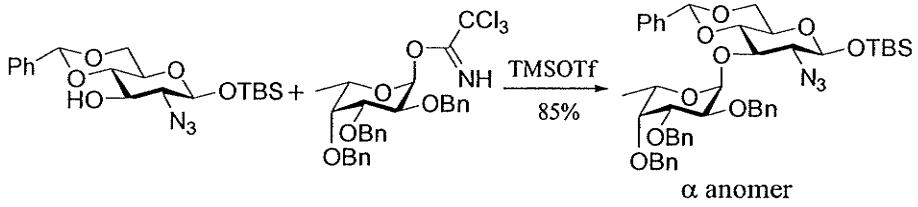
Scheme 5.4 Preparation of α and β glycosyl imidate donors

First, both α and β glycosyl imidates are easily prepared. The choice of base determines the preferred configuration for the glycosyl imidates. Scheme 5.4 describes the preparation of these anomers.[116] When the reaction is catalyzed by a mild base such as K_2CO_3, the β glycosyl imidate product is formed preferentially. This is a kinetically-controlled process because the β alkoxide is more reactive than the α alkoxide. The higher nucleophilicity of the β alkoxide comes from unfavorable dipole-dipole interactions resulting from repulsion of the lone electron pairs of the negative charge exocylic oxygen atom and the endocyclic ring oxygen atom. On the other hand, if the

reaction is initiated by a stronger base such as NaH or DBU, it is under thermodynamic control and the α glycosyl imidate predominates because the α isomeric product is thermodynamically more stable because of the anomeric effect.

Second, the glycosyl imidates are relatively stable. They can be chromatographed and stored over a long period of time. But the β isomers will slowly transform to their α isomers over time due to the anomeric effect. Therefore, they are generally prepared freshly before use.[128,129]

Third, the glycosyl imidates are easily activated by Lewis acid promoters such as TMSOTf and $BF_3 Et_2O$. The reactions are usually performed at relatively low temperatures and give good stereoselectivity and high yields.[118] Scheme 5.5 depicts the synthesis of a disaccharide using the glycosyl imidate method. The reaction stereoselectively afforded 85% of the α disaccharide anomer.



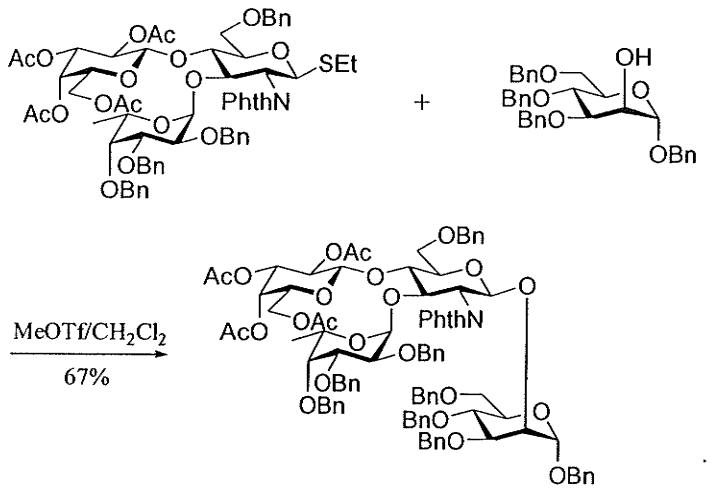
Scheme 5.5 Glycosylation with a glycosyl imidate donor

5.2.3 Thioglycosides

Thioglycosides are also very useful glycosyl donors in modern carbohydrate synthesis. Although they had been introduced in the early twentieth century, they were not widely used in carbohydrate synthesis until the 1970s. They are not only easy to prepare, but also very stable. They also tolerate a variety of reagents and reaction conditions.[130]

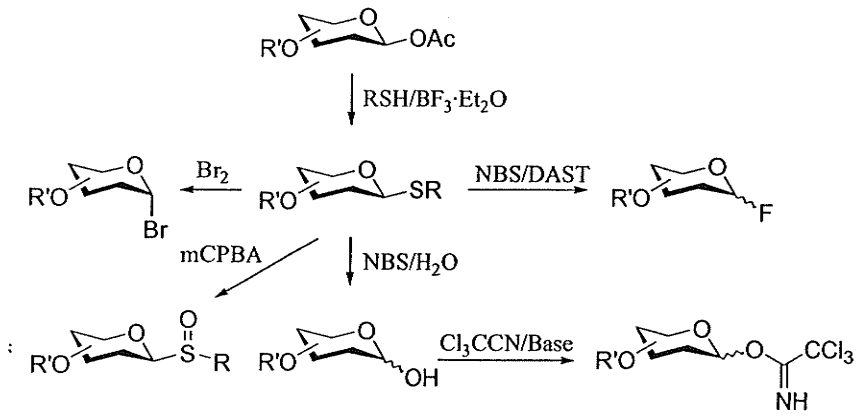
Thioglycosides are easily activated by promoters as well. MeOTf, DMTST [dimethyl(methylthio)sulfonium triflate] and NIS-TfOH are commonly used promoters for thioglycosides. The reactions generally give good stereoselectivity and high yield.[118]

Scheme 5.6 depicts the synthesis of a tetrasaccharide by the thioglycoside method. The reaction produced 67% of the β anomeric product, largely due to the bulky amino protecting phthalimide group.



Scheme 5.6 Glycosylation with thioglycoside

Thioglycosides are also versatile reagents. They can be transformed to a wide range of other glycoside donors, including halides, imidates and sulfones (Scheme 5.7). Thus, thioglycosides are particularly useful in carbohydrate synthesis.[116]



Scheme 5.7 Transformation of thioglycosides to other glycosyl donors

5.2.4 Other glycosyl donors

Besides the three most widely used types of glycosyl donors discussed in the above sections, other glycosyl donors such as glycosyl phosphates, glycosyl sulphoxides, glycosyl acetates, etc. are frequently encountered in the synthesis of disaccharides and oligosaccharides. Each glycosyl donor has its unique features. To decide whether or not to use a specific glycoside as a glycosyl donor in a synthetic sequence usually depends on the following factors:

(1) Whether or not it is easily prepared, handled, purified and stored;

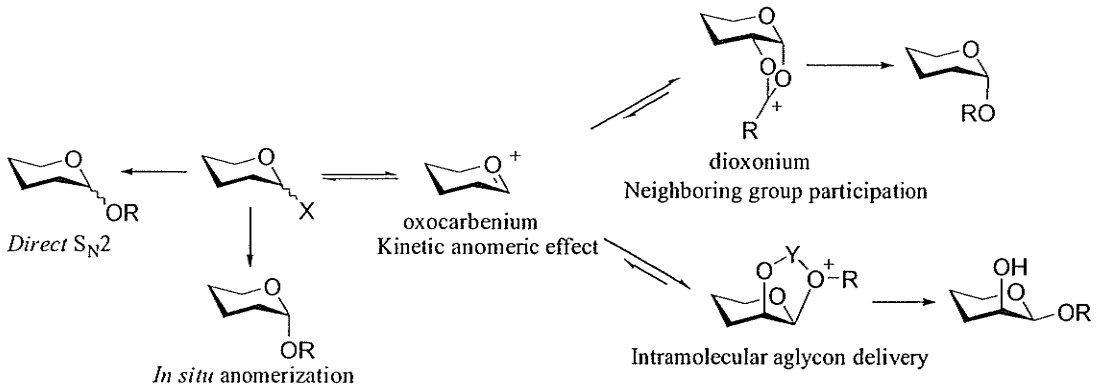
(2) Whether or not it undergoes glycosylations under mild conditions and does not interfere with other functionalities;

(3) Whether or not the reaction gives good stereoselectivity and high yield;

(4) Whether or not it is compatible with the next glycosylation sequence.

5.3 Stereoselective control of glycosylation

The previous section 5.2 deals with glycosylation strategies; this section will discuss the stereoselectivity of glycosylation. The stereochemistry of glycosylation often depends on reaction conditions such as donors, acceptors, promoters, solvents, temperatures, etc. Several options can be adopted to favor producing a desired glycosidic linkage stereoselectively, including neighboring group participation, intramolecular aglycon delivery, direct S_N2 substitution and *in situ* anomerization (Scheme 5.8). Both neighboring group participation and intramolecular aglycon delivery go through an oxocarbenium intermediate. The formation of the oxocarbenium ions is affected by the kinetic anomeric effect.



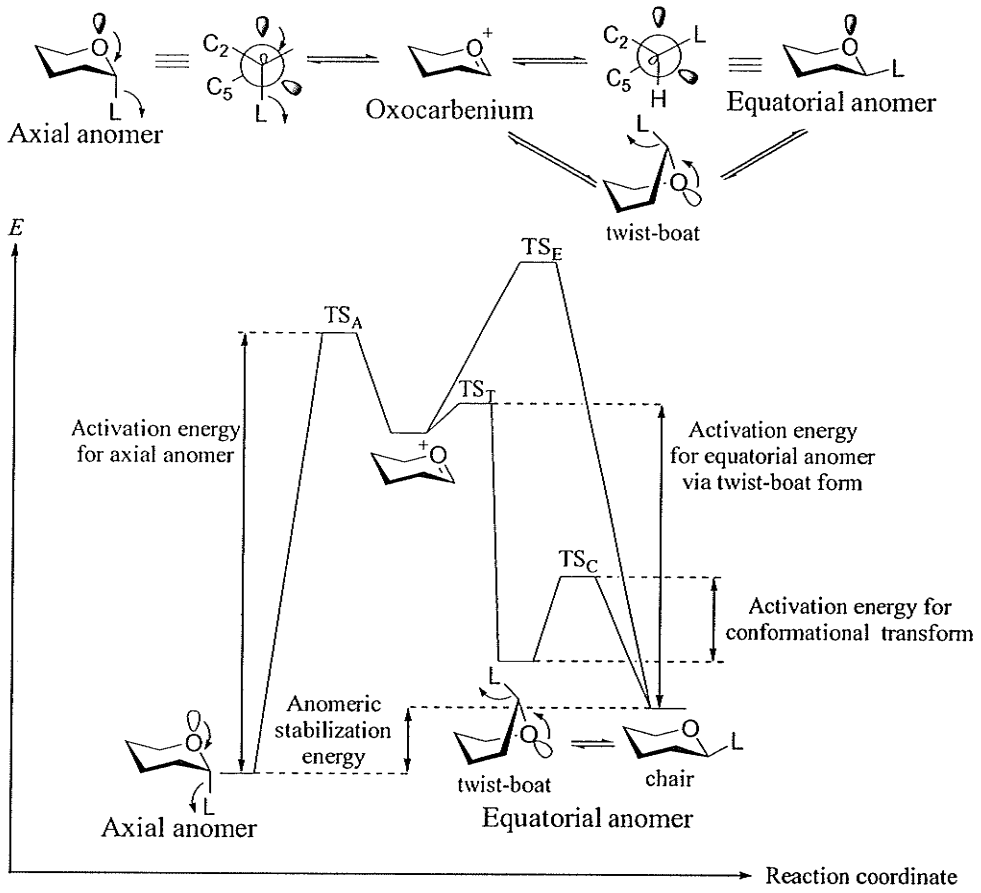
Scheme 5.8 Stereoselective control of glycosylation

5.3.1 Kinetic anomeric effect

Stereoelectronic effects have been proposed to govern reactions at the anomeric center.[132] Many examples can be found in which an axial leaving group is more reactive than an equatorial leaving group. For instance, Eliel and Nader observed in 1970 that the axial methyl 2-alkoxy-1,3-dioxane reacted readily with Grignard reagents, but the equatorial isomer was unreactive.[131] These observations can be explained by the kinetic anomeric effect (Scheme 5.9).[119] As discussed in section 4.4, the nonbonding electron lone pair of the endocyclic oxygen is antiperiplanar to the axial leaving group of an α *O*-glycoside, and is delocalized into the σ* orbital of the anomeric C-O_{exo} bond. Thus, the anomeric effect will stabilize the transition state of the axial *O*-glycoside when it becomes an oxocarbenium ion. Such stabilization cannot be provided when the anomeric leaving group is in an equatorial orientation.

However, the kinetic anomeric effect became controversial when some experiments could not be explained by this concept.[119] For example, it was observed that hydrolysis of axial glycoside esters under acid catalysis proceeds more slowly than hydrolysis of equatorial glycoside esters. If the kinetic anomeric effect existed, the results should be the opposite. A number of hypotheses have been proposed to explain the experimental results.[119] Kirby argued that ring flipping may have made the equatorial glycosides hydrolyze more rapidly.[132] A energy diagram corresponding to this hypothesis is illustrated in Scheme 5.9.[133] In this case, the equatorial anomers transform from a

normal chair conformation to a twist-boat form by overcoming a small amount of conformational energy. The lone pair of the ring oxygen in the twist-boat conformation is antiperiplanar to the aglycon leaving group. Therefore, the equatorial anomers will face a net activation energy to become oxocarbenium ions that is lower than that faced by the axial anomers.



Scheme 5.9 The kinetic anomeric effect

If equatorial anomers were rigid enough to resist conformational equilibrium, then axial glycosides should hydrolyze faster under acid catalysis than equatorial anomers. In other words, the kinetic anomeric effect could be observed under such circumstances. In order to test this theory, a series of rigid glycosides was prepared and subjected to acid hydrolysis (Figure 5.2).[133] Measurement of their hydrolysis rates shows that all of these axial isomers hydrolyzed much faster than their equatorial counterparts. These results

allow one to conclude that the cleavage of the anomeric leaving groups is affected by the kinetic anomeric effect.

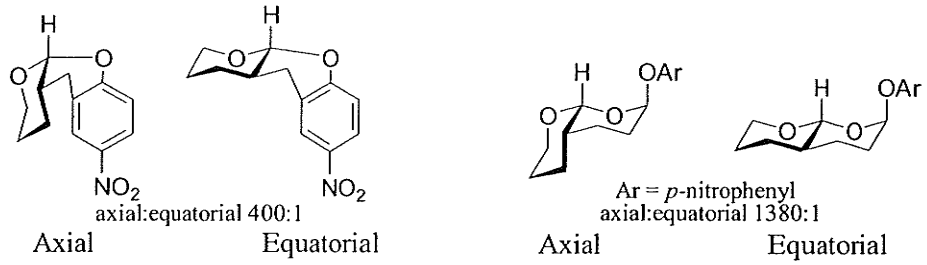
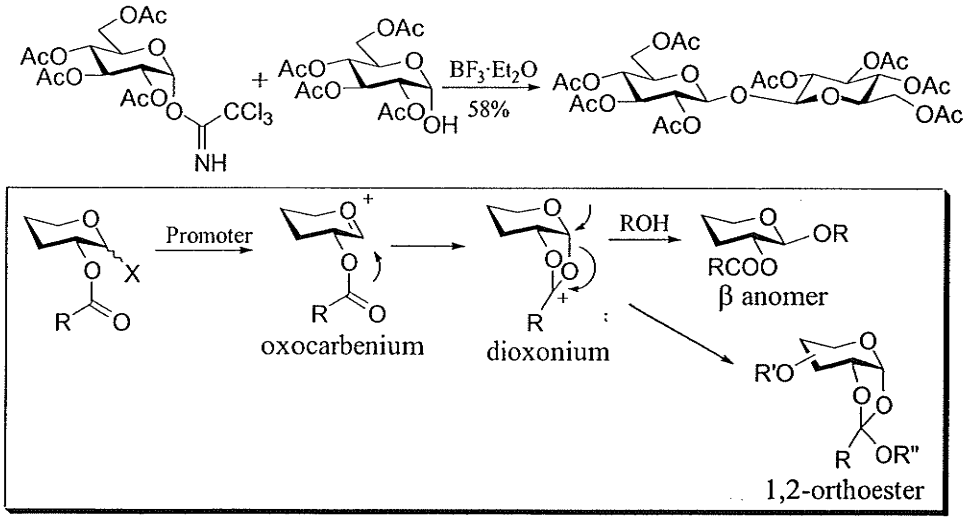


Figure 5.2 Acid hydrolysis of various glycoside esters

5.3.2 Neighboring group assistance

In these types of glycosylation, an oxocarbenium intermediate is first generated by cleavage of the anomeric leaving group that is assisted by a promoter (Scheme 5.10). A neighboring group such as acetate or benzolate then adds to the oxocarbenium to form a more stable dioxonium intermediate. This is because the positive charge of the dioxonium ion is stabilized by two adjacent oxygen atoms while the oxocarbenium ion is stabilized by only one oxygen atom. Subsequent substitution of the dioxonium by an acceptor results in the formation of a 1,2-*trans* glycoside.[116]

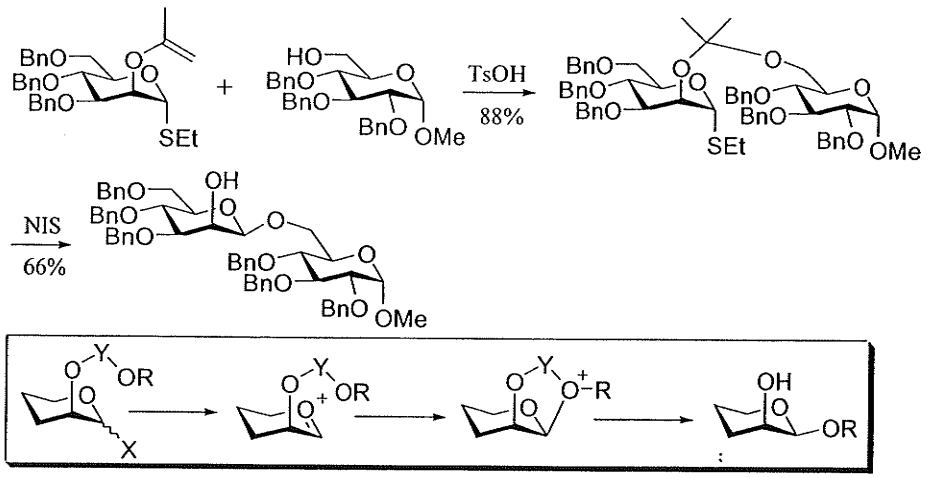


Scheme 5.10 Glycosylation with neighboring group assistance

Glycosylations involving neighboring group participation generally give products in relatively good yields and with high stereoselectivity. However, side reactions can occur. For example, attack on the dioxolane by the nucleophile can lead to a 1,2-orthoester byproduct. Scheme 5.10 depicts the synthesis of a disaccharide with this method. The low yield is due to the side reactions.[134]

5.3.3 Intramolecular aglycon delivery

The intramolecular aglycon delivery concept was first introduced by Stork and Barresi in the early 1990s.[135,136] In this approach, the sugar alcohol (ROH) is linked to the C-2 hydroxyl of a mannosyl donor through a tether Y (Y = CH_2 or $SiMe_2$). When the anomeric leaving group is activated by a promoter to generate an oxocarbenium ion, the aglycon is delivered to the β face of the donor to the anomeric center. The tether hydrolyses after the work-up, giving the β glycoside product (Scheme 5.11). Because the aglycon can only be delivered to the β face, the reaction generally affords the β anomer product.

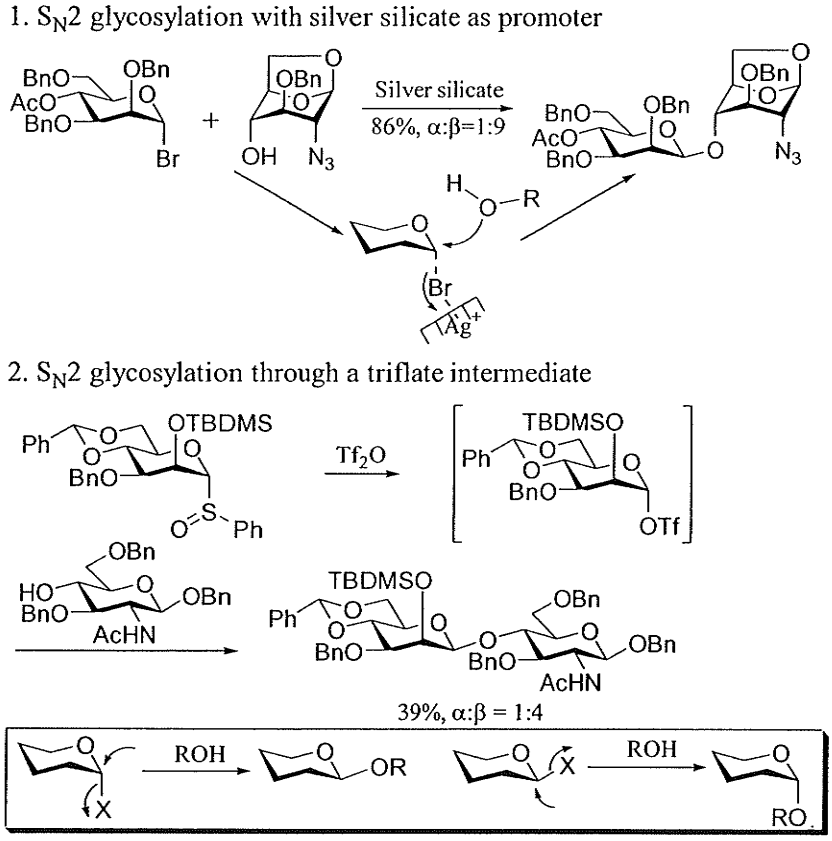


Scheme 5.11 Glycosylation by intramolecular aglycon delivery

The tether can be a silicon, an acetal, a methylene acetal, or a ketal.[123] The promoter depends on the donor used. In the example of Scheme 5.11, the promoter was NIS because the donor was a thioglycoside. The reaction produced exclusively a β disaccharide.[123]

5.3.3 *Direct* S_N2 glycosylation

Direct S_N2 glycosylation leads to configuration inversion at the anomeric carbon center (Scheme 5.12). In order to achieve *direct* S_N2 glycosylation, no neighboring participation groups should be present in the glycosyl donors because the neighboring-group participation often competes with the *direct* S_N2 glycosylation. *Direct* substitution is especially valuable in the preparation of β-linked mannosides because β-linked mannosides cannot be obtained through neighboring-group participation and *in situ* anomerization.[116]



Scheme 5.12 *Direct* S_N2 glycosylation

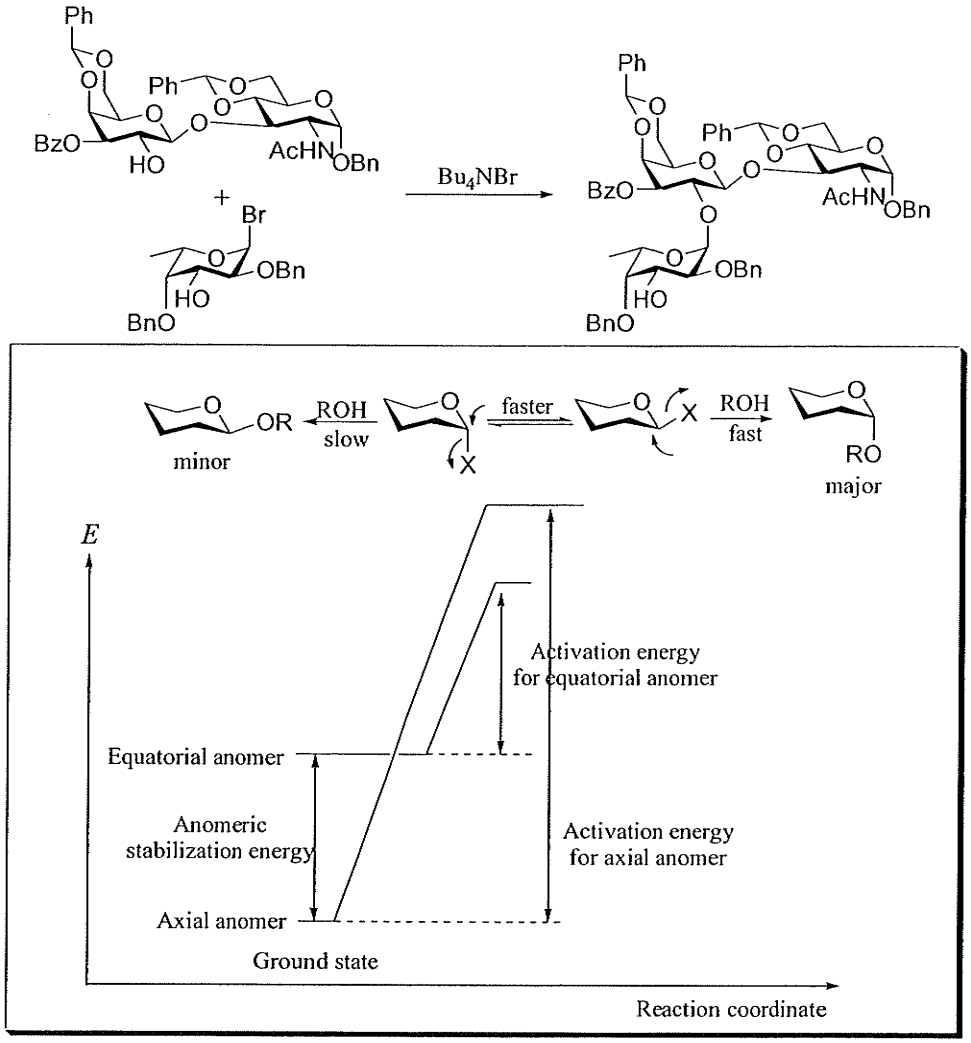
Direct S_N2 glycosylation may encounter competition from *in situ* anomerization. There are two ways to suppress *in situ* anomerization. The first method is to use insoluble promoters (Scheme 5.12). Insoluble promoters activate the leaving groups so that the *direct* substitution occurs before *in situ* anomerization. The commonly used insoluble

promoters are silver silicate and silver silicate-aluminum. For instance, glycosylation of an α glycoside bromide in the presence of an insoluble silver salt produces mainly the β disaccharide product. This is because the bromide interacts with the solid support and the formation of oxocarbenium is prevented.[137]

The second method used to suppress *in situ* anomerization is to begin with a glycosyl triflate intermediate because the glycosyl triflate is inclined to react by *direct* substitution (Scheme 5.12). However, preparation of glycosyl triflates is extremely difficult due to the high reactivity of the triflate group. But the glycosyl triflate intermediates can be generated *in situ* by treatment of glycosyl sulfoxides with triflic anhydride. The triflate group is preferentially formed in the axial orientation due to the anomeric effect. Displacement of the triflate group by a nucleophile in an S_N2 fashion mainly gives the β glycosides.[138]

5.3.4 *In situ* anomerization

In situ anomerization is a kinetically-controlled process. This concept was first introduced by Lemieux in 1965.[139,140] He observed that a rapid equilibrium is established between α and β isomers of the glycosyl bromide by addition of tetra-*n*-butyl ammonium bromide. At equilibrium, there is a shift toward the α bromide because the α anomer is stabilized by the anomeric effect. However, the β bromide is much more reactive towards nucleophilic attack by an alcohol than the more stable α bromide. Thus, glycosylation takes place preferentially on the β bromide. Because the reaction happens in a S_N2 fashion, the α-glycoside is the major product. Besides glycosyl bromides, glycosyl imidates, thioglycosides and glycosyl fluorides also undergo *in situ* anomerization.[116] Solvents also play a major role in the *in situ* anomerization reactions. It is essential that the *in situ* anomerization be conducted in a low polarity solvent. Otherwise the polar solvents would favor the formation of oxocarbenium ions, thus reducing the stereoselectivity.[116] Scheme 5.13 depicts the synthesis of a trisaccharide by this method.



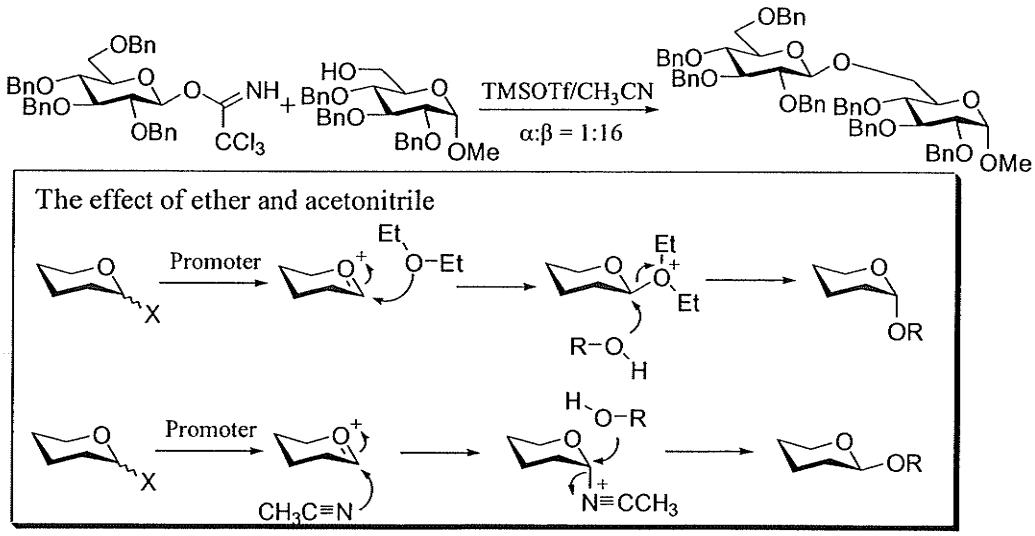
Scheme 5.13 *In situ* anomerization

The important requirement for this reaction is that the rate of equilibration is much faster than that of the glycosylation. When α and β bromides are in fast equilibrium, the product ratio is not determined by the reactant ratio, but by the relative net activation energies for the reactants (Scheme 5.13).[116] Because the α anomer has a higher activation energy and is less reactive than the β anomer because it is thermodynamically more stable due to the anomeric effect, the reaction mainly proceeds through the β anomer.

5.3.5 Solvent effect

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 the α selectivity because *in situ* anomerization is facilitated and the formation of oxocarbenium ions is suppressed.[116]

Some solvents also participate in glycosylation.[116] 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 is depicted in Scheme 5.14. When diethyl ether complexes with the oxocarbenium ion, it forms an intermediate in which the diethyl oxonium group 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 favors the equatorial position and the nitrilium prefers an axial orientation. The example given in Scheme 5.14 achieved a 16:1 ratio of β to α isomers with acetonitrile as solvent.[141]



Scheme 5.14 Solvent effect

5.4 Concluding remarks

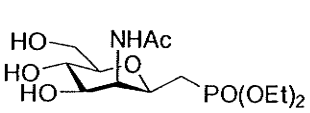
This chapter has reviewed the methodologies for the preparation of disaccharides and oligosaccharides. Three most commonly used glycosylation approaches are glycosyl halide, glycosyl imidate and thioglycoside methods. The choice of glycosylation approaches depends on the overall synthetic strategy. The considerations in deciding on a specific glycosylation method for the synthesis of a targeted carbohydrate molecule include: (1) how easily the donors and acceptors can be prepared; (2) the yield and stereoselectivity generally obtained by a given method; (3) whether or not the donors and acceptors interfere with other functionalities; (4) whether or not the approach makes the whole synthetic procedure more efficient.

This chapter has also discussed the stereoselectivity of glycosylation. The selectivity can be achieved by adopting neighboring group participation, intramolecular aglycon delivery, direct S_N2 substitution and *in situ* anomerization. Solvents also play a role in determining the stereochemical outcome.

Chapter 6

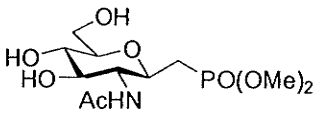
Objectives of this Ph.D. Thesis

The objective of this Ph.D. research was to prepare the following carbohydrate-based compounds as potential glycosyltransferase inhibitors: (1) β *N*-acetyl D-mannosamine and β *N*-acetyl D-glucosamine 1-phosphonates **49** and **50**; (2) α *N*-acetyl D-glucosamine 2-hydroxy and 2-keto phosphonates **51** and **52**; (3) α-L-Rha-(1→3)-α-D-GlcNAc disaccharide analogues **53**. (Figure 6.1)



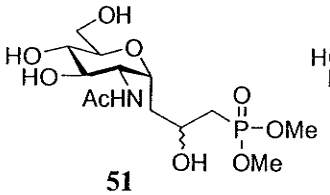
49

Analogue to β *N*-acetyl D-mannosamine 1-phosphate

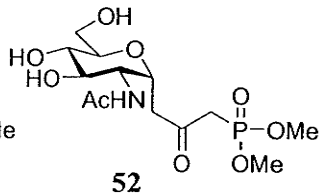


50

Analogue to β *N*-acetyl D-glucosamine 1-phosphate

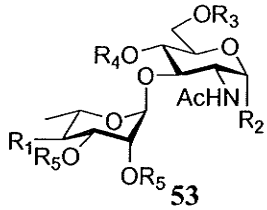


51



52

Analogues to α *N*-acetyl D-glucosamine pyrophosphate



53

R_1: H, OH, OMe, OBz
R_2: OBn, COOMe, $CH_2PO(OEt)_2$
R_3: H, OAc
R_4: H, OAc
R_3, R_4: PhCH
R_5: H, OBz

Analogues to α-L-Rha-(1→3)-α-D-GlcNAc linker

Figure 6.1 Target compounds of the Ph.D. research

Compounds **49** and **50** are β *C*-glycosyl anologues of *N*-acetyl D-glucosamine and *N*-acetyl D-mannosamine 1-phosphates. Their syntheses have not been reported. A decision was made to prepare them from an amino sugar D-GlcNAc.

Both compounds **51** and **52** are close mimics of *N*-acetyl D-glucosamine pyrophosphate. As discussed in chapter 2, one of the key features in the glycosyltransferase-catalyzed reactions is the binding that exists between the diphosphate moiety of the glycosyl donors and the divalent metal cation present at the active site of the enzymes. The current sugar nucleotide analogues that mimic the pyrophosphate moiety have not taken this binding feature fully into account.[34,35] Therefore, we decided to design and synthesize *C*-glycosyl 2-hydroxy and 2-keto phosphonates **51** and **52** as close mimics to sugar pyrophosphate nucleotides.

Compounds **53** mimic the α-L-Rha-(1→3)-α-D-GlcNAc linker found in the mycobacterial cell wall. The linker has not been targeted for drug design so far. A decision was made to prepare a series of disaccharide analogues based on this linker structure and to test their biological activity against the mycobacterial glycosyltransferases *in vitro*. The test results would reveal not only the structure-activity relationships of these compounds, but also would provide information for the design of more potent and selective inhibitors in the future.

Part II: Ph.D. Research

Chapter 7

Synthesis of β *N*-acetyl *C*-mannosaminyl Phosphonate

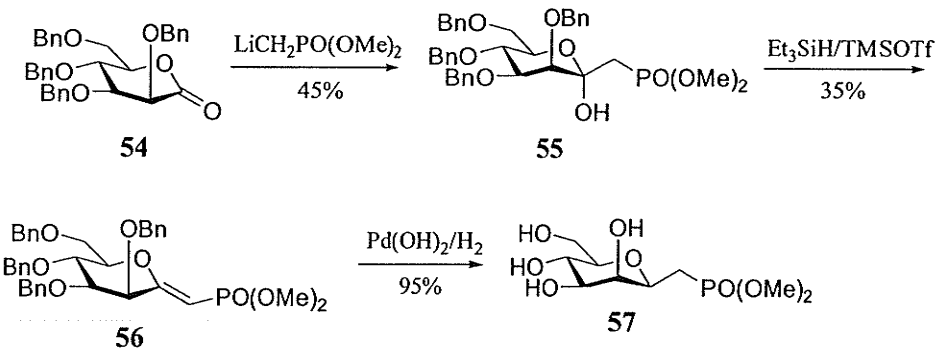
7.1 Introduction

This chapter will describe the synthesis of a β *C*-glycosidic analogue of *N*-acetyl D-mannosamine 1-phosphate **49** from the readily available amino sugar *N*-acetyl D-glucosamine via a Horner-Emmons/Michael reaction. The key step in this synthesis was a zinc-mediated olefination. No desired olefin was formed if $ZnBr_2$ was not added to the reaction or was added enough. This approach is short and efficient in comparison with the reported syntheses of other *N*-acetyl *C*-mannosyl compounds.

7.2 Synthetic strategy

7.2.1 Background review

Even though the synthesis of a β *C*-glycosidic analogue of *N*-acetyl mannosamine 1-phosphate has not been reported so far, the preparations of two closely related compounds have been accomplished.

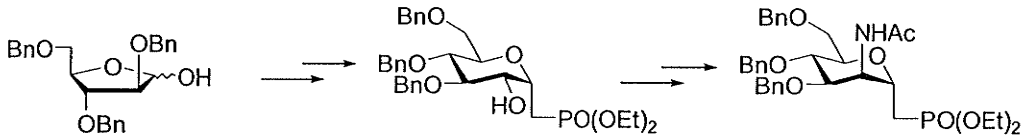


Scheme 7.1 Synthesis of *C*-mannosyl phosphonate **57**

β *C*-mannosyl phosphonate **57** was prepared by Dondoni in 2000 (Scheme 7.1).[142] The synthesis of **57** started from a gluconolactone **54**. Addition of a phosphonate

nucleophile $LiCH_2PO(OMe)_2$ to **54** afforded the ketomannosyl phosphonate **55** in 45% yield. Attempted ionic reduction with Et_3SiH/TMSOTf did not deoxygenate the anomeric hydroxyl of **55**, but instead produced the *C*-mannosyl olefin **56** in 35% yield. 34% of unreacted **55** was recovered from the reaction mixture. Hydrogenation of **56** with $Pd(OH)_2$ as catalyst afforded the phosphonate **57** in good yield. The overall yield of **57** was 15%. The drawback of this synthetic route is the low yields in the first two steps of the synthesis.

A second compound, α *N*-acetyl *C*-mannosaminyl phosphonate **60**, was reported by Nicotra in 1995 and 1996.[25,106] The synthesis of **60** was a very lengthy process (9 steps), which started from a D-arabinose derivative **58**. The phosphonate moiety was introduced into the arabinose molecule in multiple steps, affording the *C*-glucosyl phosphonate **59**. Then the amino group was installed into the sugar ring by several additional steps, furnishing the desired compound **60**. Although the yield was relatively good for each step in the synthesis, the overall yield of **60** was less than 30%. The drawback of this synthetic strategy is that it did not start from a readily available amino sugar.



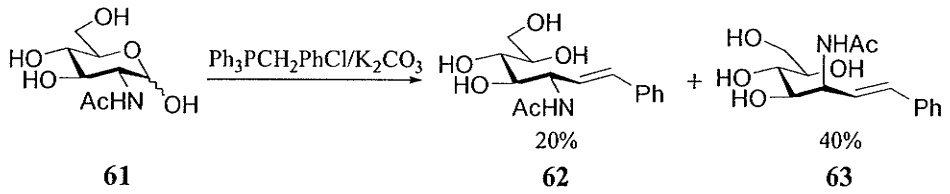
58 **59** **60**

Scheme 7.2 Synthesis of α *N*-acetyl *C*-mannosyl phosphonate **60**

7.2.2 Horner-Emmons/Michael reaction of *N*-acetyl glucosamine

As discussed in chapter 4, the Horner-Emmons/Michael reaction is a successful method for the preparation of amino *C*-glycosides. It has been known for some time that *N*-acetyl *C*-mannosaminyl derivatives can be prepared via a Horner-Emmons reaction or Horner-Emmons/Michael reaction of *N*-acetyl D-glucosamine.

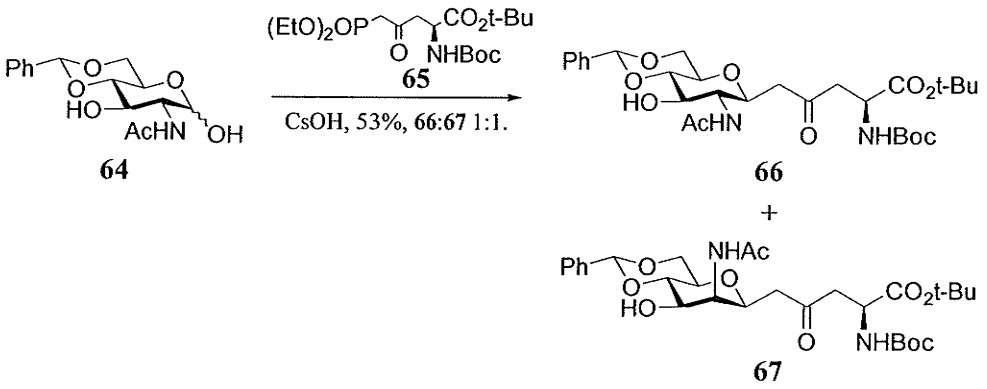
Konrad filed a patent in 1990 that described the preparation of *N*-acetyl *C*-mannosaminyl derivative **63** from amino sugar *N*-acetyl D-glucosamine via a Horner-Emmons reaction (Scheme 7.3).[143] The reaction gave two diastereomeric products – *gluco* and *manno* derivatives **62** and **63** – in a 1:2 ratio. Although the *manno* derivative **63** was the major product, it was formed in low yield (40%).



61 **62** **63**

Scheme 7.3 Synthesis of *N*-acetyl *C*-mannosaminyl derivative **63**

Davis reported in 1998 that a large amount of the *manno* derivative **67** was always isolated from the reaction of the D-glucosamine derivative **64** and a phosphonate nucleophile **65** although various bases such as Li*O*t-Bu and CsOH were used (Scheme 7.4).[100] Using CsOH as the base, the reaction gave **67** in 27% yield. The *manno* derivative **67** came from the epimerization of the *N*-acetyl group of **64** followed by a Horner-Emmons/Michael reaction.



64 **66** **67**

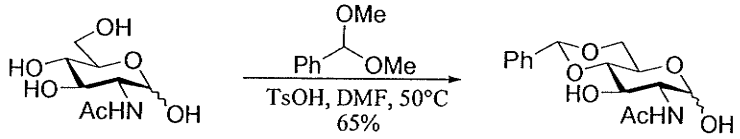
Scheme 7.4 The synthesis of *C*-manno derivative **67** from the Horner-Emmons/Michael reaction

From the above two examples, it was concluded that *N*-acetyl D-*C*-mannosaminyl derivatives could be prepared through Horner-Emmons/Michael reaction of *N*-acetyl D-

glucosamine. The yield and stereoselectivity might be improved if the reaction conditions were optimized.

7.3 Results and discussion

The synthesis of β *N*-acetyl *C*-mannosaminyl phosphonate **49** started from a commercially available *N*-acetyl D-glucosamine **61**. The 4- and 6-hydroxyls of **61** were protected with a benzylidene group by following a procedure described by Macher (Scheme 7.5).[144] *N*-acetyl D-glucosamine **61** was treated with benzaldehyde dimethyl acetal at 50°C in the presence of an acid catalyst TsOH to give 4,6-*O*-benzylidene *N*-acetyl glucosamine **64** as a mixture of α and β anomers. This method is generally applicable for selective protection of 1,3 diols of sugars.[120] Benzylidene glucosamine **64** was purified by crystallization from methanol and obtained in 65% yield.



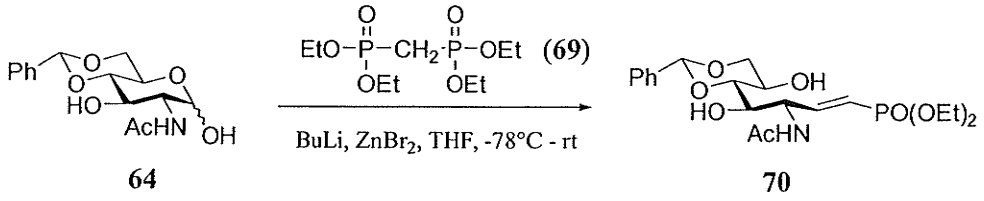
61 **64**

Scheme 7.5

Glucosamine **64** was then converted to the olefin **70** (Scheme 7.6). One equivalent of butyl lithium was added to a THF solution of diphosphonate **69** at –78°C. After stirring for 10 min at –78°C and 20 min at room temperature, half of an equivalent of anhydrous $ZnBr_2$ in THF was added to the solution. After 40 min of stirring, **64** was added to the reaction. The reaction mixture was stirred overnight at room temperature. Table 7.1 lists the yields of the olefin product **70** under various conditions.

The results shown in Table 7.1 indicated that $ZnBr_2$ was essential to this reaction. No desired product was detected if $ZnBr_2$ was absent from the reaction, and most of the starting compound was recovered from the reaction (entry 1). When the reaction temperature was raised to 50°C in this reaction, 28% of an α,β-unsaturated phosphonate **74** was isolated from the reaction after 4 hours, along with unreacted starting material.

The unexpected product **74** probably came from a *retro-aldol* reaction followed by Horner-Emmons olefination (Scheme 7.7).



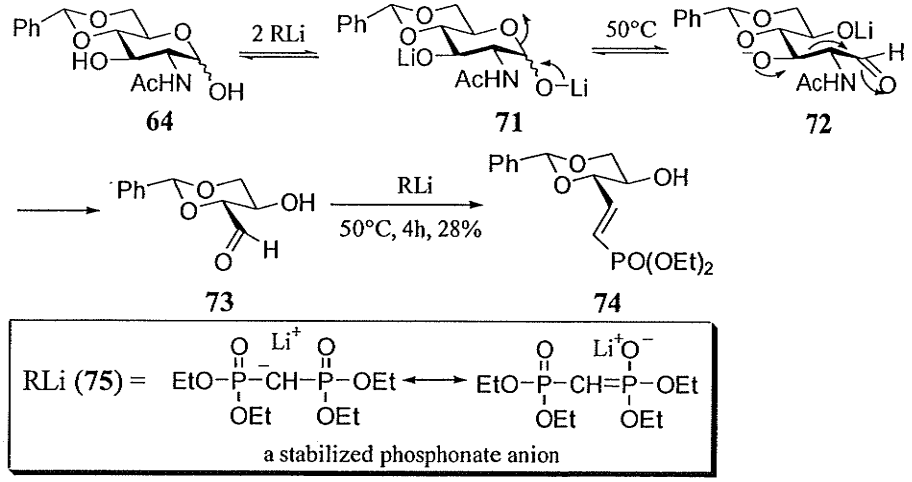
64 70

Scheme 7.6 Olefination of the glucosamine **64**

Table 7.1 The yield of olefin **70** under various conditions

Exp. No.	Reaction conditions	Yield (%)
1	**64**: **69** = 1: 6.0, no $ZnBr_2$, THF, -78°C – rt, overnight.	0
2	**64**: $ZnBr_2$: **69** = 1: 1.5: 3.0, THF, -78°C – rt, overnight.	0
3	**64**: $ZnBr_2$: **69** = 1: 3.0: 6.0, THF, -78°C – rt, overnight.	85
4	**64**: $ZnBr_2$: **69** = 1: 3.4*: 6.7, THF, -78°C – rt, overnight.	98

*Recycled.



64 71 72 73 74

Scheme 7.7

Although glycose **64** is only sparingly soluble in THF, it gradually dissolved into the reaction solution when it was stirred with **75** overnight at room temperature (Scheme

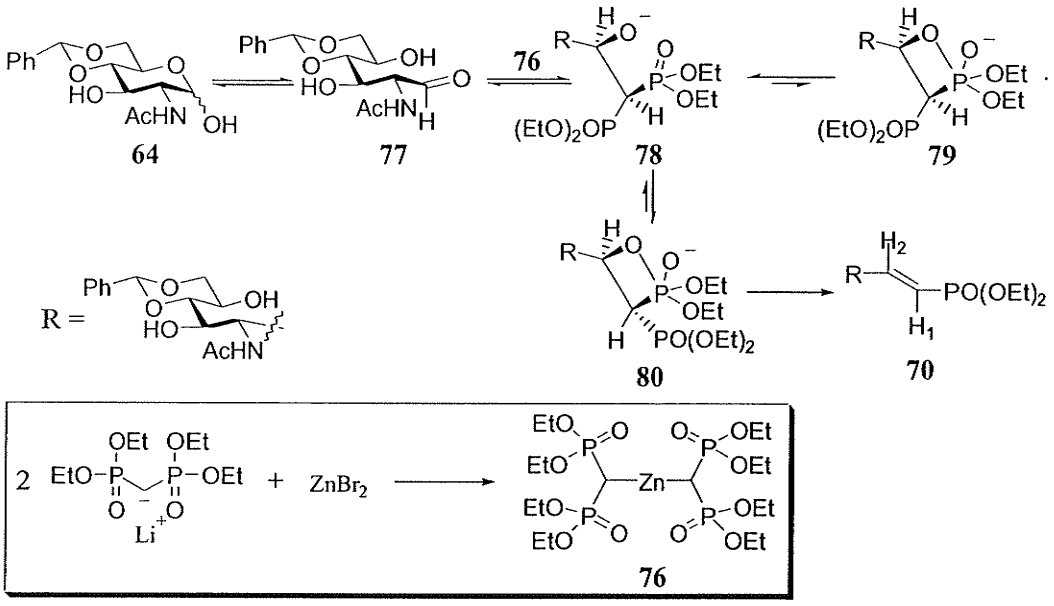
7.7) The pKa value of the lithium-diphosphonate **75** (about 26 in DMSO) is lower than that of the alkoxides in **71** (about 28-29 in DMSO), indicating that the cluster structure of **71** might help to stabilize the alkoxide structure.[115] It is well known that organolithium molecules tend to aggregate into stable cluster structures in solution.[145] Isomerization of **71** to the opened ring aldehyde **72** would need energy to break up the cluster structure. This is presumably the reason why the diphosphonate anion **75** did not react with **64** at room temperature. Higher temperature would break up the lithium alkoxide aggregate structure and shift the equilibrium to **72**. The aldehyde **72** was so unstable at the elevated temperature that it decomposed to an aldehyde **73** by a *retro-aldol* reaction before the lithium-diphosphonate **75** added to it.

It is not unusual for a glycose to undergo a *retro-aldol* reaction under basic conditions. Giannis and coworkers reported a *retro-aldol* reaction of 2-acylamino-2-deoxy-4,6-*O*-ethylidene glucose with the ylides Ph_3P=CHR (R = H, CH_3 and $C_{15}H_{31}$) in 1988.[146] Costantino and coworkers reported an elimination caused by a *retro-aldol* reaction of 6-*O*-triisopropylsilyl-3,4-di-*O*-benzyl-2-deoxygalactose and 3,4,6-tri-*O*-benzyl-2-deoxygalactose with the ylide $Ph_3P(CH_2)_{11}CH_3Br$ in 2001, as well.[147]

These disappointing initial results led us to explore the effect of $ZnBr_2$ on the reaction. The amount of $ZnBr_2$ added to the olefination of **64** was found to be critical (entries 2-4 in Table 7.1). This reaction required a very large excess of $ZnBr_2$. Even one and a half equivalents of $ZnBr_2$ present in the reaction were not enough to produce any detectable amount of the desired product (entry 2). Only after three or more equivalents of $ZnBr_2$ were added into the reaction was a good yield of the olefin product **70** obtained (entries 3 and 4). No *retro-aldol* product **74** was found in these reactions.

Based on the ratio of $ZnBr_2$ and diphosphonate **69** added, the intermediate diorganozinc **76** might form in the reaction, because diorganozinc reagents (R_2Zn) are often prepared by transmetallation of two equivalents of organolithium reagents with one equivalent of zinc halide (Scheme 7.8).[148] A zinc-diphosphonate complex such as **76** has not been reported so far. The only zinc-phosphonate complex studied so far is

[(diethoxyphosphinyl)difluoromethyl]zinc bromide, $(EtO)_2P(O)CF_2ZnBr$, which is a stable monoorganozinc compound.[149] Therefore, the structure and properties of **76** are not clear. Most of the known diorganozincs are less basic than their corresponding organolithium compounds. They are monomers in solution and have a weak affinity to ethereal solvents like THF because the 3*d* electron shell of the zinc atom is completely filled.[150] Thus, a stable zinc complex analogous to **71** would not form in the $ZnBr_2$-catalyzed reaction, and the aldehyde **77** would not undergo *retro-aldol* decomposition either. But the 3- and 5-hydroxyls of **77** would probably consume some **76** because organozincs readily react with alcohols.[147] This is probably the reason that an excess of $ZnBr_2$ was necessary for the reaction.



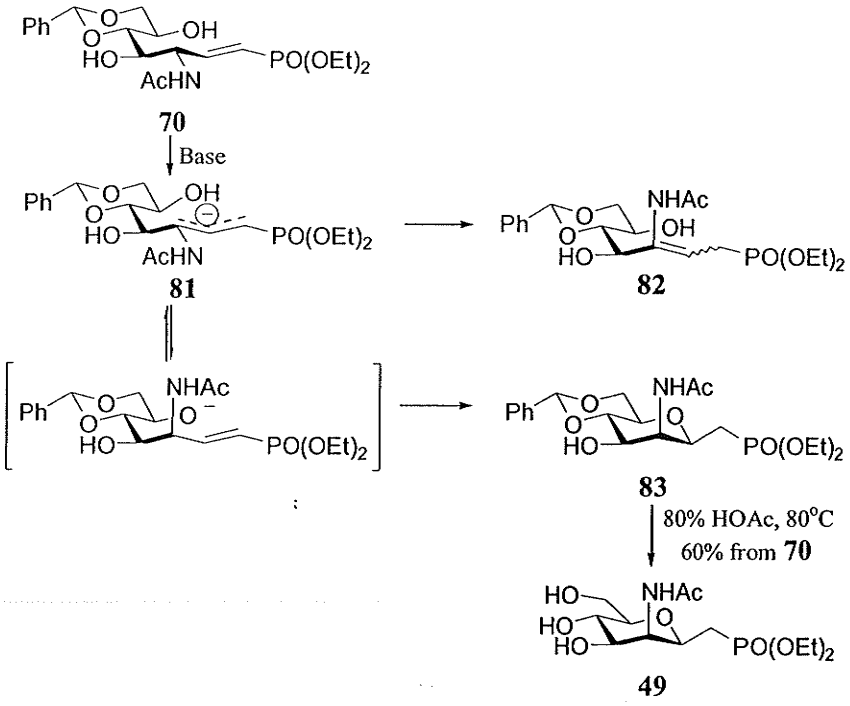
Scheme 7.8 Possible mechanism for the olefination of **64**

Addition of zinc-diphosphonate **76** to the aldehyde **77** gave the adduct **78** via one of two possible transient four-centered intermediates, **79** and **80**. The *trans* isomer **80** would be thermodynamically favored over **79**.[151] Decomposition of **80** would generate the olefin **70** with a *trans* configuration. The large ^1H coupling constant $J_{H1\text{-}2}$ (17.2 Hz) seen in the NMR spectrum of **70** supported this conclusion.

The olefination of glycoses with organozinc reagents was also studied by Boschetti and coworkers.[152] They investigated the vinylation of aldopentoses with divinylzinc, and found that a large excess of diorganozinc (10 equivalents) was needed for the reaction.

It is worth noting that diphosphonate **69** could be recycled because it was used in excess (entry 4 in Table 7.1). The NMR spectrum of the recovered **69** was the same as that of the commercial sample.

Olefin **70** underwent Michael cyclization when it was treated with K_2CO_3 in methanol (Scheme 7.9). An anion **81** was probably formed in the reaction because the estimated pKa value of 3-H in **70** (about 15) is comparable to that of methanol (15.2).[86] The isomer **82** could be isolated as a mixture of two diastereomers if the reaction was quenched at an early stage. The ratio of these diastereomers depended on the reaction time, varying from 1:1.6 to 1:2.5.



Scheme 7.9 Michael cyclization of the olefin **70**

After stirring overnight at room temperature followed by deacetylation, the target β *C*-manno phosphonate **49** was obtained as the major product. Giannis and Sandhoff also studied a Michael cyclization of ethyl-4-acetamido-2,3,4-trideoxy-6,8-ethylidene-D-gluco-oct-2-(E, Z)-enoate by treatment with 0.01 M NaOEt, finding that a short reaction time (5-15 min) gave the kinetic product, an α *C*-glycoside; but a longer reaction time (more than 24 hours) produced exclusively the thermodynamic product, the β *C*-glycoside.[153] No α anomeric products were found in the reaction of **70**, indicating that **83** was the thermodynamically more stable product. Other basic conditions were also tried for Michael cyclization of **70**. Stronger bases such as KO*t*Bu, NaOEt and NaOH gave lower yields of **49**. Although the overall yield of **49** was modest (38%), this route used a cheap and readily available starting material and was very short.

The configuration and conformation of **49** were determined by NOE correlation experiments (Figure 7.1). NOE experiments showed that the NH had a 2% correlation with $H_{4'}$, $H_{1'}$ had a 3% correlation with $H_{3'}$, and $H_{2'}$ had a 3% correlation with $H_{1'}$ and a 1% correlation with $H_{5'}$, indicating that the *N*-acetyl group was located at an axial position and the mannosyl molecule was in a $^4C_1$ conformation. The correlations of $H_{1'}$ to $H_{3'}$ (3%) suggested that the phosphonate moiety was in a β configuration.

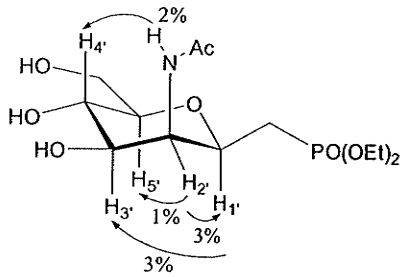


Figure 7.1 NOE correlations of **49**

7.4 Concluding remarks

β *N*-acetyl *C*-mannosaminyl phosphonate **49**, the β analogue of *N*-acetyl D-mannosamine 1-phosphate was successfully prepared from the readily available amino

sugar *N*-acetyl D-glucosamine via Horner-Emmons/Michael reaction. Compared with the synthetic routes for closely related compounds, this approach is short and efficient.

Chapter 8

Synthesis of β *N*-acetyl *C*-glucosaminyl Phosphonate

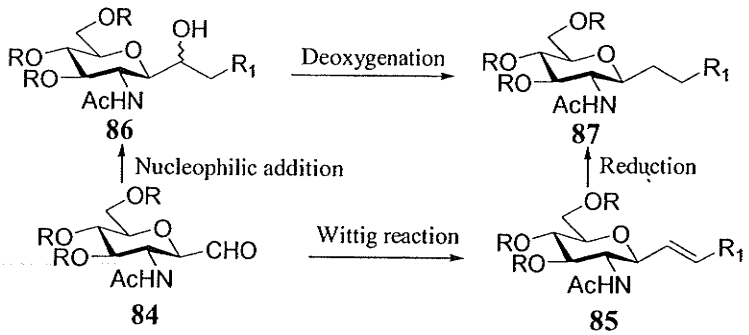
8.1 Introduction

This chapter will describe two approaches to preparing the β *C*-glycosidic analogue of *N*-acetyl D-glucosamine 1-phosphate **50**. The first approach started from a glycosyl aldehyde. Addition of a phosphite nucleophile to this glycosyl aldehyde produced a 1-hydroxy phosphonate intermediate. However, deoxygenation of this phosphonate was problematic. The second approach began with a gluconolactone and featured an olefin phosphonate intermediate in the synthesis. Reduction of this olefin afforded the target compound in excellent yield.

8.2 Synthetic strategy

8.2.1 Literature review

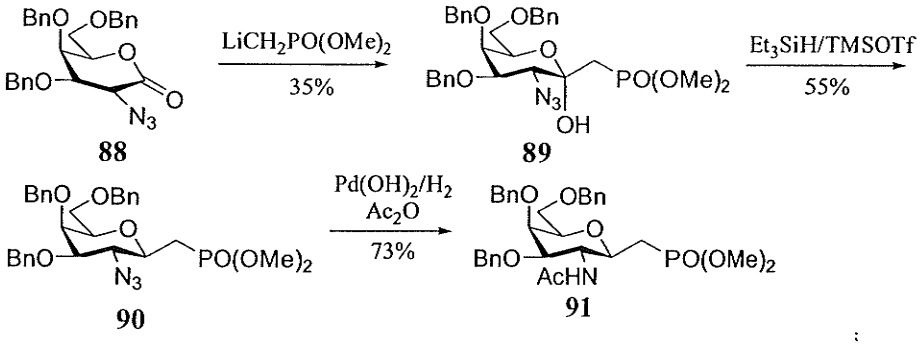
As discussed in chapter 5, several *C*-glycosylation approaches have been successfully applied to the preparation of amino *C*-glycosides. These approaches may be useful for the preparation of compound **50**.



Scheme 8.1 Glycosyl aldehyde **84** as synthon

One approach is to use a glycosyl aldehyde as a synthon (Scheme 8.1). Glycosyl aldehyde **84** is a versatile intermediate. It can be converted to the olefin **85** by Wittig reaction and to the alcohol **86** by nucleophilic addition.[154] Both **85** and **86** can be transformed to the alkane **87** by reduction and deoxygenation respectively. However, this glycosyl aldehyde synthon has not yet been used for the preparation of a glycosaminyl phosphonate.

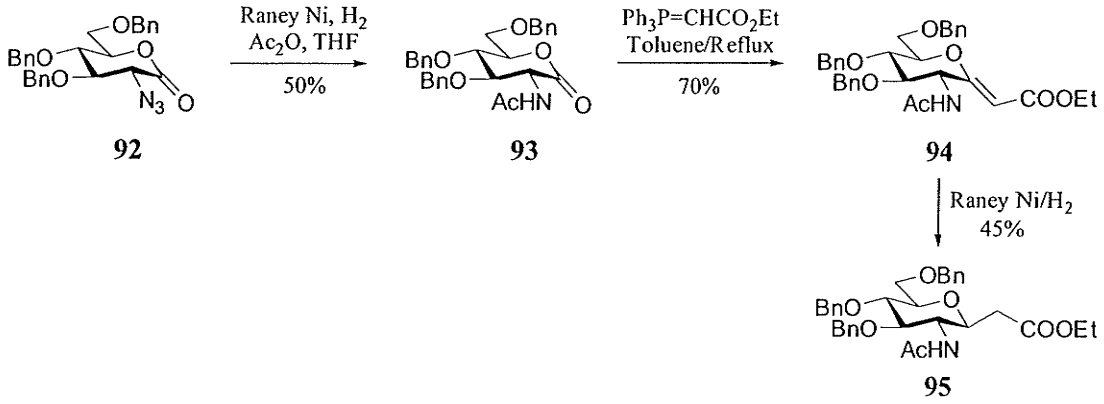
The other approach is to use a glyconolactone as a synthon. Several compounds that are closely related to **50** have been prepared by this method. The first one, β *N*-acetyl *C*-galactosaminyl phosphonate **91**, was reported in 2000 (Scheme 8.2).[142] An azido group was used as a masking function for the amino group in this approach. The glyconolactone **88** was converted to keto phosphonate **89** in 35% yield by nucleophilic addition of lithium dimethyl methylphosphonate. Deoxygenation of **89** with reducing agents triethylsilane and TMSOTf produced the β *C*-galactosyl phosphonate **90** in 55% yield. The azido group was then converted to the *N*-acetyl group by hydrogenation using palladium hydroxide as catalyst followed by acetylation. Although the whole synthetic sequence was straightforward, there were two low yielding reactions in the synthesis. The overall yield for the target compound **91** was less than 15%.



Scheme 8.2 Preparation of β *N*-acetyl *C*-galactosaminyl phosphonate **91**

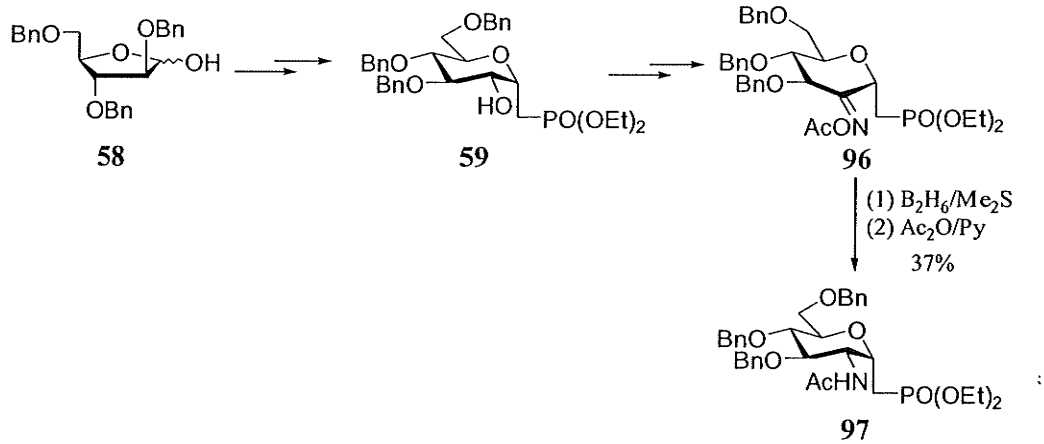
The second compound of this type prepared via a glyconolactone is β *N*-acetyl *C*-glucosamine ethyl ester **95,** reported in 1999 (Scheme 8.3).[101] The strategy used for its synthesis was different from that for compound **91**. In this approach, the azido group was converted to the *N*-acetyl group by hydrogenation with Raney Ni as catalyst in the

beginning of the synthesis. However, this step suffered from low yield (50%) due to the epimerization of the *N*-acetyl group of **93**. The other 50% of **92** was converted to the *manno* lactone. The *gluco* lactone **93** was then converted to the glycosyl olefin **94** in 70% yield by a Wittig reaction with the ylide, (carbethoxymethylene) triphenylphosphorane, in refluxing toluene. The final *C*-glycoside **95** was obtained by hydrogenation of **94** with Raney Ni as catalyst in 45% yield. Although this approach featured a Wittig olefination for direct access to *C*-glycoside, there was an overall low yield in the synthesis (16%).



92 **93** **94** **95**

Scheme 8.3 Preparation of *C*-glycoside **95**



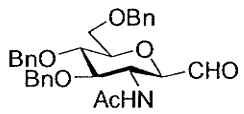
58 **59** **96** **97**

Scheme 8.4 Preparation of α *N*-acetyl *C*-glucosaminyl phosphonate **97**

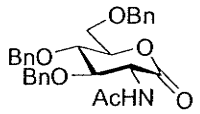
Another compound closely related to **50** is α *N*-acetyl *C*-glucosaminyl phosphonate **97**, reported in 1996, which is the α *C*-glycosyl analogue of α *N*-acetyl D-glucosamine 1-phosphate (see section 10.4.2.3).[25] The synthesis of **97** started from a D-

arabinose derivative **58** (Scheme 8.4). The synthesis first introduced the phosphonate moiety into the arabinose **58** in a multi-step sequence, giving the *C*-glycosyl phosphonate **59**. Compound **59** was converted to the acetyl oxime **96** in a few more steps. The *N*-acetyl group was introduced by reduction of **96** with diborane followed by acetylation, however, this key step was low yielding due to poor stereoselectivity in the reduction. The overall yield of the target compound **97** was less than 9% in 11 steps.

From the above discussion, it was concluded that there were not general and efficient synthetic strategies for preparing *N*-acetyl *C*-glucosaminyl phosphonates. However, glycosyl aldehyde and gluconolactone approaches might be worthwhile to pursue for the preparation of **50** if the reaction conditions could be optimized. Therefore, part of the objective of this Ph.D. research was to prepare synthons **84** and **93**, and to evaluate and optimize their reactions for the preparation of **50** (Figure 8.1).



Glycosidic aldehyde synthon
84



Glyconolactone synthon
93

Figure 8.1 Synthons **84** and **93**

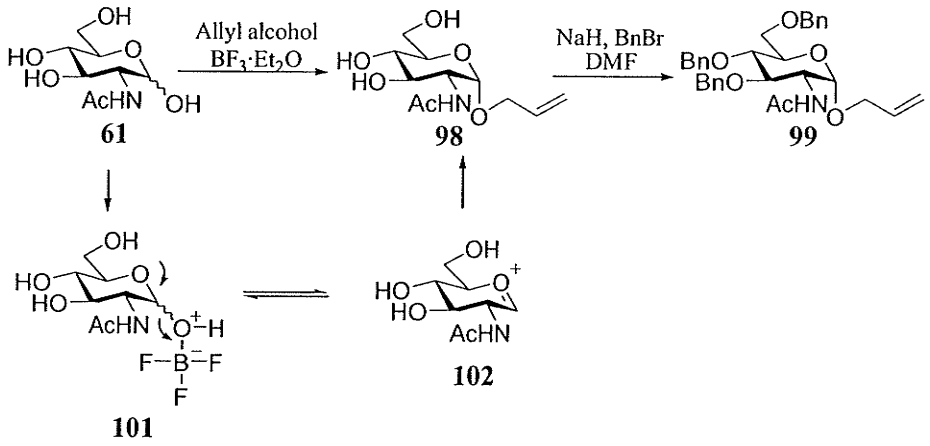
8.3 Results and discussion

8.3.1 Glycosyl aldehyde strategy

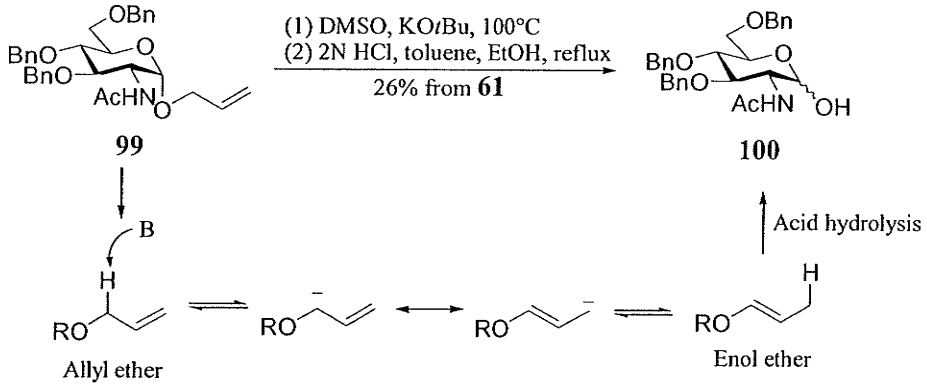
8.3.1.1 Preparation of the aldehyde 84

The synthesis of **84** started from the readily available and inexpensive amino sugar *N*-acetyl D-glucosamine **61.** The fully protected *N*-acetyl D-glucosamine **100** was prepared according to Hoffmann's procedure, which was a continuous process without purifying the intermediates.[87] First, the anomeric hydroxyl of glucosamine **61** was first protected by an allyl group. Allyl glycoside **98** was prepared by stirring in refluxing allyl alcohol in the presence of $BF_3 Et_2O$ for 1 hour (Scheme 8.5). Allyl ethers have been a useful protecting group for carbohydrates for more than half a century. They can be used

to protect both anomeric and non-anomeric hydroxyls. An allylation mechanism is proposed in Scheme 8.5.[125]



Scheme 8.5



Scheme 8.6

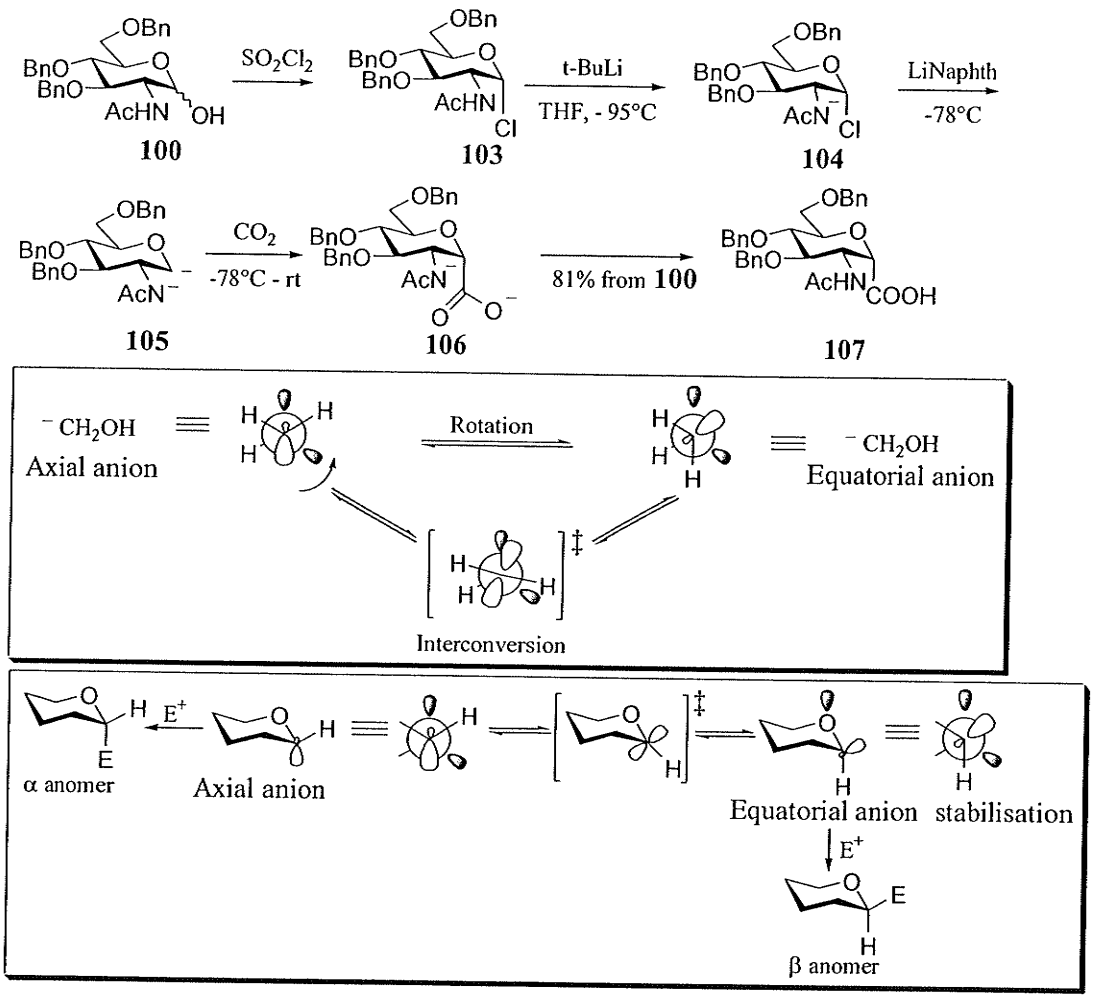
Then, the remaining hydroxyls in **98** were protected with benzyl groups by treatment with sodium hydride and benzyl bromide in DMF for 1 hour at 0°C, generating the fully protected allyl glycoside **99**. The allyl group of **99** was then cleaved by treatment with the strong base KO*t*Bu at 100°C in DMSO, followed by acid hydrolysis in a refluxing mixture of toluene, ethanol and 2N HCl, affording the glycose **100** (Scheme 8.6). The isomerization of allyl ethers under KO*t*Bu/DMSO conditions has been studied by Taskinen in 1993 and 2001.[155,156] The thermodynamic calculations show that an allyl ether is thermodynamically less stable than its isomeric enol ether (alkyl prop-1-enyl ether). The free energy of isomerization of allyl methyl ether is –19.9 kJ/mol at 298.15 K,

which corresponds to an equilibrium constant of 3100. With the increasing bulkiness of alkyl substituents, the stabilization of the enol ether isomer is further enhanced. The strong stabilization of the enol ether isomer is thought to come from the p-π conjugation of the oxygen atom and the double bond. Acid hydrolysis of the enol ether produced the glycose **100** as a mixture of α and β anomers. Recrystallization of **100** from methanol gave it as a fluffy white solid. The overall yield of **100** was 24% in 4 steps from the starting compound **61**.

The next step was to prepare the carboxylic acid **107** by using a dianion strategy developed by Kessler (Scheme 8.7).[87,157,158] To generate the dianion intermediate **105**, glycose **100** was first converted to the glycosyl chloride **103** by treatment with thionyl chloride in a mixture of CH_2Cl_2 and toluene for 30 min at room temperature. The glycosyl chloride was dissolved in THF, and *t*-butyl lithium was added at –95°C to deprotonate the *N*-acetyl group, generating a monoanion **104**. Lithiation of the chloride **104** with freshly made lithium naphthalenide at -78°C gave the dianion **105**. The reason for using such a sequence for generating the dianion is that the anomeric carbanion is far more basic than the amide anion. The pKa of hydrocarbons is estimated to be more than 35, while the pKa of an amide is only about 17.[86] Carbon dioxide was bubbled into the reaction mixture for 1 hour at -78°C, and then the reaction solution was warmed to room temperature. *C*-Glycosidic carboxylic acid **107** was obtained in 81% yield. Kessler and Burkhart reported that this reaction was very stereoselective and only α *C*-galactosamine carboxylic acid was obtained.[158] There are two reasons for this.

One reason is that although equatorial glycosyl carbanions are more stable than axial glycosyl carbanions, interconversion from the axial glycosyl carbanions to the equatorial glycosyl carbanions, or ring flipping, requires overcoming a larger energy barrier. An *ab initio* study of the species of $^{-}CH_2OH$ done by Lehn and Wipff[159] in 1976 shows that although the equatorial-like carbanion of $^{-}CH_2OH$ is much more stable than its axial-like carbanion due to stronger electron-nuclear attractions, the barriers for rotation about the C-O bond and inversion from axial- to equatorial-like carbanions are

very large (Scheme 8.7). They are 10.6 and 20.5 kcal/mol respectively for the $^{-}CH_2OH$ species.[160]



Scheme 8.7

The angle strain contributes to the energy barrier for the glycosyl anion **105** to convert from α to β configurations (Scheme 8.7). In the interconversion, **105** has to go through a transition state in which the anomeric carbon center is planar. Such a conversion from a stable pyramidal geometry to an unfavorable planar one would require a large activation energy. The above arguments have been supported by Cohen's experiments.[162,163] In his studies, α-lithio ethers were prepared by reductive lithiation of α-phenylthio tetrahydrofurans or tetrahydropyrans by lithium 1-(dimethylamino) naphthalenide. Epimerization of α carbanions into β carbanions were recorded by

trapping with the electrophile benzaldehyde. The epimerization occurred only after the temperature was warmed up from –78°C to –30°C.

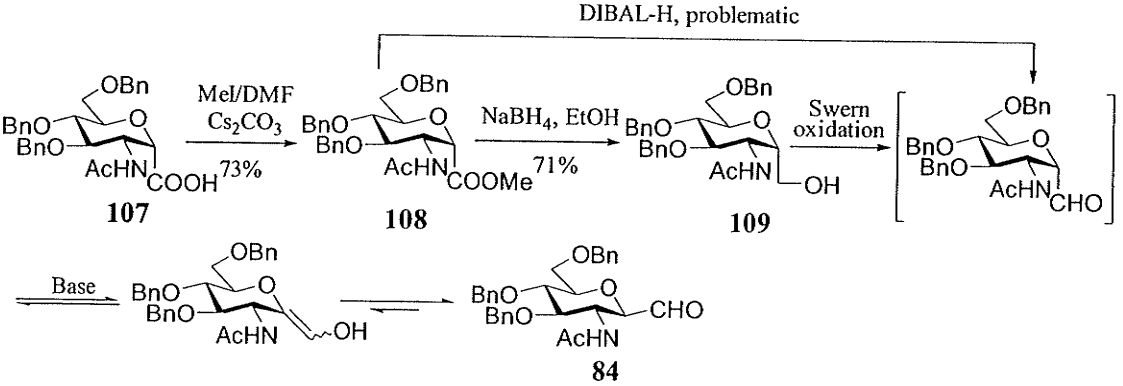
The other reason for kinetic control in reaction of glycosyl carbanions is that the solvent THF is a less favorable environment for carbanion interconversion due to its low dielectric constant. Retention of the anion configuration is often seen in solvents of low dielectric constant, while inversion occurs in solvents of high dielectric constant.[145]

As discussed in section 4.3, the interference of the 2-*N*-acetyl group to *C*-glycosylation of amino sugars is a serious problem. One purpose of using a dianion approach is to suppress such interference. Because the anomeric carbanion is far more nucleophilic than the amide anion, it added to carbon dioxide first. The amide anion might react with carbon dioxide as well due to the excess of carbon dioxide present in the reaction, but the newly formed carbamate would hydrolyse in the workup, giving back the *N*-acetyl group.[113]

The carboxylic acid **107** was methylated with methyl iodide and cesium carboxylate in DMF for 4 hours at room temperature, leading to the methyl ester **108** in 73% yield (Scheme 8.8). Reduction of **108** with sodium borohydride in ethanol gave the desired glycosyl alcohol **109** in 71% yield. Attempts to prepare **109** by addition of the dianion **105** to formaldehyde were unsuccessful. Wittman and coworkers also attempted to prepare the glycosyl alcohol by addition of the dianion from 3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride to formaldehyde, but they found that the formaldehyde was largely polymerized in the reaction and only a very small amount of the desired product was isolated.[164]

The glycosyl aldehyde **84** (uncharacterized) was prepared by Swern oxidation of **109** (Scheme 8.8). Because α glycosyl aldehydes are inclined to epimerize to the thermodynamically more stable β isomers,[165] **84** was expected to be a β glycosyl aldehyde. However, identification of the configuration of **84** was problematic. Aldehyde **84** could not be purified by chromatography, indicating that it might not be stable.

Petrusova and coworkers prepared a fully unprotected **84** in 1996 by a β *C*-glycosidic nitromethane approach.[154] They found that this aldehyde was readily oxidized by air. The ^{1}H NMR spectrum of one of the fractions from the chromatography showed a major doublet peak at 9.55 ppm with ^3J = 2.6 Hz and a minor broad singlet peak at 9.58 ppm. It also showed a 5:1 ratio of two separated NH peaks. ^{13}C DEPT experiments of this fraction showed only a CH peak at 197.8 ppm. These NMR data indicated that the glycosyl aldehyde was produced, but in a mixture of α and β isomers.



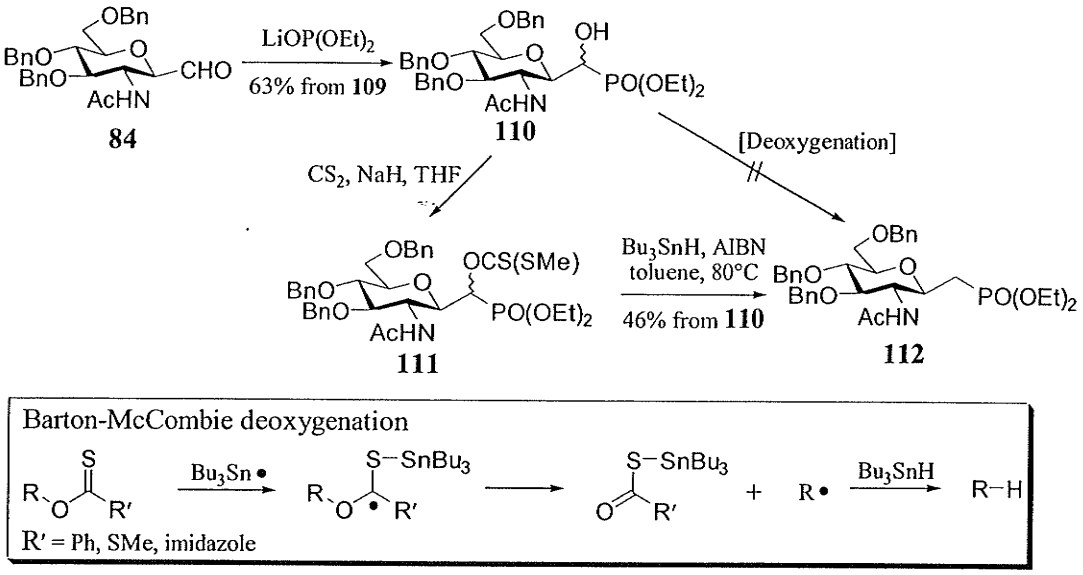
Scheme 8.8 Preparation of the glycosyl aldehyde **84**

It is worth noting that the β *C*-glycosidic nitromethane approach for **84** employed by Petrusova and coworkers[154] is more stereoselective and straightforward than our synthetic route. The reason why our approach was initially chosen is that the intermediates **108** and **109** were important starting compounds for other projects (see section 10.4.2.2 for **108** and section 10.4.2.4.1 for **109**) and the β *C*-glycosidic nitroethane approach did not produce both compounds.

Attempts to prepare **84** from the methyl ester **108** by reduction with diisobutylaluminum hydride (DIBAL-H) were unsuccessful because the reduction was difficult to control (Scheme 8.8). The reduction was very slow when the temperature was below 0°C, but when the temperature was raised to room temperature, a large amount of over-reduced product **109** was found in the reaction mixture.

8.3.1.2 Preparation of β *N*-acetyl *C*-glucosaminyl phosphonate

C-glycosyl 1-hydroxy phosphonate **110** was prepared by addition of a phosphite nucleophile, $LiOP(OEt)_2$, to aldehyde **84** (Scheme 8.9). Phosphonate **110** was obtained as a single isomer in 63% yield (from **109**). The NOE correlation of H_1 to $H_{2'}$ (2%) and a relatively large ^1H coupling constant $^3J_{1',2'}$ (7.0 Hz) in the NMR spectrum of **110** indicated that the phosphonate moiety of **110** occupied an equatorial position, and this compound was mostly in a $^4C_1$ conformation.



Scheme 8.9

Most of our attempts to remove the hydroxyl of **110** were unsuccessful. Reduction with Zn/HOAc, $NaBH_4$/TFA and SmI_2/Ac_2O did not produce the desired product **112**. Attempts to convert the hydroxyl of **110** into a halide or a thiocarbonyl imidazole derivative were also unsuccessful. Finally **110** was deoxygenated by the Barton-McCombie method (Scheme 8.9). Stirring of **110** with carbon disulfide, sodium hydride and methyl iodide in THF overnight at room temperature gave a thiocarbonyl derivative **111**. Reduction of **111** with tributyltin hydride in 80°C toluene for 20 min furnished the desired β *C*-glycoside **112** in 46% yield.

Barton and McCombie first employed thionobenzoate, *S*-methyl xanthate and thiocarbonylimidazolide derivatives for deoxygenation of carbohydrate secondary alcohols in 1975.[166] Since then, this method has been extensively applied in the preparation of deoxy sugars. The mechanism is shown in Scheme 8.9.[166] An α-stabilized thiocarbon radical is formed when thiocarbonyl derivatives are attacked by tributyltin radical. The thiomethyl group stabilizes the radical. β Scission of the radical followed by hydrogen transfer from the stannane affords the desired deoxygenated products.

This glycosyl aldehyde route is quite lengthy and inefficient (10 steps and less than 6% overall yield), and it would be difficult to scale up. In order to prepare the target compound **50** in a more efficient way, the lactone approach was chosen as the next strategy.

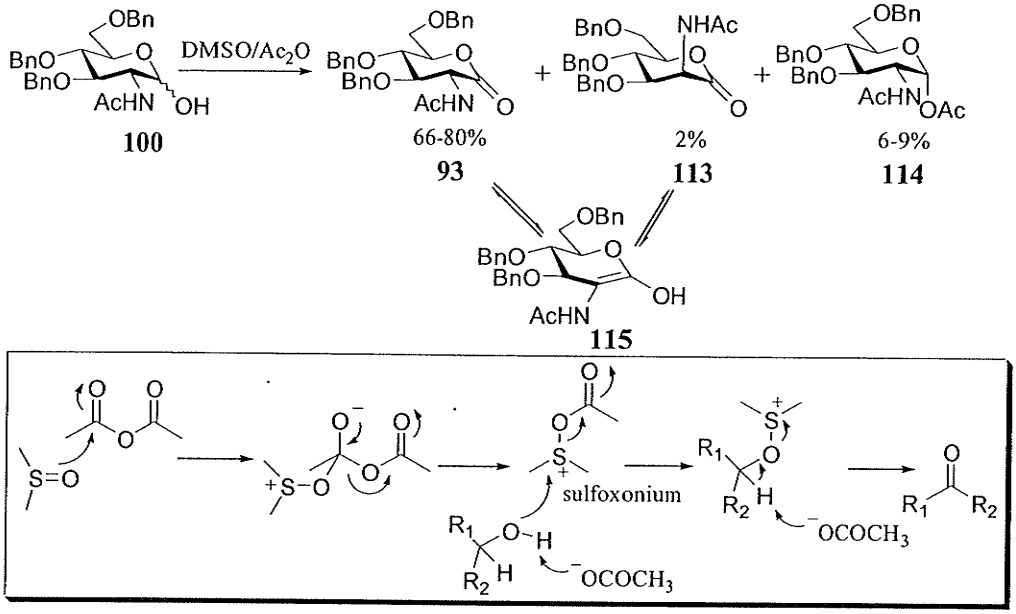
8.3.2 Gluconolactone strategy

8.3.2.1 Preparation of the gluconolactone 93

Gluconolactone **93** was first prepared by Pravdic and Fletcher in 1971 by oxidation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose **100** with Ac_2O/DMSO in 92% yield.[167] In 1996, Ayadi and coworkers prepared **93** by oxidation of **100** with TPAP/NMO in 90% yield.[168] However, this research group reported in 1999 that a pure sample of **93** had not been obtained, part of the product being epimerized to a *manno* lactone.[101] They proposed an alternative route to **93**, in which an azido group was used as a masking group of the acetamido group. However, hydrogenation of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluco-1,5-lactose with Raney Ni in Ac_2O only generated 50% of **93**, the rest of the product was epimerized to the *manno* lactone. Based on the above information, Pravdic's method was chosen for preparing **93**.

Glycose **100** was dissolved in a mixture of DMSO and Ac_2O, and the solution was kept overnight at room temperature (Scheme 8.10). Unlike Pravdic's exclusive production of **93**, *gluco* lactone **93** (66-80%), *manno* lactone **113** (about 2%) and acetyl glycoside **114** (6-9%) were obtained in our experiments. The *manno* lactone **113**

probably came from the *gluco* lactone **93** by tautomerization through the intermediate **115**. On one occasion, the solvents were evaporated by raising the temperature to 80°C. 34% of the *manno* lactone **113** was isolated from that experiment, indicating that the acetamido group of **93** was prone to epimerization.

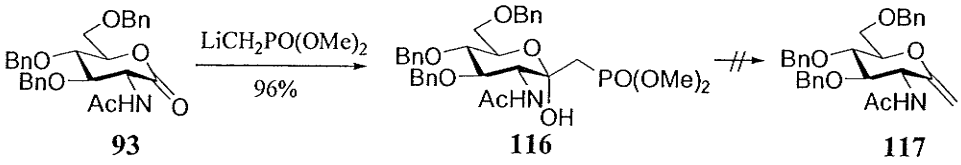


Scheme 8.10 Preparation of the lactone **93**

Oxidation of alcohols by Ac_2O/DMSO was first reported by Albright and Goldman in 1967.[169] In their work, yohimbine was oxidized to the ketone in 85% yield at room temperature in 24 hours. A mechanism for oxidation of secondary alcohols is shown in Scheme 8.10. The key step in the mechanism is the formation of a sulfoxonium species, which is a strong oxidizing agent.

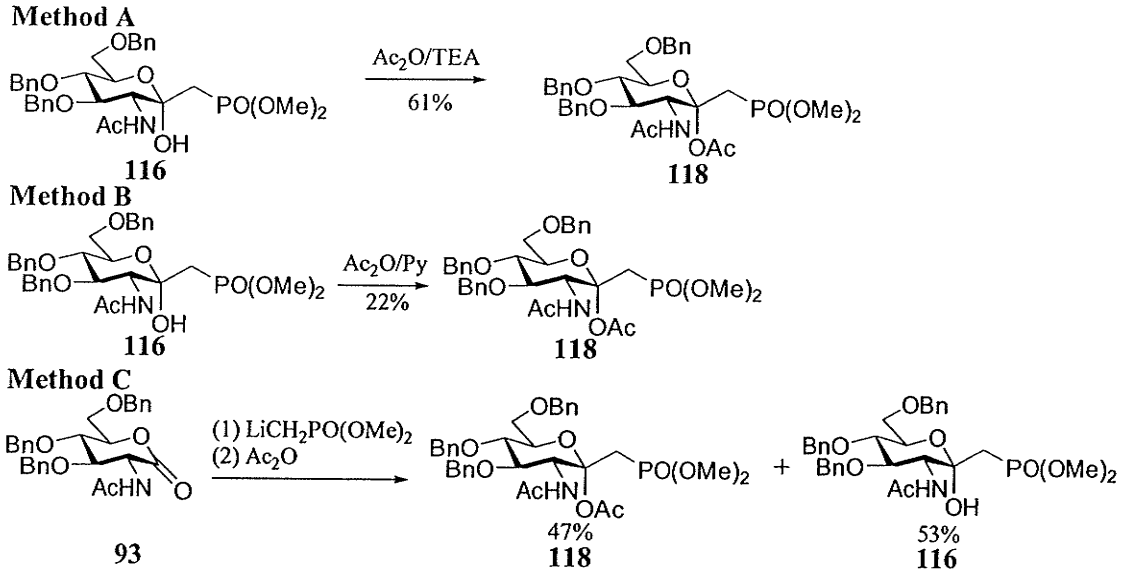
8.3.2.2 Preparation of β *N*-acetyl *C*-glucosaminyl phosphonate

Addition of the phosphonate nucleophile, $LiCH_2PO(OMe)_2$, to lactone **93** gave the 2-hydroxy phosphonate **116** (also called a keto phosphonate) in 96% yield (Scheme 8.11). This reaction was highly stereoselective and only a single diastereomer was isolated. The hydroxyl in **116** preferred an α configuration due to the anomeric effect. Although **116** was an intermediate of the Horner-Emmons reaction, it was very stable. It would not decompose to the olefin **117** even when it was heated to 100°C.



Scheme 8.11

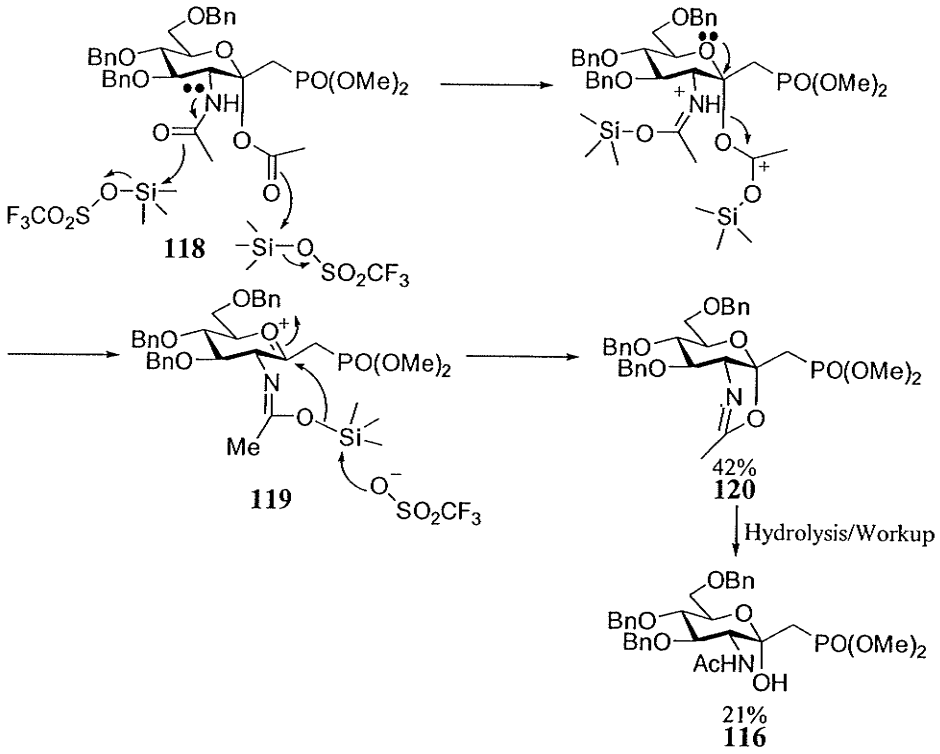
Although direct deoxygenation of the anomeric hydroxyl with ionic reduction was feasible for other *C*-glycosides,[142,170] this method was not successful applied to **116**. Attempted reductions of **116** with $Et_3SiH/BF_3{\cdot}Et_2O$, Et_3SiH/TFA, Et_3SiH/TMSOTf, Ph_3SiH/Et_2AlCl and $NaBH_4$/TFA did not yield the desired product. Ionic reduction has also been found not to be applicable to the *C*-glycosides 3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-dimethoxyphosphoryl-α-D-manno-2-heptulopyranose[142] and 3,4,5,7-tetra-*O*-benzyl-1-benzyl-1-deoxy-α-D-gluco-2-heptulopyranose,[71] indicating that our results are not unprecedented.



Scheme 8.12

The anomeric hydroxyl of **116** was acetylated. Three acetylation methods were tried (Scheme 8.12). The first one was to treat **116** with Ac_2O and triethylamine, giving the desired acetylated product **118** in 61% yield. The unreacted starting compound (39%) was recovered from the reaction. The second reaction used pyridine as the base for the

acetylation, producing only 22% of **118**. The third method was to add Ac_2O into the reaction of **93** with $LiCH_2PO(OMe)_2$ during the workup, isolating 47% of **118** and 53% of the un-acetylated compound **116**. Comparing these three methods, acetylation with Ac_2O/TEA was the best one.

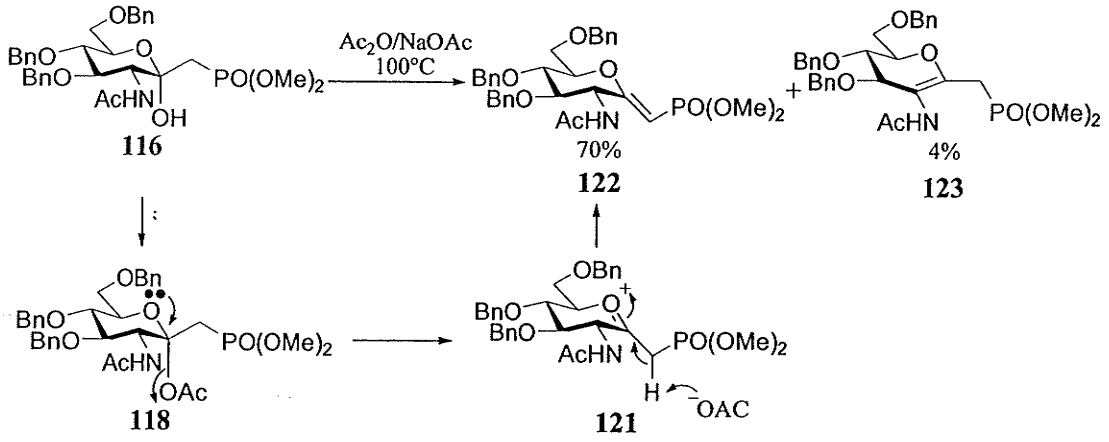


Scheme 8.13

C-glycosides have reportedly been prepared by deoxygenation of anomeric acetates using ionic reduction methods such as Et_3SiH/TMSOTf.[171] The reduction occurs when a hydride is transferred from the Si-H bond to carbocations that are formed in the presence of TMSOTf.[172] However, reduction of **118** with TMSOTf/Et_3SiH did not produce the deoxygenated product, but instead, an oxazoline **120** and a deacetylated product **116**, were isolated in 42% and 21% yields respectively, along with the unreacted **118**. A possible reaction mechanism is proposed in Scheme 8.13. It is believed that the amide and ester moieties of **118** readily react with TMSOTf at room temperature, forming eniminium and dioxonium ions respectively.[173] An oxocarbenium **119** is generated by cleavage of the axial dioxonium, and cyclizes to the oxazoline **120** before accepting a hydride. The oxazoline **120** is very resistant to ionic reduction. Treatment of

120 with Et_3SiH/TFA or Ph_3SiH/TfOH did not yield any reduced product. The deacetylated product **116** probably came from hydrolysis of **120** during the workup. Another attempted deoxygenation of **118**, with $Zn(BH_4)_2$/TMSOTf, generated 59% of **120** and 41% of **116**. The configuration of **120** was confirmed by NOE experiments since no NOE correlations were found between H_1 and $H_{2'}$ or H_1 and $H_{5'}$.

Although $SOCl_2$/pyridine and methyl oxalyl chloride/pyridine have reportedly been successful in transforming anomeric hydroxyls to α,β-unsaturated phosphonates,[28] treatment of **116** with $SOCl_2$/pyridine, oxalyl chloride/pyridine or $POCl_3$/pyridine led to complex mixtures that could not be fully identified. Treatment of **116** with sodium acetate in Ac_2O at 100°C for 2 h generated the olefin **122** in good yield (70%), along with a minor byproduct **123** in 4% yield. No oxazoline byproduct was found in this reaction. The mechanism for formation of **122** can be explained by the Scheme 8.14. The acetylated derivative **118** is probably generated first because a large amount of it was isolated in the early stages of the reaction. The oxocarbenium **121** is formed from **118** by cleavage of the acetyl group. Then **121** is converted to **122** by losing a proton. The oxocarbenium **121**, unlike the oxocarbenium **119** in Scheme 8.13, does not cyclize because there is not a base present in the reaction mixture to activate the acetamido group. When the reaction temperature was lower than 100°C or the stronger base, K_2CO_3, was used, compound **122** was obtained in a lower yield.



Scheme 8.14 Preparation of the α,β-unsaturated phosphonate **122**

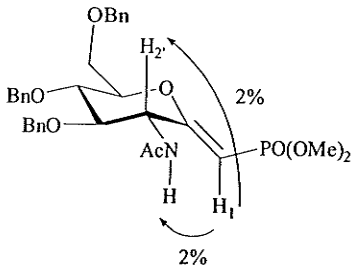


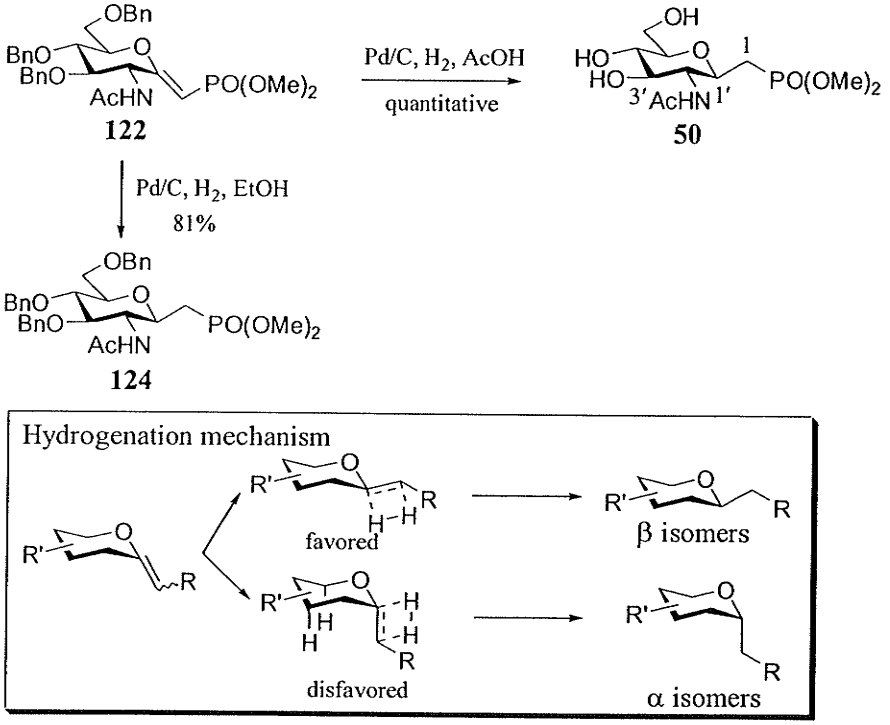
Figure 8.2 NOE correlations of the olefin **122**

The configuration of **122** was determined by NOE correlation experiments (Figure 8.2). The strong correlations of H_1 with both $H_{2'}$ and N-H indicated that the phosphonate moiety was *cis* to the ring oxygen.

Hydrogenation of **122** with Pd/C as catalyst in EtOH in the presence of a catalytic amount of acetic acid selectively reduced the olefin, and left the benzyl ethers intact, affording the product **124** in 81% yield (Scheme 8.15). Hydrogenation of **122** in glacial acetic acid with Pd/C as catalyst removed the benzyl protecting groups as well as reduced the alkene, quantitatively furnishing the desired final product **50**. Both hydrogenations were highly stereoselective, and only one diasteromer was isolated. The conformation and configuration of **124** and **50** were assigned based on the ^1H coupling constants between 1′- and 2′-protons. The dihedral angle dependence of the coupling constants between protons on vicinal carbon atoms is given by the Karplus correlation.[175] The observed coupling constants for axial-axial vicinal protons in carbohydrates are 8-10 Hz, and axial-equatorial and equatorial-equatorial proton coupling constants are 2-3 Hz.[176] The large ^1H coupling constants $J_{H1'-2'}$ 9.7 Hz for **124** and 8.7 Hz for **50** found in their NMR spectra indicated that both of them were in the β anomeric configuration and the $^4C_1$ conformation.

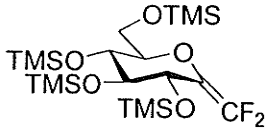
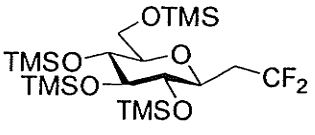
The mechanism accounting for the hydrogenation stereoselectivity is not completely clear, because the heterogeneous hydrogenation (catalysts like Pd/C are insoluble in the reaction medium) is not well understood.[177] A thorough literature search shows that hydrogenation of pyranosyl *exo*-glycals gives exclusively β *C*-glycosides

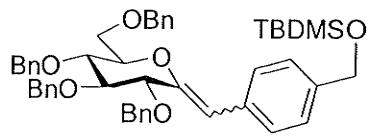
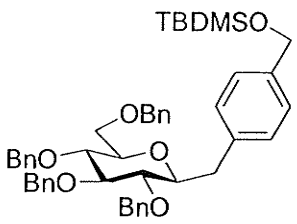
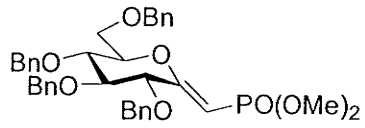
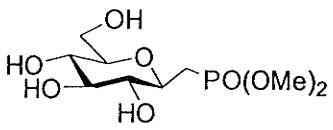
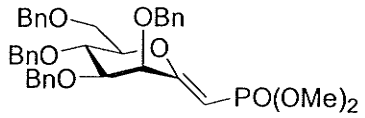
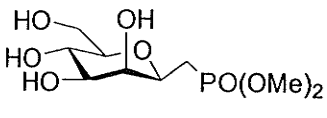
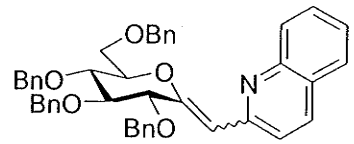
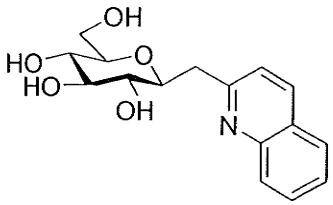
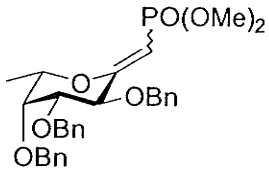
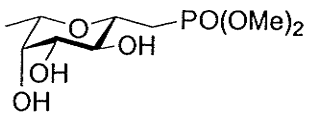
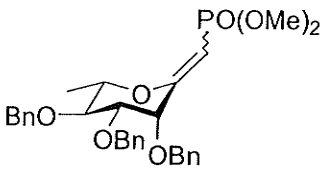
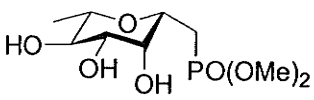
regardless of their structures (Table 8.1). It is known that in most cases, both hydrogen atoms are added to the double bond in a *syn* manner.[172] There would be two possible transition states formed in the reduction of **122**, hydrogen approaching from either the top face of the double bond or from the bottom of the double bond (Scheme 8.15). Addition from the top face would be disfavored due to 1,3-diaxial interactions. Therefore, only the β anomeric products are produced in the hydrogenation of these compounds.



Scheme 8.15 Hydrogenation of olefin **122**

Table 8.1 Hydrogenation of pyranosyl *exo*-glycals

Starting Compounds	Catalyst	Products	Yield	Ref.
	Pd/C		78%	178

	Pd 5% on alumina		95%	179
	$Pd(OH)_2$		NA	142
	$Pd(OH)_2$		95%	142
	Pd/C		NA	180
	Pd/C		Quant.	28
	Pd/C		NA	28

NA: not available

The overall yield of **50** from the glycose **100** was 44%. Compared with the aldehyde method, this lactone approach is much more efficient.

8.4 Concluding remarks

β *N*-acetyl *C*-glucosaminyl phosphonate **50**, the analogue of *N*-acetyl D-glucosamine 1-phosphate could be prepared from either the aldehyde or the gluconolactone strategies. But the aldehyde approach gave lower yields of the products. In contrast, the gluconolactone approach successfully produced the target molecule through an olefin intermediate in good yield.

Chapter 9

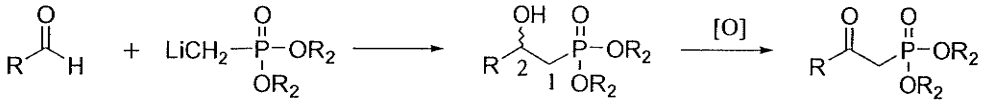
Synthesis of Analogues of α *N*-Acetyl Glucosamine Pyrophosphate

9.1 Introduction

This chapter will describe the synthesis of α *C*-glycosyl 2-hydroxy and 2-keto phosphonates **51** and **52** as analogues of α *N*-acetyl D-glucosamine pyrophosphate. Preparation of 2-hydroxy and 2-keto phosphonates could start from a wide range of starting compounds such as esters, aldehydes and epoxides. This chapter will examine the feasibility and efficiency of these strategies.

9.2 Synthetic strategies

The key step in the preparation of phosphonate analogues **51** and **52** is expected to be the introduction of the phosphonate moiety into the *N*-acetyl D-glucosamine fragment. There are three strategies.

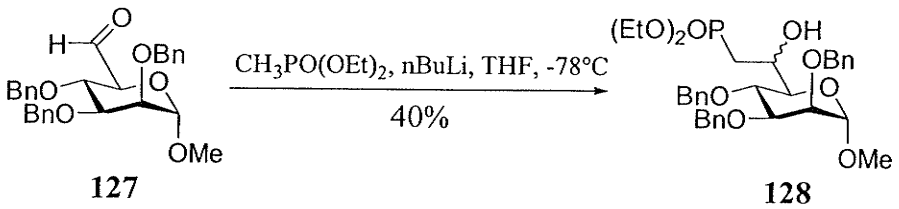


125 **126** **51** **52**

Scheme 9.1 The aldehyde approach

The first strategy is an aldehyde approach, shown in Scheme 9.1. Addition of a phosphonate nucleophile **126** to the glycosyl aldehyde **125** would generate a 2-hydroxy phosphonate **51**, and the keto phosphonate **52** would be obtained by oxidation of **51**. The preparation of 2-hydroxy phosphonates from aldehydes is a common method for preparing Horner-Emmons reagents.[181,182] Although it is an intermediate of the Horner-Emmons reaction, **51** is very stable because it does not have an electron-withdrawing group at C-1. Such a structural feature would disfavor elimination, because elimination normally requires a decreased negative charge at C-1.[182] The only example of this

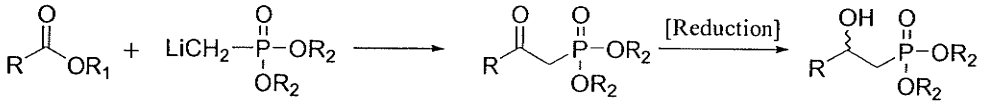
approach in carbohydrate chemistry is the synthesis of D-mannose 6-phosphate analogue **128** done by Vidal and coworkers in 2000 (Scheme 9.2).[183] Addition of $LiCH_2PO(OEt)_2$ to aldehyde **127** gave **128** in 40% yield.



127 **128**

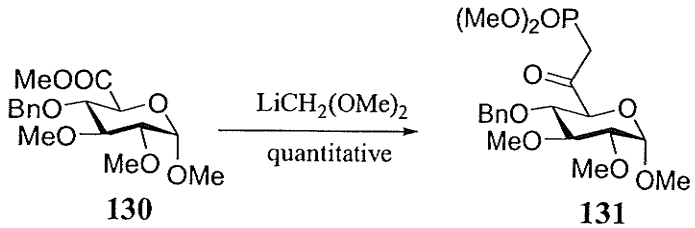
Scheme 9.2

The second strategy begins with a sugar ester **129** (Scheme 9.3). Addition of the phosphonate nucleophile **126** to a glycosyl ester **129** would give the 2-keto phosphonate **52**. Reduction of **52** would then generate the 2-hydroxy phosphonate **51**. A few 2-keto phosphonate sugars have been prepared by this method.[184-187] One of the examples is **131** prepared by Narkunan and Nagarajan in 1994 (Scheme 9.4).[184] Addition of **126** to the sugar methyl ester **130** produced **131** in quantitative yield.



129 **126** **52** **51**

Scheme 9.3 The ester appoach

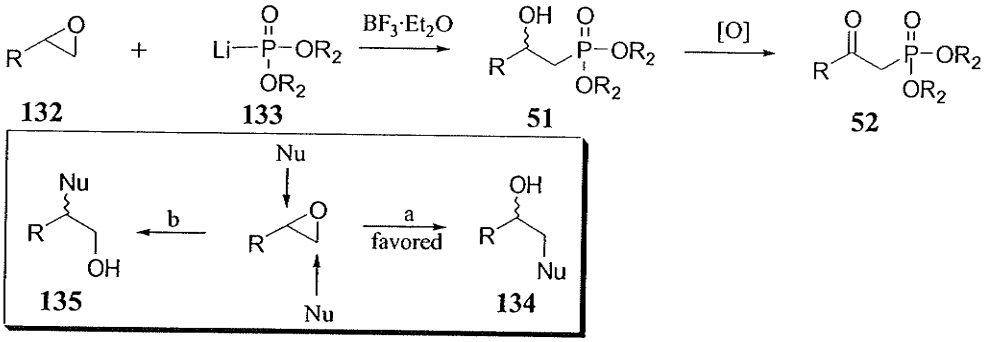


130 **131**

Scheme 9.4

The third strategy starts from a sugar epoxide **132** (Scheme 9.5). Addition of a phosphite nucleophile **133** to the glycosyl epoxide **132** in the presence of $BF_3 Et_2O$ would generate the 2-hydroxy phosphonate **51**. Oxidation of **51** would produce the 2-keto

phosphonate **52**. The addition step has been systematically studied by Li and coworkers.[188] $BF_3 Et_2O$ is essential for the addition, presumably as it is able to promote the opening of epoxides by nucleophiles.[189] Without $BF_3 Et_2O$, the addition would require harsh conditions such as refluxing in NaOEt/EtOH and lead to poorer yields.[190] A lot of functionalities, such as benzyl ethers, bromides and carbamates, can survive the reactions when $BF_3 Et_2O$ is used as catalyst.[188] There are two possible products generated in addition of the nucleophiles to **132**. One comes from the attack at the terminal site of the oxirane ring, the other from the attack at the most substituted site of the oxirane ring. The experiments done by Li and coworkers show that this reaction is highly regiospecific and the nucleophiles always attack at the least hindered site of the oxirane ring due to steric effects.[188] However, this method has not been applied to the preparation of sugar phosphonates.



Scheme 9.5 The epoxide approach

In this Ph.D. research, these three strategies for the preparation of **51** and **52** were all evaluated.

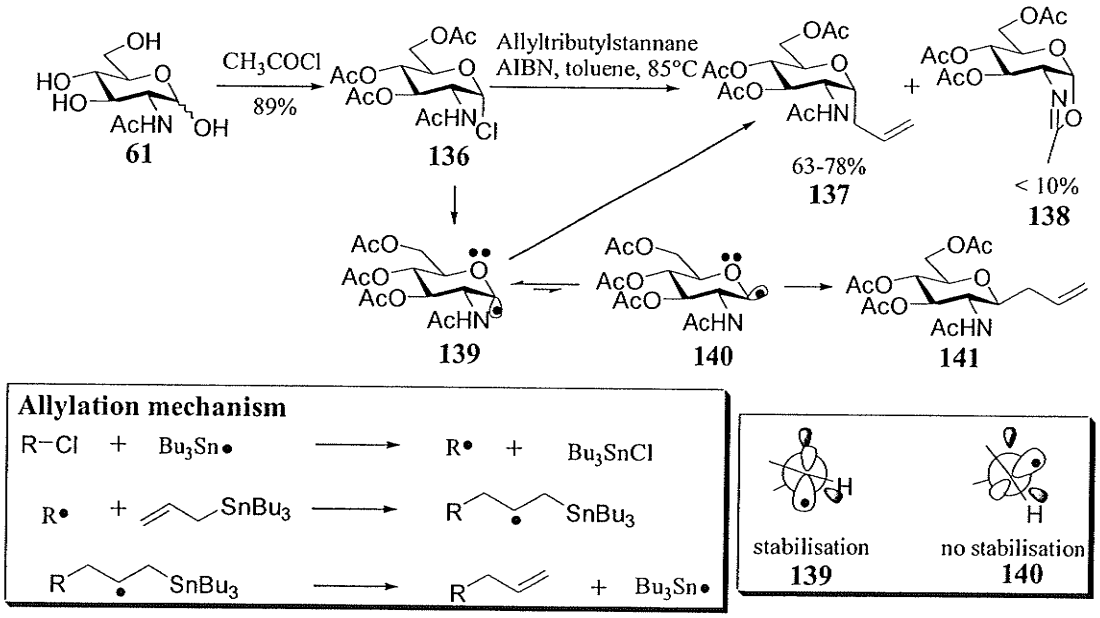
9.3 Results and discussion

9.3.1 The aldehyde approach

The aldehyde approach started with the preparation of an allyl *N*-acetyl *C*-glucosamine **137**, which was synthesized by following a procedure described by Bertozzi[191] and Horton (Scheme 9.6).[103] The readily available amino sugar **61** was converted to the glycosyl chloride **136** by stirring in acetyl chloride at room temperature

overnight. The chloride **136** was recrystallized from ether, giving a colorless solid in 89% yield.[192] The rest of the product was peracetyl *N*-acetyl glucosamine. Compound **136** was not stable and gradually decomposed over time when open to the air. Thus, it was freshly prepared only when it was needed.

The chloride **136** was suspended in a toluene solution of allyltributylstannane and AIBN (radical initiator). The mixture was stirred at 85°C for 3 hours. 63-78% of the α allyl *C*-glycoside **137** and about 10% of the oxazoline byproduct **138** were isolated. The rest of the product was probably polymerized.[103] Our results are in good agreement with those of Bertozzi and Horton.[103,191] A general allylation mechanism is shown in Scheme 9.6.[193] The chloride is abstracted by the tributyltin radical, generating a radical R: The radical R· then adds to allyltributylstannane. Fragmentation of the adduct generates the allyl compound and the tributyltin radical.

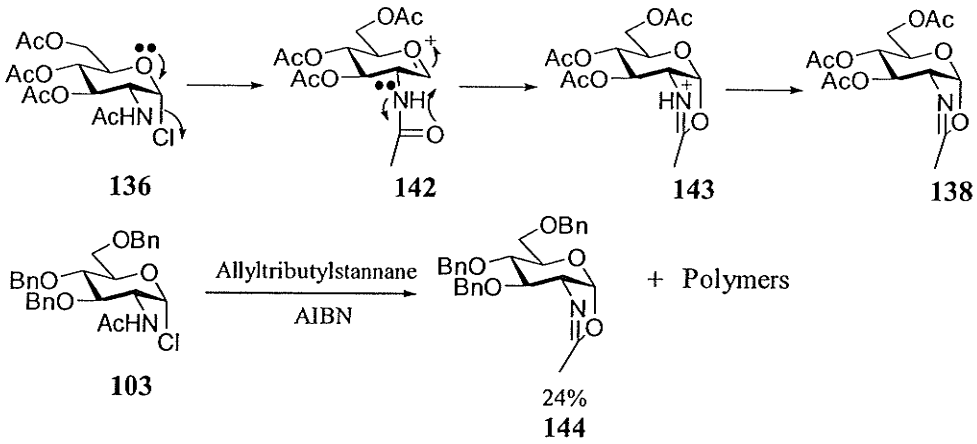


Scheme 9.6

The significance of this allylation is its stereoselectivity. Bertozzi obtained a 10:1 ratio of α/β *C*-glycoside isomers **137** and **141**.[191] Both Horton[103] and we, were unable to isolate the β isomer **141**. It is well known that the unpaired electron in most carbon-centered radicals occupies an orbital that has mainly *p* character, allowing attack from

both sides.[126] In order to achieve high stereoselectivity, the radicals have to either react from a preferred conformation or be attacked predominantly from one side.[194] Both experimental data and molecular modeling show that the conformation of a radical like **139** is strongly influenced by stereoelectronic effects.[194] Just like the anomeric effect in *O*-glycosides discussed in section 4.4, the unpaired electron at the anomeric carbon center is stabilized by the ring oxygen and prefers to adopt an axial conformation both in the ground state and the transition state. During the axial attack at radical **139**, the single electron involved in forming the new bond and the electron pair at the ring oxygen remain in one plane so that a stabilizing interaction can occur (Scheme 9.6).[194,195] Therefore, the glycosyl radical **139** reacts predominately at the axial position.

The cyclization of **136** leading to the oxazoline byproduct **138** is believed to go by an oxocarbenium mechanism (Scheme 9.7).[103] At elevated temperatures, the cleavage of the Cl-C bond is driven by the kinetic anomeric effect, generating the oxocarbenium ion **142**. Intermediate **142** is then converted to a more stabilized ion **143** by neighboring group participation. Ion **143** gives the oxazoline **138** by losing a proton.

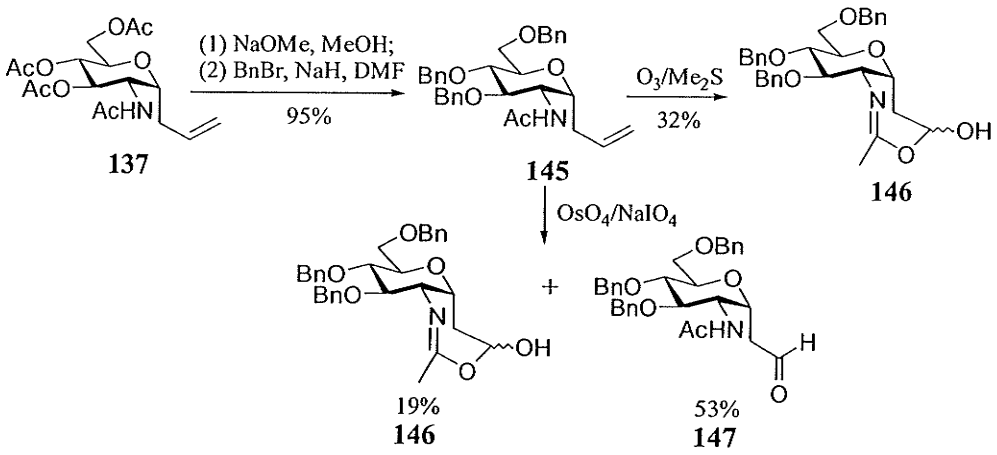


Scheme 9.7

Attempts to prepare **137** from a benzylated glycosyl chloride **103** (its preparation is in section 8.3.1.1) by reaction with allylbromide using the dianion approach were unsuccessful. The reaction produced complex products that could not be purified and identified, probably due to polymerization. Attempts to prepare the allyl product by

radical coupling of **103** with allyltributylstannane were not successful, either (Scheme 9.7). The reaction gave 24% of the oxazoline byproduct **144**. The other products could not be purified and identified, probably due to polymerization as well.

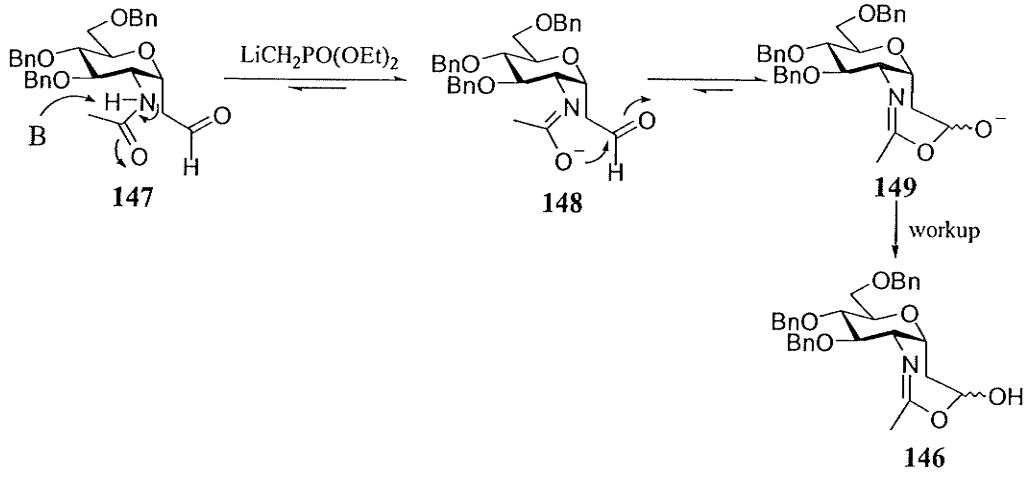
Olefin **137** was converted to the benzylated compound **145** in 95% yield by removal of the acetyl groups by methanolysis, followed by benzylation with NaH/BnBr (Scheme 9.8). The olefin **145** was initially oxidized with ozone at −78°C, giving the cyclized product **146** in 32% yield. The other products could not be purified or identified. A free aldehyde **147** was obtained in 53% yield, along with 19% of the cyclized product **146**, when **145** was oxidized by $NaIO_4/OsO_4$, a procedure described by Grugier and coworkers.[35] Grugier and coworkers also obtained a mixture of **146** and **147** from this reaction.



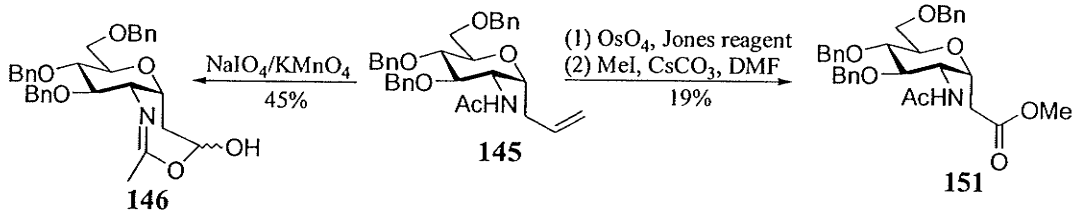
Scheme 9.8

Addition of the phosphonate anion, $LiCH_2PO(OEt)_2$, to aldehyde **147** was unsuccessful (Scheme 9.9). The reaction gave exclusively the cyclized product **146**. The phosphonate anion probably served as a base in the reaction instead of as a nucleophile because it is more basic than the amide anion. The pKa value of a phosphonate is about 27 in DMSO and the pKa for amides is about 25 in DMSO.[115] Deprotonation of the acetamido group of **147** would generate the anion **148**. Intramolecular addition of the anion to the aldehyde would produce the alkoxide **149**. The equilibrium would favor **149** because the shift was driven by the conversion of a highly basic amide anion to a less

basic alkoxide anion. Since the aldehyde method failed to produce the desired 2-hydroxy phosphonate, the ester approach was pursued.



Scheme 9.9



Scheme 9.10

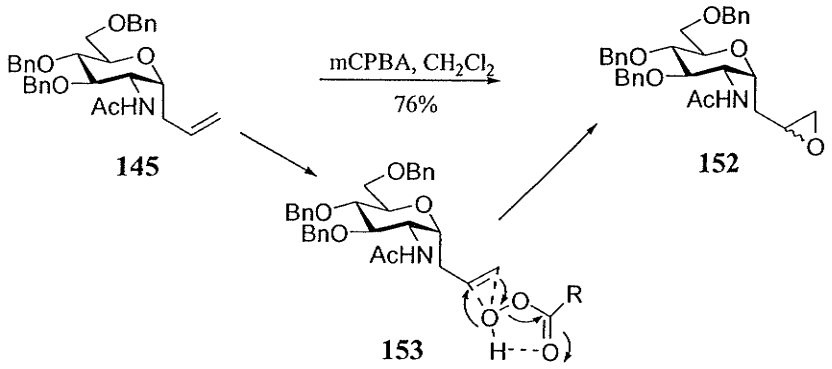
9.3.2 The ester approach

The ester approach was as problematic as the aldehyde approach. Oxidation of the double bond of **145** with $NaIO_4/KMnO_4$ gave 45% of the cyclized lactol **146** and other unidentified products. Oxidation of **145** with $KMnO_4$/acetone, $KMnO_4/CuSO_4$ and $KMnO_4/Bu_4NBr$ failed to produce any of the desired carboxylic acid products. The best approach that has been found so far was oxidation with OsO_4/Jones reagent followed by methylation with methyl iodide and cesium carboxylate (Scheme 9.10). The reaction gave 19% of the desired methyl ester **151**. Addition of the phosphonate nucleophile, $LiCH_2PO(OEt)_2$, to **151** failed to generate the desired 2-keto phosphonate product. The addition did not occur at room temperature, and led to unidentified products at elevated

temperatures. Since the ester method also failed to give the desired 2-keto phosphonate product, the epoxide approach was pursued.

9.3.3 The epoxide approach

The most general way to prepare an epoxide is by epoxidation of an alkene with a peroxycarboxylic acid such as *m*-chloroperoxybenzoic acid.[161] Stirring **145** in CH_2Cl_2 with *m*CBPA at 0°C for 4 hours gave a 1:1 diasteromeric mixture of epoxides **152** in 76% yield (Scheme 9.11).

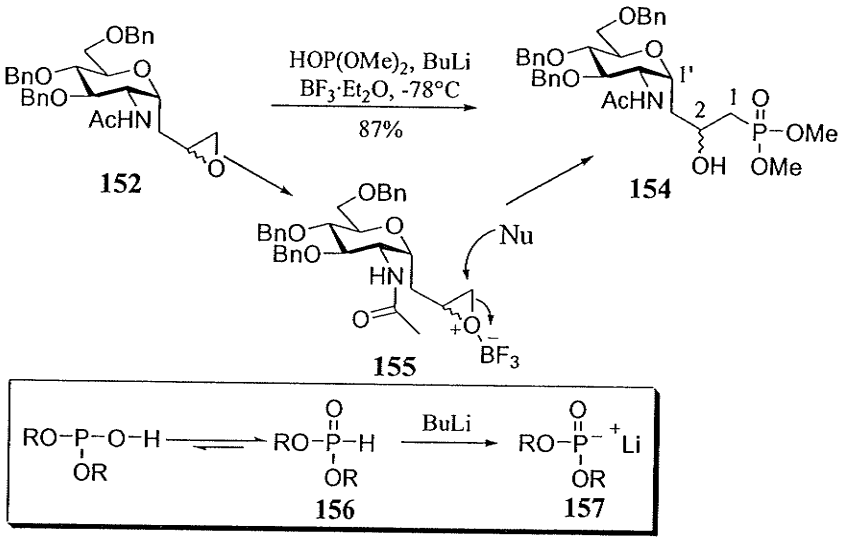


Scheme 9.11

Opening epoxides with a phosphite nucleophile has been studied by Li and Azuhata.[188,197] Their results show that Lewis acid $BF_3 Et_2O$ is essential for the addition. Other Lewis acids such as zinc iodide, zinc, iron(III), tin(II) and tin(IV) chlorides require a special phosphite nucleophile $TMSP(OR)_2$, but for $BF_3 Et_2O$, the simple phosphite $(RO)_2POH$ is satisfatory.[188] The 2-hydroxy phosphonate **154** was prepared based by Li's procedure (Scheme 9.12).

BuLi was added dropwise to a THF suspension of diethyl phosphite at –78°C. It is known that dialkyl phosphites are in tautomeric equilibrium with their phosphoryl forms **156**, with more than 95% of dialkyl phosphites being in the phosphoryl forms.[198] Therefore, the ion **157** was formed when diethyl phosphite was deprotonated. After stirring for 20 min, epoxide **152** and $BF_3 Et_2O$ were added to the reaction. The reaction solution was stirred 30 min at –78°C and 1 hour at room temperature. The desired 2-

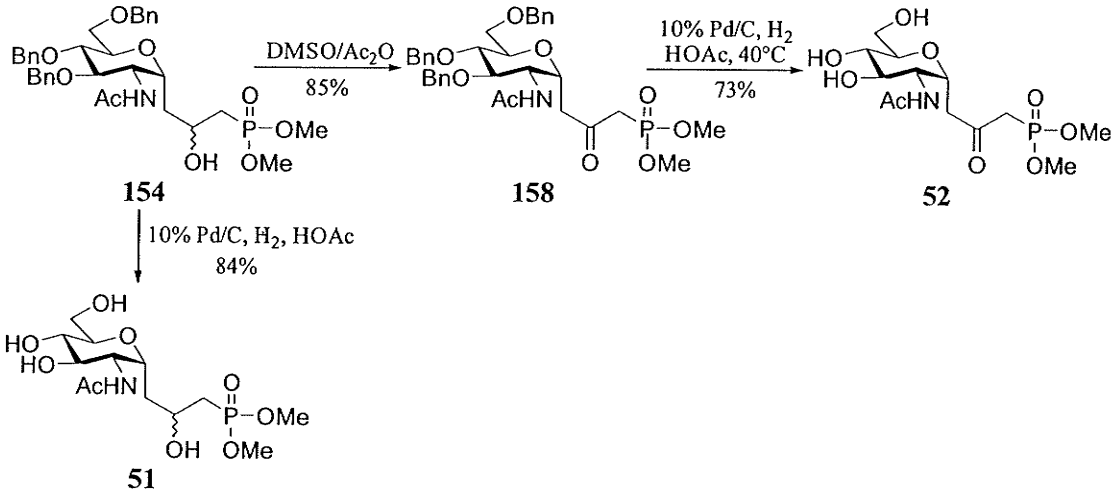
hydroxy phosphonate **154** was obtained as a 1:1 diastereomeric mixture in 87% yield. These stereoisomers could not be separated by chromatography. Two CH carbon signals appeared at 63.4 and 66.0 ppm with C-P coupling constants $^{2}J_{C\text{-}P}$ about 4 Hz in their ^{13}C NMR spectra indicated that, these signals arose from C-2 carbons of the two diastereomers. This confirmed that the phosphite nucleophile had attacked the epoxide at the terminal site, otherwise the C-2 signals of the adduct would have beeen located much further upfield.



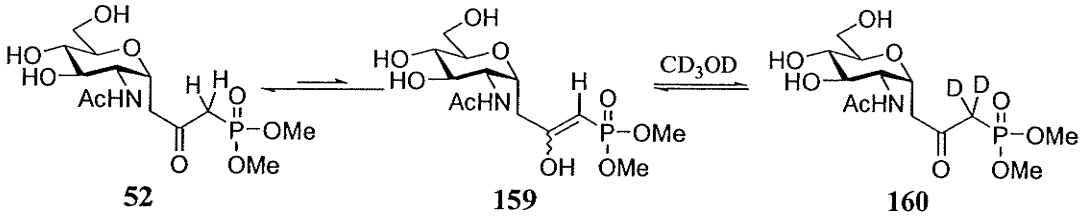
Scheme 9.12

One of the greatest challenges in manipulating the anomeric side chain of 2-acetamido *C*-glycosides is the interference of the amide group. Such interference was encountered in the reduction of **118** (Scheme 8.13) and the preparation of 2-hydroxy phosphonate using the aldehyde approach (Scheme 9.9). Nicotra and coworkers also experienced such problems in their synthesis of *C*-glycosyl phosphonates.[25,105] Surprisingly, the acetamido group in **152** did not add to the epoxide although the electrophilicity of the epoxide was significantly increased by the Lewis acid $BF_3\,Et_2O$ (Scheme 9.12). This may be because the reaction medium was neutral and the acetamido group could not be activated.

Deprotection of **154** by hydrogenation over Pd/C at room temperature afforded the *N*-acetyl α-D-glucosamine diphosphate analogue **51** in 84% yield (Scheme 9.13). Oxidation of **154** with Ac_2O/DMSO gave 2-keto phosphonate **158** in 85% yield. Removal of the benzyl protecting groups from **158** by hydrogenation at 40°C afforded the 2-keto analogue **52** in 73% yield. The hydrogenation of **158** was more sluggish than the corresponding reaction of **154**, and did not proceed to any significant extent at room temperature.



Scheme 9.13



Scheme 9.14

The ketophosphonate **52** was observed to undergo rapid deuterium/hydrogen exchange at the C-1 position when NMR spectra were aquired in deuteriomethanol solution (Scheme 9.14). Neither the protons nor the carbon at this position were observed in the ^1H or ^{13}C NMR spectrum. However, the signals from C-1 could be observed by ^{13}C NMR when the spectrum was aquired in a 5:1 $CDCl_3/CH_3OH$ solution. A strong CH_2

signal at the C-1 position obtained by DEPT experiments (no obvious CH signal was recorded at C-1) indicated that the keto form was favored over the enol form. These observations suggest that **52** may be able to access either tautomeric form as needed to interact with a glycosyltransferase active site.

9.4 Concluding remarks

Three strategies - the ester, aldehyde and epoxide approaches – were explored to prepare the *C*-glycosyl analogues of α *N*-acetyl D-glucosamine pyrophosphate **51** and **52**. The first two methods have failed to produce the target molecules. The aldehyde approach failed because the aldehyde intermediate was easily converted to its cyclic form, thus losing its electrophilicity toward the phosphonate nucleophile. The problem with the ester approach was that the preparation of the ester intermediate was extremely difficult, and that the reaction of the ester with a phosphonate nucleophile did not result in the desired 2-keto phosphonate product.

The epoxide approach has successfully led to the target molecules. Addition of the phosphite nucleophile to the epoxide intermediate regioselectively gave the 2-hydroxy phosphonate. Removal of the benzyl protecting groups furnished the final product **51** in good yield. The 2-keto phosphonate was prepared by oxidation of the 2-hydroxy phosphonate with DMSO/Ac_2O.

Chapter 10

Synthesis of Analogues of the α-L-Rha-(1→3)-α-D-GlcNAc Linker

10.1 Introduction

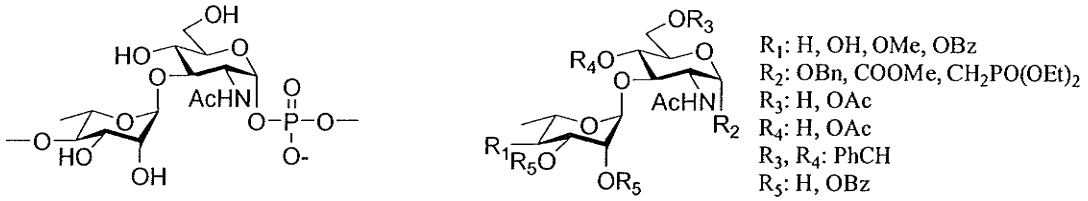
This chapter will describe the design and synthesis of analogues of the α-L-Rha-(1→3)-α-D-GlcNAc linker as potential inhibitors of the mycobacterial glycosyltransferases. The design was based on this linker's structure. These disaccharide analogues were synthesized using a thioglycoside strategy. Seventeen analogues were tested in a collaborator's laboratory using an *in vitro* glycosyltransferase assay. Three of them showed inhibitory activity.

10.2 Design of the analogues

The α-L-Rha-(1→3)-α-D-GlcNAc linker provides a novel structure for design of analogues. Three sites of this linker were modified, shown in Figure 10.1. The first site was C-4′ on the L-rhamnopyranose moiety. This site is very important because the mycobacterial glycosyltransferases use this site to link to the AG. This site could be blocked using an OMe or OBz substituent, or simply by deoxygenation of C-4′. The purpose of choosing various substituents was to test which substituent would lead to active inhibition.

The second site was C-1 on the *N*-acetyl D-glucosamine moiety. This site is also very important because it is used by the mycobacterial glycosyltransferases to link to the peptidoglycan through a phosphate linker. This site was modified by a variety of substituents. The first one was OBn. OBn would prevent the mycobacterial glycosyltransferases from using it as a glycosyl donor. The second and third substituents were COOMe and $CH_2PO(OEt)_2$. Both substituents are *C*-glycosides and close mimics of

the phosphate moiety. The third would be a hydroxyl. It would be interesting to see how a reducing end at C-1 affects the activity.



α-L-Rha-(1→3)-α-D-GlcNAc linker

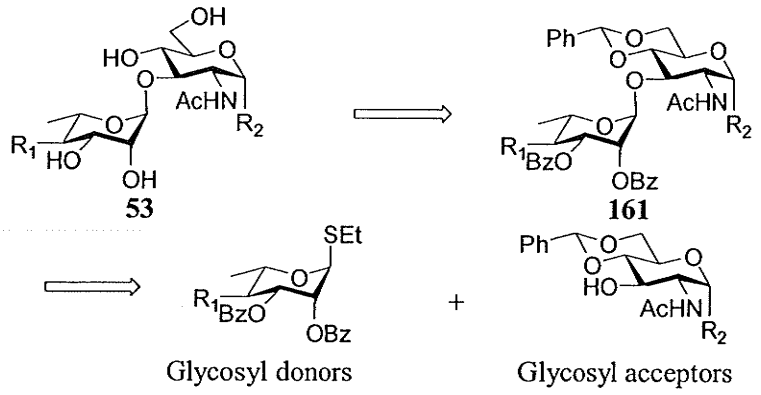
Analogues of the α-L-Rha-(1→3)-α-D-GlcNAc linker

Figure 10.1 Analogues of the α-L-Rha-(1→3)-α-D-GlcNAc linker

The third modification site was the hydroxyls of both the L-rhamnose and *N*-acetyl D-glucosamine moieties. As seen from the discussion in chapter 3, the protecting groups may play an important role in the activity of those analogues. However, there are not any general rules on which protecting group is the best for activity. Thus, several protecting groups such as benzylidene, acetate and benzolate were chosen as substituents. Also, unsubstituted sugars were used for comparison.

10.3 Retro-synthetic analysis

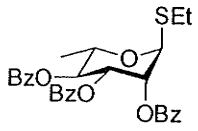
As reviewed in chapter 5, many approaches are available for the preparation of disaccharides and oligosaccharides. Thioglycosides, glycosyl imidates and glycosyl halides are the most commonly used glycosylation donors. The thioglycoside approach was selected for preparing the disaccharide analogues in this thesis.



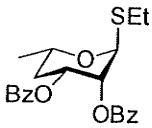
Scheme 10.1 Retro-synthetic analysis of disaccharide analogues

Scheme 10.1 shows the retro-synthetic analysis of the disaccharide analogues. The target disaccharides **53** could be prepared from the protected disaccharides **161** by removal of the protecting groups. Disaccharides **161** could in turn be synthesized by the coupling of glycosyl donors and acceptors. The glycosyl donors could be prepared as benzolated thioglycosides so that the α glycoside linkage could be synthesized by the thioglycoside approach using neighboring group participation. Figure 10.2 lists the donors and acceptors whose synthesis would be attempted.

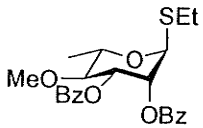
Glycosyl donors



162

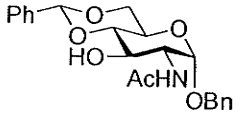


163

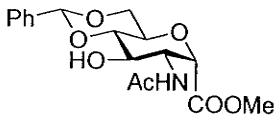


164

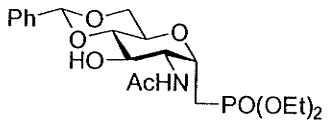
Glycosyl acceptors



165



166



167

Figure 10.2 Glycosyl donors and acceptors

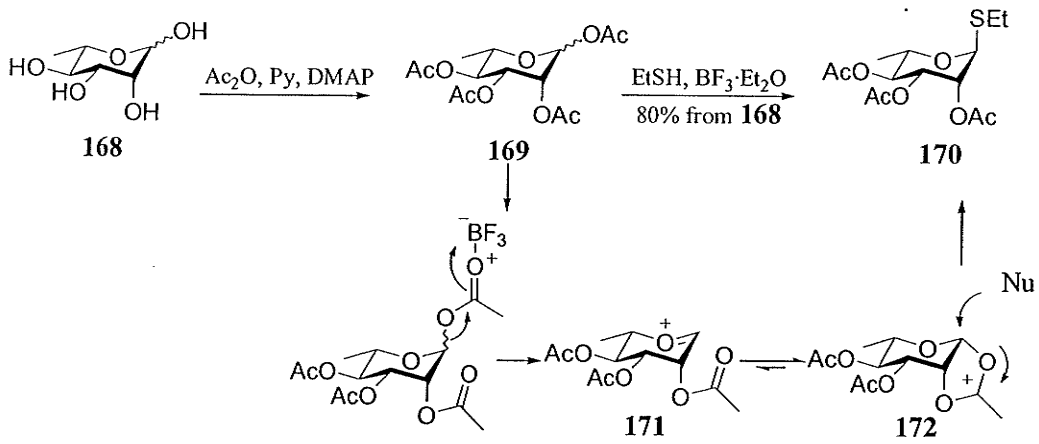
10.4 Results and discussion

10.4.1 Glycosyl donors

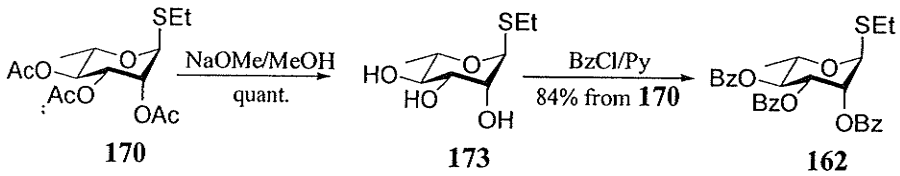
10.4.1.1 4-*O*-Bz-L-rhamnoside donor 162

Preparation of thioglycosides often starts from peracetylated aldoses.[116] L-Rhamnose **168** was converted to the fully acetylated rhamnose **169** by treatment with Ac_2O, pyridine and DMAP at room temperature for 3 hours (Scheme 10.2). Compound **169** was obtained as a mixture of α and β diastereomers. The thioglycoside **170** was prepared from **169** based on Bundle's procedure.[199] A suspension of **169**, 3 Å powdered molecular sieves and $BF_3{\cdot}Et_2O$ in ethanethiol was stirred for 2 hours at room temperature, giving **170** as a 9:1 mixture of α and β anomers. The α and β anomers were separated by flash chromatography. The overall yield of the α anomer **170** from **168** was 80%.

The reaction mechanism for preparing thiogoglycosides from peracetylated aldoses has been studied (Scheme 10.2).[200] An oxocarbenium **171** is first formed when the exocyclic acetyl group is cleaved by the assistance of $BF_3 \cdot Et_2O$. The more stable dioxonium **172** is generated from **171** due to neighboring group participation (neighboring group participation mechanism is discussed in section 5.3.2.). Nucleophilic attack of a thiol on **172** in a S_N2 fashion gives the α thioglycoside **170**. The β anomer probably comes from the *in situ* anomerization of its α anomer (*in situ* anomerization is discussed in section 5.3.4). This is because thioglycosides are reported to undergo *in situ* anomerization without bond breaking when $BF_3 \cdot Et_2O$ is present, and the ratio of α and β isomers is governed by the anomeric effect.[201] This explains why the α anomer **170** was the predominant product.



Scheme 10.2



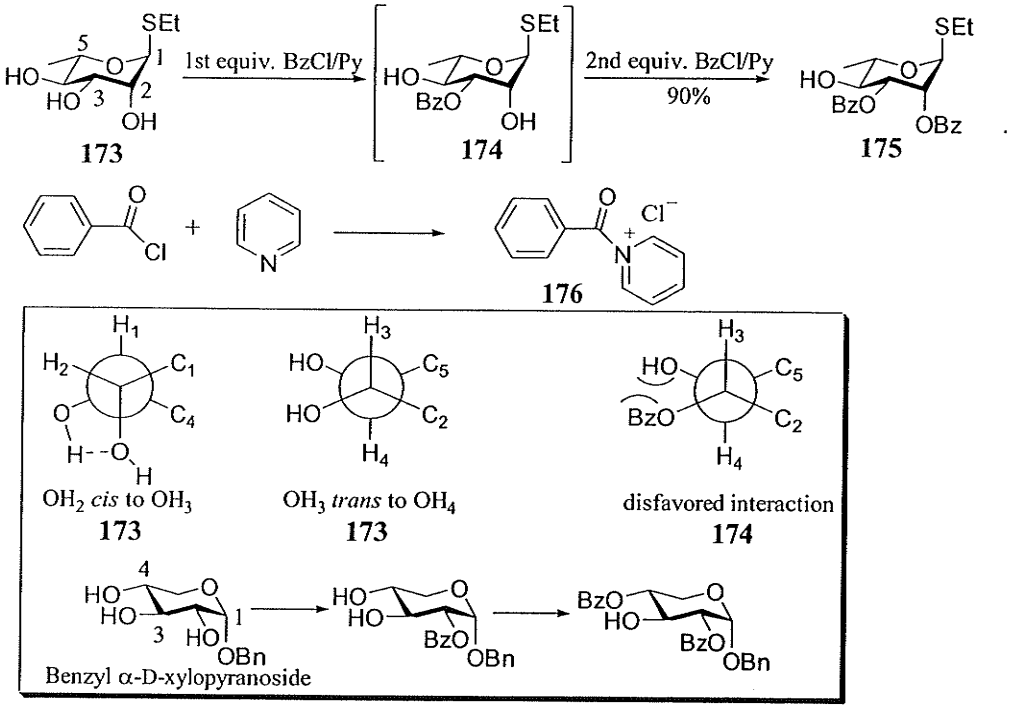
Scheme 10.3

Compound **170** was then converted to the benzolated donor **162** according to the synthetic strategy laid out in section 10.3 (Scheme 10.3). The acetate groups in **170** were cleaved by treatment with sodium methoxide in methanol for 15 min at room

temperature. The free hydroxyls were then benzolated by treatment with BzCl and pyridine for 1.5 hours at room temperature. The overall yield of **162** was 75%.

10.4.1.2 4-Deoxy-L-rhamnoside donor 163

Preparation of the donor **163** was based on a procedure described by Hultin and Buffie in our laboratory, which started from the thioglycoside **173** (Scheme 10.4).[202] Treatment of **173** with two equivalents of BzCl and pyridine in CH_2Cl_2 at –40°C for 30 min regioselectively generated 2,3-dibenzolated thioglycoside **175** in good yield (Scheme 10.4).

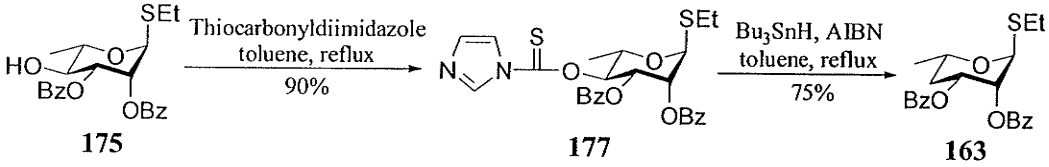


Scheme 10.4

Regioselective acylation of monosaccharides was extensively studied two decades ago.[203] The pyridinium salt **176** is believed to first form in the reaction, and is reactive towards hydroxyls that have intramolecular hydrogen-bonding.[203] Equatorial hydroxyls are generally more reactive than axial hydroxyls.[203] There are two equatorial hydroxyls in **173**. The 3-hydroxyl is *cis* to the 2-hydroxyl, forming an H-bond with it. However, the 4-hydroxyl is *trans* to the 3-hydroxyl, and cannot form a H-bond due to its position.[204]

Therefore, the 3-hydroxyl would be benzolated first, giving the monobenzolated thioglycoside **174**. The 4-hydroxyl in **174** would be less reactive than the 2-hydroxyl because it has a disfavored interaction with 3-benzoyloxy group. This argument is supported by the regioselective benzoylation of benzyl α-D-xylopyranoside. The reactivity of the hydroxyls in this sugar is: 2-OH > 4-OH > 3-OH. The 3-hydroxyl is the least reactive because it suffers from an unfavorable *gauche* interaction with the 2-benzoyloxy group after the 2-hydroxyl is benzolated.[203] Thus, the 2-hydroxyl of **174** would be benzolated by the second equivalent of BzCl.

The 4-hydroxyl of **175** was deoxygenated by Barton-McCombie method, shown in Scheme 10.5 (the mechanism has been discussed in Scheme 8.5). The hydroxyl was converted to the thiocarbonylimidazolide in 90% yield by treatment with thiocarbonyldiimidazole in refluxing toluene. Reduction of **177** with tributyltin hydride in refluxing toluene afforded the desired donor **163** in 75% yield. The overall yield of **163** was 68%.



175 **177** **163**

Scheme 10.5

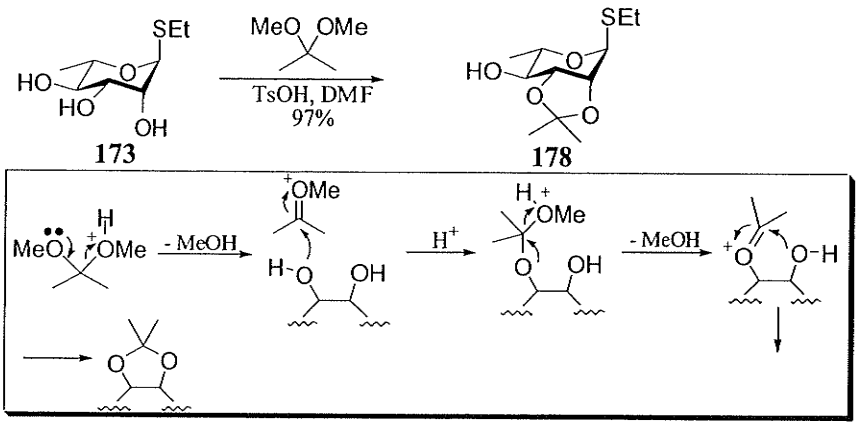
10.4.1.3 4-*O*-Me-L-rhamnoside donor 164

The initial attempts to prepare the donor **164** by reaction of **175** with MeI/NaH did not cleanly give a methylated product, but instead, many spots were visible on the TLC plate. The various products might come from migration of the benzoyl groups. Thus, an alternate synthetic route was chosen.

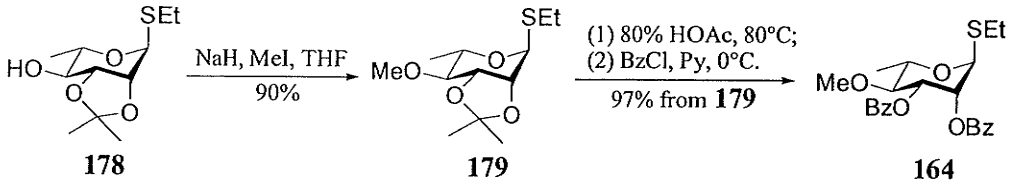
The alternative started from preparing the protected thioglycoside **178**, shown in Scheme 10.6. Treatment of **173** with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in DMF for 30 min at room temperature selectively protected the 2,3-diol with an isopropylidene group. The 2,3-isopropylidene ketal is formed preferentially over the 3,4-

isopropylidene ketal because the *cis*-fused ketal is more thermodynamically stable than the *trans*-fused ketal.[120] A reaction mechanism is proposed in Scheme 10.6.

Thioglycoside **178** was methylated by stirring with MeI and NaH in THF at room temperature for 4 hours, giving the methylated thioglycoside **179** in 90% yield (Scheme 10.7). Acid hydrolysis of **179**, followed by benzoylation, afforded the desired donor **164** in 85% overall yield.



Scheme 10.6



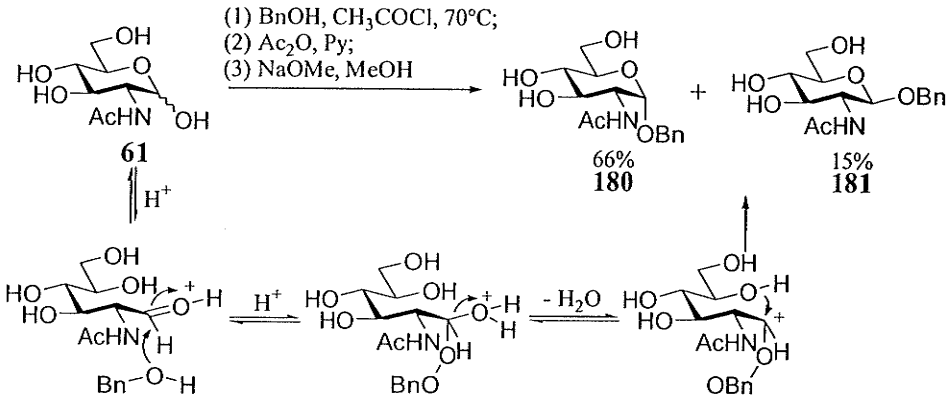
Scheme 10.7

10.4.2 Synthesis of glycosyl acceptors

10.4.2.1 1-*O*-Bn-D-GlcNAc acceptor 165

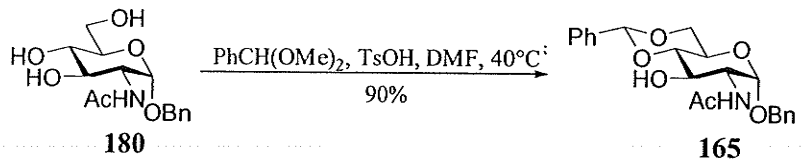
The synthesis of **165** started from the free *N*-acetyl D-glucosamine **61**. Acetyl chloride was added to a solution of **61** in benzyl alcohol, and the reaction mixture was stirred for 1 hour at 70°C. The anomeric hydroxyl was regioselectively benzylated, producing a 4.3:1 ratio mixture of α and β anomers **180** and **181** in 81% yield. The

mechanism is illustrated in Scheme 10.8.[125] The reaction is acid-catalyzed and reaction of acetyl chloride and the solvent produces hydrochloric acid. This reaction is under thermodynamic control, and the α anomer is produced as the major product due to the anomeric effect. These two isomers could not be separated from one another by flash chromatography. The mixture was fully acetylated with Ac_2O and pyridine, and was separated by flash chromatography. Cleavage of the acetate groups by methanolysis gave the α glycoside **180** in 66% yield from **61**.



Scheme 10.8

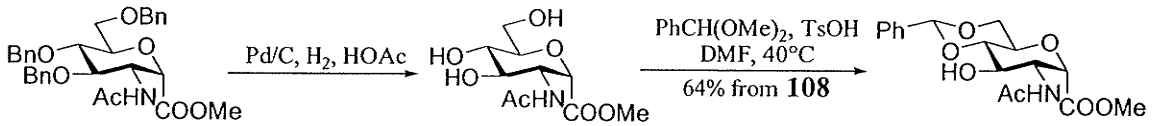
The 4- and 5-hydroxyls of **180** were then protected by treating with benzaldehyde dimethyl acetal and acid catalyst TsOH in DMF at 40°C for 1.5 hours, generating the acceptor **165** in 90% yield (Scheme 10.9). This reaction was very selective and only a single diastereomer was isolated. This is because the newly formed 1,3-dioxane in **165** is thermodynamically favored over the 1,3-dioxolane alternative, and the phenyl group strongly favored an equatorial orientation.[120] The overall yield of **165** was 59%.



Scheme 10.9

10.4.2.2 *C*-GlcNAc methyl ester acceptor 166

The synthesis of **166** started from the methyl ester *C*-glycoside **108** (its preparation is in Scheme 8.8), shown in Scheme 10.10. Hydrogenation of **108** with Pd/C as catalyst in glacial acetic acid overnight at room temperature afforded the free *C*-glycoside **182**. The hydrogenation was very sluggish when the reduction was conducted in ethanol. Compound **182** was converted to the acceptor **166** by reaction with benzaldehyde dimethyl acetal in DMF in the presence of acid catalyst TsOH. The acceptor **166** was obtained in 64% overall yield.



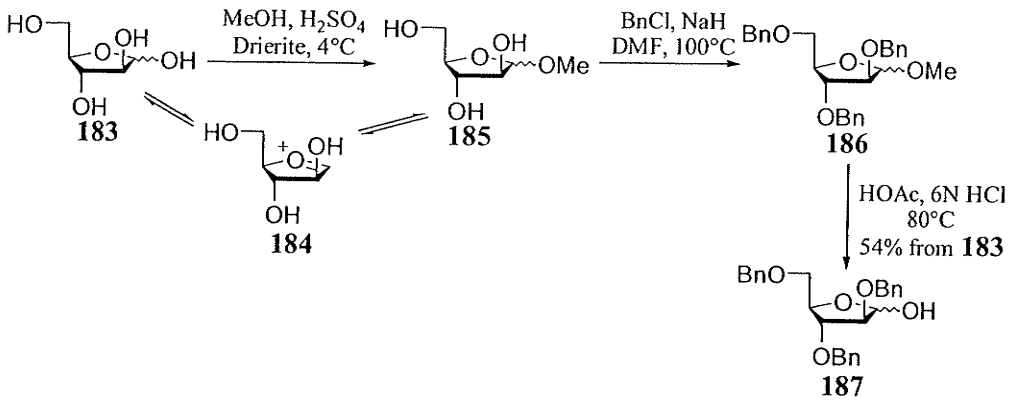
108 **182** **166**

Scheme 10.10

10.4.2.3 *C*-GlcNAc phosphonate acceptor 167

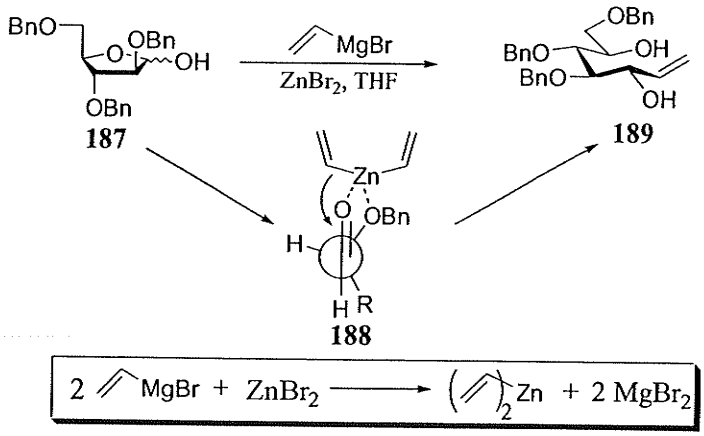
The synthesis of **167** was based on a modified procedure developed by Casero and coworker.[25] The strategy they used was to install the amino group and the phosphonate moiety in a sequence.

The synthesis started from a protected D-arabinose **187**, which was prepared by a procedure described by Tejima and Fletcher and the intermediates in the reaction were not characterized (Scheme 10.11).[205] D-Arabinose **183** was stirred in anhydrous methanol containing Drierite and concentrated sulfuric acid at 4°C overnight, producing the methyl arabinofuranoside **185** as a mixture of α and β anomers. This reaction probably went through an oxocarbenium intermediate **184**. Benzylation of **185** with BnCl/NaH in 100°C DMF for 5 hours gave the fully benzylated arabinoside **186**. Demethylation of **186** by acid hydrolysis in aqueous acetic acid and 6 N hydrochloric acid at 80°C for 30 min furnished **187** in 54% yield from **183**.



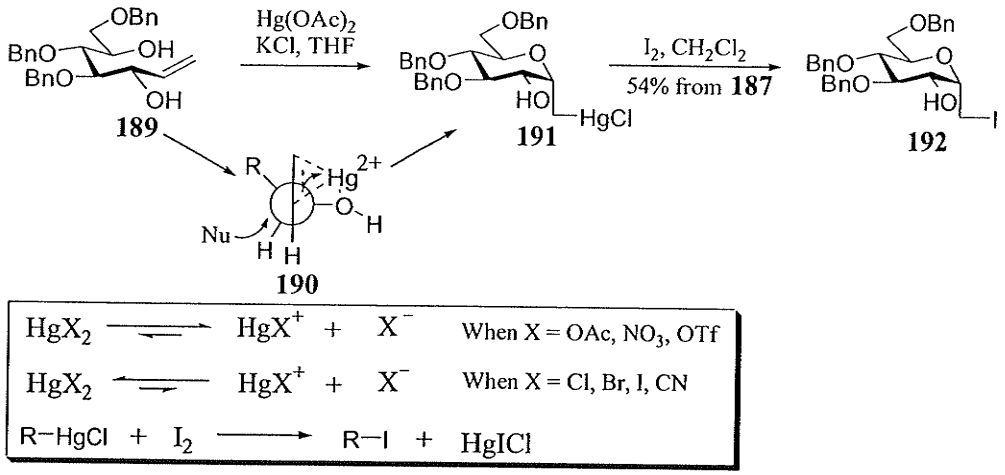
Scheme 10.11

The conversion of **187** to **202** was based on Casero's procedure.[25] Arabinose **187** was converted to an enitol **189** by vinylation with divinylzinc (Scheme 10.12). Divinylzinc was prepared by reacting two equivalents of vinylmagnesium bromide with one equivalent of $ZnBr_2$. The vinylation gave **189** as a single diastereomer. This reaction probably went through transition state **188**, in which the zinc atom chelated the aldehyde oxygen and the adjacent benzyl ether oxygen, and the vinyl group approached the aldehyde from the least hindered side (Cram's rule). Boschetti and coworkers have studied the vinylation of aldopentoses by vinylmagnesium bromide and divinylzinc, and found that although divinylzinc was less reactive than the Grignard reagent, it afforded much better stereoselectivity.[152]



Scheme 10.12

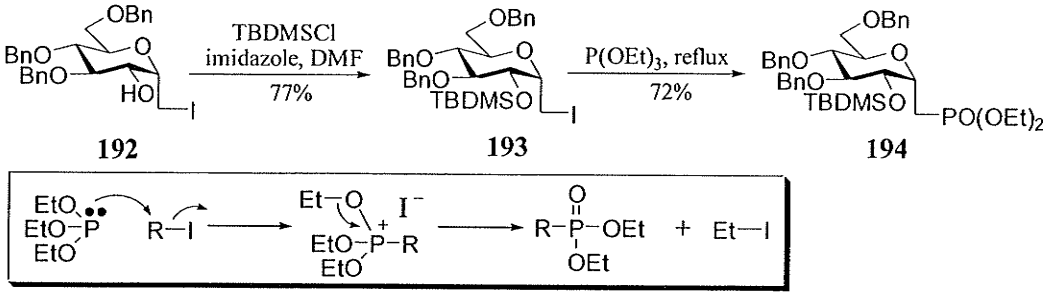
As discussed in chapter 4, Michael cyclization is an attractive method to prepare *C*-glycosides. However, the conventional base catalysis often leads to β C-glycosides because β *C*-glycosides are thermodynamically more stable.[50,206] In order to prepare an α *C*-glycoside, mercuric acetate was chosen for the cyclization of **189** (Scheme 10.13). When X is OAc, NO_3 or OTf, mercurium salt HgX_2 favors the dissociated forms HgX^+ and X^- in the solution.[207] HgX^+ is a strong soft electrophile.[231] When $HgOAc^+$ approached the double bond of **189**, the allyl alcohol would direct it into transition state **190**.[174] Backside attack of **190** by the 6-hydroxyl would give the *C*-glycoside **191** in an α configuration. Boschetti and coworkers used mercuric acetate to cyclize aldopentoses, and obtained good stereoselectivity in most cases.[152] KCl was added to the reaction before the workup, transforming RHgOAc to the less reactive species RHgCl to avoid side reactions. Treatment of **191** with iodine cleaved the mercury and yielded the iodide **192**. The iodide **192** was obtained in 54% yield from **187**.



Scheme 10.13

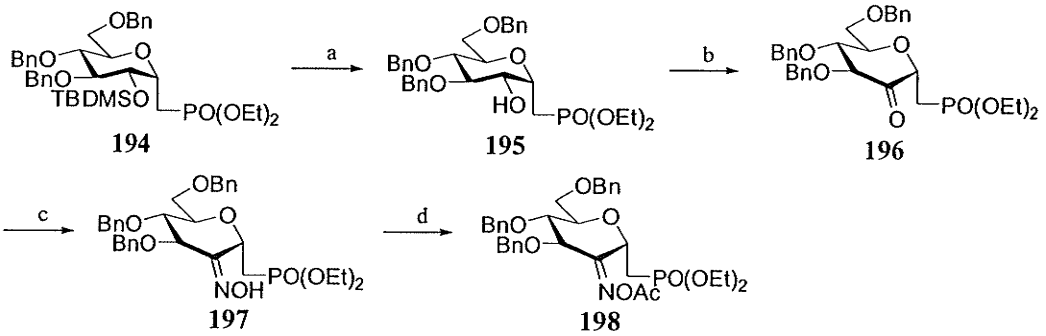
The 2-hydroxyl of **192** was protected by a TBDMS group before **192** was converted to the corresponding phosphonate (Scheme 10.14). Silylation of **192** with TBDMSCl and imidazole in DMF for two days gave **193** in 77% yield. Reflux of **193** in $P(OEt)_3$ for 5 hours produced the phosphonate **194** in 72% yield. This reaction is also called the Arbuzov reaction. The Arbuzov reaction is a versatile method for the formation of C-P bonds by treating alkyl halides in a refluxing trialkyl phosphite.[208] A mechanism

for this reaction is proposed in Scheme 10.14. The phosphorus of the phosphite is very nucleophilic because it has a lone pair of electrons. It first attacks the alkyl group of the alkyl halide, then an alkyl group of the phosphite dissociates to give the P=O bond and the alkyl group is eliminated as a new alkyl halide. The driving force of Arbuzov reaction is the transformation of the trivalent phosphorus to the pentavalent phosphorus, which releases 32 to 65 kcal/mol of energy.[208]

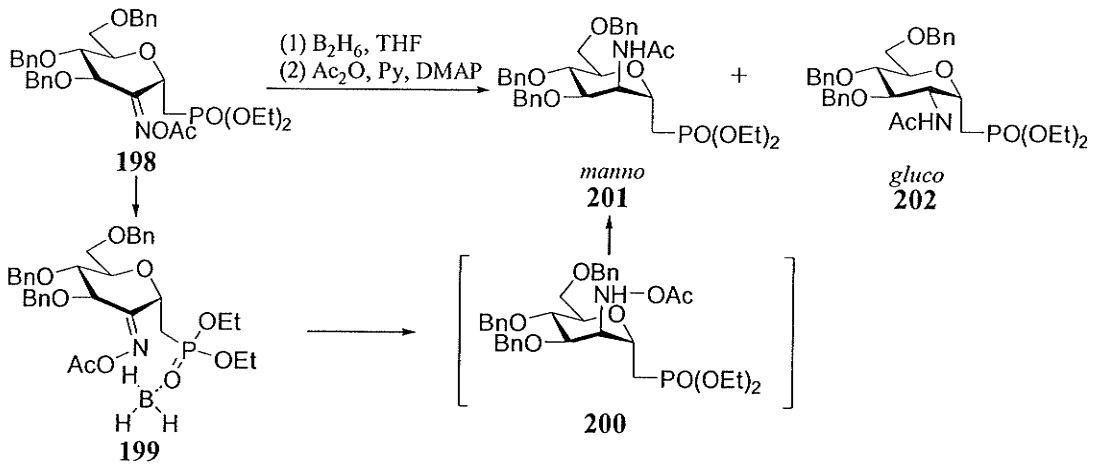


Scheme 10.14

Conversion of **194** to **198** was straightforward, and is shown in Scheme 10.15. The silyl protecting group of **194** was cleaved by treatment with trifluoroacetic acid. The free 2-hydroxyl was then oxidized to a carbonyl group by Ac_2O/DMSO. The keto group was transformed into an oxime by treating with hydroxy amine at pH 4.5. Acetylation of **197** gave the acetyl oxime **198** in good yield.



Scheme 10.15 Reagents and conditions: (a) CF_3COOH(aq.), CH_2Cl_2, rt, overnight, 80%; (b) DMSO, Ac_2O, rt, overnight, 87%; (c) NH_2OH, pH 4.5, rt, 3 h; (d) Ac_2O, pyridine, DMAP, rt, 2 h, 84% from **196**.



Scheme 10.16

Table 10.1 Reduction of acetyl oxime **198**

Exp. No.	Conditions for reduction	Ratio of **201** to **202**	Overall yield (%)
Lit.[25]	4.0 equiv. B_2H_6, -5°C - rt, until **198** disappeared	1: 4.5	45
1	1.0 equiv. B_2H_6, -15°C - rt, 22 h	1: 0.91	63
2	2.0 equiv. B_2H_6, -15°C - rt, 60 h	1: 0.66	86
3	3.9 equiv. B_2H_6, -5°C - rt, overnight	1: 0.54	54
4	4.1 equiv. B_2H_6, -5°C - rt, 20 h	1: 0.83	51

The effectiveness of Casero's synthetic sequence rests on the stereoselective reduction of the acetyl oxime. Stereoselective reduction of acetyl oximes is reportedly successful when diborane is used as reducing agent.[121] The reduction of **198** was conducted with various amounts of diborane, and the reduction products were acetylated directly without isolation (Scheme 10.16). Table 10.1 gives the reduction results in comparison with Casero's.[25] Good diastereoselectivity could not be achieved for the reduction of **198** for the *gluco* phosphonate **202** in any of our experiments although the overall yields were better than Casero's. Even though Casero observed a relatively good stereoselectivity for **202**, the overall yield was very poor. The relatively good selectivity reported by Casero might come from incomplete reduction. Our results showed that a

large excess of diborane resulted in neither good selectivity nor high overall yield (entries 3 and 4). In contrast, a small excess of diborane produced a much better overall yield although the selectivity fluctuated (entries 1 and 2).

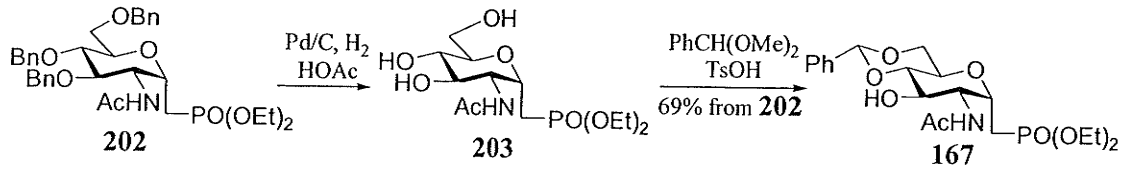
Neighboring ester-directed reduction

Scheme 10.17

It is reported that the stereoselective reduction of acetyl oximes is directed by a neighboring ester group. The ester group can coordinate the diborane to deliver the hydride preferentially to one face over the other face. In the example illustrated in Scheme 10.17, the hydride is only delivered to the top face because the bottom face is hindered by the 4-hydrogen.[121] However, the problem associated with **198** is that it does not have such a directing group. The neighboring benzyl ether at C-3 may not provide enough coordination to diborane. The axial phosphonate would probably offer better coordination, but it directs the hydride to the bottom face, thus increasing the formation of the *manno* phosphonate **201**. This explains why the *manno* product was isolated as the major product.

It is unfortunate to have poor selectivity and a low yielding step at the last stage of a multiple step synthesis. This synthetic route was chosen because no other alternatives were available (see the following section 10.4.2.4).

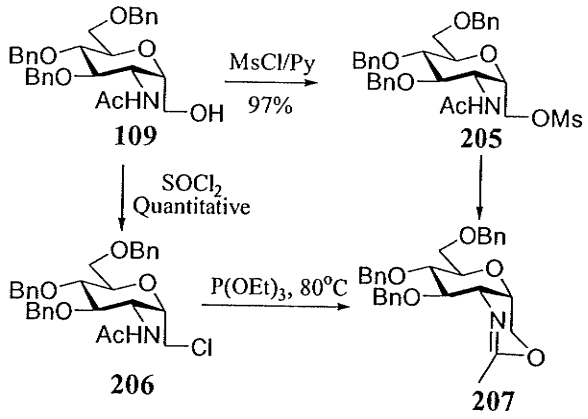
The phosphonate **202** was then transformed to the desired acceptor **167** in 69% yield by removal of the benzyl groups by hydrogenation followed by benzylidenation (Scheme 10.18). The overall yield for the synthesis of **167** was less than 2% from D-arabinose.



Scheme 10.18

10.4.2.4 Attempts to prepare 202 from amino sugars

There are two drawbacks associated with Casero's strategy in the preparation of **202**. (1) This procedure did not start from a readily available amino sugar. Starting from an amino sugar might make the synthesis more efficient. (2) The overall yield of **202** was too low due to the poor selectivity and low yield in the reduction of **198**. In order to improve the synthetic efficiency, a different strategy, which would start from the derivatives of the readily available amino sugar *N*-acetyl D-glucosamine **61**, was chosen.



Scheme 10.19

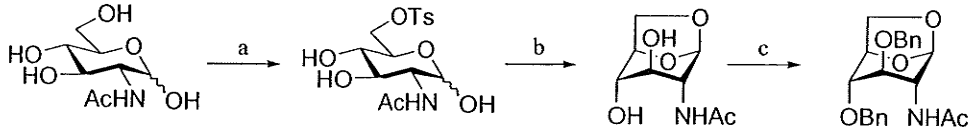
10.4.2.4.1 Direct substitution

The first attempt to prepare **202** was the direct substitution using the Arbuzov reaction (Scheme 10.19).[208] The *C*-glycoside alcohol **109** was prepared in section 8.3.1.1. It was converted to the mesylate derivative **205** in 97% yield by stirring with mesyl chloride and pyridine for 5 hours at room temperature. Reflux of **205** in $P(OEt)_3$ with KI did not produce the desired phosphonate. Instead, a cyclized product **207** was detected in the reaction mixture. Compound **109** was quantitatively transformed into the chloride **206** by stirring with thionyl chloride at room temperature for 30 min. Treatment of **206** with

$P(OEt)_3$ at 80°C also produced **207**. This result was not unexpected because the *N*-acetyl group of amino sugars is well known for its ability to participate in cyclization.[25]

10.4.2.4.2 1,6-anhydrosugar approach

The second attempt to prepare **202** used the 1,6-anhydrosugar approach. Examples of carbon-carbon bond formation by the substitution of an acetal with a carbon nucleophile have been reported.[209-211] The nucleophiles used include organocopper compounds and Grignard reagents, and the Lewis acid catalysts include $TiCl_4$ and $BF_3 Et_2O$. The synthesis of an α *C*-glycoside from a 1,6-anhydrosugar has also been reported.[212] The nucleophile and Lewis acid used in this case were lithium (trimethylsilyl) acetylide and trimethylaluminium respectively.



61 **208** **209** **210**

Scheme 10.20 Reagents and conditions: (a) TsCl, pyridine, 0°C, 5 h; (b) DBU, EtOH, rt, overnight, 64% over the last two step; (c) BnBr, BaO, $Ba(OH)_2$, DMF, 50°C, 3 h, 79%.

The preparation of the 1,6-anhydride of *N*-acetyl D-glucosamine **210** was based on a procedure described by Lafont and coworkers[213] in 1989, shown in Scheme 10.20. The primary alcohol of the free *N*-acetyl D-glucosamine **61** was regioselectively tosylated by stirring with *p*-toluenesulfonyl chloride and pyridine at 0°C for 5 hours, producing the tosylate derivative **208**. Intramolecular substitution of **208** under basic catalysis gave the 1,6-anhydrosugar **209** in 64% yield from **61**. Comparing the conformation of **209** and **208**, **209** seems to be more strained than **208** because two hydroxyls and one NHAc group are in axial positions. Nevertheless, **209** is strongly stabilized by a favorable anomeric effect.[214] Formation of anhydrosugars from intramolecular substitution by other alcohols is possible, but they would not benefit from the anomeric effect to offset the strain caused by the axial substituents. Benzylation of **209** was accomplished using a

procedure described by Tailler and coworkers.[215] Compound **209** was stirred in a suspension of BnBr, $Ba(OH)_2$ and BaO in DMF at 50°C for 3 hours, affording the desired anhydrosugar **210** in 79% yield.

Our preliminary experiments showed that **210** would not react with the phosphonate nucleophile $LiCH_2PO(OMe)_2$ even in the presence of Lewis acids such as $AlCl_3$ or $BF_3 Et_2O$. This may be because **210** is a relatively soft electrophile and $LiCH_2PO(OMe)_2$ is a relatively hard nucleophile. Our results are in agreement with other people's observations that most alkyllithium reagents normally do not react with acetals.[209]

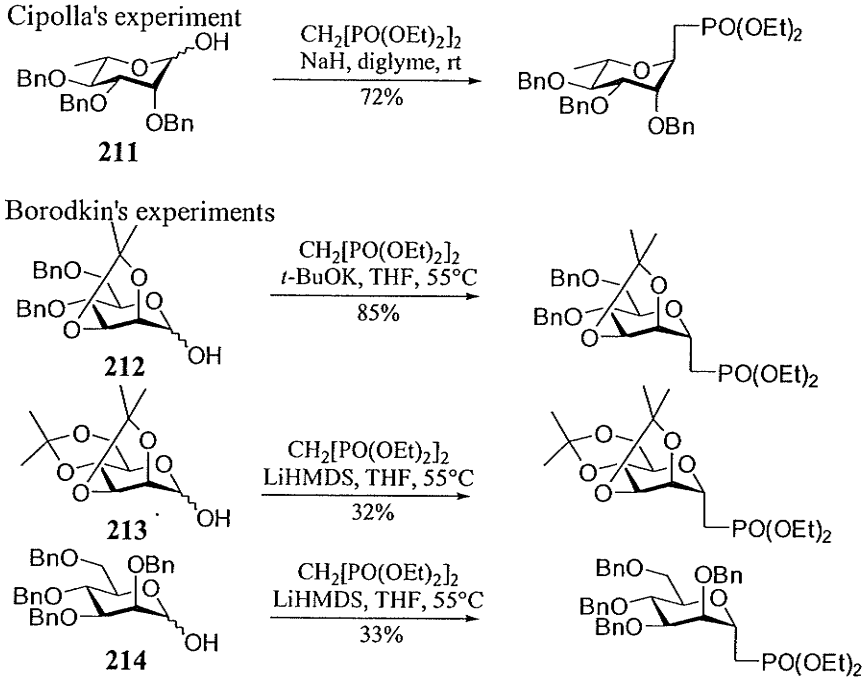
10.4.2.4.3 One-step Horner-Emmons/Michael approach

As discussed in chapter 4, the one-step Horner-Emmons/Michael approach is one of the most successful methods for the preparation of amino *C*-glycosides. The α *C*-glycoside methanephosphonates have been successfully prepared by this method by Cipolla and Borodkin in 1998 and 2001 respectively, shown in Scheme 10.21.[29,216] However, the one-step Horner-Emmons/Michael reaction is also influenced by the substituents on the sugars. Borodkin and coworkers have studied three types of D-mannopyranoses, and found that D-mannopyranose **212** afforded the α *C*-glycoside methanephosphonate in the best yield, the other two D-mannopyranoses **213** and **214** produced the desired products in about 30% yield .[216]

Encouraged by their success, 2 *N*-acetyl D-glucosamine derivatives were chosen as starting material for evaluation of this method for the preparation of **202** (Figure 10.3). Compounds **100** has been prepared in sections 8.3.1.1.

The synthesis of **215** started from **64** (Scheme 10.22). Compound **64** was converted to the acetylated glucosamine **217** by stirring in Ac_2O, pyridine and DMAP at room temperature for 2.5 hours. The large coupling constant $^3J_{1,2}$ (8.7 Hz) seen in the ^1H NMR spectra of **216** indicated that it was a β isomer. Selective cleavage of the anomeric

acetate with magnesium oxide in methanol afforded **215** in excellent yield.[217] The overall yield of **215** was 85%.



Scheme 10.21

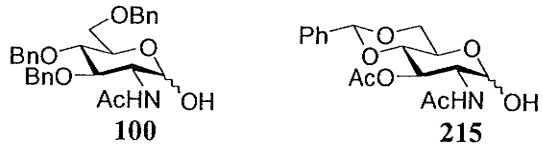
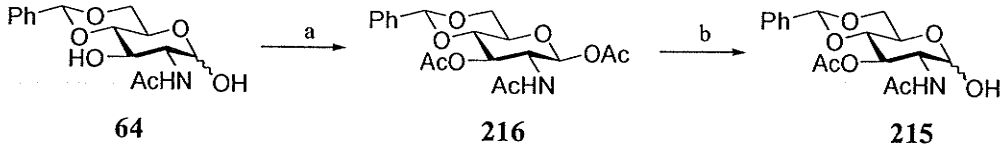


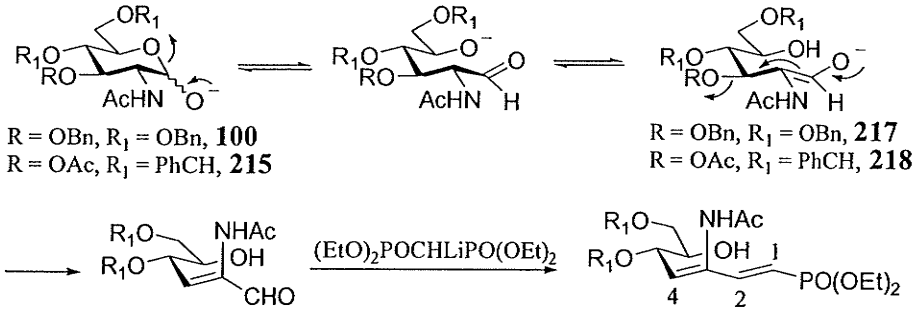
Figure 10.3 *N*-acetyl D-glucosamine derivatives chosen for the one-step Horner-Emmons/Michael reaction



Scheme 10.22 Reagents and conditions: (a) Ac_2O, pyridine, DMAP, rt, 2.5 h, 89%; (b) MgO, MeOH, rt, 1 h, 96%.

Unfortunately, reactions of these 2 *N*-acetyl D-glucosamine compounds with tetraethyl methylenediphosphonate in the presence of a base did not result in any of the desired products. Different combination of bases and solvent systems were tried, such as NaH/diglyme, NaH/THF, NaH/CH_3CN and LiHMDS/THF. None of the desired phosphonate products were identified in these reactions. Instead, by-products **221** and **222** were isolated.

The mechanism for the reactions of **100** and **215** with $(EtO)_2POCHLiPO(OEt)_2$ is illustrated in Scheme 10.23. The diphosphonate anion acted as a base at the begining of the reactions, and the intermediates **217** and **218** underwent elimination after being deprotonated, producing the aldehyde intermediates **219** and **220**. The Horner-Emmons reactions of **219** and **220** with the diphosphonate nucleophile generated the α,β,γ,δ-unsaturated phosphonates **221** and **222**. The large ^1H coupling constants $^3J_{1,2}$ (17.1 Hz for **221** and 17.6 Hz for **222**) seen in their NMR spectra indicated that these newly formed olefins adopted a 1,2-*trans* configuration.



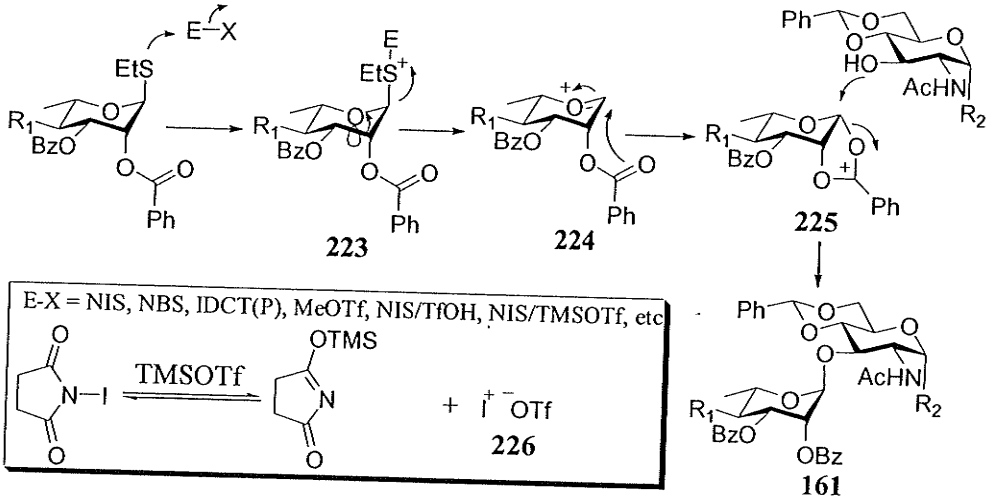
Scheme 10.23 Reaction mechanisms of **100** and **215** with $(EtO)_2POCHLiPO(OEt)_2$

10.4.2.4.4 Conclusions and future work

Althought attempts to prepare compound **202** directly from the amino sugar derivatives were unsuccessful, this does not necessarily mean that all the efforts have been exhausted. There are some aspects that have not been explored yet. Chapter 12 will discuss some suggestions for further research.

10.4.3 Synthesis of disaccharide analogues

At this stage, the glycosyl donors and acceptors were ready for the preparation of the disaccharide analogues. As discussed in section 5.2.3, thioglycosides as glycosylation donors have been extensively studied. A widely accepted glycosylation mechanism for thioglycosides is illustrated in Scheme 10.24.[218]

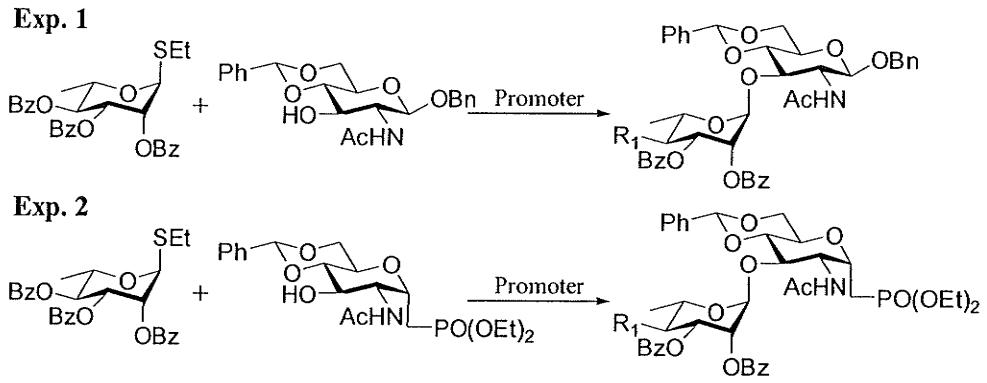


Scheme 10.24

The sulfur in thioglycoside donors first adds to the soft electrophiles E–X during glycosylation. The electrophile could be a single activator such as NIS, or a combination of two promoters such as NIS/TMSOTf.[218] In the case of two promoters such as NIS/TMSOTf and NIS/TfOH, the reactive species is IOTf (**226**).[219] The choice of promoters depends on reaction conditions. The breaking of the sulfur-carbon bond is driven by the kinetic anomeric effect to generate an oxocarbenium ion **224** (the kinetic anomeric effect is discussed in section 5.3.1). Neighboring group participation would generate a more stable cyclic dioxonium ion **225** (neighboring group participation is discussed in section 5.3.2). Nucleophilic attack of glycoside acceptors on **225** would exclusively produce the axial disaccharides **161**.

10.4.3.1 Synthesis of disaccharide analogues with 1-*O*-Bn-D-GlcNAc acceptor 165

Before preparing the disaccharide analogues, the glycosylation conditions were studied first. Table 10.2 lists the reaction results of the experiments shown in Scheme 10.25.



Scheme 10.25

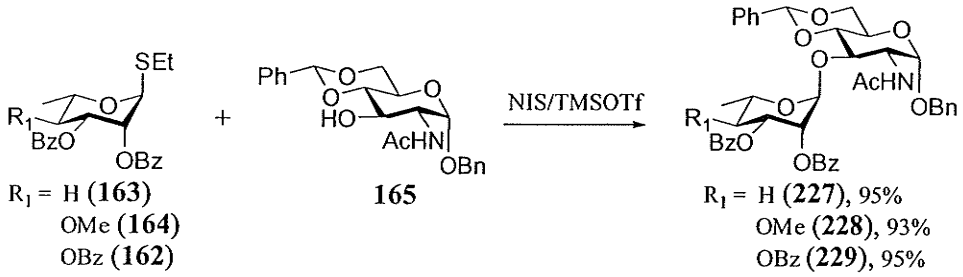
Table 10.2 Glycosylation results

No.	Experiment	Reaction conditions	Yield of the disaccharide (%)
1	Exp.1	IDCP, CH_2Cl_2	0
2	Exp.1	NIS, TMSOTf, collidine, CH_2Cl_2	0
3	Exp.1	NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2	20-39
4	Exp.2	NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2	41
5	Exp.2	NIS, TMSOTf, 4 Å molecular sieves, CH_2Cl_2	92

IDCP seemed unsuitable to our glycosylation (entry 1). Addition of an acid scavenger collidine was not good either (entry 2). A combination of promoters NIS/TfOH only gave low yields of the products (entries 3 and 4). The best result was obtained from the promoters NIS/TMSOTf.

Scheme 10.26 illustrates the preparation of the disaccharide analogues **227** to **229** from the donors **162** to **164** and the accepter **165**. All of the reactions gave almost

quantitative yields of the disaccharide products. Furthermore, just as expected, all of these reactions were very stereoselective. Only a single diastereomer was isolated from these reactions. The configurations and conformations of these disaccharide analogues were determined by NOE correlation experiments (Figure 10.4 and Table 10.3).



R_1 = H (**163**)
OMe (**164**)
OBz (**162**)

165

R_1 = H (**227**), 95%
OMe (**228**), 93%
OBz (**229**), 95%

Scheme 10.26

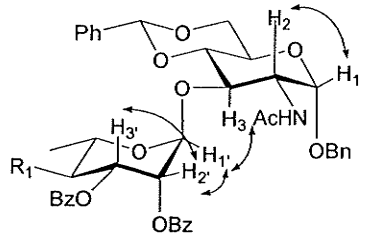


Figure 10.4 NOE correlations of disaccharide analogues **227-229**

Table 10.3 NOE correlations (percentage) of **227-229**

Compounds	$^4C_1$ conformation of GlcNAc		α Anomeric configuration of Rha				$^1C_4$ conformation of Rha	
	H_1-H_2	H_2-H_1	$H_{1'}$-H_3	H_3-$H_{1'}$	$H_{1'}$-$H_{2'}$	$H_{2'}$-$H_{1'}$	$H_{2'}$-$H_{3'}$	$H_{3'}$-$H_{2'}$
227	5	6	7	9	5	5	7	6
228	11	NA	4	NA	NA	NA	3	NA
229	10	NA	8	NA	5	4	5	NA

NA: not available

The conformations of the *N*-acetyl D-glucosamine and L-rhamnose moieties were determined by the correlations of H_1-H_2 and $H_{2'}$-$H_{3'}$. The configuration of the rhamnose

anomeric center was assigned by the correlations of $H_{1'}$-H_3 and $H_{1'}$-$H_{2'}$. No NOE correlations were found between $H_{1'}$ and $H_{3'}$ or $H_{5'}$ and H_1 and H_3 or H_5 in the three disaccharides. The data shown in Table 10.3 strongly supported the conclusion that the L-rhamnose and *N*-acetyl D-glucosamine moieties in these disaccharides adopted chair conformations $^1C_4$ and $^4C_1$ respectively, and the rhamnose anomeric carbon was in an α configuration.

The small ^{1}H coupling constants $^3J_{H1\text{-}H2}$ for **227-229** and $^3J_{H1'\text{-}H2'}$ for **228** and **229** found in their NMR spectra also supported the conformations and configurations of **227-229** assigned by NOE experiments (Table 10.4).

Table 10.4 Coupling constants in ^{1}H NMR spectra of **227-229**

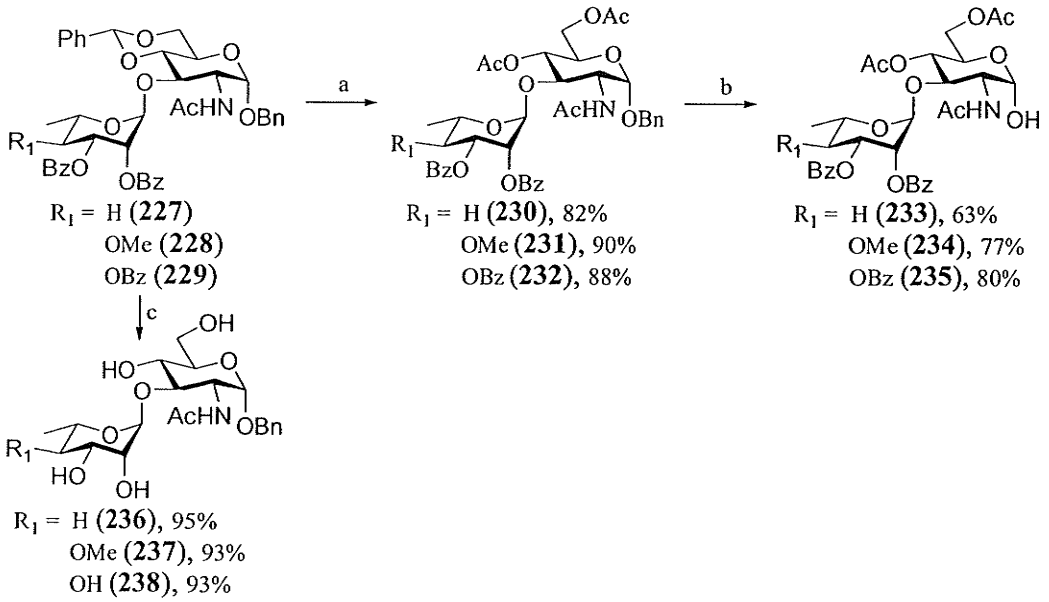
Compound	$^3J_{H1\text{-}H2}$ (Hz)	$^3J_{H1'\text{-}H2'}$ (Hz)
227	3.7	NA
228	3.7	1.7
229	3.7	1.6

NA: not available

Analogues **227-229** were further derivatized according to the requirements discussed in section 10.2. Scheme 10.27 depicts these derivatization steps. Removal of the benzylidene protecting groups from **227-229** by acid hydrolysis at 70°C for 1-2 hour followed by acetylation with Ac_2O/pyridine gave the disaccharide analogues **230-232** in good yields (82%, 90% and 88% respectively). Hydrogenation of **230-232** with Pd/C as catalyst in glacial acetic acid generated the reducing disaccharide analogues **233-235** in moderate yields (63%, 77% and 80%, respectively), indicating that these disaccharides were sluggish in being fully debenzylated. The configurations of the newly formed anomeric hydroxyls of **233-235** was assigned as axial based on the small ^{1}H coupling constants $^3J_{1,2}$ (2.8-3.3 Hz) found in the NMR spectra of **233-235**.

The disaccharides **227-229** were also transformed into the disaccharides **236-238** by acid-catalyzed removal of the benzylidene protecting groups followed by cleavage of

the benzoate groups with sodium methoxide and methanol. These disaccharide analogues were obtained almost in quantitative yield.



R_1 = H (**227**)
OMe (**228**)
OBz (**229**)

R_1 = H (**230**), 82%
OMe (**231**), 90%
OBz (**232**), 88%

R_1 = H (**233**), 63%
OMe (**234**), 77%
OBz (**235**), 80%

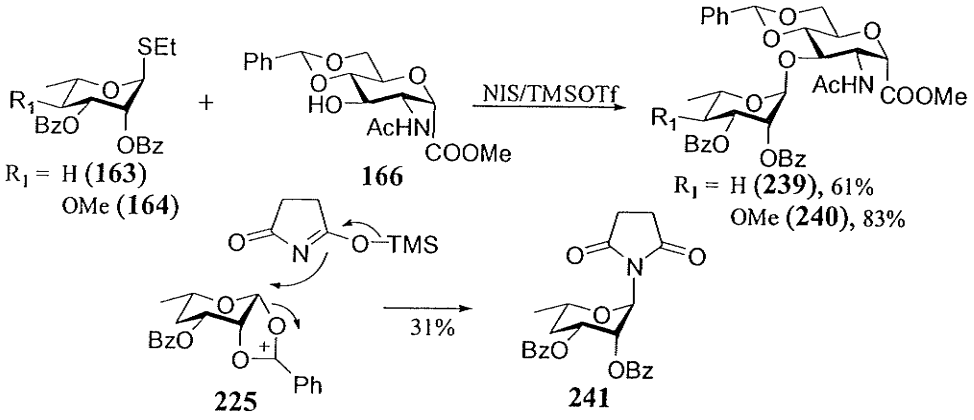
R_1 = H (**236**), 95%
OMe (**237**), 93%
OH (**238**), 93%

Scheme 10.27 Reagents and conditions: (a) (1) 80% HOAc, 70°C, 1-2 h; (2) Ac_2O, pyridine, DMAP, rt, 2 h – overnight; (b) Pd/C, H_2, HOAc, rt, 24 h; (c) (1) 80% HOAc, 70°C, 1-2 h; (2) NaOMe, MeOH, rt, 1 h.

10.4.3.2 Synthesis of analogues with *C*-GlcNAc methyl ester acceptor 166

Both the disaccharide analogues **239** and **240** were prepared from the donors **163** and **164** and the acceptor **166** by the thioglycoside glycosylation method, shown in Scheme 10.28. Compounds **239** and **240** were isolated in surprisingly low yields compared with disaccharides **227-229**. A succinimide byproduct **241** was isolated in 31% yield in the reaction of **163** and **166**. Isolation of succinimide byproducts from thioglycoside glycosylation is not unprecedented because there is always an equivalent amount of succinimide generated in the reaction mixture when NIS/TMSOTf or NIS/TfOH is used as the promoter.[220,221] Although the nitrogen of the succinimide is a poor nucleophile, in the case of highly unreactive acceptors, it can compete effectively for the dioxonium intermediate **225**. In a particular case, Zhang and coworkers found that such a side reaction seriously affected their synthesis.[220] In our case, the diminished

nucleophilicity of the hydroxyl on **166** may be due to the strongly electron-withdrawing ester group.



Scheme 10.28

These glycosylations were also highly stereoselective. Both **239** and **240** were obtained as a single diastereomer. NOE correlations listed in Table 10.5 indicated that both the L-rhamnose and *N*-acetyl D-glucosamine moieties in each disaccharide were in the expected chair conformations, and that the rhamnose moiety possessed the desired axial anomeric configuration. This assessment was supported by the large ^{1}H coupling constants $^{3}J_{H3\text{-}H4}$ (9.8 Hz) found in the NMR spectrum of **239**, and the large ^{1}H coupling constants $^{3}J_{H3\text{-}H4}$ (9.8 Hz) and the small ^{1}H coupling constant $^{3}J_{H1'\text{-}H2'}$ (1.6 Hz) in the NMR spectrum of **240**.

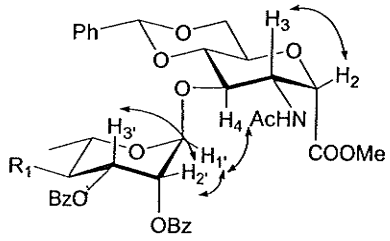


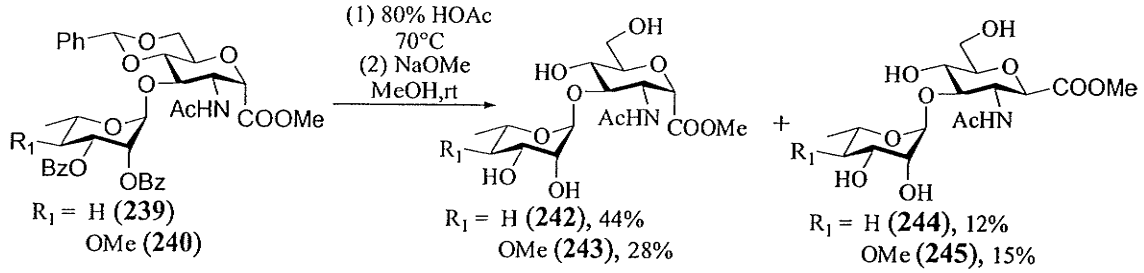
Table 10.5 NOE correlations (percentage) of **239** and **240**

Compounds	$^{4}C_1$ conformation of GlcNAc		α Anomeric configuration of Rha				$^{1}C_4$ conformation of Rha	
	H_2-H_3	H_3-H_2	$H_{1'}$-H_4	H_4-$H_{1'}$	$H_{1'}$-$H_{2'}$	$H_{2'}$-$H_{1'}$	$H_{2'}$-$H_{3'}$	$H_{3'}$-$H_{2'}$

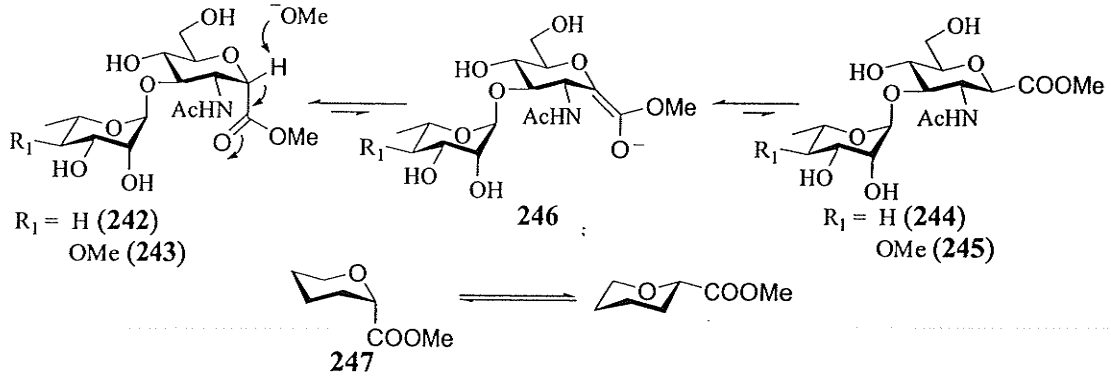
239	5	4	8	5	NA	NA	4	NA
240	4	NA	8	NA	5	4	5	5

NA: not available

The deprotected disaccharides **242** and **243** were prepared by removal of the benzylidene protecting groups of **239** and **240**, followed by cleavage of the benzoate groups, shown in Scheme 10.29. However, **242** and **243** were isolated only in 44% and 28% yields, respectively. Two byproducts **244** and **245** were obtained in 12% and 15% yields respectively. The yield loss might come from the hydrolyzed carboxylic acids that we were not able to isolate. Both byproducts probably came from the anomerization of the desired disaccharides via an enolate intermediate **246** formed by deprotonation (Scheme 10.30).

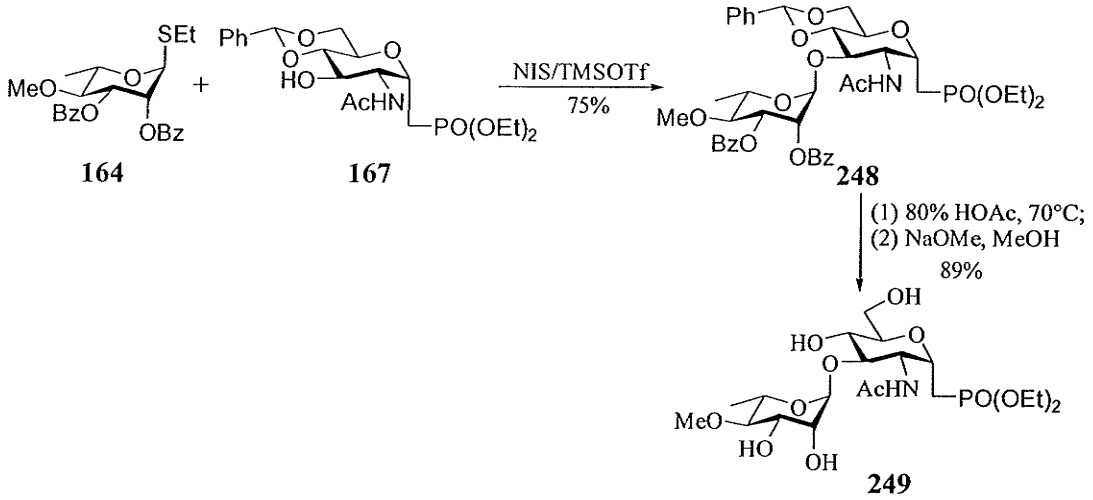


Scheme 10.29



Scheme 10.30

The free energy associated with the anomeric effect of an ester group has been studied.[222] Axial esters and equatorial esters (**242** and **244**, **243** and **245**) differ only in the orientation of the ester groups, therefore, the free energy $\Delta G°$ for their interconversion would be mainly affected by the anomeric effect and the solvent effects. A 2-substituted oxane **247** would be an appropriate model because it does not have any substituents except for the ester group. The free energy contributed by the anomeric effect to **247** is about 2.4 kcal/mol (Scheme 10.30).[222] Because polar solvents like methanol would stabilize equatorial esters better than axial esters,[222] the free energy change for conversion of **242** to **244** and **243** to **245** would be somewhat lower than 2.4 kcal/mol. Even so, $\Delta G°$ would still be positive, therefore the equilibria would favor **242** and **243**.



Scheme 10.31

10.4.3.3 Synthesis of analogues with *C*-GlcNAc phosphonate acceptor 167

The disaccharide analogue **248** was prepared from the coupling of the donor **164** and the *C*-glycoside phosphonate acceptor **167** using the promoter NIS/TMSOTf, shown in Scheme 10.31. Compound **248** was obtained as a single diastereomer in 75% yield. The conformation of the D-GlcNAc phosphonate moiety in **248** could not be determined due to the serious overlap of the proton signals in its ^{1}H NMR spectrum, but NOE correlation experiments indicated that the L-rhamnose moiety of **248** had a $^1C_4$ conformation. (Figure 10.5) Removal of the benzylidene and benzoyl protecting groups

by acid hydrolysis and base-catalyzed transesterification furnished the desired disaccharide analogue **249** in good yield.

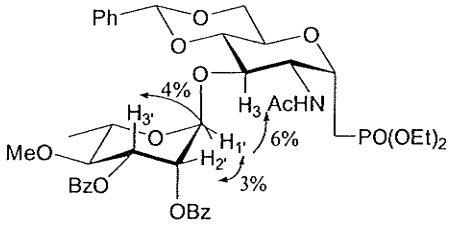


Figure 10.5 NOE correlations of **248**

10.4.4 Biological test against mycobacterial glycosyltransferases

Seventeen disaccharide analogues were sent for biological testing against mycobacterial glycosyltransferases (Figure 10.6). The tests were done by our collaborators Dr. D. C. Crick and Dr. P. J. Brennan at Colorado State University.

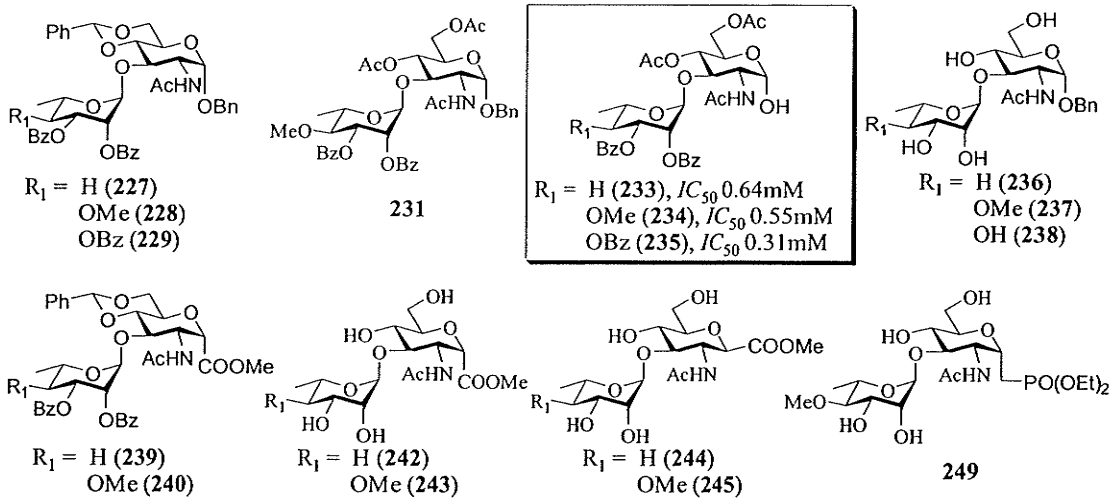


Figure 10.6

The glycosyltransferase activity was assayed in mixtures of [^{3}H]UDP-Gal, UDP-Gal*p*, UDP-Gal*p* mutase, dTDP-Rha, UDP-GlcNAc and a cell wall enriched fraction.[223] The cell wall enriched fraction was prepared by removing the cell fluid. It contained the mycobacterial glycosyltransferases and the polyprenol phosphate. The purpose of using UDP-Gal*p* mutase is to transform the galactopyranose fragment of UDP-Gal*p* into galactofuranose. The radiolabeled reaction products were extracted with $CHCl_3/CH_3OH$,

quantified by liquid scintillation spectrometry and identified by TLC. The membrane and cell wall enriched fraction is able to catalyze the transfer of *N*-acetyl glucosamine 1-phosphate (GlcNAc-1-P) and L-rhamnose (Rha) from their respective nucleotide donors to endogenous polyprenyl phosphate (Pol-P), yielding Pol-P-P-GlcNAc (GL-1) and Pol-P-P-GlcNAc-Rha (GL-2). Galactosyl transferase-1 (Galtase-1) mediates the transfer of a galactofuranosyl (Gal*f*) residue from UDP-Gal*f* to GL-2, giving rise to Pol-P-P-GlcNAc-Rha-Gal*f* (GL-3) (see Scheme 1.1). Our compounds were designed to disrupt the conversion of GL-2 to GL-3.

The test results showed that three disaccharide analogues **233-235** had an inhibitory effect on the mycobacterial glycosyltransferases. Their IC_{50} values from the enzyme assay were 0.64, 0.55 and 0.31 mM respectively.[248] Compared with other disaccharide analogues in terms of IC_{50}, **235** has been the best mimics so far (Table 10.6).

Table 10.6 Activity comparison

Disaccharide analogues	Target	IC_{50} (mM)	Ref.
Literature:			
Ara*f*-(1→5)-Ara*f*, **21b**	Arabinosyltransferase	1.12	11
Ara*f*-(1→5)-Gal*f*, **24c**	Galactosyltransferase	1.16	45
Ara*f*-(1→5)-Gal*f*, **24d**	Galactosyltransferase	3.20	45
Gal*f*-(1→6)-Gal*f*, **27c**	Galactosyltransferase	3.65	13
Gal*f*-(1→5)-Gal*f*, **28d**	Galactosyltransferase	3.32	13
Ours:			
Rha-(1→3)-GlcNAc, **233**	Galactosyltransferase-1	0.64	248
Rha-(1→3)-GlcNAc, **234**	Galactosyltransferase-1	0.55	248
Rha-(1→3)-GlcNAc, **235**	Galactosyltransferase-1	0.31	248

The disaccharide analogues **233-235** have some features in common. First, they all have free anomeric hydroxyls in the *N*-acetyl D-glucosamine moiety. This reducing end seems to be very important for their activity because other compounds that do not possess such a reducing end have no activity. Second, they all have the hydroxy groups protected by esters. Other compounds that have their hydroxyls free do not have any inhibitory activity. However, it is not clear at this moment whether the protection is necessary for the activity. The difference among these compounds arises from the various substituents at C-4′ of the L-rhamnose moiety. It seems that these substituents do not affect activity of these compounds. The test results provided us with information for studying the relationship between analogue structure and the activity, and ideas for designing more potent inhibitors.

10.5 Concluding remarks

This chapter has reviewed the design and synthesis of the analogues of a unique mycobacterial cell wall linker as potential mycobacterial glycosyltransferase inhibitors. This disaccharide linker provides a novel structure for modifications. The C-1 position of its *N*-acetyl D-glucosamine moiety and C-4′ of its L-rhamnose moiety are the two most important modification sites since they are the outlets to link to other cell wall components. Hydroxyls on these two sugar molecules are also useful sites because some of them, if not all, may be involved in binding with the target enzymes.

These disaccharide analogues were prepared using a thioglycoside approach. For this purpose, a series of glycosyl donors and acceptors have been synthesized. Most of them were successfully prepared in high yields and good selectivities except for the *C*-glycosyl phosphonate acceptor. Attempts to improve its preparation have not been successful. Most of the disaccharide analogues were obtained in good yields and with high selectivities. Seventeen analogues have been tested in a mycobacterial glycosyltransferase assay. Three compounds possessed inhibitory activity. This result has shed light on ideas for the design of the future glycosyltransferase inhibitors.

Chapter 11

Conclusions

The objective of this Ph.D. work was to synthesize carbohydrate-based compounds as potential glycosyltransferase inhibitors. Three types of glycosyltransferase inhibitors were prepared during the course of this thesis work.

β *N*-Acetyl *C*-mannosaminyl phosphonate **49** and β *N*-acetyl *C*-glucosaminyl phosphonate **50** were the first type of carbohydrate mimics synthesized. They were analogues of *N*-acetyl D-mannosamine and D-glucosamine 1-phosphates. The *C*-glycosyl phosphonate **49** was successfully synthesized from the readily available and inexpensive amino sugar *N*-acetyl D-glucosamine via a Horner-Emmons/Michael reaction. Compared with other synthetic routes, this approach was short and efficient. Two approaches were evaluated for the preparation of 50. The glycosyl aldehyde approach proceeded through a 1-hydroxy phosphonate intermediate, but deoxygenation of this hydroxyl was difficult. In contrast, the gluconolactone approach was successful in preparing the target compound in a few steps via an olefin intermediate.

α *N*-Acetyl D-glucosaminyl 2-hydroxy and 2-keto phosphonates **51** and **52** were the second type of glycosyltransferase inhibitors prepared. Three synthetic strategies – the ester, aldehyde and epoxide approaches, were explored. The first two methods failed to produce any desired target molecules, but the epoxide approach was successful for the preparation of the target analogues. Addition of a phosphonate nucleophile to the glycosyl epoxide led to the regioselective formation of a 2-hydroxy phosphonate. Removal of the benzyl protecting groups by hydrogenation furnished the target 2-hydroxy phosphonate **51** in good yield. The 2-keto phosphonate **52** was prepared from the 2-hydroxy phosphonate by oxidation with Ac_2O/DMSO followed by cleavage of the benzyl groups.

α-L-Rha-(1→3)-α-D-GlcNAc disaccharide derivatives were the third type of glycosyltransferase inhibitors prepared. In order to prepare the disaccharide compounds, a series of glycosyl donors and acceptors was synthesized. Most of the syntheses were successful giving high yield and good selectivity, except for the *C*-glycosyl phosphonate acceptor, which required a lengthy procedure to introduce the phosphonate and amino group moieties. Attempts to improve its preparation were unsuccessful. The glycosyl donors and acceptors were coupled to give the disaccharide compounds in good yield and with high selectivity. The disaccharide compounds were further derivatized, generating seventeen final disaccharide analogue products. These disaccharide analogues were tested in a mycobacterial glycosyltransferase assay. Three disaccharide compounds had inhibitory activity. The results provided new insight for future drug design.

Chapter 12

Future work

12.1 Introduction

Although this Ph.D. research accomplished its goal of preparing a number of interesting carbohydrate-based compounds as potential inhibitors of mycobacterial glycosyltransferases and other glycosyltransferases, there are still some questions that have not been answered and there are issues that are still waiting for further exploration.

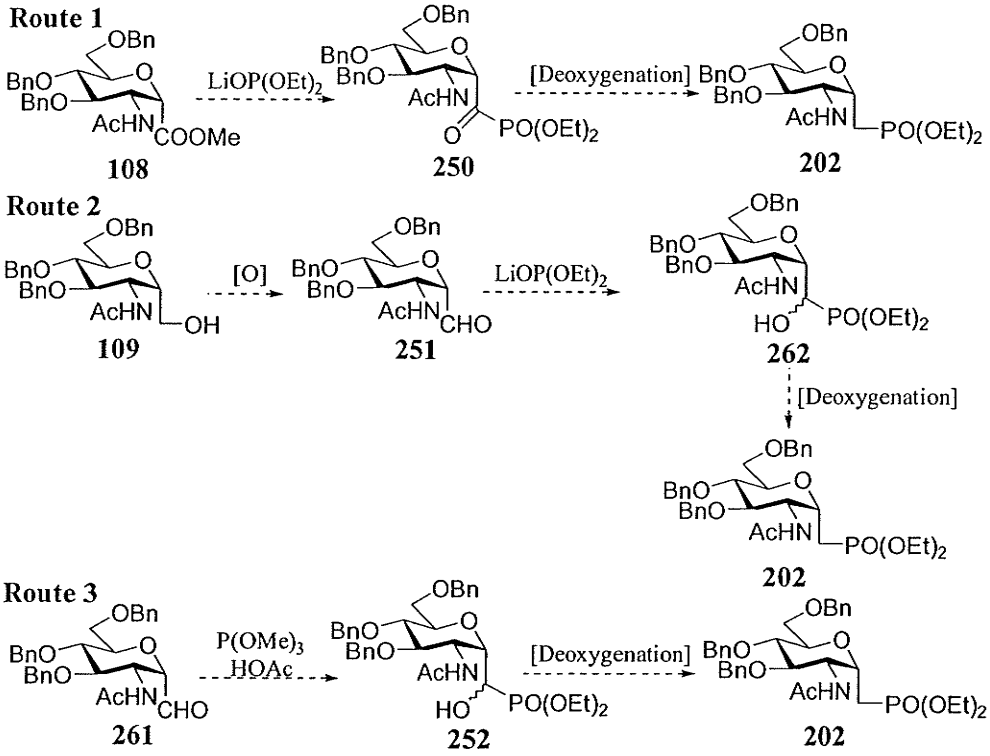
There are a few things that I would like to point out for future work. One is to prepare the α *C*-glucosamine phosphonate **202** in a more efficient way. Although the initial attempts were not successful (see section 10.4.2.4), not all ideas have been explored. Another area of future work is to learn more about the application scope of the zinc-diphosphonate complex **76**. The third area is to further study the structure-activity relationship of the disaccharide analogues. Although some key structural features related to their activities are revealed, there are still unanwsered questions. Further research on the structure-activity relationship of these disaccharide analogues is warranted.

12.2 Preparation of α *C*-glucosamine phosphonate

12.2.1 Addition approach

As discussed in section 4.3, one of the greatest challenges for the preparation of amino *C*-glycosides is the interference of the neighboring amino group with the manipulation of the anomeric side chain. An acetamido group can be activated as a nucleophile in the presence of (1) a strong base (2) a strong Lewis acid such as TMSOTf, and (3) heat. The nucleophile $LiOP(OEt)_2$ fulfills the task of adding to the electrophile on anomeric side chain without incurring interference from the acetamido group (see section 9.3.3). Based on these observations, three synthetic routes are proposed for preparation of **202** from simple glucosamine derivatives, shown in Scheme 12.1.

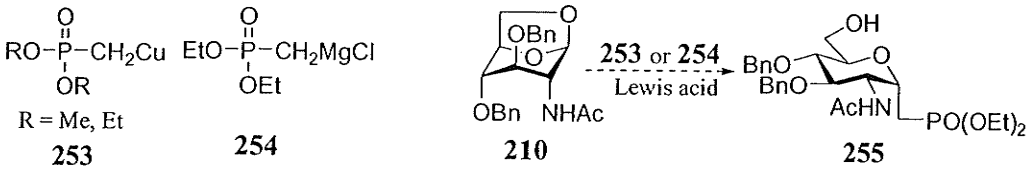
Compound **250** in the first route could be reduced to a 1-hydroxy phosphonate during the workup if it were unstable. As to the second route, preparation of the aldehyde **251** should be under neutral conditions to avoid epimerization of the anomeric carbon. Route 3 is a similar approach to route 2, but uses an acid-catalyzed reaction.[224-226]



Scheme 12.1

12.2.2 Acetal approach

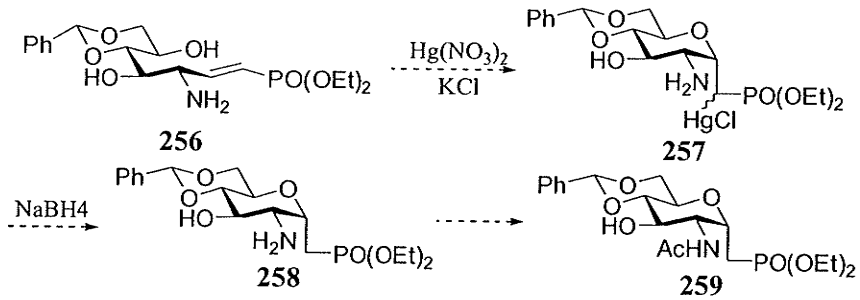
It has been well documented that organocopper and Grignard reagents react with acetals in the presence of a Lewis acid such as $BF_3 Et_2O$.[209-211,227,228] Cuprophosphonate **263** has been prepared and shown to have good reactivity toward acid chlorides.[229] The phosphonate Grignard reagent **254** has also been prepared, and readily reacts with allyl bromide, benzyl bromide and benzaldehyde.[230] The 1,6-anhydrosugar **210** is a highly strained acetal because two bulky benzyl groups are in axial positions. It would probably react with the nucleophiles **253** and **254** in the presence of a strong Lewis acid such as $BF_3 Et_2O$ (Scheme 12.2).



Scheme 12.2

12.2.3 Michael cyclization

Mercuric salts are useful reagents for the preparation of α *C*-glycosides through intermolecular cyclization of enitols directed by an allylic alcohol (see Scheme 10.13). An allylic amino group may have the same directing effect on mercuration of olefins as the allylic alcohol (Scheme 12.3). The electron-withdrawing phosphonate group in **256** might diminish the nucleophilicity of the olefin to some extent. In this case, a more reactive mercuric salt such as $Hg(NO_3)_2$ or $Hg(OTf)_2$ is needed.

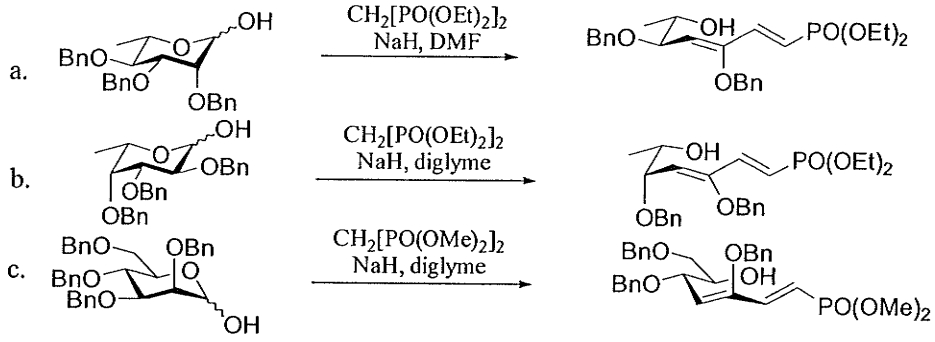


Scheme 12.3

12.3 The application of zinc diphosphonate

Dialkylzincs, which are generated *in situ* and used directly, have demonstrated excellent reactivities toward glycoses both in the literature[152] and in the experiments discussed in section 7.3. Zinc diphosphonate **76** could be used to prepare *C*-glycosides that cannot be prepared by a normal Horner-Emmons/Michael reaction. Scheme 12.4 lists some examples of the failed Horner-Emmons/Michael reactions for the preparation of α *C*-glycoside phosphonates.[29,216]

It is also interesting to explore the effect of Lewis acids on reactions of glycoses and zinc diphosphonate. Lewis acids have been found to be able to dramatically promote nucleophilic addition of organozinc compounds to carbonyl compounds.[232] However, such a strategy has not yet applied to the preparation of *C*-glycosides.



Scheme 12.4 Some failed Horner-Emmons reactions reported in the literature

12.4 Structure-activity relationship of disaccharide analogues

The last two to three years have witnessed a growing number of attempts to prepare carbohydrate-based compounds that specifically target mycobacterial glycosyltransferases (see the discussion in chapter 3). The biggest challenge in designing an analogue is that a detailed knowledge of the targeted enzyme is not available. Without such knowledge, it is necessary to investigate a much wider range of inhibitor structures in hopes of finding strong activity.

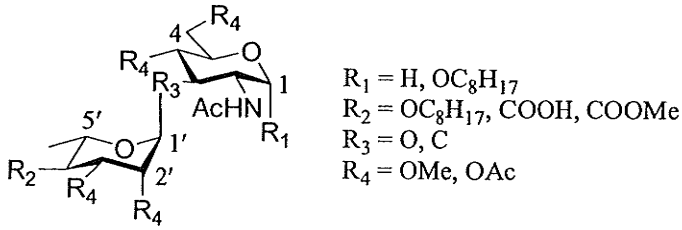


Figure 12.1

Figure 12.1 shows some modifications of the disaccharide structures proposed for furture work. (1) To put an OC_8H_{17} group in the C-1 position of the D-GlcNAc moiety. This is because the octyl group is a good mimic of the polyprenol moiety of mycobacterial sugar donors. (2) To put an octyl group on the 4′-hydroxyl position of the

L-rhamnose moiety. Right now it is not clear why a bulky group like benzoyl group on that position is better for activity. It would be interesting to see how a lipophilic octyl group on that position would affect the activity. (3) To turn the *O*-disaccharide analogues into *C*-disaccharides. The advantage of using a *C*-linkage is that it resists hydrolysis. (4) To block the hydroxy groups of both sugar units with one methyl or acetate group at a time to assess which hydroxyl binds to the target enzymes. Only after knowing their role in binding with the enzymes can we simplify the sugar structure and design a second generation of analogues.

Experimental

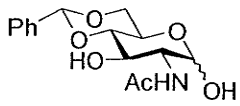
Experimental

12.1 General methods

All the reactions were performed under a nitrogen atmosphere using oven-dried glassware except as noted. Reagents were purchased from Aldrich Chemical Co. and were used as received. All solvents were purified or dried according to standard procedures.[233] Reactions were monitored by TLC. Flash column chromatography was performed on silica gel 60 (230-400 mesh) for product purification. NMR spectra were recorded on a Bruker AM 300 instrument at 300 MHz for ^{1}H, 75.5 MHz for ^{13}C and 121.5 MHz for ^{31}P. Melting points were determined on a capillary apparatus with the thermometer uncorrected. Optical rotations were measured at ambient temperature using a Rudolph Research Autopol III polarimeter. Mass spectra were obtained by electrospray ionization on a Micromass Quattro LC instrument.

12.2 Synthesis of β *N*-acetyl *C*-mannosaminyl phosphonate

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranose (64)

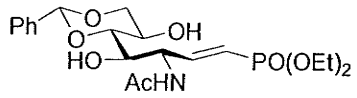


64

Compound **64** was prepared according to a procedure described by Macher.[144] A solution of **61** (5.00 g, 22.6 mmol), benzaldehyde dimethyl acetal (5.20 ml, 33.9 mmol) and *p*-toluenesulfonic acid monohydrate (500 mg) in dry DMF (10 ml) were stirred for 4 h at 50°C. After the reaction solution cooled down, triethylamine (2 ml) was added. The solid product was washed thoroughly with water, methanol and ether. Compound **64** was isolated as a white powder (4.51 g, 65%). NMR data showed that it was a mixture of α and β anomers.

Mp 239-240°C, lit.[234] 247-248°C. $[\alpha]_D$ –24 (*c* 0.50, DMSO). lit.[234] –24 (*c* 1.0, DMSO).

Diethyl (4*R*, 5*S*, 6*R*)-3-acetamido-5,7-*O*-benzylidene-4,6-dihydroxy-1-heptene phosphonate (70)



70

n-Butyllithium (2.0 M, 2.9 ml, 5.8 mmol) was added dropwise to a stirred solution of **69** (1.5 ml, 5.8 mmol) in THF (4 ml) at –78°C. After stirring for 10 min at –78°C and 20 min at room temperature, a solution of $ZnBr_2$ (0.65 g, 2.9 mmol) in THF (2 ml) was added. After stirring for 40 min, **64** (300 mg, 0.97 mmol) was added to the reaction, and the stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with saturated NH_4Cl, dried and concentrated. The crude product was purified by flash chromatography (1:10 hexane:ethyl acetate to 10:1 ethyl acetate:methanol) to give **70** as a thick colorless oil (366 mg, 85%).

$[\alpha]_D$ –12.0 (*c* 1.10, CH_2Cl_2).

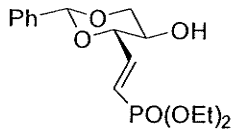
^{1}H NMR (CD_3COCD_3) δ 1.22 (dt, 3H, *J* = 16.0 and 7.0 Hz, OCH_2CH_3), 1.27 (dt, 3H, *J* = 16.0 and 7.0 Hz, OCH_2CH_3), 1.87 (s, 3H, CH_3CO), 3.59 (dd, 1H, *J* = 10.4 and 10.4 Hz, H-7), 3.69 (dd, 1H, *J* = 9.2 and 2.1 Hz, H-5), 3.92 (dddd, 1H, *J* = 10.4, 9.2, 6.2 and 5.4 Hz, H-6), 3.85-4.20 (m, 4H, 2 × OCH_2CH_3), 4.14 (ddd, 1H, *J* = 8.2, 8.0 and 2.1 Hz, H-4), 4.21 (dd, 1H, *J* = 10.4 and 5.4 Hz, H-7), 4.33 (d, 1H, *J* = 8.0 Hz, OH_4), 4.82 (d, 1H, *J* = 6.2 Hz, OH_6), 4.89 (dddd, 1H, *J* = 8.3, 8.2, 5.6 and 1.5 Hz, H-3), 5.50 (s, 1H, PhC*H*), 5.92 (ddd, 1H, *J* = 18.8, 17.2 and 1.5 Hz, H-1), 6.86 (ddd, 1H, *J* = 22.3, 17.2 and 5.6 Hz, H-2), 7.30-7.53 (m, 5H, aromatic), 7.56 (d, 1H, *J* = 8.3 Hz, N*H*Ac).

^{13}C NMR (CD_3COCD_3) δ 16.38 (*J* = 2.9 Hz), 16.46 (*J* = 2.9 Hz), 22.83 (CH_3CO), 55.55 (*J* = 21.8 Hz, C-3), 61.38 (C-6), 61.87 (*J* = 4.9 Hz), 61.94 (*J* = 4.9 Hz), 70.26 (C-4), 71.78 (C-7), 82.46 (C-5), 101.13 (PhCH), 118.05 (*J* = 186.4 Hz, C-1), 150.89 (*J* = 5.8 Hz, C-2), 170.28 (CO).

^{31}P NMR (CD_3COCD_3) δ 19.24.

ESI-MS (m/z): $C_{20}H_{30}NO_8P$, [M+H$^+$] calc., 444.44; found, 444.42.

Diethyl (3*S*,4*R*)-3,5-*O*-benzylidene-4-hydroxy-1-*E*-pentene phosphonate (74)



74

LiHMDS (1.0 M, 0.69 ml, 0.69 mmol) was added to a solution of **69** (0.20 ml, 0.69 mmol) in THF at –78°C. After stirring for 10 min at –78°C and 40 min at room temperature, **64** (215 mg, 0.69 mmol) was added to the reaction. The mixture was stirred for 4 h at 50°C. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (ether:methanol from 100:1 to 50:1) to give **74** (67 mg, 28%) as a colorless viscous oil.

$[\alpha]_D$ –54.5 (*c* 1.05, CH_2Cl_2).

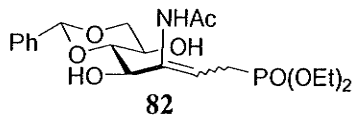
^{1}H NMR ($CDCl_3$): δ 1.33 (dt, 6H, *J* = 7.1 and 0.8 Hz, 2 × OCH_2CH_3), 3.62 (dddd, 1H, *J* = 10.1, 8.8, 5.0 and 4.1 Hz, H-4), 3.68 (dd, 1H, *J* = 10.1 and 10.1 Hz, H-5), 4.00-4.14 (m, 4H, 2 × OCH_2CH_3), 4.23 (ddd, 1H, *J* = 8.8, 3.2 and 2.0 Hz, H-3), 4.32 (dd, 1H, *J* = 10.1 and 4.1 Hz, H-5), 5.44 (d, 1H, *J* = 5.0 Hz, O*H*), 5.58 (s, 1H, PhC*H*), 6.11 (ddd, 1H, *J* = 21.6, 17.2 and 2.0 Hz, H-1), 7.26 (ddd, 1H, *J* = 23.3, 17.2 and 3.2 Hz, H-2), 7.34-7.57 (m, 5H, aromatic).

^{13}C NMR ($CDCl_3$): δ 16.69 (*J* = 6.5 Hz), 62.44 (*J* = 5.6 Hz), 62.46 (*J* = 5.5 Hz), 65.36 (*J* = 1.9 Hz, C-4), 72.00 (C-5), 81.36 (*J* = 20.5 Hz, C-3), 101.10, 116.36 (*J* = 188.9 Hz, C-1), 149.99 (*J* = 6.7 Hz, C-2).

^{31}P NMR ($CDCl_3$): δ 20.41.

ESI-MS (m/z): $C_{16}H_{23}O_6P$, [M+H$^+$] calc. 343.33, found 343.34.

Diethyl (4*R*, 5*S*, 6*R*)-3-acetamido-5,7-*O*-benzylidene-4,6-dihydroxy-2-heptene phosphonate (82)



82

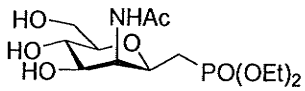
A pair of diastereomers **82** was isolated as intermediates from the preparation of **83** (the procedure is described in the preparation of **49**). The ratio of these diastereomers depended on reaction time, varying from 1:1.6 to 1:2.5. The following NMR data correspond to the major diastereomer.

^{1}H NMR (CD_3COCD_3): δ 1.24-1.36 (m, 6H), 1.95 (s, 3H), 2.58 (dd, 2H, J = 21.5 and 7.8 Hz, H-2), 3.50-3.60 (m, 1H, H-7), 3.60-3.75 (m, 1H, H-6), 3.90-4.10 (m, 4H), 4.20-4.30 (m, 2H, H-4,5), 5.45 (s, 1H), 5.56 (t, 1H, J = 21.5 Hz), 7.25-7.55 (m, 5H), 8.14 (s, 1H, NHAc).

^{13}C NMR (CD_3COCD_3): δ 16.25, 23.39, 25.40 (C-1, J = 139 Hz), 61.24, 64.55, 68.71, 71.02, 82.62, 101.08, 112.80 (C-2, J = 11.3 Hz), 137.41 (C-3, J = 35.9 Hz), 172.04.

^{31}P NMR (CD_3COCD_3): δ 29.10.

Diethyl *C*-(2-acetamido-2-deoxy-β-D-mannopyranosyl) methanephosphonate (49)



49

K_2CO_3 (42 mg, 0.30 mmol) was added to a solution of **70** (89 mg, 0.20 mmol) in EtOH (5 ml) and the mixture was stirred overnight at room temperature. Amberlite IR-120 (H^+) was added to neutralize the solution. The Amberlite was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. Aqueous HOAc (80% v/v, 2 ml) was added to dissolve the residue and the mixture was stirred for 1 h at 80°C. The solvent was evaporated, and the crude product was purified by flash chromatography (CH_2Cl_2 to 5:1 CH_2Cl_2:methanol) to give **49** as a glassy solid.

$[\alpha]_D$ + 40.2 (*c* 1.65, CH_2Cl_2).

^{1}H NMR (CD_3CN): δ 1.28 (t, 6H, *J* = 7.1 Hz, 2 × CH_3), 1.93 (s, 3H, CH_3CO), 1.90-2.04 (m, 1H, H-1), 2.26 (ddd, 1H, *J* = 16.1, 11.8 and 11.8 Hz, H-1), 3.35 (bdd, 1H, *J* = 7.4 and 7.4 Hz, H-5′), 3.61 (dd, 1H, *J* = 8.2 and 7.4 Hz, H-4′), 3.60-3.75 (m, 1H, H-3′), 3.60-3.80 (m, 2H, H-6′), 3.83-3.92 (m, 1H, H-2′), 4.08 (q, 4H, 2 × CH_2), 4.41 (dddd, 1H, *J* = 11.8, 4.8, 4.8 and 3.3 Hz, H-1′), 6.95 (d, 1H, *J* = 7.9 Hz, NHAc).

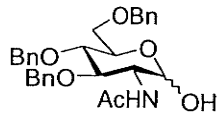
^{13}C NMR (CD_3CN): δ 15.26, 15.34, 21.80, 23.52 (*J* = 142.8 Hz, C-1), 52.77 (*J* = 14.1 Hz, C-2′), 60.66 (C-6′), 61.40 (*J* = 20.9Hz), 61.49 (*J* = 20.9 Hz), 67.27 (*J* = 5.4 Hz, C-1′), 70.20 (C-4′), 70.24 (C-5′), 74.75 (C-3′), 170.46.

^{31}P NMR (CD_3CN): δ 31.21.

ESI-MS (m/z): $C_{13}H_{26}NO_8P$, [M+H$^+$] calc., 356.33; found, 356.30.

12.3 Synthesis of β *N*-acetyl *C*-glucosaminyl phosphonate

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (100)



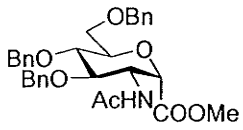
100

Compound **100** was prepared by a modification of the procedure described by Hoffmann.[87] A suspension of **61** (100 g, 452 mmol), allyl alcohol (1.4 L) and BF_3 Et_2O (13 ml) was refluxed for 2 h. The reaction mixture was concentrated to dryness under reduced pressure. The residue was dissolved in DMF (250 ml), and THF (500 ml) was added to the solution. The solution was cooled to 0°C, and then tetrabutylammoniun iodide (4 g) and sodium hydride (72.4 g, 1.80 mol) were added. Within 30 min, heat was evolved and a gray solid precipitated. Benzyl bromide (213 ml, 1.80 mol) was added dropwise slowly into the reaction, causing a gradual exothermic dissolution of the solid. The temperature was raised to room temperature and the stirring was continued for 1 h. The reaction mixture was filtered through a Celite pad. The solvent was evaporated to give a yellow solid. A suspension of the yellow solid and potassium *t*-butoxide (60 g) in DMSO (600 ml) was stirred for 4 h at 100°C. After the solvent was evaporated again, the

residue was refluxed in a mixture of ethanol, toluene and 2 N HCl (7:3:1, 400 ml). A white solid precipitated within 45 min. The solid was washed thoroughly with water. Recrystallization from methanol afforded **100** as a white fluffy solid (53 g, 24%). NMR data showed that it was a mixture of α and β anomers.

Mp 219-219.5°C, lit. 212-214°C,[87] 216-219°C.[235,236] $[\alpha]_D$ +28 (*c* 0.50, DMSO).

3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (108)



108

Compound **108** was prepared according to Schafer's procedure.[26] Thionyl chloride (12 ml) was added to a suspension of **100** (2 g, 4.1 mmol) in a mixture of CH_2Cl_2 and toluene (1:1, 24 ml). After stirring for 30 min at room temperature, the solvents were evaporated. The residue was dissolved in dry THF and cooled to –95°C. Butyllithium (2.5 M, 2.0 ml, 5.0 mmol) was added, and the solution was stirred for 5 min. A freshly prepared lithium naphthalenide solution in THF (1.0 M, 9.6 ml, 9.6 mmol) was added and the soluction was stirred until the color changed from dark green to dark brown. Carbon dioxide was bubbled through the reaction mixture for 1 h at –78°C. After the solution was warmed to room temperature, it was diluted with brine and ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated. Purification of the residue with chromatography (ethyl acetate:methanol:acetic acid from 20:2:1 to 2:2:1) gave **107** as a colorless oil (5.0 g, 81%). Its NMR data are consistent with the literature results.[26]

Cs_2CO_3 (0.82 g) and methyl iodide (0.16 ml, 2.5 mmol) were added to a solution of **107** (1.09 g, 2.1 mmol) in DMF (10 ml). The mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The residue was suspended in water and extracted with ethyl acetate. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated. Column

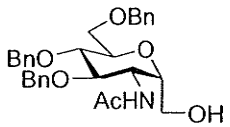
chromatography of the residue (1:2 hexane:ethyl acetate) gave **108** as a white solid (0.81 g, 73%).

$[\alpha]_D$ +29.3 (*c* 0.75, CH_2Cl_2). lit.[26] +41.4 (*c* 0.37, acetone).

^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, CH_3CO), 3.68 (m, 1H, H-6), 3.74 (s, 3H, OCH_3), 3.76-3.82 (m, 3H, H-7, 4), 4.19 (dd, 1H, *J* = 9.8 and 5.7 Hz, H-5), 4.48-4.68 (m, 8H, H-2, H-3, 3 × CH_2Ph), 6.45 (d, 1H, *J* = 9.6 Hz, *NH*Ac), 7.20-7.40 (m, 15H, aromatic).

The NMR data are in agreement with the literature results.[26]

3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-1-hydroxy-D-*glycero*-D-*ido*-heptitol (109)



109

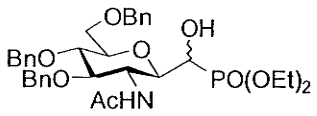
Compound **109** was prepared according to Schafer's procedure.[26] Sodium borohydride (80 mg, 1.4 mmol) was added to a solution of **108** (0.37 g, 0.69 mmol) in dry ethanol-THF (2.5:1, 7 ml). The mixture was stirred for 3 h at room temperature. The solvent was evaporated and the residue was diluted with brine and CH_2Cl_2. The organic layer was dried and concentrated. Flash chromatography of the residue (hexane:ethyl acetate from 1:2 to 1:4) gave **109** as an amorphous white solid (0.25 g, 71% yield).

$[\alpha]_D$ +20.3 (*c* 1.22, acetone). lit.[26] +20.1 (*c* 1.22, acetone).

^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3H, CH_3CO), 3.40 (dd, 1H, *J* = 11.6 and 8.7 Hz, H-1), 3.64 (dd, 1H, *J* = 11.6 and 5.7 Hz, H-1), 3.66-3.70 (m, 1H, H-4), 3.73-3.77 (m, 1H, H-5), 3.78 (dd, 1H, *J* = 10.1 and 7.1 Hz), 3.89 (dd, 1H, *J* = 9.9 and 7.1 Hz, H-7), 4.08 (ddd, 1H, *J* = 8.7, 5.8 and 0.9 Hz, H-2), 4.25-4.36 (m, 2H, H-6, 3), 4.48 (dd, 2H, *J* = 11.4 and 8.6 Hz, $PhCH_2$), 4.54 (s, 2H, $PhCH_2$), 4.46 (d, 2H, *J* = 11.5 and 11.5 Hz, $PhCH_2$), 7.08 (d, 1H, *J* = 8.8 Hz, *NH*Ac), 7.22-7.42 (m, 15H, aromatic).

The NMR data are in agreement with the literature results.[26]

Diethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-β-D-glucopyranosyl) methanolphosphonate (110)



110

Oxalyl chloride (0.23 ml, 2.6 mmol) was added to a solution of DMSO (0.18 ml, 2.6 mmol) in CH_2Cl_2 at –78°C. The mixture was stirred for 15 min. Then a solution of **109** (538 mg, 1.1 mmol) in CH_2Cl_2 (5 ml) was added and the reaction mixture was stirred for another 15 min at –78°C. Then, triethylamine (0.615 ml, 4.4 mmol) was added. After stirring for 15 min at –78°C and 20 min at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine. The organic layer was dried and concentrated to give a yellow oil that was used directly for the next reaction.

n-BuLi (2.5 M, 0.86 ml, 2.2 mmol) was added to a solution of $HOP(OEt)_2$ (0.28 ml, 2.1 mmol) in THF (5 ml) at –78°C, and the solution was stirred for 5 min. Then a solution of the yellow oil in THF (5 ml) was added. After stirring for 2 h at –78°C and 20 min at room temperature, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated NH_4Cl, water, brine, dried and concentrated. The residue was purified by flash chromatography (1:50 hexane:ethyl acetate to 20:1 ethyl acetate:methanol) to give **110** as a colorless thick oil that was found to be a single isomer (453 mg, 66%).

$[\alpha]_D$ +6.7 (*c* 0.60, CH_2Cl_2).

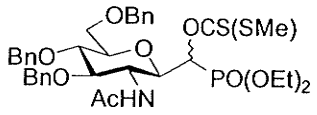
^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, *J* = 8.1 Hz, OCH_2CH_3), 1.32 (t, 3H, *J* = 8.1 Hz, OCH_2CH_3), 1.90 (s, 3H, CH_3CO), 3.59 (ddd, 1H, *J* = 15.2, 10.7 and 5.1 Hz, H-1), 3.72-3.78 (m, 2H, H-3′, 4′), 3.85 (dd, 1H, *J* = 9.4 and 5.6 Hz, H-6′), 4.02 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-6′), 4.00-4.30 (m, 4H, 2 × OCH_2CH_3), 4.33 (ddd, 1H, *J* = 10.3, 9.4 and 5.6 Hz, H-5′), 4.37 (dd, 1H, *J* = 10.7 and 7.0 Hz, H-1′), 4.30-4.40 (m, 1H, H-2′), 4.50-4.85 (m, 6H, 3 × $PhCH_2$), 5.18 (dd, 1H, *J* = 29.1 and 5.1 Hz, OH), 7.20-7.40 (m, 16H, aromatic and NHAc).

^{13}C NMR ($CDCl_3$): δ 16.88, 16.95, 23.49, 46.33 (J = 12.6), 62.66 (J = 6.6 Hz), 63.33 (J = 6.6 Hz), 68.42 (C-6′), 69.08 (C-5′), 72.33, 72.41, 73.28 (C-3′), 73.70, 73.89 (C-4′), 75.36 (C-1′), 172.50.

^{31}P NMR ($CDCl_3$): δ 25.78.

ESI-MS (m/z): $C_{34}H_{44}NO_9P$, [M+H$^+$] calc., 642.70; found, 642.68.

Diethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-β-D-glucopyranosyl) 1-methylthiocarbonate methanephosphonate (112)



112

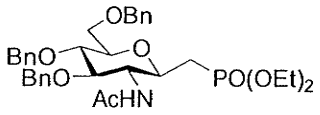
Compound **112** was prepared according to a procedure described by Chen.[237] Sodium hydride (60%, 6.2 mg, 0.16 mmol) and carbon disulfide (0.50 ml, 8.3 mmol) were added to a solution of **110** (78 mg, 0.12 mmol) in THF. After stirring for 1.5 h at room temperature, methyl iodide (19 μl, 0.31 mmol) was added. After stirring overnight, the solvent was evaporated under reduced pressure. Column chromatography of the residue (hexane:ethyl acetate from 1:1 to 1:10) gave **112** as a light yellow oil.

^{1}H NMR ($CDCl_3$) δ 1.26 (dt, 6H, J = 7.1 and 3.0 Hz, 2 × OCH_2CH_3), 1.84 (s, 3H, CH_3CO), 2.59 (s, 3H, SCH_3), 3.50 (ddd, 1H, J = 10.3, 8.3 and 7.0 Hz, H-2′), 3.61-3.68 (m, 2H, H-4′, 5′), 3.72-3.80 (m, 2H, H-6′), 4.04-4.27 (m, 4H, 2 × OCH_2CH_3), 4.11 (dd, 1H, J = 7.0 and 7.0 Hz, H-3′), 4.42 (ddd, 1H, J = 10.3, 3.9 and 1.8 Hz, H-1′), 4.50-4.68 (m, 4H, 2 × $PhCH_2$), 4.82 (d, 2H, J = 11.1 Hz, $PhCH_2$), 5.95 (d, 1H, J = 8.3 Hz, NHAc), 6.51 (dd, 1H, J = 12.6 and 1.8 Hz, H-1), 7.18-7.37 (m, 15 H, aromatic).

^{13}C NMR ($CDCl_3$): δ 16.30 (d, J = 7.3 Hz), 16.44 (d, J = 7.3 Hz), 19.43 (SCH_3), 23.76 (CH_3CO), 52.47 (d, J = 9.6 Hz, C-2′), 62.88 (d, J = 6.5 Hz), 63.76 (d, J = 6.5 Hz), 69.07 (C-6′), 73.33, 74.48 (d, J = 165 Hz, C-1), 74.76, 75.23, 75.60 (C-1′), 78.58 (C-4′), 79.53 (C-5′), 82.49 (d, J = 1.7 Hz, C-3′), 170.20, 216.13 (d, J =4.7 Hz).

^{31}P NMR ($CDCl_3$): δ 16.84.

Diethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-β-D-glucopyranosyl) methanephosphonate (111)



111

A suspension of tributyltin hydride (0.17 ml, 0.61 mmol), AIBN (6 mg) and **112** (89 mg, 0.12 mmol) in toluene (2 ml) was stirred for 20 min at 80°C under argon atmosphere. The solvent was evaporated. The residue was dissolved in CH_3CN and the solution was extracted with hexane to remove the tin compounds. The CH_3CN solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate) to give **111** as a colorless oil (35 mg, 46%).

$[\alpha]_D$ –2.5 (*c* 1.25, CH_2Cl_2).

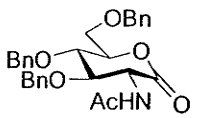
^1H NMR ($CDCl_3$): δ 1.22 (t, 3H, *J* = 7.1 Hz), 1.28 (t, 3H, *J* = 7.1 Hz), 1.86 (s, 3H, CH_3CO), 2.02-2.14 (m, 2H), 3.52 (ddd, 1H, *J* = 9.5, 3.3 and 3.3 Hz), 3.55-3.63 (m, 1H), 3.60-3.68 (m, 1H), 3.35-3.75 (m, 1H), 3.35-3.70 (m, 2H), 3.87-3.95 (m, 1H), 3.90-4.10 (m, 4H), 4.44-4.88 (m, 6H), 6.26 (d, 1H, *J* = 9.2 Hz), 7.16-7.40 (m, 15H).

^{13}C NMR ($CDCl_3$): δ 16.64 (*J* = 3.2 Hz), 16.73 (*J* = 2.9 Hz), 23.35, 29.02 (*J* = 142 Hz), 55.26 (*J* = 14.5 Hz), 61.78 (*J* = 6.6 Hz), 62.68 (*J* = 6.2 Hz), 69.05, 74.9 (*J* = 4.7 Hz), 75.08, 75.22, 78.41, 79.00, 83.43 (*J* = 2.5 Hz), 170.61.

^{31}P NMR ($CDCl_3$): δ 30.39.

ESI-MS (m/z): $C_{34}H_{44}NO_8P$, [M+H^+] calc., 626.70; found, 626.70.

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (93)



93

Ac_2O (5 ml) was added to a solution of **100** (0.99 g, 2.0 mmol) in DMSO (7ml). The solution was stirred overnight at room temperature. The reaction mixture was diluted with aqueous saturated $NaHCO_3$ and ethyl acetate. The organic layer was dried and

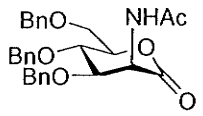
concentrated. Column chromatography of the residue (hexane:ethyl acetate from 1:1 to 1:50) gave **93** as a white solid (649 mg, 66%).

Mp 144.5-145°C, lit. 140-141°C,[168] 141-142°C.[167] $[\alpha]_D$ +122 (*c* 0.60, CH_2Cl_2), lit. + 125.7 (*c* 1, CH_2Cl_2),[168] +123.3 (*c* 0.60, $CHCl_3$).[167]

^{1}H NMR ($CDCl_3$): δ 1.85 (s, 3H, C*H*$_3$CO), 3.77 (dd, 2H, *J* = 2.3 and 2.3 Hz, H-6), 3.96-4.08 (m, 3H, H-2, 3, 4), 4.41-4.48 (m, 1H, H-5), 4.49 (d, 1H, *J* = 12.1 Hz, PhC*H*$_2$), 4.59 (d, 1H, *J* = 12.1 Hz, PhC*H*$_2$), 4.65 (dd, 2H, *J* = 9.1 and 9.1 Hz, PhC*H*$_2$), 4.79 (s, 1H, PhC*H*$_2$), 4.83 (s, 1H, PhC*H*$_2$), 6.22 (d, 1H, *J* = 6.1 Hz, N*H*Ac), 7.15-7.40 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 22.61, 55.48, 67.84, 73.62, 74.56, 74.58, 76.11, 78.59, 79.62, 168.71, 170.44.

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-mannono-1,5-lactone (113)



113

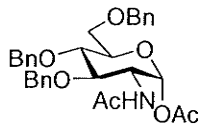
A minor product **102** (2%) was isolated from the preparation of **93**.

Amorphous white solid. $[\alpha]_D$ +134 (*c* 0.85, CH_2Cl_2), lit.[101] $[\alpha]_D$ +112.3 (*c* 0.60, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3H, C*H*$_3$CO), 3.60-3.80 (m, 2H, H-6), 3.90 (dd, 1H, *J* = 7.8 and 0.9 Hz, H-4), 4.17 (dd, 1H, *J* = 3.3 and 0.9 Hz, H-3), 4.38 (ddd, 1H, *J* = 7.8 3.7 and 3.7 Hz, H-5), 4.30-4.64 (m, 6H, 3 × PhC*H*$_2$), 5.04 (dd, 1H, *J* = 7.1 and 3.3 Hz, H-2), 6.44 (d, 1H, *J* = 7.1 Hz, N*H*Ac), 7.16-7.40 (m, 15H, aromatic).

The NMR data are in agreement with the literature results.[101]

Acetyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranoside (114)



114

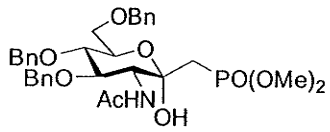
A minor product **114** (6-9%) was isolated from the preparation of **93**.

Amorphous white solid. $[\alpha]_D$ +87.4 (*c* 5.40, CH_2Cl_2).

^1H NMR ($CDCl_3$): δ 1.79 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 3.63-3.92 (m, 5H, H-3, 4, 5, 6), 4.52 (d, 1H, *J* = 11.9 Hz, $PhCH_2$), 4.60 (d, 1H, *J* = 10.6 Hz, $PhCH_2$), 4.63 (d, 1H, *J* = 11.9 Hz, $PhCH_2$), 4.67 (d, 1H, *J* = 11.9 Hz, $PhCH_2$), 4.82 (d, 1H, *J* = 10.6 Hz, $PhCH_2$), 4.89 (d, 1H, *J* = 11.9 Hz, $PhCH_2$), 5.06 (d, 1H, *J* = 8.6 Hz, NHAc), 6.15 (d, 1H, *J* = 3.5 Hz, H-1), 7.16-7.44 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 20.94, 23.19, 51.49, 68.14, 73.38, 73.58, 74.58, 75.16, 77.88, 79.04, 91.60, 168.94, 169.90.

Dimethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranose) methanephosphonate (116)



116

n-BuLi (2.5 M, 0.55 ml) was added to a solution of $CH_3P(O)(OMe)_2$ (0.15 ml, 1.4 mmol) in THF (2.5 ml) at –78°C. After stirring for 30 min, **93** (150 mg, 0.31 mmol) in THF (1.5 ml) was dropped in, and the solution was stirred for 1 h at –78°C. The cold bath was then removed and the temperature rose slowly to 0°C. Aqueous saturated NH_4Cl was added to the reaction. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with water and brine, dried and concentrated. The crude product was purified by flash chromatography (1:50 hexane: ethyl acetate to ethyl acetate) to give **116** as a white solid (180 mg, 96%).

Mp 128-129°C, $[\alpha]_D$ +29.8 (*c* 0.50, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C*H*$_3$CO), 2.23 (dd, 2H, *J* = 18.3 and 1.3 Hz, H-1), 3.63 (bd, 1H, *J* = 10.2 Hz, H-6′), 3.74 (dd, 1H, *J* = 10.2 and 4.3 Hz, H-6′), 3.66 (d, 3H, *J* = 10.7 Hz, OC*H*$_3$), 3.69 (d, 3H, *J* = 10.7 Hz, OC*H*$_3$), 3.71 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-3′), 3.75 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 4.04 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-2′), 4.05 (dd, 1H, *J* = 10.1 and 10.1 Hz, H-5′), 4.49 (s, 2H, PhC*H*$_2$), 4.60 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.67 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.83 (d, 1H, *J* = 2.8 Hz, PhC*H*$_2$), 4.86 (d, 1H, *J* = 2.8 Hz, PhC*H*$_2$), 5.75 (d, 1H, *J* = 9.6 Hz, N*H*Ac), 6.00 (bs, 1H, OH), 7.20-7.40 (m, 15H, aromatic).

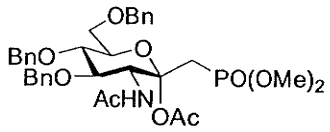
^{13}C NMR ($CDCl_3$): δ 23.39, 33.44 (*J* = 135.1 Hz, C-1), 52.21 (*J* = 7.1 Hz), 54.13 (*J* = 5.8 Hz), 57.29 (*J* = 14.5 Hz, C-2′), 69.08 (C-6′), 71.80 (C-5′), 73.70, 75.28, 75.36, 78.91 (C-4′), 81.17 (*J* = 4.2 Hz, C-3′), 97.32 (*J* = 8.0 Hz, C-1′), 170.61.

^{31}P NMR ($CDCl_3$): δ 32.22.

ESI-MS (m/z): $C_{32}H_{40}NO_9P$, [M+H$^+$] calc., 614.64; found, 614.69.

Anal. Calcd for $C_{32}H_{40}NO_9P$: C, 62.63; H, 6.57; N, 2.28; Found: C, 62.24; H, 6.92; N, 2.27.

Dimethyl *C*-(acetyl 2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranoside) methanephosphonate (118)



118

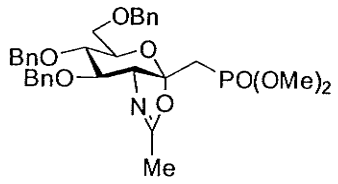
Acetylation of **116** was based on a procedure reported by Dondoni.[238] Ac_2O (0.15 ml, 1.6 mmol) and triethylamine (0.25 ml, 1.8 mmol) were added to a solution of **116** (96 mg, 0.16 mmol) in CH_2Cl_2 (0.5 ml). The mixture was stirred at room temperature overnight. The reaction solution was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with 1.2 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried and evaporated to dryness. Column chromatography of the residue (1:10 hexane:ethyl acetate to 50:1 ethyl acetate:methanol) gave **118** as a colorless oil (61 mg, 61%). The unreacted **116** (36 mg) was recovered from the reaction.

^{1}H NMR ($CDCl_3$): δ 1.92 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.78 (dd, 1H, J = 45.8 and 16.0 Hz, H-1), 2.88 (dd, 1H, J = 46.3 and 16.0 Hz, H-1), 3.67 (dd, 6H, J = 11.1 and 5.6 Hz, 2 × OCH_3), 3.69 (ddd, 1H, J = 11.1, 9.8 and 9.3 Hz, H-5′), 3.70 (dd, 1H, J = 9.3 and 9.3 Hz, H-6′), 3.74 (d, 1H, J = 9.8 and 9.8 Hz, H-3′), 3.77 (dd, 1H, J = 11.1 and 9.3 Hz, H-6′), 4.35 (dd, 1H, J = 9.8 and 9.8 Hz, H-2′), 4.58 (dd, 2H, J = 11.7 and 11.7 Hz, PhCH_2), 4.57 (d, 1H, J = 11.7 Hz, PhCH_2), 4.68 (d, 1H, J = 11.7 Hz, PhCH_2), 4.82 (dd, 2H, J = 11.1 and 11.1 Hz, PhCH_2), 5.94 (d, 1H, J = 9.8 Hz, N*H*Ac), 7.20-7.40 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 22.02, 23.64, 30.99 (J = 140.9 Hz, C-1), 52.39 (J = 7.0 Hz), 52.87 (J = 6.1 Hz), 56.91 (J = 2.8 Hz, C-2′), 68.24 (C-6′), 73.47, 73.91 (C-3′), 75.17, 75.20, 77.59 (C-4′), 80.74 (J = 4.2 Hz, C-5′), 103.27 (J = 1.6 Hz, C-1′), 169.40, 170.43.

^{31}P NMR ($CDCl_3$): δ 25.94.

Dimethyl *C*-(3′,4′,6′-tri-*O*-benzyl-2′-deoxy-1′,2′-(3a-methyl-[3,2-d]oxazol)-α-D-glucopyranoside) methanephosphonate (120)



120

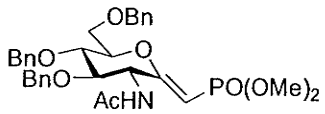
Compound **120** was produced from the reaction of **118** and TMSOTf/Et_3SiH. It was obtained as a colorless oil (42%).

^{1}H NMR ($CDCl_3$): δ 2.03 (d, 3H, J = 1.8 Hz, CH_3), 2.56 (dd, 1H, J = 56.6 and 15.7 Hz, H-1), 2.61 (dd, 1H, J = 56.6 and 15.7 Hz, H-1), 3.46-3.68 (m, 4H, H-4′, 5′, 6′), 3.70 (d, 3H, J = 10.7 Hz, OCH_3), 3.74 (d, 3H, J = 10.9 Hz, OCH_3), 3.99 (dd, 1H, J = 2.3 and 2.3 Hz, H-3′), 4.24 (d, 1H, J = 12.0 Hz, PhCH_2), 4.45 (dd, 1H, J = 2.3 and 1.5 Hz, H-2′), 4.46-4.56 (m, 4H, 2 × PhCH_2), 4.67 (d, 1H, J = 12.0 Hz, PhCH_2), 7.12-7.38 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 14.28, 35.60 (J = 142.6 Hz, C-1), 52.29 (J = 6.3 Hz), 52.74 (J = 6.3 Hz), 67.99 (J = 3.6 Hz, C-2′), 69.68 (C-6′), 71.29, 71.51 (C-4′), 71.76, 73.26, 74.49 (C-5′), 76.50 (C-3′), 106.59 (J = 1.6 Hz, C-1′), 164.87.

^{31}P NMR ($CDCl_3$): δ 26.16.

Dimethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-D-glucopyranosyl) methylenephosphonate (122)



122

A suspension of **116** (0.868 g) and anhydrous NaOAc (0.40 g) in Ac_2O (4 ml) was stirred for 2 h at 100°C. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (ether:methanol from 50:1 to 10:1) to give **108** as a white needle crystal (0.584 g, 70%).

Mp 166-167°C. $[\alpha]_D$ +70.8 (c 0.85, CH_2Cl_2).

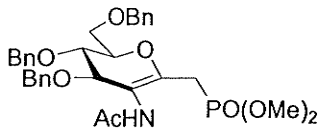
^{1}H NMR ($CDCl_3$): δ1.89 (s, 3H, C*H*$_3$CO), 3.62-3.63 (m, 1H, H-3′), 3.65 (d, 3H, J = 11.5 Hz, OC*H*$_3$), 3.67 (d, 3H, J = 11.5 Hz, OC*H*$_3$), 3.73-3.83 (m, 2H, H-6′), 3.97-3.99 (m, 2H, H-4′,5′), 4.71-4.72 (m, 1H, H-2′), 4.52-4.81 (m, 6H, C*H*$_2$Ph), 4.98 (dd, 1H, J=11.9 and 1.3 Hz, H-1), 6.33 (d, 1H, J=8.7 Hz, N*H*Ac), 7.20-7.40 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ23.1, 52.4 (J = 5.8 Hz), 52.7 (J = 14 Hz, C-2′), 53.1 (J = 5.8 Hz), 68.7 (C-6′), 73.9, 74.4, 74.6, 76.2 (C-5′), 80.0 (C-3′), 80.4 (C-4′), 93.8 (J = 192, C-1), 167.4 (C-1′), 170.3.

^{31}P NMR ($CDCl_3$): δ 20.6.

ESI-MS (m/z): $C_{32}H_{38}NO_8P$, [M+H$^+$] calc. 595.63, found 596.65.

Dimethyl 3-acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-D-*xylo*-2-*E*-heptenitol phosphonate (123)



123

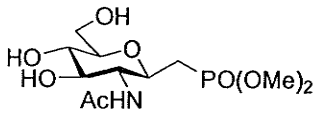
Compound **123** was the minor product (4%) in the preparation of **122**. It was obtained as a colorless oil.

^{1}H NMR ($CDCl_3$): δ1.97 (s, 3H, CH_3CO), 2.74 (dd, 1H, J = 123.7 and 15.0 Hz, H-1), 2.81(dd, 1H, J = 124.8 and 15.0 Hz, H-1), 3.63 (dd, 1H, J = 11.4 and 5.0 Hz, H-7), 3.71 (d, 3H, J = 7.5 Hz, OCH_3), 3.75 (d, 3H, J = 7.6 Hz, OCH_3), 3.52 (dd, 1H, J = 11.4 and 5.2 Hz, H-7), 3.89 (dd, 1H, J = 6.7 and 5.2 Hz, H-5), 4.23-4.32 (m, 1H, H-6), 4.46-4.80 (m, 7H, H-4, 3 × $PhCH_2$), 7.20-7.40 (m, 15H, aromatic), 7.69 (bs, 1H, N*H*Ac).

^{13}C NMR ($CDCl_3$): δ 23.32, 28.21 (J = 138.8 Hz, C-1), 52.66 (J = 6.8 Hz), 53.64 (J = 6.7 Hz), 68.11 (C-7), 72.58, 72.82, 73.24, 74.09 (C-4), 74.15 (C-5), 77.24 (C-6), 112.09 (J = 10.2, C-2), 141.69 (J = 11.3, C-3), 169.69.

^{31}P NMR ($CDCl_3$): δ 28.78.

Dimethyl *C*-(2′-acetamido-2′-deoxy-β-D-glucopyranosyl) methanephosphonate (50)



50

Palladium on carbon (10%, 35 mg) was added to a solution of **122** (35 mg) in glacial acetic acid (0.5 ml). The mixture was flushed with hydrogen gas by balloon. After stirring vigorously under a hydrogen atmosphere overnight at room temperature, the Pd/C solid was removed by filtration. The filtrate was evaporated to dryness, and column chromatography of the residue (ether to 2:1 ether:methanol) gave **50** as a glassy solid (25 mg, quantitative yield).

$[\alpha]_D$ +5.6 (c 1.0, CH_3OH).

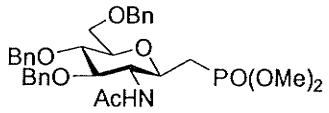
^{1}H NMR (D_2O): δ 2.04 (s, 3H, CH_3CO), 2.10-2.30 (m, 2H, H-1), 3.41 (ddd, 1H, J = 8.7, 3.8 and 2.2 Hz, H-5′), 3.52 (dd, 1H, J = 8.7 and 8.7 Hz, H-3′), 3.48 (dd, 1H, J = 8.7 and 8.7 Hz, H-4′), 3.70 (dd, 1H, J = 8.7 and 8.7 Hz, H-2′), 3.65-3.72 (m, 1H, H-1′), 3.76 (dd, 1H, J = 13.1 and 3.8 Hz, H-6′a), 3.76 (d, 3H, J = 11.0 Hz, OCH_3), 3.78 (d, 3H, J = 11.0 Hz, OCH_3), 3.89 (dd, 1H, J = 13.1 and 2.2 Hz, H-6′b).

^{13}C NMR (D_2O): δ22.6, 27.3 (J = 141 Hz, C-1), 53.5 (J = 6.4 Hz), 53.8 (J = 6.4 Hz), 56.4 (J = 15.5 Hz, C-1′), 61.0 (C-6′), 70.1 (C-4′), 73.7 (J = 6.0 Hz, C-2′), 75.3 (J = 2.8 Hz, C-3′), 80.0 (C-5′), 175.1.

^{31}P NMR (D_2O): δ 35.7.

ESI-MS (m/z): $C_{11}H_{22}NO_8P$, [M+H$^+$] calc. 328.28, found 328.43.

Dimethyl *C*-(2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-β-D-glucopyranosyl) methanephosphonate (124)



124

Palladium on carbon (10%, 100 mg) was added to a solution of **122** (83 mg) in ethanol (1.0 ml) containing a catalytic amount of acetic acid. The mixture was flushed with hydrogen gas by balloon. After stirring vigorously under a hydrogen atmosphere for 5 h at room temperature, the Pd/C solid was removed by filtration and the solvent was evaporated to dryness. Column chromatography of the residue (10:1 ether:methanol) gave **124** as a thick colorless oil (67 mg, 81%).

$[\alpha]_D$ +7.8 (*c* 0.55, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, CH_3CO), 2.00-2.20 (m, 2H, H-1), 3.45-3.55 (m, 1H, H-5′), 3.59 (dd, 1H, J = 9.7 and 9.7 Hz, H-3′), 3.66 (dd, 1H, J = 9.7 and 9.7 Hz, H-4′), 3.65-3.71 (m, 3H, H-1′, 6′), 3.65 (d, 3H, J = 11.0 Hz, OCH_3), 3.66 (d, 3H, J = 11.0 Hz, OCH_3), 3.91 (ddd, 1H, J = 9.7, 9.7 and 9.6 Hz, H-2′), 4.48 (d, 1H, J = 12.1, $PhCH_2$), 4.52 (d, 1H, J = 12.1, $PhCH_2$), 4.57 (d, 1H, J = 10.9, $PhCH_2$), 4.67 (d, 1H, J = 10.9,

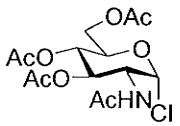
PhCH_2), 4.79 (d, 1H, J = 12.1, PhCH_2), 4.82 (d, 1H, J = 12.1, PhCH_2), 6.0 (d, 1H, J = 9.6, NHAc), 7.15-7.35 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 23.34, 28.32 (J = 141.9Hz, C-1), 51.90 (J = 6.7 Hz), 52.96 (J = 6.1 Hz), 55.22 (J = 6.7 Hz, C-2′), 68.94 (C-6′), 73.36, 74.47 (J = 4.9 Hz, C-1′), 74.65, 74. 86, 78.49 (C-4′), 78.98 (C-5′), 83.19 (C-3′), 170.56.

^{31}P NMR ($CDCl_3$): δ 32.50.

12.4 Synthesis of analogues of α *N*-acetyl D-glucosamine pyrophosphate

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (136)

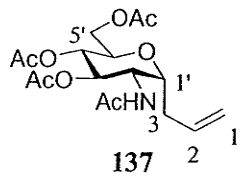


136

Compound **136** was prepared according to a procedure described by Horton.[192] *N*-acetyl D-glucosamine **61** (5 g) was suspended in acetyl chloride (10 ml) for 12 h at room temperature. The viscous solution was diluted with CH_2Cl_2 (40 ml) and the solution was poured into ice water (50 ml) with stirring. The organic layer was washed with saturated aqueous $NaHCO_3$, dried and concentrated. Crystallization of the crude product in ether gave **136** as a light amber solid (7.33 g, 89%).

^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 4.05-4.15 (m, 1H, H-6a), 4.19-4.30 (m, 2H, H-5, 6b), 4.51 (ddd, 1H, J = 10.7, 8.7 and 3.8 Hz, H-2), 5.17 (dd, 1H, J = 9.6 and 9.6 Hz, H-4), 5.30 (dd, 1H, J = 10.7 and 9.6 Hz, H-3), 5.99 (d, 1H, J = 8.7 Hz, NHAc), 6.16 (d, 1H, J = 3.8 Hz, H-1).

3-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-α-D-glucopyranosyl)-1-propene (137)



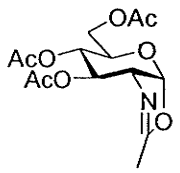
137

Compound **137** was prepared by following a procedure described by Horton.[103] The freshly prepared **136** (6.23 g, 17.0 mmol), allyltributyltin (25 ml, 78 mmol) and AIBN (0.82 g) were stirred in toluene (10 ml) at 85°C for 3 h under an argon atmosphere. After cooling down, the reaction mixture was diluted with acetonitrile (450 ml) and extracted with *n*-pentane (5 × 150 ml). The acetonitrile layer was concentrated under reduced pressure. Column chromatography of the residue (1:4 hexane:ethyl acetate) gave **137** as an amorphous white solid (4.94 g, 78%).

^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H, CH_3CO), 2.03 (s, 6H, 2 × CH_3CO), 2.04 (s, 3H, CH_3CO), 2.19-2.47 (m, 2H, H-3), 3.86 (ddd, 1H, J = 6.6, 6.6 and 3.6 Hz, H-5′), 4.08 (dd, 1H, J = 12.0 and 3.6 Hz, H-6′), 4.14-4.31 (m, 3H, H-1′,2′,6′), 4.90 (dd, 1H, J = 7.0 and 7.0 Hz, H-4′), 4.96-5.16 (m, 3H, H-1,3′), 5.72 (tdd, 1H, J = 17.0, 10.2 and 6.8 Hz, H-2), 6.00 (d, 1H, J = 8.1 Hz, N*H*Ac).

The NMR data are in agreement with the literature results.[103]

3,4,6-Tri-*O*-acetyl-1,2-dideoxy-2′-methyl-α-D-glucopyranoso[2,1-d]-oxazoline (138)



138

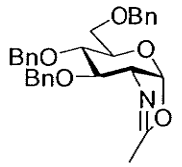
Compound **138** was the minor product in the preparation of **137**. It was obtained as a colorless oil. Although it has been prepared in the literature, NMR data were not available.[239-241]

^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 3.56 (dt, 1H, J = 9.0 and 4.4 Hz, H-5), 4.13 (d, 2H, J = 4.4 Hz, H-6), 4.21 (dd, 1H, J = 7.4 and 2.5

Hz, H-2), 4.88 (dd, 1H, J = 9.0 and 2.5 Hz, H-4), 5.22 (dd, 1H, J = 2.5 and 2.5 Hz, H-3), 5.92 (d, 1H, J = 7.4 Hz, N*H*Ac).

^{13}C NMR ($CDCl_3$): δ 13.89, 20.68, 20.77, 20.85, 63.31 (C-6), 64.94 (C-2), 67.49 (C-5), 68.37 (C-4), 70.35 (C-3), 99.35 (C-1), 166.57, 169.15, 169.47, 170.52.

3,4,6-Tri-*O*-benzyl-1,2-dideoxy-2′-methyl-α-D-glucopyranoso[2,1-d]-oxazoline (144)



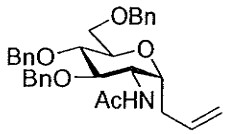
144

Compound **144** was isolated as a colorless oil from the reaction of **103**, allytributylstannane and AIBN at 80°C.

^{1}H NMR ($CDCl_3$): δ 2.05 (d, 3H, J = 1.7 Hz, C*H*$_3$), 3.48-3.62 (m, 3H, H-4, 6), 3.69 (ddd, 1H, J = 8.8, 2.4 and 1.2 Hz, H-5), 4.01 (dd, 1H, J = 2.8 and 2.8 Hz, H-3), 4.22 (ddd, 1H, J = 7.7, 2.8 and 1.7 Hz, H-2), 4.31 (d, 1H, J = 11.7, PhC*H*$_2$), 4.50 (d, 1H, J = 12.3, PhC*H*$_2$), 4.56 (d, 1H, J = 12.3, PhC*H*$_2$), 4.57 (d, 1H, J = 12.0, PhC*H*$_2$), 4.60 (d, 1H, J = 12.0, PhC*H*$_2$), 4.71 (d, 1H, J = 11.7, PhC*H*$_2$), 6.03 (d, 1H, J = 7.7 Hz, C-1), 7.14-7.42 (m, 15H, aromatic).

The NMR data are in agreement with the literature results.[240]

3-(2′-Acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)-1-propene (145)



145

Compound **145** was prepared by following a procedure reported by Xie.[35] Compound **137** (1.00 g, 2.69 mmol) was added into a sodium methoxide solution in methanol (1.0 M, 10.0 ml, 10.0 mmol), and the mixture was stirred for 30 min at room temperature. Then Amberlite IR-120 (acidic) was added to neutralize the reaction

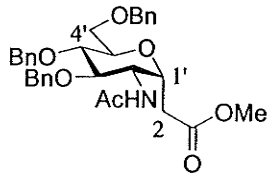
solution. The Amberlite was removed by filtration and the filtrate was concentrated under reduced pressure. Sodium hydride (60%, 340 mg) was added to the solution of the residue in DMF (10 ml) at 0°C. After stirring 10 min at 0°C, benzyl bromide (1.0 ml, 8.47 mmol) was added to the solution. After stirring 1 h at room temperature, the reaction mixture was diluted with ethyl acetate (300 ml). The solid in the reaction mixture was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography of the residue (hexane:ethyl acetate from 3:1 to 1:1) gave **145** as a white solid (1.32 g, 95%).

$[\alpha]_D$ +12.4 (*c* 0.90, CH_2Cl_2), lit. $[\alpha]_D$+11.5 (*c* 0.98, CH_2Cl_2),[35] +4.4 (*c* 1.0, $CHCl_3$).[242]

^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3H, CH_3CO), 2.12-2.34 (m, 2H, H-3), 3.58-3.63 (m, 1H, H-4′), 3.69-3.74 (m, 1H, H-3′), 3.77 (dd, 1H, *J* = 9.9 and 7.0 Hz, H-6′), 3.88 (dd, 1H, *J* = 9.9 and 6.8 Hz, H-6′), 3.98 (ddd, 1H, *J* = 7.6 and 5.6 and 1.8 Hz, H-1′), 4.18-4.32 (m, 2H, H-2′,5′), 4.45 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.51 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.54 (s, 2H, PhCH_2), 4.60 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.63 (d, 1H, *J* = 11.5 Hz, PhCH_2), 5.02-5.16 (m, 2H, H-1), 5.74-5.92 (m, 1H, H-2), 6.54 (d, 1H, *J* = 9.7 Hz, N*H*Ac), 7.22-7.40 (m, 15H, aromatic).

The NMR data are in agreement with the literature results.[35,242]

Methyl (2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl) ethanoate (151)



151

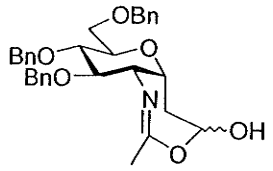
Jones reagent (3 ml) and osmium tetraoxide (2.5% in *t*-butanol, 5 drops) were added to a solution of **145** (182 mg, 0.353 mmol) in acetone (3 ml) at 0°C. After stirring 1.5 h at 0°C, saturated aqueous sodium thiosulfite was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was concentrated to dryness. Cesium

carbonate (0.20 g) and methyl iodide (0.11 ml, 1.77 mmol) were added to the solution of the residue in DMF (2 ml). After stirring 2 h at room temperature, the reaction mixture was diluted with water (30 ml) and CH_2Cl_2. The organic layer was dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate from 2:1 to 1:2) gave **151** as a white solid (36 mg, 19%).

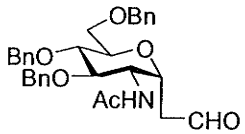
^{1}H NMR ($CDCl_3$): δ 1.84 (s, 3H, CH_3CO), 2.45 (bs, 1H, H-2), 2.47 (d, 1H, J = 2.4 Hz, H-2), 3.60-3.64 (m, 1H, H-3′), 3.66 (s, 3H, OCH_3), 3.66-3.70 (m, 1H, H-4′), 3.85 (d, 1H, J = 2.7 Hz, H-6′), 3.87 (d, 1H, J = 3.1 Hz, H-6′), 4.20-4.28 (m, 2H, H-2′, 5′), 4.36-4.68 (m, 7H, 3 × $PhCH_2$, H-1′), 6.68 (d, 1H, J = 9.7 Hz, NHAc), 7.20-7.40 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 23.25, 36.94 (C-2), 47.23 (C-2′), 51.74 (OCH_3), 65.29 (C-1′), 67.93 (C-6′), 71.75, 72.25, 72.82 (C-3′), 73.36, 74.11 (C-4′), 75.21 (C-5′), 169.86, 171.44.

2-(2′-Acetylamino-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl) ethanal (147) and its cyclized form **(146)**



146

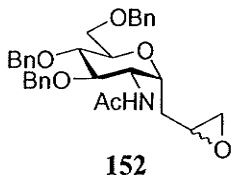


147

Compound **147** was prepared according to a procedure described by Xie.[35] Osmium tetraoxide (2.5% in *t*-butanol, 5 drops) and $NaIO_4$ (93 mg, 0.44 mmol) were added to a solution of **145** (154 mg, 0.29 mmol) in THF/H_2O (1:1, 10ml). After stirring for 2 h at room temperature, a white solid precipitated. The solid was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography of the residue (hexane:ethyl acetate from 1:2 to 1:4) gave both **146** and **147** as glassy white solids (**147**, 57 mg, 53%; **146**, 20 mg, 19%). Compound **147** could not be obtained in a pure form, but a proton peak shown at 9.72 ppm in its ^{1}H NMR spectrum and a CHO signal at 200.01 ppm in the DEPT experiments indicated that it was

the correct product. The NMR spectrum of **146** could not be completely assigned because the compound was formed as an inseparable 1:1 mixture of diastereomers. Neither of CHO nor NH peaks were observed in the ^{1}H NMR spectrum of **146**, supporting that it was in a cyclized form. Our observations are consistent with the literature results.[35]

2′-Acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl 1,2-propylene oxide (152)



152

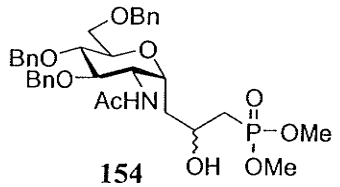
mCPBA (332 mg, 1.7 mmol) was added to a CH_2Cl_2 solution (4 ml) of **145** (298 mg, 0.58 mmol) at 0 °C. After 9 h stirring at rt, the reaction was quenched by adding saturated $Na_2S_2O_3$. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with saturated $NaHCO_3$, water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (2:1 to 1:4 hexane- ethyl acetate) to give a 1:1 mixture of diastereomers of **152** as an amorphous white solid (233 mg, 76%).

^{1}H NMR ($CDCl_3$) δ 1.42 (ddd, 1H, J = 14.5, 7.9 and 3.0 Hz, H-3), 1.68-1.78 (m, 2H, H-3), 1.78-1.90 (m, 1H, H-3), 1.84 (s, 3H, CH_3CO), 1.85 (s, 3H, CH_3CO), 2.49 (dd, 1H, J = 5.0 and 2.7 Hz, H-1), 2.54 (dd, 1H, J = 5.0 and 2.7 Hz, H-1), 2.69 (dd, 1H, J = 4.70 and 4.0 Hz, H-1), 2.76 (dd, 1H, J = 4.70 and 4.0 Hz, H-1), 3.00-3.07 (m, 1H, H-2), 3.07-3.15 (m, 1H, H-2), 3.57-3.64 (m, 2H, H-3′), 3.67-3.73 (m, 2H, H-4′), 3.73-3.95 (m, 4H, H-6′), 4.07-4.33 (m, 6H, H-1′,2′,5′), 4.40-4.66 (m, 12H, OCH_2Ph), 6.65 (d, 2H, J = 9.6 Hz, NHAc), 7.20-7.42 (m, 30H, aromatic).

^{13}C NMR ($CDCl_3$) δ 23.3 (CH_3CO), 34.1 (C-3), 35.1 (C-3), 46.7 (C-1), 47.5 (C-1), 48.1 (C-5′), 49.3 (C-2), 49.5 (C-2), 65.6 (C-2′), 66.0 (C-2′), 67.7 (C-6′), 68.0 (C-6′), 71.8, 71.9, 72.1, 72.2, 73.2, 73.1 (C-3′), 73.2 (C-3′), 74.3 (C-4′), 75.0 (C-1′), 75.1 (C-1′), 169.80, 169.83.

Anal. Calcd for $C_{32}H_{37}NO_6$: C, 72.29; H, 7.01. Found: C, 71.94; H, 6.89.

Dimethyl (2′-acetamido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl) 2-propanolphosphonate (154)



154

n-BuLi (2.5 M in hexane, 1.1 ml) was added to a THF solution (4 ml) of $HOP(OMe)_2$ (0.24 ml, 2.6 mmol) at –78°C. After 20 min of stirring at –78°C, **152** (277 mg, 0.52 mmol) and $BF_3 Et_2O$ (0.33 ml, 2.6 mmol) were added into the reaction. After stirring 30 min at –78°C and 1 h at room temperature, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated NH_4Cl and brine, dried and concentrated. The residue was purified by flash chromatography (ether to 10:1 ether:methanol) to give a 1:1 mixture of diastereomers **154** as an amorphous white solid (280 mg, 84%).

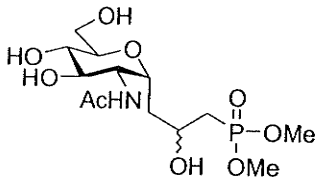
^{1}H NMR ($CDCl_3$) δ 1.52-1.76 (m, 4H, H-3), 1.83 (s, 3H, C*H*$_3$CO), 1.84 (s, 3H, C*H*$_3$CO), 1.90-2.00 (m, 4H, H-1), 3.40-3.50 (m, 2H, H-5′), 3.54-3.62 (m, 4H, H-6′), 3.68 (bd, 6H, J = 10.9 Hz, OC*H*$_3$), 3.70 (d, 3H, J = 10.9 Hz, OC*H*$_3$), 3.71 (d, 3H, J = 10.9 Hz, OC*H*$_3$), 3.93-4.10 (m, 2H, H-3′), 4.14-4.32 (m, 6H, H-2,1′,2′), 4.36-4.68 (m, 12H, OCH_2Ph), 6.57 (d, 1H, J = 9.6 Hz, N*H*Ac), 6.65 (d, 1H, J = 9.6 Hz, N*H*Ac), 7.20-7.38 (m, 30H, aromatic).

^{13}C NMR ($CDCl_3$) δ 23.1 (*C*H_3CO), 23.2 (*C*H_3CO), 33.0 (d, J = 139 Hz, C-1), 33.1 (d, J = 139 Hz, C-1), 38.0 (d, J = 13 Hz, C-3), 39.2 (d, J = 16 Hz, C-3), 47.8 (C-2′), 47.9 (C-2′), 52.1 (d, J = 7 Hz, O*C*H_3), 52.2 (d, J = 6 Hz, O*C*H_3), 52.4 (d, J = 7 Hz, O*C*H_3), 52.5 (d, J = 6 Hz, O*C*H_3), 63.4 (d, J = 4 Hz, C-2), 64.2 (C-3′), 66.0 (d, J = 4 Hz, C-2), 67.0 (C-6′), 67.1 (C-6′), 67.3 (C-3′), 71.8, 72.0, 72.2, 73.1, 73.3, 73.1 (C-5′), 73.2 (C-5′), 73.9 (C-4′), 74.0 (C-4′), 74.7 (C-1′), 74.8 (C-1′), 169.8, 169.9.

^{31}P NMR ($CDCl_3$) δ 32.8, 33.0.

Anal. Calcd for $C_{34}H_{44}NO_9P$: C, 63.64; H, 6.91; N, 2.18. Found: C, 63.99; H, 7.26; N, 2.17.

Dimethyl (2′-acetamido-2′-deoxy-α-D-glucopyranosyl) 2-propanol phosphonate (51)



51

Pd/C (10 %, 99 mg) was added to a glacial HOAc solution (0.5 ml) of the compound **154** (99 mg, 0.15 mmol) and the suspension was flushed with hydrogen gas. After stirring vigorously overnight under hydrogen (balloon) pressure at room temperature, the reaction mixture was filtered through a Celite pad, and then concentrated. The residue was purified by flash chromatography (2:1 ether:methanol) to give a 1:1 ratio of diastereomers of **51** as a glass like solid (48 mg, 84%).

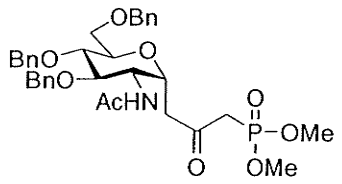
^{1}H NMR (D_2O) δ 1.56 (ddd, 1H, J = 13.0, 10.2 and 2.5 Hz), 1.73 (ddd, 1H, J = 10.2, 6.6 and 3.7 Hz), 2.03 (bs, 6H), 1.96-2.28 (m, 6H), 3.37-3.46 (m, 2H), 3.47-3.54 (m, 2H), 3.56-3.65 (m, 2H), 3.74 (d, 3H, J = 0.4 Hz), 3.75 (d, 3H, J = 1.0 Hz), 3.78 (d, 3H, J = 0.4 Hz), 3.79 (d, 3H, J = 1.0 Hz), 3.70-3.79 (m, 2H), 3.81 (dd, 1H, J = 4.9 and 2.5 Hz), 3.86 (dd, 1H, J = 4.9 and 2.5 Hz), 3.96 (ddd, 2H, J = 16.2, 10.3 and 5.8 Hz), 4.04-4.17 (m, 2H), 4.18-4.33 (m, 2H).

^{13}C NMR (D_2O) δ 22.24, 22.26, 30.65 (d, J = 139.8 Hz), 32.02 (d, J = 137.6 Hz), 32.92 (d, J = 12.6 Hz), 33.48 (d, J = 13.3 Hz), 53.31, 53.39, 53.46, 53.47, 53.54, 53.64, 61.24, 61.38, 62.45 (d, J = 4.0 Hz), 64.70 (d, J = 5.8), 70.31, 70.89, 70.99, 71.03, 71.18, 71.42, 73.36, 73.60174.76.

^{31}P NMR (D_2O) δ 36.23, 35.84.

ESI-MS (m/z): $C_{13}H_{26}NO_9P$, [M+H$^+$] calc., 372.33; found, 372.38.

Dimethyl (2′-acetamido -3′,4′,6′-tri-*O*-benzyl-2′-deoxy--α-D-glucopyranosyl) 2-propanone phosphonate (158)



158

Compound **154** (126 mg) was added to a mixture of DMSO/Ac_2O (1.4:1.0, 1.2 ml), and the solution was stirred overnight at room temperature. The reaction mixture was then added to ice water and extracted with ethyl acetate. The organic layer was then washed with aqueous saturated $NaHCO_3$, water and brine, dried and concentrated. Column chromatography of the residue (ether to 10:1 ether:methanol) gave **158** as a colorless oil (107 mg, 85%).

$[\alpha]_D$ +7.3 (*c* 0.55, CH_2Cl_2).

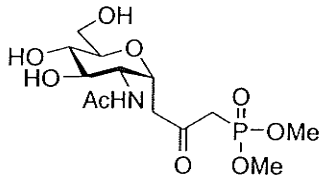
^{1}H NMR ($CDCl_3$) δ 1.84 (s, 3H, C*H*$_3$CO), 2.65 (dd, 1H, *J* = 17.1 and 5.2 Hz, H-3), 2.77 (dd, 1H, *J* = 17.1 and 7.9 Hz, H-3), 3.12 (dd, 2H, *J* = 22.2 and 2.2 Hz, H-1), 3.44-3.58 (m, 1H, H-5′), 3.64-3.67 (m, 1H, H-4′), 3.75 (d, 6H, *J* = 10.0 Hz, 2 × OC*H*$_3$), 3.78 (dd, 1H, *J* = 17.3 and 9.5 Hz, H-6′), 3.87 (dd, 1H, *J* = 17.3 and 9.5 Hz, H-6′), 4.20 (dd, 1H, *J* = 7.1 and 7.1 Hz, H-3′), 4.19 (bdd, 1H, *J* = 9.5 and 7.1 Hz, H-2′), 4.49-4.53 (m, 1H, H-1′), 4.38-4.67 (m, 6H, 3 × PhC*H*$_2$), 6.73 (d, 1H, *J* = 9.5 Hz, N*H*Ac), 7.20-7.40 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$) δ 23.27, 41.76 (*J* = 129.2 Hz, C-1), 45.70 (*J* = 1.0 Hz), 47.20 (C-2′), 52.92 (*J* = 6.9), 53.00 (*J* = 6.9), 64.32 (C-1′), 67.75 (C-6′), 71.87, 71.96, 73.10 (C-5′), 73.31, 73.96 (C-4′), 75.18 (C-3′), 169.95, 199.37 (*J* = 6.4).

^{31}P NMR ($CDCl_3$) δ 23.38.

Anal. Calcd for $C_{34}H_{42}NO_9P$: C, 63.84; H, 6.62; N, 2.19. Found: C, 63.61; H, 6.81; N, 2.20.

Dimethyl (2′-acetamido-2′-deoxy-α-D-glucopyranosyl) 2-propanone phosphonate (52)



52

Pd/C (10 %, 10 mg) was added to a glacial acetic acid solution (0.5 ml) of the compound **158** (12 mg, 0.019 mmol) and hydrogen was flushed into the reaction flask with vigorous stirring. After stirring 2h at 40°C, the reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash chromatography (5:1 ethyl acetate:methanol) to give **52** as a glassy solid (5 mg, 73%).

$[\alpha]_D$ +60.0 (*c* 0.6, CH_3OH).

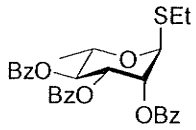
^1H NMR (CD_3OD) δ 1.98 (bs, 3H, CH_3CO), 2.78 (dd, 1H, J = 16.6 and 4.5 Hz, H-3), 3.02 (dd, 1H, J = 16.6 and 9.1 Hz, H-3), 3.35 (dd, 1H, J = 8.0 and 8.0 Hz, H-4′), 3.49-3.58 (m, 1H, H-5′), 3.58 (dd, 1H, J = 9.7 and 8.0 Hz, H-3′), 3.71-3.77 (m, 2H, H-6′), 3.80 (d, 3H, J = 11.2 Hz, OCH_3), 3.80 (d, 3H, J = 11.2 Hz, OCH_3), 3.97 (dd, 1H, J = 9.7 and 5.2 Hz, H-2′), 4.67 (ddd, 1H, J = 9.1, 5.2 and 4.5 Hz, H-1′). (H-1 signal disappears due to hydrogen exchange)

^{13}C NMR (CD_3OD) δ 22.67, 43.03 (d, J = 2.3 Hz, C-3), 53.84 (d, J = 6.5 Hz), 53.86 (d, J = 6.5 Hz), 54.30 (C-2′), 62.66 (C-6′), 70.16 (C-1′), 72.04 (C-3′), 72.21 (C-4′), 76.41 (C-5′), 173.62, 201.41 (d, J = 6.2 Hz). (C-1 is at 40.25 with $J_{C,P}$ = 130 Hz when 5:1 $CDCl_3$/MeOH is used as solvent)

^{31}P NMR (CD_3OD) δ 25.56.

12.5 Synthesis of analogues to the α-L-Rha-(1→3)-α-D-GlcNAc linker

Ethyl 2, 3, 4-tri-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (162)



162

Ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**170**) was prepared according to the procedure described by Bundle.[199] Ethyl 1-thio-α-L-rhamnopyranoside (**173**) was obtained quantitatively from **170** by treatment with sodium methoxide in methanol (1 M) for 30 min at room temperature. BzCl (1.86 ml, 16.1 mmol) was added to a solution of **173** (0.955 g, 4.59 mmol) in pyridine (10 ml) at 0°C. After stirring 1.5 h, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with 1.2 N HCl, water and brine, dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate 9:1) gave **162** as a thick colorless oil (2.0 g, 84%). **162** has been prepared by Ley, but no NMR data were available.[243]

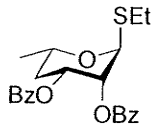
$[\alpha]_D$ +96.6 (*c*1.08, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ1.38 (t, 3H, *J* = 7.4 Hz, C*H*$_3$), 1.38 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), 2.65-2.83 (m, 2H, SC*H*$_2$), 4.60 (dd, 1H, *J* = 8.7 and 6.2 Hz, H-5), 5.53 (s, 1H, H-1), 5.74-5.83 (m, 3H, H-2, 3, 4), 7.21-8.15 (m, 15H, 3 × *Ph*).

^{13}C NMR ($CDCl_3$): δ15.04 (*C*H$_3$), 17.72 (*C*H$_3$), 25.76 (*C*H$_2$), 67.44 (*C*H), 70.55 (*C*H), 72.14 (*C*H), 72.73 (*C*H), 82.29 (*C*H), 165.44 (*C*=O), 165.57 (*C*=O), 165.81 (*C*=O).

Anal. Calcd for $C_{29}H_{28}O_7S$: C, 66.91; H, 5.42. Found: C, 66.42; H, 5.50.

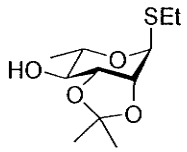
Ethyl 2,3-di-*O*-benzoyl-4,6-dideoxy-1-thio-α-L-*lyxo*-hexopyranoside (163)



163

Compound **163** was prepared according to the procedure described by Hultin.[202] The NMR data are consistent with the published results.

Ethyl 2, 3-*O*-propylidene-1-thio-α-L-rhamnopyranoside (178)



178

2,2-Dimethoxypropane (1.57 ml, 12.8 mmol) and a catalytic amount of TsOH were added to a solution of **173** (1.77 g, 8.50 mmol) in acetone (10 ml). After stirring 30 min at room temperature, triethylamine (1 ml) was added to quench the reaction. The solvent was evaporated under reduced pressure. Column chromatography of the residue (hexane:ethyl acetate 3:1) gave **178** as a thick colorless oil (2.05 g, 97%).

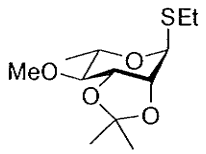
$[\alpha]_D$ –87.7 (*c*1.14, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ1.27 (d, 3H, *J* = 7.1 Hz, C*H*$_3$), 1.29 (t, 3H, *J* = 7.1 Hz, C*H*$_3$), 1.33, 1.52 (2 × s, 6H, 2 × C*H*$_3$), 2.53 (dq, 1H, *J* = 13.1 and 7.4 Hz, SC*H*$_2$), 2.66 (dq, 1H, *J* = 13.1 and 7.4 Hz, SC*H*$_2$), 3.42 (dd, 1H, *J* = 9.7 and 7.6 Hz, H-4), 3.95 (dq, 1H, *J* = 7.6 and 7.1 Hz, H-5), 4.03 (dd, 1H, *J* = 9.7 and 5.6 Hz, H-3), 4.16 (dd, 1H, *J* = 5.6 and 0.6 Hz, H-2), 5.50 (ws, 1H, H-1).

^{13}C NMR ($CDCl_3$): δ14.65, 17.27 (2 × *C*H$_3$), 24.43 (S*C*H$_2$CH$_3$), 26.36, 28.20 (2 × *C*H$_3$), 66.02 (C-5), 75.31 (C-4), 76.82 (C-2), 78.47 (C-3), 79.45 (C-1), 109.55 (*C*(CH$_3$)$_2$).

Anal. Calcd for $C_{11}H_{20}O_4S$: C, 53.20; H, 8.12. Found: C, 53.70; H, 8.50.

Ethyl 2, 3-*O*-propylidene-4-*O*-methyl-1-thio-α-L-rhamnopyranoside (179)



179

Sodium hydride (60%, 380 mg) and methyl iodide (0.7 ml, 11.3 mmol) were added to a solution of **178** (1.87 g, 7.52 mmol) in THF (10 ml). After stirring 4 h at room temperature, the solvent was evaporated. Column chromatography of the residue (hexane:ethyl acetate 20:1) gave **179** as a thick colorless oil (1.77 g, 90%).

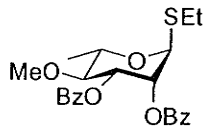
$[\alpha]_D$ –98.9 (*c*1.10, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ1.26 (d, 3H, *J* = 6.3 Hz, C*H*$_3$), 1.29 (t, 3H, *J* = 7.4 Hz, C*H*$_3$), 1.34, 1.54 (2 × s, 2 × C*H*$_3$), 2.52 (dq, 1H, *J* = 13.1 and 7.4 Hz, SC*H*$_2$), 2.65 (dq, 1H, *J* = 13.1 and 7.4 Hz, SC*H*$_2$), 3.02 (dd, 1H, *J* = 9.7 and 7.2 Hz, H-4), 3.53 (s, 3H, OC*H*$_3$), 3.93 (dq, 1H, *J* = 9.7 and 6.3 Hz, H-5), 4.10 (dd, 1H, *J* = 7.2 and 5.7 Hz, H-3), 4.15 (dd, 1H, *J* = 5.7 and 0.5 Hz, H-2), 5.48 (ws, 1H, H-1).

^{13}C NMR ($CDCl_3$): δ14.67, 17.73 (2 × *C*H$_3$), 24.43 (S*C*H$_2$CH$_3$), 26.44, 28.14 (2 × *C*H$_3$), 59.54 (O*C*H$_3$), 65.35 (C-5), 76.92 (C-4), 78.09 (C-2), 79.53 (C-3), 84.07 (C-1), 109.18 (*C*(CH$_3$)$_2$).

Anal. Calcd for $C_{12}H_{22}O_4S$: C, 54.93; H, 8.45. Found: C, 54.39; H, 8.50.

Ethyl 2, 3-di-*O*-benzoyl-4-*O*-methyl-1-thio-α-L-rhamnopyranoside (164)



164

Compound **179** was stirred in aqueous acetic acid solution (80% v/v, 20 ml) at 80°C for 1 h. The solution was then concentrated to dryness under reduced pressure. Pyridine (5 ml) and BzCl (2.20 ml, 19 mmol) were added to the solution of the residue in CH_2Cl_2 (10 ml) at 0°C. After stirring 1 h at 0°C, the reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with 1.2N HCl, water and brine, dried and

concentrated. Column chromatography of the residue (hexane:ethyl acetate 20:1) gave **164** as a thick colorless oil (2.64 g, 97%).

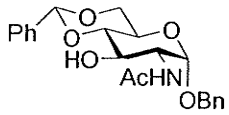
$[\alpha]_D$ +16.0 (*c*1.04, $CHCl_3$), lit.[244] +15.0 (*c*1.0, $CHCl_3$).

^1H NMR ($CDCl_3$): δ1.33 (t, 3H, *J* = 7.4 Hz, CH_3), 1.44 (d, 3H, *J* = 6.2 Hz, CH_3), 2.60-2.78 (m, 2H, SCH_2), 3.51 (s, 3H, OCH_3), 3.53 (dd, 1H, *J* = 9.6 and 9.4 Hz, H-4), 4.25 (dq, 1H, *J* = 9.4 and 6.1 Hz, H-5), 5.37 (d, 1H, *J* = 1.8 Hz, H-1), 5.56 (dd, 1H, *J* = 9.6 and 3.4 Hz, H-3), 5.68 (dd, 1H, *J* = 3.4 and 1.8 Hz, H-2), 7.36-8.07 (m, 10H, 2 × *Ph*).

^{13}C NMR ($CDCl_3$): δ14.98 (CH_3), 17.94 (CH_3), 25.60 (CH_2), 60.74 (OCH_3), 68.38 (*C*H), 72.58 (*C*H), 72.96 (*C*H), 81.18 (*C*H), 82.05 (*C*H), 165.32 (*C*=O), 165.46 (*C*=O).

Anal. Calcd for $C_{23}H_{26}O_6S$: C, 64.17; H, 6.09. Found: C, 64.16; H, 6.36.

Benzyl 2-acetamido-4, 6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (165)



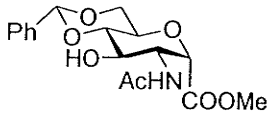
165

Benzaldehydrate dimethyl acetal (2.25 ml, 15.1 mmol) and TsOH (89 mg) were added to a solution of **180** (2.67 g, 8.58 mmol) in DMF (10 ml). After stirring 2.5 h at 40°C, a white solid precipitated. After the reaction mixture cooled down, triethylamine (5 ml) was added to quench the reaction. The solid was washed with water and recrystallized from methanol. **165** was obtained as a white solid (2.84 g, 90%).

Mp 261-262°C, lit. 263-264°C,[245] 262°C.[246] $[\alpha]_D$ +124.0 (*c*0.50, DMSO), lit. +120.0 (*c*1.0, pyridine),[245] +114.0(*c*1.0, pyridine).[246]

^1H NMR ($CDCl_3$): δ2.00 (s, 3H, CH_3C=O), 3.61 (t, 1H, *J* = 9.2 Hz), 3.77 (t, 1H, *J* 10.1 Hz), 3.87 (dd, 1H, *J* = 9.3 and 4.8 Hz), 3.96 (t, 1H, *J* = 9.3 Hz), 4.20-4.30 (m, 2H), 4.50 (d, 1H, *J* = 11.8Hz), 4.76 (d, 1H, *J* = 11.8 Hz), 4.94 (d, 1H, *J* = 3.9Hz), 5.77 (s, 1H), 5.79 (d, 1H, *J* = 8.6 Hz), 7.35-7.50 (m, 10H).

3-Acetamido-2, 6-anhydro-5, 7-*O*-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (166)



166

Palladium on carbon (10%, 0.5 g) was added to a solution of **108** (0.74 g, 1.39 mmol) in glacial acetic acid (10 ml), and the mixture was flushed with hydrogen from a balloon. After vigorously stirring overnight under a hydrogen atmosphere at room temperature, the reaction mixture was filtered and concentrated. Benzaldehydrate dimethyl acetal (0.25 ml, 1.67 mmol) and a catalytic amount of TsOH were added to the solution of the residue in DMF (5 ml). After stirring 2 h at 40°C, a white solid was precipitated. After the reaction mixture cooled down, triethylamine (5 ml) was added to quench the reaction. The solid was washed with water and crystallized in methanol. **166** was obtained as a white solid (0.31 g, 64%).

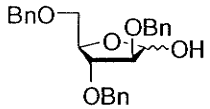
Mp 260.5-261.0°C. $[\alpha]_D$ +74.9 (*c*0.35, DMSO).

^{1}H NMR ($CDCl_3$): δ2.04 (s, 3H, C*H*$_3$C=O), 2.73 (d, 1H, *J* = 3.0 Hz, H-OH), 3.53 (ddd, 1H, *J* = 9.7, 9.3 and 4.2 Hz, H-6), 3.59 (t, 1H, *J* = 9.3 Hz, H-5), 3.74 (t, 1H, *J* = 9.7 Hz, H-7), 3.83 (s, 3H, OC*H*$_3$), 4.05 (dt, 1H, *J* = 9.3 and 3.0 Hz, H-4), 4.33 (dd, 1H, *J* = 9.7 and 4.2 Hz, H-7), 4.48 (ddd, 1H, *J* = 9.3, 8.7 and 5.7 Hz, H-3), 4.60 (d, 1H, *J* = 5.7 Hz, H-2), 5.55 (s, 1H, PhC*H*), 6.62 (d, 1H, *J* = 8.7 Hz, N*H*Ac), 7.36-7.49 (m, 5H, *Ph*).

^{13}C NMR ($CDCl_3$): δ23.36 (*C*H$_3$C=O), 51.60 (C-3), 52.71 (O*C*H$_3$), 67.98 (C-6), 68.66 (C-7), 70.08 (C-4), 74.49 (C-2), 82.25 (C-5), 101.96 (Ph*C*H), 170.87 (*C*=O), 171.04 (*C*=O).

Anal. Calcd for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02;N, 3.99. Found: C, 58.34; H, 6.20; N, 4.11.

2,3,5-Tri-*O*-benzyl-β-D-arabinofuranose (187)



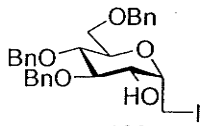
187

Compound **187** was prepared according to a precedure described by Tejima and Fletcher for the preparation of 2,3,5-tri-*O*-benzyl-β-L-arabinofuranose.[205] Powdered D-arabinose (20 g, 0.13 mol) was dissolved in anhydrous methanol (400 ml), and the solution, after the addition of concentrated sulfuric acid (2.0 ml), was stirred at room temperature overnight. After passing a column containing IR-150 (150 ml), the reaction mixture was concentrated *in vacuo*, giving **185** as a light yellow sirup. The sirup was dissolved in DMF (100 ml), and THF (600 ml) was added to the solution. After sodium hydride (24 g, 0.60 mol) was added and the suspension was stirred at 0°C for 30 min, benzyl chloride (69 ml, 0.60 mol) was added, and the reaction mixture was stirred for 30 min at 0°C, another 30 min at room temperature and 1 h in refluxing. The mixture was filtered, and the filtrate was concentrated *in vacuo*, giving **186** as a red sirup. The crude sirup was dissolved in glacial acetic acid (260 ml) and 6 N hydrochloric acid (40 ml). It was heated at 1 h at 80°C, concentrated *in vacuo*. Column chromatography of the residue (hexane:ethyl acetate from 9:1 to 6:1) afforded **187** as a white solid (30 g, 54%).

M.p. 85.5-86.5°C.

^{1}H NMR ($CDCl_3$): δ 3.51 (dd, 1H, J = 10.1 and 4.4 Hz), 3.82 (d, 1H, J = 10.0 Hz), 4.01 (dd, 1H, J = 4.4 and 4.4 Hz), 4.09 (dd, 1H, J = 4.4 and 4.4 Hz), 4.53-4.58 (m, 5H), 4.64 (dd, 1H, J = 11.6 and 11.6 Hz), 5.33 (dd, 1H, J = 10.1 and 4.4 Hz), 7.22-7.38 (m, 15H).

***C*-(2-Hydroxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-iodomethane (192)**



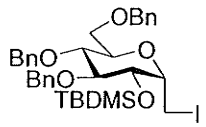
192

Compound **192** was prepared by a procedure described by Casero.[25] Vinylmagnesium bromide (1 M in THF, 6.0 ml, 6.0 mmol) was added to a solution of $ZnBr_2$ (0.67, 3.0 mmol) in THF, and the mixture was stirred for 30 min at room temperature. A solution of **187** (0.50 g, 1.2 mmol) in THF was added to the reaction. After 4 h, a saturated NH_4Cl solution was added and the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with 1.2 HCl, saturated $NaHCO_3$, water and brine, and then dried and concentrated, giving **189** as a light yellow oil.

$Hg(OAc)_2$ (0.39 g, 1.2 mmol) was added to a solution of the yellow oil in THF, the mixture was stirred for 8 h at room temperature. KCl (0.13 g, 1.8 mmol) dissolved in minimium amount of water was added. After 30 min, the reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate 3:1) gave **191** as a pale yellow oil.

A solution of iodine (0.37 g, 1.4 mmol) in CH_2Cl_2 was added to a solution of **191** in CH_2Cl_2. After 4 h, a saturated $Na_2S_2O_3$ solution was added. The organic layer was washed with water and brine, dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate 9:1) gave **192** (0.32 g, 54%) as a colorless oil. Compound **192** was confirmed by comparison of its NMR spectra with the literature data.[25]

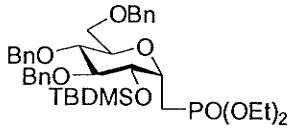
C-(2-O-(t-Butyldimethylsilyl)-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-iodomethane (193)



193

Compound **193** was prepared by a procedure described by Casero.[25] Imidazole (24.9 g, 366 mmol) and TBDMSCl (20 g, 133 mmol) were added to a sulotion of **192** (13.2 g, 26.6 mmol) in DMF (100 ml). After overnight, DMF was removed *in vacuo*, and the residue was purified by column chromatography (hexane:ethyl acetate 20:1), affording **193** (14.2 g, 77%) as a colorless oil. Compound **193** was confirmed by comparison of its NMR spectra with the literature data.[25]

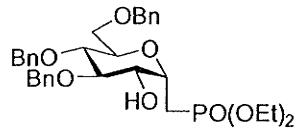
Diethyl C-(2-O-(t-butyldimethylsilyl)-3,4,6-tri-O-benzyl-α-D-glucopyranosyl) methanephosphonate (194)



194

Compound **193** was prepared by a procedure described by Casero.[25] Compound **193** (16.4 g, 23.7 mmol) was refluxed in $P(OEt)_3$ (100 ml) for 5 h. $P(OEt)_3$ was evaporated *in vacuo*. Column chromatography of the residue (hexane:ethyl acetate 20:1) gave **194** (12.0 g, 72%) as a light yellow oil. Compound **194** was confirmed by comparison of its NMR spectra with the literature data.[25]

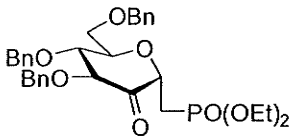
Diethyl *C*-(3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl) methanephosphonate (195)



195

Compound **195** was prepared by a procedure described by Casero.[25] A mixture of CF_3COOH/H_2O (9:1, 14 ml) was added to a solution of **194** (12.0 g, 17.2 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water and brine, dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate 1:2) gave **195** (8.0 g, 80%) as a light yellow oil. Compound **195** was confirmed by comparison of its NMR spectra with the literature data.[25]

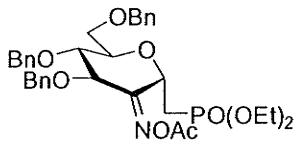
Diethyl *C*-(3,4,6- tri-*O*-benzyl-α-D-*arabino*-hexosulopyranosyl) methanephosphonate (196)



196

Compound **196** was prepared by a procedure described by Casero.[25] A mixture of **195** (0.83 g, 0.68 mmol) and $DMSO/Ac_2O$ (3:2, 7 ml) was stirred overnight. The reaction was quenched by adding ice-water and extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water and brine, dried and concentrated. Purification of the residue with chromatography (hexane:ethyl acetate 1:1) gave **196** (0.72 g, 87%) as a light yellow oil. Compound **195** was confirmed by comparison of its NMR spectra with the literature data.[25]

Diethyl *C*-(3,4,6- tri-*O*-benzyl-α-D-*arabino*-hexosulopyranosyl) methanephosphonate acetyloxime (198)

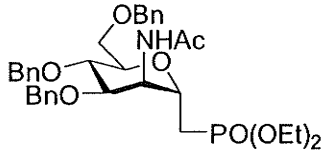


198

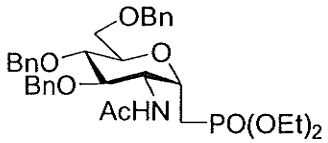
Compound **198** was prepared by a procedure described by Casero.[25] A mixture of **196** (99 mg, 0.17 mmol) in THF/MeOH (1:1, 4 ml) and a pH 4.5 buffer solution of $NH_2OH\,HCl$ (0.18 g/ml, 2.8 ml, 7.3 mmol) was stirred 4 h at room temperature. The reaction solution was extracted by CH_2Cl_2. The organic layer was washed by saturated $NaHCO_3$, water and brine, dried and concentrated, giving **197** as a colorless oil.

Catalytic DMSO, pyridine (0.32 ml, 4.0 mmol) and Ac_2O (0.19 ml, 2.0 mmol) were added to a solution of the residue in CH_2Cl_2 (5 ml). After stirring for 5 h at room temperature, the reaction solution was diluted by CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water and brine, dried and concentrated. The crude product was purified by chromatography (hexane:ethyl acetate 1:1), affording **198** (91 mg, 84%) as a colorless oil. Compound **198** was confirmed by comparison of its NMR spectra with the literature data.[25]

Diethyl *C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl) methanephosphonate (201) and **Diethyl *C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl) methanephosphonate (202)**



manno
201

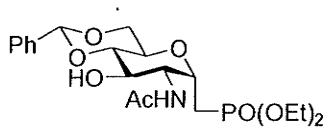


gluco
202

Compound **202** was prepared by a procedure described by Casero,[25] and **201** was a byproduct of this reaction. Diborane solution (1 M) was added to a solution of **198** in THF. The reaction was stirred until the disappearance of the starting material. The

detailed reaction conditions were described in Table 10.1. Methanol was added and the solution was concentrated *in vacuo*. Pyridine, Ac_2O and a catalytic amount of DMAP were added to a solution of the residue in CH_2Cl_2. After stirring overnight at room temperature, the reaction solution was diluted by CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water and brine, dried and concentrated. The crude products were purified by chromatography (ethyl acetate:CH_2Cl_2:mathanol 1:1:0.1), affording **201** and **202** as colorless oils. Their identification was confirmed by comparison of their NMR specta with the literature data.[25]

Diethyl *C*-(4, 6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate (167)



167

Palladium on carbon (10%, 400 mg) was added to a vigorously stirred suspension of **202** (449 mg, 0.72 mmol) in glacial acetic acid (1.5 ml). The mixture was flushed with hydrogen from a balloon. The suspension was stirred for 3 h at room temperature under a hydrogen atmosphere. Then the Pd/C powder was removed by filtration and the filtrate was concentrated. *p*-Toluenesulfonic acid (20 mg) and benzaldehyde methyl acetal (0.25 ml, 1.7 mmol) were added to a solution of the residue in DMF (1 ml). After stirring at 40°C for 1 h, triethylamine was added to quench the reaction. The mixture was concentrated under reduced pressure. Column chromatography of the residue (ethyl acetate:CH_2Cl_2:methanol 1:1:0.05 to 1:1:0.2) gave **167** as a white solid (184 mg, 58%).

M.p. 188.5-189.2°C. $[\alpha]_D$ +3.8 (*c*1.0, $CHCl_3$).

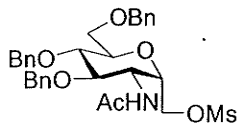
^{1}H NMR (CD_3COCD_3, 500MHz): δ 1.28 (t, 6H, J = 7.1 Hz, 2 × CH_3CH_2), 1.92 (s, 3H, CH_3C=O), 2.09-2.13 (m, 1H, PCH_2), 2.43 (ddd, 1H, J = 16.1, 16.1 and 11.3 Hz, PCH_2), 3.55 (dd, 1H, J = 9.1 and 9.1 Hz, H-4), 3.66-3.80 (m, 2H, H-5, 6), 3.90 (ddd, 1H, J = 10.5, 9.1 and 4.6 Hz, H-3), 4.04-4.10 (m, 4H, 2 × OCH_2CH_3), 4.16-4.20 (m, 1H, H-6), 4.53-4.61 (m, 1H, H-1), 5.62 (s, 1H, PhC*H*), 7.30-7.54 (m, 6H, *Ph* and N*H*Ac).

^{13}C NMR (CD_3COCD_3, 125.8 MHz): δ16.86 (d, J = 3.3 Hz, CH_3CH_2), 16.90 (d, J = 3.1 Hz, CH_3CH_2), 23.03 (CH_3C=O), 24.49 (d, J = 142.9 Hz, PCH_2), 55.56 (d, J = 12.9 Hz, C-2), 61.90 (d, J = 5.9 Hz, CH_3CH_2), 62.37 (d, J = 5.9 Hz, CH_3CH_2), 65.26 (C-5), 68.85 (C-3), 69.76 (C-6), 71.81 (d, J = 4.8 Hz, C-1), 84.26 (C-4), 102.37 (PhCH), 170.82.

^{31}P NMR ($CDCl_3$): δ 29.20.

Anal. Calcd for $C_{20}H_{32}NO_8P$: C, 53.93; H, 7.24; N, 3.15. Found: C, 54.34; H, 7.31;N, 3.20.

Methanesulfonic 3-acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-1-hydroxy-D-*glycero*-D-*ido*-heptitol ester (205)



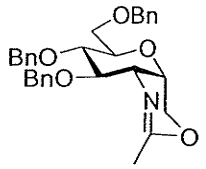
205

Methanesulfonyl chloride (21.4 μl, 0.277 mmol) and pyridine (31.8 μl, 0.396 mmol) were added to a solution of **109** (100 mg, 0.198 mmol) in CH_2Cl_2 (2 ml). After stirring 5 h at room temperature, the reaction mixture was diluted with water and CH_2Cl_2. The organic layer was washed with 1.2 N HCl, saturated aqueous $NaHCO_3$ and brine, dried and concentrated. Column chromatography of the residue (hexane:ethyl acetate 1:4) afforded **205** as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 1.83 (s, 3H, CH_3CO), 2.99 (s, 3H, CH_3), 3.55-3.60 (m, 1H, H-5), 3.63-3.73 (m, 2H, H-4,7), 3.91 (dd, 1H, J = 10.2 and 7.6 Hz, H-7), 4.20-4.38 (m, 5H, H-1,2,3,6), 4.44 (d, 1H, J = 11.4 Hz, PhCH_2), 4.48 (d, 1H, J = 11.4 Hz, PhCH_2), 4.51 (s, 2H, PhCH_2), 4.61 (d, 1H, J = 11.4 Hz, PhCH_2), 4.62 (d, 1H, J = 11.4 Hz, PhCH_2), 6.68 (d, 1H, J = 9.6 Hz, NHAc), 7.20-7.50 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 23.23, 37.72, 45.71, 66.85, 67.29, 70.43, 71.96, 72.24, 72.95, 73.37, 73.78, 75.27, 169.88.

3-Amino-4,5,7-tri-*O*-benzyl-1,3-deoxy-1-*O*,3-*N*-(ethan-1-ylide)-D-*glycero*-D-*ido*-heptitol (207)



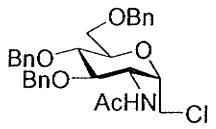
207

Compound **205** (90 mg, 0.15 mmol) and KI (51 mg, 0.31 mmol) were refluxed in $P(OEt)_3$ for 1.5 h. The mixture was evaporated to dryness and the residue was purified by flash chromatography (hexane:ethyl acetate from 1:2 to 1:4). Compound **207** (42 mg, 58%) was obtained as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 1.94 (d, 3H, J = 1.3 Hz, CH_3), 3.58-3.62 (m, 1H, H-3), 3.66 (dd, 1H, J = 10.5 and 3.5 Hz, H-7), 3.71 (dd, 1H, J = 6.7 and 6.7 Hz, H-5), 3.78 (dd, 1H, J = 10.5 and 5.2 Hz, H-7), 3.84 (dd, 1H, J = 6.7 and 6.0 Hz, H-4), 3.92 (ddd, 1H, J = 6.7, 5.2 and 3.5 Hz, H-6), 4.02-4.07 (m, 1H, H-1a), 4.10-4.30 (m, 2H, H-1b, 2), 4.50 (d, 1H, J = 11.6, $PhCH_2$), 4.50 (d, 1H, J = 12.0, $PhCH_2$), 4.58 (d, 1H, J = 12.0, $PhCH_2$), 4.68 (d, 1H, J = 11.6, $PhCH_2$), 4.76 (d, 1H, J = 11.6, $PhCH_2$), 4.82 (d, 1H, J = 11.6, $PhCH_2$), 7.20-7.50 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 21.37, 54.21 (C-3), 62.57 (C-2), 65.25 (C-1), 68.83 (C-7), 73.12, 73.19, 73.29, 74.37 (C-6), 74.99 (C-5), 81.61 (C-4), 174.64.

3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-1-chloro-3-deoxy-D-*glycero*-D-*ido*-heptitol (206)



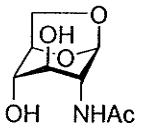
206

Thionyl chloride (0.5 ml) was added to a solution of the compound **109** (123 mg, 0.244 mmol) in CH_2Cl_2 (4 ml). The mixture was stirred 30 min at room temperature. Evaporation of the solvent gave **206** as a white foam. **206** was used without purification.

^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, C*H*$_3$CO), 3.46-3.49 (m, 1H, H-3), 3.49 (dd, 1H, *J* = 10.8 and 5.1 Hz, H-7), 3.88-3.92 (m, 1H, H-5), 3.90 (dd, 1H, *J* = 10.8 and 8.4 Hz, H-7), 4.14-4.29 (m, 1H, H-4), 4.21-4.29 (m, 1H, H-6), 4.35-4.60 (m, 6H, 2 × PhC*H*$_2$, H-1), 4.69 (d, 1H, *J* = 11.3 Hz, PhC*H*$_2$), 4.73-4.77 (m, 1H, H-2), 4.88 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 7.30-7.55 (m, 15H, aromatic).

^{13}C NMR ($CDCl_3$): δ 19.27, 48.71 (C-5), 56.49 (C-4), 66.10 (C-7), 70.41 (C-3), 71.17 (C-2), 71.40 (C-1), 72.30, 73.10, 73.70, 75.82 (C-6), 172.09.

2-Acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (209)



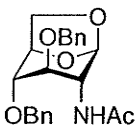
209

Compound **209** was prepared by a procedure reported by Boullanger.[213] A solution of *p*-toluenesulfonyl chloride (3.2 g, 16.8 mmol) in pyridine (20 ml) was added to a suspension of **61** (3.1 g, 14 mmol) in pyridine (14 ml) at 0°C. After stirring 5 h at 0°C, methanol (2 ml) was added to quench the reaction and the solvent was evaporated under reduced pressure. DBU (3.0 ml, 24 mmol) was added to the solution of the residue in ethanol (60 ml). After stirring overnight at room temperature, the solvent was evaporated *in vaccuo*. Column chromatography of the residue (ethyl acetate: CH_2Cl_2:Methanol 1:1:0.1 to 1:1:0.2) afforded **209** (1.78 g, 64%) as a white solid.

^{1}H NMR (D_2O): δ 2.04 (s, 3H, C*H*$_3$CO), 3.63-3.68 (m, 1H, H-4), 3.72-3.75 (m, 1H, H-3), 3.78 (dd, 1H, *J* = 7.6 and 7.6 Hz, H-6), 3.75-3.80 (m, 1H, H-2), 4.19 (dd, 1H, *J* = 7.6 and 0.6 Hz, H-6), 4.65 (bd, 1H, *J* = 5.2 Hz, H-2), 5.44 (bs, 1H, H-1).

^{13}C NMR (D_2O): δ 22.29, 52.62 (C-2), 65.74 (C-6), 71.04 (C-3), 71.41 (C-4), 76.50 (C-5), 100.74 (C-1).

2-Acetamido-1,6-anhydro-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranose (210)



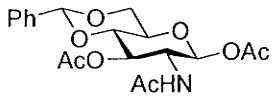
210

Compound **210** was prepared by following a procedure described by Garegg.[247] BaO (2.4 g), $Ba(OH)_2$ (4.8 g) and benzyl bromide (0.9 ml, 7.5 mmol) were added to a solution of the compound **209** (610 mg, 3.0 mmol) in DMF (20 ml). After stirring 3 h at 50°C, the solid in the reaction mixture was removed by filtration and the solvent was evaporated. Column chromatography of the residue (hexane:ethyl acetate 4:1 to 1:4) afforded **210** as a light yellow oil (905 mg, 79%).

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C*H*$_3$CO), 3.36 (d, 1H, *J* = 1.2 Hz, H-3), 3.46 (dd, 1H, *J* = 1.2 and 1.2 Hz, H-4), 3.70 (dd, 1H, *J* = 7.2 and 6.1 Hz, H-6), 4.18 (dd, 1H, *J* = 7.2 and 0.5 Hz, H-6), 4.22 (dd, 1H, *J* = 9.7 and 1.2 Hz, H-2), 4.36-4.50 (m, 3H, PhC*H*$_2$), 4.55 (bd, 1H, *J* = 6.1 Hz, H-5), 4.74 (d, 1H, *J* = 12.1 Hz, PhC*H*$_2$), 5.34 (bs, 1H, H-1), 6.12 (d, 1H, *J* = 9.7 Hz, N*H*Ac), 7.15-7.35 (m, 10H, aromatic).

^{13}C NMR ($CDCl_3$): δ 23.20, 47.68 (C-2), 65.03 (C-6), 71.18, 71.56, 73.95 (C-5), 75.77 (C-4), 75.81 (C-3), 100.69 (C-1), 169.33.

2-Acetamido-1,3-di-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose (216)



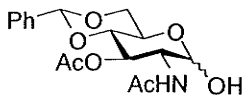
216

Ac_2O (1.2 ml, 11.6 mmol) and DMAP (100 mg) were added to a suspension of the compound **64** (0.598 g, 1.93 mmol) in pyridine (5 ml). After stirring 2.5 h at room temperature, the reaction mixture was poured into icy water and extracted with ethyl acetate. The organic layer was washed with 1.2N HCl, aqueous saturated $NaHCO_3$ and brine, dried and concentrated. Column chromatography of the residue afforded **216** as a white solid (0.674 g, 89%).

^{1}H NMR (DMSO-d): δ 1.78 (s, 3H, C*H*$_3$CO), 1.98 (s, 3H, C*H*$_3$CO), 2.04 (s, 3H, C*H*$_3$CO), 3.62 (ddd, 1H, *J* = 9.7, 9.7 and 4.9 Hz, H-5), 3.78 (dd, 1H, *J* = 9.7 and 9.7 Hz, H-6), 3.83 (dd, 1H, *J* = 9.7 and 9.7 Hz, H-4), 3.98 (ddd, 1H, *J* = 9.7, 9.3 and 8.7 Hz, H-2), 4.25 (dd, 1H, *J* = 9.7 and 4.9 Hz, H-6), 5.24 (dd, 1H, *J* = 9.7 and 9.7 Hz, H-3), 5.65 (s, 1H, PhC*H*), 5.77 (d, 1H, *J* = 8.7 Hz, H-1), 7.38 (s, 5H, aromatic), 7.99 (d, 1H, *J* = 9.3 Hz, N*H*Ac).

^{13}C NMR (DMSO-d): δ 20.49, 22.61, 52.63 (C-2), 66.41 (C-5), 67.36 (C-6), 71.43 (C-3), 77.71 (C-4), 92.22 (C-1), 100.32, 168.88, 169.46, 169.63.

2-Acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose (215)

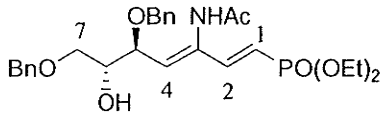


215

Compound **215** was prepared according to a procedure reported by Herzig.[217] Magnesium oxide (1 g) was added to a solution of the compound **217** (0.417 g, 1 mmol) in methanol (15 ml). After stirring 1 h at room temperature, the solid in the reaction mixture was removed by filtration and the filtrate was concentrated. The crude product **215** was obtained as a white solid (336 mg, 96%), and was used for the next reaction

without purification. Both TLC and NMR showed that it was a mixture of α and β anomers.

Diethyl (5*S*,6*R*)-3-*N*-acetyl-5,7-di-*O*-benzyl-6-hydroxy-hepta-1,3-diene phosphonate (221)



221

Sodium hydride (60%, 32 mg, 0.80 mmol) was added to a solution of tetraethyl methanediphosphate (**69**) (0.20 ml, 0.80 mmol) in diglyme (10 ml) at 0°C. After 30 min of stirring at 0°C, **100** (66 mg, 0.13 mmol) was added. The mixture was stirred for 2 h at room temperature. The reaction was then treated with a saturated solution of NH_4Cl, and the water layer was extracted with CH_2Cl_2. The organic layer was dried and concentrated. Flash chromatography of the residue (hexane:ethyl acetate 9:1 to ethyl acetate) gave **221** (83 mg, 64%) as a thick colorless oil.

$[\alpha]_D$ +56.3 (*c* 0.16, CH_2Cl_2).

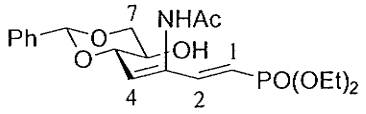
^{1}H NMR ($CDCl_3$): δ 1.24-1.30 (m, 6H, 2 × OCH_2CH_3), 1.81 (s, 3H, CH_3CO), 3.54 (bd, 2H, *J* = 4.8 Hz, H-7), 3.76 (d, 1H, *J* = 5.8 Hz, O*H*), 3.87 (ddt, 1H, *J* = 8.5, 5.8 and 4.8 Hz, H-6), 4.16 (dd, 1H, *J* = 9.0 and 8.5 Hz, H-5), 4.00-4.20 (m, 4H, 2 × OCH_2CH_3), 4.28 (d, 1H, *J* = 11.8 Hz, $PhCH_2$), 4.46 (d, 2H, *J* = 3.0 Hz, $PhCH_2$), 4.54 (d, 1H, *J* = 11.8 Hz, $PhCH_2$), 5.73 (dd, 1H, *J* = 17.4 and 17.1 Hz, H-1), 5.79 (d, 1H, *J* = 9.0 Hz, H-4), 7.00 (dd, 1H, *J* = 21.6 and 17.1 Hz, H-2), 7.18-7.31 (m, 10H, aromatic), 7.85 (s, 1H, N*H*Ac).

^{13}C NMR ($CDCl_3$): δ 16.57, 16.76, 23.16, 62.25 (*J* = 5.0 Hz), 62.32 (*J* = 4.8 Hz), 71.34, 71.79 (C-7), 72.58 (C-6), 73.84, 74.83 (C-5), 115.64 (*J* = 190.8 Hz, C-1), 132.21 (C-4), 137.13 (*J* = 25.4 Hz, C-3), 145.68 (*J* = 7.6 Hz, C-2), 169.72.

^{31}P NMR ($CDCl_3$): δ 19.41.

ESI-MS (m/z): $C_{27}H_{36}NO_7P$, [M+H$^+$] calc. 518.56, found 518.61.

Diethyl (5*S*,6*R*)-3-*N*-acetyl-5,7-*O*-benzylidene-6-hydroxy-hepta-1,3-diene phosphonate (222)



222

Sodium hydride (60%, 21 mg, 0.53 mmol) was added to a solution of tetraethyl methanediphosphate (**69**) (0.15 ml, 0.53 mmol) in THF (2 ml) at 0°C. After stirring for 20 min, **215** (154 mg, 0.44 mmol) was added. The mixture was stirred for 2 h at 0°C. The reaction was then treated with a saturated solution of NH_4Cl, and the water layer was extracted with CH_2Cl_2. The organic layer was dried and concentrated. Flash chromatography of the residue (ether:methanol from 100:1 to 50:1) gave **222** (38 mg, 20%) as a thick colorless oil.

^{1}H NMR ($CDCl_3$): δ 1.24-1.34 (m, 6H, 2 × OCH_2CH_3), 2.13 (s, 3H, CH_3CO), 3.64 (dd, 1H, J = 10.1 and 10.1 Hz, H-7), 3.70 (ddd, 1H, J = 10.1, 8.3 and 4.1 Hz, H-6), 3.98-4.10 (m, 4H, 2 × OCH_2CH_3), 4.33 (dd, 1H, J = 10.1 and 4.1 Hz, H-7), 4.39 (dd, 1H, J = 8.3 and 8.3 Hz, H-5), 5.16 (bs, 1H, O*H*), 5.51 (s, 1H, PhC*H*), 5.82 (dd, 1H, J = 17.6 and 17.6 Hz, H-1), 5.89 (d, 1H, J = 8.3 Hz, H-4), 6.95 (dd, 1H, J = 21.5 and 17.6 Hz, H-2), 7.31-7.50 (m, 5H, aromatic), 8.67 (s, 1H, N*H*Ac).

^{13}C NMR ($CDCl_3$): δ 16.25, 16.32, 23.63, 62.65 (J = 5.7 Hz), 62.70 (J = 5.7 Hz), 66.12 (C-6), 72.05 (C-7), 78.72 (C-5), 101.27, 115.71 (J = 190.7 Hz, C-1), 130.78 (C-4), 135.38 (J = 25.7 Hz, C-3), 146.11 (J = 7.1 Hz, C-2), 170.27.

^{31}P NMR ($CDCl_3$): δ 18.94.

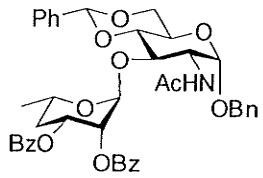
ESI-MS (m/z): $C_{20}H_{28}NO_7P$, [M+H$^+$] calc. 426.42, found 426.38.

General procedure A for the synthesis of 227-229, 239, 240 and 248

The *N*-acetyl D-glucosamine acceptor and freshly activated powdered 4 Å molecular sieves were added to a solution of the thioglycoside donor in CH_2Cl_2. The mixture was vigorously stirred for 1 h. *N*-Iodosuccinimide and TMSOTf were added, and the suspension was stirred at room temperature until TLC indicated the completion of the

reaction. The reaction mixture was diluted with CH_2Cl_2 and filtered through a Celite pad. The filtrate was washed with saturated $Na_2S_2O_3$, saturated $NaHCO_3$, water and brine, then dried and concentrated. The residue was purified by flash column chromatography with a mixture of hexane and ethyl acetate as the eluent.

Benzyl 2′,3′-di-*O*-benzoyl-4′-deoxy-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (227)



227

According to the general procedure A above, **227** was obtained as a white solid (682 mg, 95% yield) from the reaction of donor **163** (388 mg, 0.968 mmol) and acceptor **165** (523 mg, 1.31 mmol) in the presence of NIS (261 mg, 1.16 mmol) and TMSOTf (30 μl).

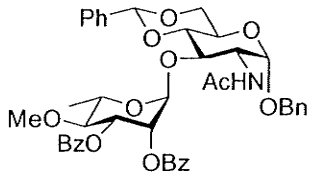
Mp 232-233°C. $[\alpha]_D$ +68.5 (*c* 1.03, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ0.77 (d, 3H, *J* = 6.2 Hz, H-6′), 1.75-1.90 (m, 2H, H-4′), 2.10 (s, 3H, C*H*$_3$C=O), 3.70 (dd, 1H, *J* = 9.5 and 9.5 Hz, H-4), 3.79 (dd, 1H, *J* = 10.2 and 10.2 Hz, H-6), 3.93 (ddd, 1H, *J* = 10.2, 9.5 and 4.7 Hz, H-5), 3.98 (dd, 1H, *J* = 9.5 and 9.5 Hz, H-3), 4.25 (dd, 1H, *J* = 10.2 and 4.7 Hz, H-6), 4.27 (m, 1H, H-5′), 4.45 (ddd, 1H, *J* = 9.9, 9.5 and 3.7 Hz, H-2), 4.49 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.74 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.87 (d, 1H, *J* = 3.7 Hz, H-1), 5.17 (m, 2H, H-2′, 1′), 5.51-5.54 (m, 1H, H-3′), 5.57 (s, 1H, PhC*H*), 5.69(d, 1H, *J* = 9.9 Hz, N*H*Ac), 7.30-8.10 (m, 20H, 4 × *Ph*).

^{13}C NMR ($CDCl_3$): δ20.35 (C-6′), 23.42 (*C*H$_3$C=O), 23.72 (C-4′), 53.12 (C-2), 63.50 (C-5), 64.28 (C-5′), 67.75 (C-3′), 68.95 (C-6), 69.47 (C-2′), 70.03 (Ph*C*H$_2$), 76.58 (C-3), 80.36 (C-4), 97.81 (C-1), 99.09 (C-1′), 102.19 (Ph*C*H), 165.45 (Ph*C*=O), 165.70 (Ph*C*=O), 170.61 (CH$_3$*C*=O).

Anal. Calcd for $C_{42}H_{43}NO_{11}$: C, 68.37; H, 5.87; N, 1.90. Found: C, 68.54; H, 6.05; N, 1.94.

Benzyl 2′,3′-di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (228)



228

According to the general procedure A above, **228** was obtained as a white solid (256 mg, 93% yield) from the reaction of donor **164** (616 mg, 1.43 mmol) and acceptor **165** (348 mg, 0.871 mmol) in the presence of NIS (332 mg, 1.48 mmol) and TMSOTf (30 μl).

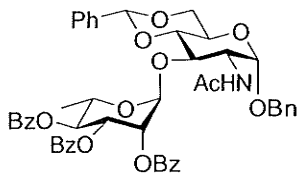
Mp 216-217°C. $[\alpha]_D$ +114 (*c* 1.07, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ0.95 (d, 3H, *J* = 6.2 Hz, H-6′), 2.09 (s, 3H, C*H*$_3$C=O), 3.39 (s, 3H, OC*H*$_3$), 3.40 (dd, 1H, *J* = 9.8 and 9.8 Hz, H-4′), 3.77 (dd, 1H, *J* = 9.8 and 9.6 Hz, H-4), 3.83 (d, 1H, *J* = 10.1 Hz, H-6), 3.94 (dd, 1H, *J* = 9.8 and 4.6 Hz, H-5), 4.01 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-3), 4.14 (dq, 1H, *J* = 9.8 and 6.2 Hz, H-5′), 4.28 (dd, 1H, *J* = 10.1 and 4.6 Hz, H-6), 4.51 (ddd, 1H, *J* = 9.8, 9.6 and 3.7 Hz, H-2), 4.52 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.76 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.90 (d, 1H, *J* = 3.7 Hz, H-1), 5.14 (d, 1H, *J* = 1.7 Hz, H-1′), 5.37 (dd, 1H, *J* = 3.4 and 1.7 Hz, H-2′), 5.60 (s, 1H, PhC*H*), 5.67 (dd, 1H, *J* = 9.8 and 3.4 Hz, H-3′), 5.74 (d, 1H, *J* = 9.8 Hz, N*H*Ac), 7.28-8.06 (m, 20H, 4 × *Ph*).

^{13}C NMR ($CDCl_3$): δ17.18 (C-6′), 23.28 (*C*H$_3$C=O), 53.02 (C-2), 59.89 (O*C*H$_3$), 63.39 (C-5), 67.56 (C-5′), 68.88 (C-6), 69.99 (PhCH$_2$), 71.05 (C-3′), 71.97 (C-2′), 75.71 (C-3), 80.33 (C-4), 89.77 (C-4′), 97.73 (C-1), 97.87 (C-1′), 101.91 (PhCH), 165.10 (Ph*C*=O), 165.42 (Ph*C*=O), 170.38 (CH$_3$*C*=O).

Anal. Calcd for $C_{43}H_{45}NO_{12}$: C, 67.26; H, 5.91; N, 1.82. Found: C, 66.91; H, 6.04; N, 1.89.

Benzyl 2′,3′,4′-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (229)



229

According to the general procedure A above, **229** was obtained as an amorphous glassy solid (736 mg, 95% yield) from the reaction of donor **162** (759 mg, 1.46 mmol) and acceptor **165** (363 mg, 0.908 mmol) in the presence of NIS (332 mg, 1.48 mmol) and TMSOTf (30 μl).

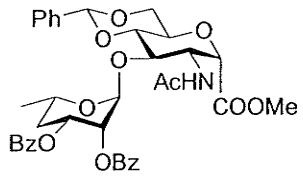
$[\alpha]_D$ +100 (*c* 1.03, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ0.86 (d, 3H, *J* = 6.2 Hz, H-6′), 2.12 (s, 3H, C*H*$_3$C=O), 3.83 (dd, 1H, *J* = 10.0 and 10.0 Hz, H-6), 3.84 (dd, 1H, *J* = 10.0 and 10.0 Hz, H-4), 3.99 (ddd, 1H, *J* = 10.0, 10.0 and 4.6 Hz, H-5), 4.09 (dd, 1H, *J* = 10.0 and 10.0 Hz, H-3), 4.32 (dd, 1H, *J* = 10.0 and 4.6 Hz, H-6), 4.48 (dq, 1H, *J* = 10.0 and 6.2 Hz, H-5′), 4.53 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.60 (ddd, 1H, *J* = 10.4, 10.0 and 3.7 Hz, H-2), 4.78 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.92 (d, 1H, *J* = 3.7 Hz, H-1), 5.27 (d, 1H, *J* = 1.6 Hz, H-1′), 5.45 (dd, 1H, *J* = 3.4 and 1.6 Hz, H-2′), 5.60 (dd, 1H, *J* = 10.0 and 10.0 Hz, H-4′), 5.67 (s, 1H, PhC*H*), 5.85 (d, 1H, *J* = 10.4 Hz, N*H*Ac), 5.90 (dd, 1H, *J* = 10.0 and 3.4 Hz, H-3′), 7.20-8.15 (m, 20H, 4 × *Ph*).

^{13}C NMR ($CDCl_3$): δ16.74 (C-6′), 23.14 (*C*H$_3$C=O), 53.20 (C-2), 63.58 (C-5), 66.82 (C-5′), 69.12 (C-6), 69.70 (C-3′), 70.39 (Ph*C*H$_2$), 71.89 (C-2′), 72.12 (C-4′), 75.70 (C-3), 80.55 (C-4), 98.20 (C-1′), 98.43 (C-1), 102.70 (Ph*C*H).

Anal. Calcd for $C_{49}H_{47}NO_{13}$: C, 68.60; H, 5.52; N, 1.63. Found: C, 68.24; H, 5.29; N, 2.07.

2′,3′-Di-*O*-benzoyl-4′-*O*-deoxy-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-5,7-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (239)



239

According to the general procedure A above, **239** was obtained as a white solid (166 mg, 61% yield) from the reaction of donor **163** (189 mg, 0.477 mmol) and acceptor **166** (140 mg, 0.397 mmol) in the presence of NIS (130 mg, 0.572 mmol) and TMSOTf (20 μl).

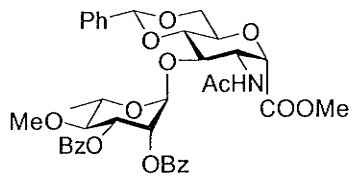
Mp 242-243°C. $[\alpha]_D$ +22.9 (*c* 1.25, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ0.78 (d, 3H, *J* = 6.2 Hz, H-6′), 1.84-2.04 (m, 2H, H-4′), 2.16 (s, 3H, C*H*$_3$C=O), 3.51 (ddd, 1H, *J* = 9.7, 9.7 and 4.8 Hz, H-6), 3.71 (dd, 1H, *J* = 9.7 and 9.7 Hz, H-5), 3.76 (dd, 1H, *J* = 10.0 and 9.7 Hz, H-7), 3.85 (s, 3H, OC*H*$_3$), 4.10 (dd, 1H, *J* = 10.0 and 9.7 Hz, H-4), 4.25 (m, 1H, H-5′), 4.34 (dd, 1H, *J* = 10.0 and 4.8 Hz, H-7), 4.51 (d, 1H, *J* = 5.6 Hz, H-2), 4.69 (ddd, 1H, *J* = 10.0, 9.9 and 5.6 Hz, H-3), 5.21-5.24 (m, 2H, H-2′, 1′), 5.50-5.58 (m, 1H, H-3′), 5.57 (s, 1H, PhC*H*), 6.39 (d, 1H, *J* = 9.9 Hz, N*H*Ac), 7.33-8.10 (m, 15H, 3 × *Ph*).

^{13}C NMR ($CDCl_3$): δ20.35 (C-6′), 23.45 (*C*H$_3$C=O), 33.70 (C-4′), 51.07 (C-3), 52.78 (O*C*H$_3$), 64.39 (C-5′), 67.72 (C-3′), 68.72 (C-2′), 68.78 (C-6), 69.29 (C-7), 75.03 (C-2), 75.93 (C-4), 80.51 (C-5), 99.13 (C-1′), 102.19 (Ph*C*H), 165.47 (Ph*C*=O), 165.75 (Ph*C*=O), 170.79 (R*C*=O), 170.96 (R*C*=O).

Anal. Calcd for $C_{37}H_{39}NO_{12}$: C, 64.43; H, 5.70; N, 2.03. Found: C, 64.15; H, 5.67; N, 2.15.

2′,3′-Di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-5,7-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (240)



240

According to the general procedure A above, **240** was obtained as an amorphous glassy solid (220 mg, 83% yield) from the reaction of donor **164** (240 mg, 0.546 mmol) and acceptor **166** (130 mg, 0.369 mmol) in the presence of NIS (150 mg, 0.655 mmol) and TMSOTf (20 μl).

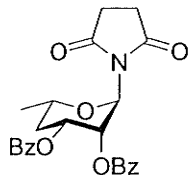
$[\alpha]_D$ +91.3 (*c* 1.05, ethyl acetate).

^{1}H NMR ($CDCl_3$): δ0.92 (d, 3H, *J* = 6.2 Hz, H-6′), 2.12 (s, 3H, CH_3C=O), 3.39 (s, 3H, OCH_3), 3.42 (dd, 1H, *J* = 9.8 and 9.8 Hz, H-4′), 3.51 (ddd, 1H, *J* = 9.8, 9.8 and 4.8 Hz, H-6), 3.77 (dd, 1H, *J* = 9.8 and 9.8 Hz, H-5), 3.77 (ddd, 1H, *J* = 10.6, 9.8 and 4.8 Hz, H-7), 3.84 (s, 3H, OCH_3), 4.10 (dd, 1H, *J* = 10.0 and 9.8 Hz, H-4), 4.10 (dq, 1H, *J* = 9.8 and 6.2 Hz, H-5′), 4.34 (dd, 1H, *J* = 10.6 and 4.8 Hz, H-7), 4.53 (d, 1H, *J* = 5.6 Hz, H-2), 4.70 (ddd, 1H, *J* = 10.0, 9.7 and 5.6 Hz, H-3), 5.17 (d, 1H, *J* = 1.6 Hz, H-1′), 5.37 (dd, 1H, *J* = 3.4 and 1.6 Hz, H-2′), 5.57 (s, 1H, PhC*H*), 5.64 (dd, 1H, *J* = 9.8 and 3.4 Hz, H-3′), 6.36 (d, 1H, *J* = 9.7 Hz, N*H*Ac), 7.32-8.04 (m, 15H, 3 × *Ph*).

^{13}C NMR ($CDCl_3$): δ17.20 (C-6′), 23.35 (CH_3C=O), 51.05 (C-3), 52.74 (OCH_3), 60.12 (OCH_3), 67.76 (C-5′), 68.57 (C-6), 68.75 (C-7), 71.22 (C-3′), 71.81 (C-2′), 74.93 (C-2), 75.65 (C-4), 80.49 (C-5), 80.79 (C-4′), 97.95 (C-1′), 101.96 (PhCH), 165.23 (PhC=O), 165.53 (PhC=O), 170.70 (RC=O), 170.87 (RC=O).

Anal. Calcd for $C_{38}H_{41}NO_{13}$: C, 63.41; H, 5.74; N, 1.95. Found: C, 63.01; H, 5.78; N, 1.95.

4′-Deoxy-2′,3′-di-benzoyl-α-L-rhamnopyranosyl pyrrole-2,5-dione (241)



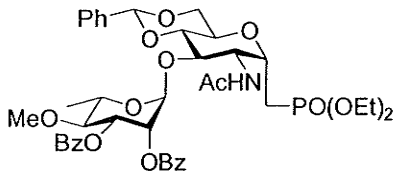
241

Byproduct **241** (65 mg, 31%) was obtained as a glassy solid from the preparation of **239**.

^{1}H NMR ($CDCl_3$): δ 1.41 (d, 3H, J = 6.5 Hz, CH_3), 2.00 (ddd, 1H, J = 12.6, 5.6 and 1.8 Hz, H-4′), 2.40 (ddd, 1H, J = 9.9, 9.9 and 5.6 Hz, H-4′), 2.68-2.72 (m, 4H, 2 × CH_2), 4.48 (ddt, 1H, J = 12.6, 9.9 and 6.5 Hz, H-5′), 6.10-6.30 (m, 2H, H-1′,2′), 6.30-6.50 (m, 1H, H-3′), 7.30-8.10 (m, 10H, aromatic).

^{13}C NMR ($CDCl_3$): δ 20.58, 28.07, 34.39, 67.13, 68.50, 69.19, 75.12, 165.20, 165.61, 176.61.

Diethyl 2′,3′-di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)- *C*-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate (248)



248

According to the general procedure A above, **248** was obtained as an amorphous white solid (44.6 mg, 75% yield) from the reaction of donor **164** (66.2 mg, 0.154 mmol) and acceptor **167** (32.7 mg, 0.0734 mmol) in the presence of NIS (45.4 mg, 0.202 mmol) and TMSOTf (6 μl).

$[\alpha]_D$ +25.0 (*c* 0.76, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ0.93 (d, 3H, J = 6.1 Hz, H-6′), 1.23 (t, 3H, J = 7.0 Hz, CH_3CH_2), 1.25 (t, 3H, J = 7.0 Hz, CH_3CH_2), 2.10 (s, 3H, CH_3C=O), 2.10-2.30 (m, 2H, PCH_2), 3.37 (s, 3H, OCH_3), 3.40 (dd, 1H, J = 9.7 and 9.7 Hz, H-4′), 3.71-3.74 (m, 3H, H-

5, 4, 6), 3.94-4.12 (m, 6H, 2 × OC*H*$_2$CH$_3$, H-3, 5′), 4.23 (m, 1H, H-6), 4.40-4.50 (m, 2H, H-2, 1), 5.24 (d, 1H, J = 1.4 Hz, H-1′), 5.42 (dd, 1H, J = 3.4 and 1.4 Hz, H-2′), 5.57 (s, 1H, PhC*H*), 5.62 (dd, 1H, J = 9.7 and 3.4 Hz, H-3′), 7.32-8.02 (m, 16H, 3 × *Ph*, N*H*Ac).

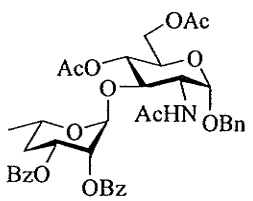
^{13}C NMR ($CDCl_3$): δ16.29 (*C*H$_3$CH$_2$), 16.37 (*C*H$_3$CH$_2$), 17.31 (C-6′), 23.03 (*C*H$_3$C=O), 25.68 (d, J = 143.4 Hz, P*C*H$_2$), 53.38 (d, J = 13.5 Hz, C-2), 60.03 (O*C*H$_3$), 62.14 (d, J = 6.5 Hz, CH$_3$*C*H$_2$), 62.32 (d, J = 6.5 Hz, CH$_3$*C*H$_2$), 65.10 (C-5), 67.60 (C-5′), 69.29 (C-6), 70.75 (d, J = 4.5 Hz, C-1), 71.30 (C-2′), 71.55 (C-3′), 74.68 (C-3), 80.85 (C-4), 81.00 (C-4′), 97.55 (C-1′), 101.92 (Ph*C*H), 165.21 (Ph*C*=O), 165.29 (Ph*C*=O), 171.17 (CH$_3$*C*=O).

^{31}P NMR ($CDCl_3$): δ 28.50.

General procedure B for the synthesis of 230-232

The disaccharides **227-229** (0.20 mmol) were suspended in 80% HOAc at 70°C for 2 h. The solution was concentrated to dryness under reduced pressure. Then pyridine (2 ml) and DMAP (20 mg) were added to a suspension of the residue in Ac_2O (1 ml). The reaction mixture was stirred at room temperature until TLC showed the completion of the reaction. The reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with 1 M HCl, saturated $NaHCO_3$, water and brine, and then was dried and concentrated. The residue was purified by flash column chromatography with a mixture of hexane and ethyl acetate as the eluent.

Benzyl 2′,3′-di-*O*-benzoyl-4′-deoxy-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (230)



230

Compound **230** was obtained as an amorphous white solid (82% yield) from the reaction of **227** according to general procedure B above.

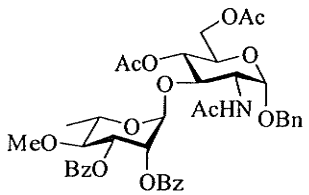
$[\alpha]_D$ +99.2 (*c* 0.65, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ 1.24 (d, 3H, *J* = 6.1 Hz, H-6′), 1.92-2.00 (m, 2H, H-4′), 2.09 (s, 6H, 2 × C*H*$_3$C=O), 2.10 (s, 3H, C*H*$_3$C=O), 3.89-3.95 (m, 2H, H-3, 5), 4.00-4.14 (m, 2H, H-6, 5′), 4.20 (dd, 1H, *J* = 12.3 and 4.6 Hz, H-6), 4.47 (ddd, 1H, *J* = 10.0, 9.7 and 3.6 Hz, H-2), 4.51 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.70 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.94 (d, 1H, *J* = 3.6 Hz, H-1), 5.10 (d, 1H, *J* = 1.6 Hz, H-1′), 5.15 (dd, 1H, *J* = 9.8 and 9.8 Hz, H-4), 5.28 (dd, 1H, *J* = 2.1 and 1.6 Hz, H-2′), 5.43-5.50 (m, 1H, H-3′), 5.91 (d, 1H, *J* = 9.7 Hz, N*H*Ac), 7.27-8.08 (m, 15H, 3 × *Ph*).

^{13}C NMR ($CDCl_3$): δ20.73 (C-6′), 21.10 (*C*H$_3$C=O), 23.26 (*C*H$_3$C=O), 33.67 (C-4′), 52.32 (C-2), 62.15 (C-6), 65.26 (C-5′), 67.38 (C-3′), 68.31 (C-5), 69.07 (C-2′), 69.93 (C-4), 70.01 (Ph*C*H$_2$), 79.98 (C-3), 96.92 (C-1), 100.73 (C-1′), 165.01 (Ph*C*=O), 165.94 (Ph*C*=O), 169.50 (CH$_3$*C*=O), 170.55 (CH$_3$*C*=O), 170.73 (CH$_3$*C*=O).

ESI-MS: $C_{39}H_{43}NO_{13}$, [M+H$^+$], 734.77; found 734.69.

Benzyl 2′,3′-di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (231)



231

Compound **231** was obtained as an amorphous white solid (90% yield) from the reaction of **228** according to general procedure B above.

$[\alpha]_D$ +137 (*c* 1.07, CH_2Cl_2).

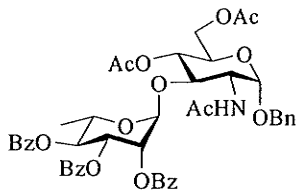
^{1}H NMR ($CDCl_3$): δ1.33 (d, 3H, *J* = 6.1 Hz, H-6′), 2.07 (s, 3H, C*H*$_3$C=O), 2.09 (s, 6H, 2 × C*H*$_3$C=O), 3.44 (dd, 1H, *J* = 9.3 and 9.3 Hz, H-4′), 3.48 (s, 3H, OC*H*$_3$), 3.90 (dq, 1H, *J* = 9.3 and 6.1 Hz, H-5′), 3.90 (ddd, 1H, *J* = 9.6, 4.6 and 2.2 Hz, H-5), 3.94 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-3), 4.02 (dd, 1H, *J* = 12.3 and 2.2 Hz, H-6), 4.15 (dd, 1H, *J* = 12.3 and 4.6 Hz, H-6), 4.45 (dd, 1H, *J* = 9.8 and 3.6 Hz, H-2), 4.50 (d, 1H, *J* = 11.8 Hz,

PhCH_2), 4.69 (d, 1H, J = 11.8 Hz, PhCH_2), 4.94 (d, 1H, J = 3.6 Hz, H-1), 5.02 (ws, 1H, H-1′), 5.15 (dd, 1H J = 9.6 and 9.6 Hz, H-4), 5.43-5.47 (m, 2H, H-2′, 3′), 5.82 (d, 1H, J = 9.6 Hz, N*H*Ac), 7.34-8.03 (m, 15H, 3 × *Ph*).

^{13}C NMR ($CDCl_3$): δ17.67 (C-6′), 20.62 (CH_3C=O), 20.94 (CH_3C=O), 23.09 (CH_3C=O), 52.08 (C-2), 60.27 (OCH_3), 62.02 (C-6), 68.20 (C-5), 68.44 (C-5′), 69.85 (C-4), 69.92 (PhCH_2), 71.22 (C-3′), 71.67 (C-2′), 79.00 (C-3), 80.29 (C-4′), 96.82 (C-1), 99.18 (C-1′), 164.78 (PhC=O), 165.60 (PhC=O), 169.29 (CH_3C=O), 170.39 (CH_3C=O), 170.65 (CH_3C=O).

Anal. Calcd for $C_{40}H_{45}NO_{14}$: C, 62.90; H, 5.94; N, 1.83. Found: C, 63.29; H, 6.18; N, 1.84.

Benzyl 2′,3′,4′-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (232)



232

Compound **232** was obtained as an amorphous white solid (88% yield) from the reaction of **229** according to general procedure B above.

$[\alpha]_D$ +184 (*c* 0.90, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ1.29 (d, 3H, J = 6.2 Hz, H-6′), 2.10 (s, 3H, CH_3C=O), 2.13 (s, 3H, CH_3C=O), 2.17 (s, 3H, CH_3C=O), 3.91-3.98 (m, 2H, H-3, 5), 4.01-4.15 (m, 1H, H-6), 4.21-4.26 (m, 2H, H-6, 5′), 4.54 (d, 1H, J = 11.8 Hz, PhCH_2), 4.55-4.63 (m, 1H, H-2), 4.73 (d, 1H, J = 11.8 Hz, PhCH_2), 4.99 (d, 1H, J = 3.6 Hz, H-1), 5.11 (d, 1H, J = 1.6 Hz, H-1′), 5.23 (dd, 1H, J = 9.6 and 9.6 Hz, H-4), 5.55 (m, 1H, H-2′), 5.62-5.71(m, 2H, H-3′, 4′), 5.91 (d, 1H, J = 9.7Hz, N*H*Ac), 7.21-8.09 (m, 20H, 4 × *Ph*).

^{13}C NMR ($CDCl_3$): δ17.26 (C-6′), 20.63 (CH_3C=O), 21.04 (CH_3C=O), 23.14 (CH_3C=O), 51.84 (C-2), 61.92 (C-6), 67.64 (C-5′), 68.20 (C-5), 69.72 (C-4′), 69.98 (C-

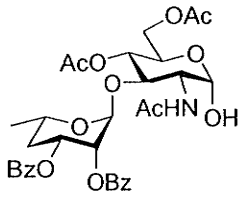
4), 70.01 (Ph*C*H_2), 70.97 (C-3′), 71.18 (C-2′), 80.64 (C-3), 96.87 (C-1), 99.84 (C-1′), 164.85 (Ph*C*=O), 165.71 (Ph*C*=O), 165.82 (Ph*C*=O), 169.43 (CH_3*C*=O), 170.55 (CH_3*C*=O), 170.62 (CH_3*C*=O).

Anal. Calcd for $C_{46}H_{47}NO_{15}$: C, 64.71; H, 5.55; N, 1.64. Found: C, 64.36; H, 5.76; N, 1.63.

General procedure C for the synthesis of 233-235

A suspension of **230-232** (0.2 mmol) and Pd/C catalyst (10%, 50 mg) in glacial acetic acid (2 ml) was flushed in hydrogen and vigorously stirred until TLC showed the completion of the reaction. The mixture was filtered through a Celite pad and the filtrate was concentrated. The residue was purified by flash column chromatography with a mixture of hexane and ethyl acetate as the eluent.

2′,3′-Di-*O*-benzoyl-4′-deoxy-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (233)



233

Compound **233** was obtained as an amorphous white solid (63% yield) from the reaction of **230** according to general procedure C above.

$[\alpha]_D$ +78.7 (*c* 1.00, CH_2Cl_2).

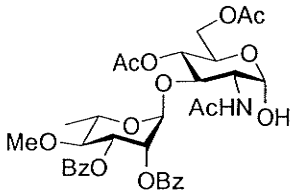
^{1}H NMR ($CDCl_3$): δ1.24 (d, 3H, *J* = 6.0 Hz, H-6′), 1.85-2.15 (m, 2H, H-4′), 2.05 (s, 3H, C*H*$_3$C=O), 2.09 (s, 3H, C*H*$_3$C=O), 2.10 (s, 3H, C*H*$_3$C=O), 4.01 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-3), 4.01-4.23 (m, 4H, H-6, 5, 5′), 4.33 (ddd, 1H, *J* = 9.4, 9.4 and 3.2 Hz, H-2), 5.07 (bs, 1H, H-1′), 5.11 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-4), 5.26 (bs, 1H, H-2′), 5.29 (d, 1H, *J* = 3.2 Hz, H-1), 5.50 (ddd, 1H, *J* = 10.7, 4.0 and 4.0 Hz, H-3′), 6.62 (d, 1H, *J* = 9.4 Hz, N*H*Ac), 7.26-8.06 (m, 10H, 2 × *Ph*).

^{13}C NMR ($CDCl_3$): δ20.77 (*C*H_3C=O), 21.13 (C-6′, *C*H_3C=O), 21.21 (*C*H_3C=O), 23.19 (C-4′), 53.23 (C-2), 62.37 (C-6), 65.33 (C-5′), 67.55 (C-3′), 67.69 (C-5), 69.18 (C-2′), 70.11 (C-4), 80.20 (C-3), 91.90 (C-1), 101.09 (C-1′), 165.32 (Ph*C*=O), 166.11 (Ph*C*=O), 169.76 (CH_3*C*=O), 171.09 (CH_3*C*=O), 171.69 (CH_3*C*=O).

ESI-MS: $C_{32}H_{37}NO_{13}$, [M+H^+], 644.65; found 644.69

Anal. Calcd for $C_{32}H_{37}NO_{13}$: C, 59.71; H, 5.79; N, 2.18. Found: C, 59.39; H, 5.72; N, 2.17.

2′,3′-Di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (234)



234

Compound **234** was obtained as an amorphous white solid (77% yield) from the reaction of **231** according to general procedure C above.

$[\alpha]_D$ +136 (*c* 0.75, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ1.35 (d, 3H, *J* = 6.1 Hz, H-6′), 2.08 (s, 3H, C*H*$_3$C=O), 2.12 (s, 3H, C*H*$_3$C=O), 2.13 (s, 3H, C*H*$_3$C=O), 3.48 (s, 3H, OC*H*$_3$), 3.44 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 3.96 (dq, 1H, *J* = 9.6 and 6.1 Hz, H-5′), 4.08 (dd, 1H, *J* = 10.0 and 9.4 Hz, H-3), 4.08-4.23 (m, 3H, H-6, 5), 4.33 (ddd, 1H, *J* = 10.0, 9.2 and 2.8 Hz, H-2), 5.02 (d, 1H, *J* = 1.3 Hz, H-1′), 5.14 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-4), 5.30 (d, 1H, *J* = 2.8 Hz, H-1), 5.44 (dd, 1H, *J* = 3.2 and 1.3 Hz, H-2′), 5.51 (dd, 1H, *J* = 9.6 and 3.2 Hz, H-3′), 6.54 (d, 1H, *J* = 9.2 Hz, N*H*Ac), 7.30-8.10 (m, 10H, 2 × *Ph*).

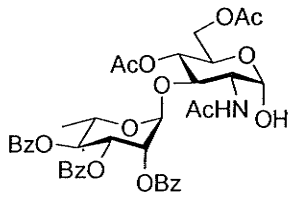
^{13}C NMR ($CDCl_3$): δ18.15 (C-6′), 21.18 (*C*H_3C=O), 21.56 (*C*H_3C=O), 23.52 (*C*H_3C=O), 53.69 (C-2), 60.98 (O*C*H_3), 62.78 (C-6), 68.00 (C-5), 69.00 (C-5′), 70.64 (C-4), 71.84 (C-2′), 72.28 (C-3′), 79.78 (C-3), 81.10 (C-4′), 92.40 (C-1), 100.07 (C-1′),

165.52 (Ph*C*=O), 166.14 (Ph*C*=O), 170.20 (CH_3C=O), 171.47 (CH_3C=O), 172.15 (CH_3C=O).

ESI-MS: $C_{33}H_{39}NO_{14}$, [M+H$^+$], 674.67; found 674.73

Anal. Calcd for $C_{33}H_{39}NO_{14}$: C, 58.84; H, 5.84; N, 2.08. Found: C, 59.21; H, 6.21; N, 2.12.

2′,3′,4′-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (235)



235

Compound **235** was obtained as an amorphous white solid (80% yield) from the reaction of **232** according to general procedure C above.

$[\alpha]_D$ +146 (*c* 0.50, CH_2Cl_2).

^{1}H NMR ($CDCl_3$): δ1.28 (d, 3H, *J* = 6.1 Hz, H-6′), 2.09 (s, 3H, CH_3C=O), 2.10 (s, 3H, CH_3C=O), 2.19 (s, 3H, CH_3C=O), 4.06-4.22 (m, 2H, H-6), 4.12-4.28 (m, 2H, H-5,5′), 4.11 (dd, 1H, *J* = 10.1 and 10.1 Hz, H-3), 4.41 (ddd, 1H, *J* = 10.1, 9.4 and 3.3 Hz, H-2), 4.60 (d, 1H, *J* = 2.8 Hz, H-OH), 5.09 (d, 1H, *J* = 1.6 Hz, H-1′), 5.22 (dd, 1H, *J* = 9.2 and 9.2 Hz, H-4), 5.38 (dd, 1H, *J* = 3.3 and 2.8 Hz, H-1), 5.52-5.53 (m, 1H, H-2′), 5.63-5.68 (m, 2H, H-3′, 4′), 6.33 (d, 1H, *J* = 9.4Hz, N*H*Ac), 7.20-8.10 (m, 15H, 3 × *Ph*).

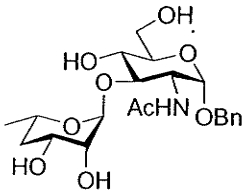
^{13}C NMR ($CDCl_3$): δ17.41 (C-6′), 20.80 (CH_3C=O), 21.25 (CH_3C=O), 23.26 (CH_3C=O), 53.21 (C-2), 62.19 (C-6), 67.93 (C-,5, 5′), 69.92 (C-4), 70.26 (C-4′), 71.21 (C-2′), 71.24 (C-3′), 80.46 (C-3), 92.34 (C-1), 100.23 (C-1′), 165.26 (*C*=O), 165.87 (*C*=O), 166.26 (*C*=O), 169.68 (*C*=O), 170.95 (*C*=O), 171.62 (*C*=O).

Anal. Calcd for $C_{39}H_{41}NO_{15}$: C, 61.33; H, 5.41; N, 1.83. Found: C, 60.91; H, 5.56; N, 1.79.

The general procedure D for the synthesis of 236-238, 242-245 and 249

The disaccharides **227-229**, **239**, **240** or **248** (0.2 mmol) were suspended in 80% aqueous acetic acid at 70°C for 2 h. The reaction mixture was concentrated to dryness. The residue was then suspended in NaOMe/MeOH solution (0.1 M, 5 ml). After stirring for 1 h at room temperature, Amberlite IR-120 (acidic) was added. The mixture was filtered and the filtrate was concentrated. The products were purified by column chromatography with a mixture of ethyl acetate, CH_2Cl_2 and methanol as the eluent.

Benzyl 4′-*O*-deoxy-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (236)



236

Compound **236** was obtained as a white solid (95% yield) from the reaction of **227** according to general procedure D above.

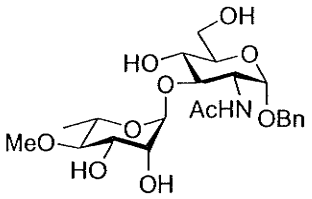
Mp 193-194°C. $[\alpha]_D$ +81.1 (*c* 0.35, CH_2Cl_2).

^{1}H NMR (D_2O): δ 1.15 (d, 3H, *J* = 6.3 Hz, H-6′), 1.55 (ddd, 1H, *J* = 11.9, 11.9 and 11.9 Hz, H-4′), 1.70 (ddd, 1H, *J* = 11.9, 2.3 and 0.7 Hz, H-4′), 2.00 (s, 3H, CH_3C=O), 3.53 (dd, 1H, *J* = 9.0 and 9.0 Hz, H-4), 3.59 (dd, 1H, *J* = 1.7 and 1.7 Hz, H-2′), 3.72-3.86 (m, 4H, H-6, 5, 3), 3.96 (ddd, 1H, *J* = 11.9, 1.7 and 0.7 Hz, H-3′), 4.02 (dd, 1H, *J* = 10.4 and 3.6 Hz, H-2), 4.20 (ddd, 1H, *J* = 11.9, 6.3 and 2.3 Hz, H-5′), 4.57 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.78 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.88 (d, 1H, *J* = 1.7 Hz, H-1′), 4.90 (d, 1H, *J* = 3.6 Hz, H-1), 7.40-7.46 (m, 5H, *Ph*).

^{13}C NMR (D_2O): δ20.25 (C-6′), 22.23 (CH_3C=O), 34.76 (C-4′), 53.51 (C-2), 60.85 (C-6), 65.48 (C-3′), 66.01 (C-5′), 68.79 (C-4), 68.82 (C-2′), 69.95 (PhCH$_2$), 72.64(C-5), 79.60 (C-3), 96.57 (C-1), 102.29 (C-1′), 174.49 (CH_3C=O).

ESI-MS: $C_{21}H_{31}NO_9$, [M+H$^+$], 442.48; found 442.59.

Benzyl 4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (237)



237

Compound **237** was obtained as a white solid (93% yield) from the reaction of **228** according to general procedure D above.

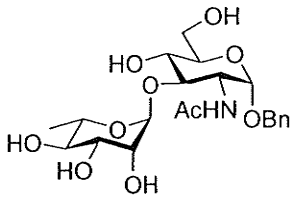
Mp 161-163°C. $[\alpha]_D$ +61.9 (*c*0.75, CH_3OH).

^1H NMR (D_2O): δ1.25 (d, 3H, *J* = 6.3 Hz, H-6′), 1.99 (s, 3H, CH_3C=O), 3.15 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 3.52 (s, 3H, OCH_3), 3.55 (dd 1H, *J* = 10.0 and 10.0 Hz, H-4), 3.75-3.85 (m, 3H, H-6, 5), 3.76 (dd, 1H, *J* = 2.9 and 1.4 Hz, H-2′), 3.77 (dd, 1H, *J* = 10.0 and 10.0 Hz, H-3), 3.78 (dd, 1H, *J* = 9.6 and 2.9 Hz, H-3′), 3.97 (dq, 1H, *J* = 9.6 and 6.3 Hz, H-5′), 4.03 (dd, 1H, *J* = 10.0 and 3.6 Hz, H-2), 4.56 (d, 1H, *J* = 11.8 Hz, $PhCH_2$), 4.76 (d, 1H, *J* = 11.8 Hz, $PhCH_2$), 4.80 (d, 1H, *J* = 1.4 Hz, H-1′), 4.89 (d, 1H, *J* = 3.6 Hz, H-1), 7.30-7.50 (m, 5H, *Ph*).

^{13}C NMR (D_2O): δ17.02 (C-6′), 22.22 (CH_3C=O), 53.53 (C-2), 60.36 (OCH_3), 60.84 (C-6), 68.21 (C-5′), 68.69 (C-4), 69.94 ($PhCH_2$), 70.30 (C-3′), 71.23 (C-2′), 72.71 (C-5), 79.58 (C-3), 82.60 (C-4′), 96.53 (C-1), 101.40 (C-1′), 174.47 (CH_3C=O).

Anal. Calcd for $C_{22}H_{33}NO_{10}$: C, 56.04; H, 7.05; Found: C, 55.51; H, 6.58.

Benzyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (238)



238

Compound **238** was obtained as a white solid (93% yield) from the reaction of **229** according to general procedure D above.

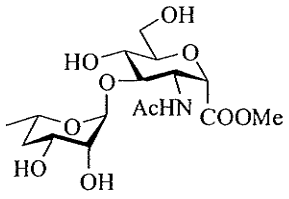
Mp 211-212°C. $[\alpha]_D$ +69.0 (*c* 0.50, CH_3OH).

^1H NMR (D_2O): δ1.21 (d, 3H, *J* = 6.3 Hz, H-6′), 1.99 (s, 3H, CH_3C=O), 3.41 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 3.55 (dd, 1H, *J* = 9.1 and 9.1 Hz, H-4), 3.75 (dd, 1H, *J* = 9.6 and 3.3 Hz, H-3′), 3.75 (dd, 1H, *J* = 3.3 and 1.2 Hz, H-2′), 3.75 (dd, 1H, *J* = 9.1 and 9.1 Hz, H-3), 3.77-3.84 (m, 3H, H-6, 5), 3.96 (dq, 1H, *J* = 9.6 and 6.3 Hz, H-5′), 4.04 (dd, 1H, *J* = 9.1 and 3.6 Hz, H-2), 4.56 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.77 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.83 (d, 1H, *J* = 1.2 Hz, H-1′), 4.89 (d, 1H, *J* = 3.6 Hz, H-1), 7.30-7.50 (m, 5H, *Ph*).

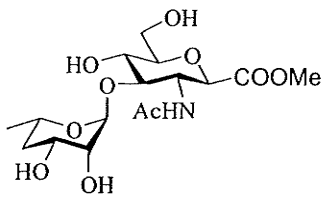
^{13}C NMR (D_2O): δ16.83 (C-6′), 22.24 (CH_3C=O), 53.54 (C-2), 60.86 (C-6), 68.72 (C-4), 69.24 (C-5′), 69.94 (PhCH_2), 70.58 (C-3′), 71.12 (C-2′), 72.27 (C-4′), 72.72 (C-3), 79.72 (C-5), 96.54 (C-1), 101.56 (C-1′), 174.47 (CH_3C=O).

Anal. Calcd for $C_{21}H_{31}NO_{10}$: C, 55.14; H, 6.83; Found: C, 54.76; H, 6.95.

4′-Deoxy-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (242) and **4′-Deoxy-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-3-deoxy-D-*glycero*-D-*gulo*-heptonic acid methyl ester (244)**



242



244

Compounds **242** (44% yield) and **244** (12% yield) were obtained as amorphous white solids from the reaction of **239** according to general procedure D above.

Compound **242**: $[\alpha]_D$ +8.2 (*c* 0.69, H_2O).

^{1}H NMR (D_2O): δ1.17 (d, 3H, *J* = 6.3 Hz, H-6′), 1.58 (q, 1H, *J* = 11.9 Hz, H-4′), 1.73 (ddd, 1H, *J* = 11.9, 4.5 and 2.2 Hz, H-4′), 2.04 (s, 3H, C*H*$_3$C=O), 3.59 (t, 1H, *J* = 7.8 Hz, H-5), 3.69 (dd, 1H, *J* = 3.2 and 1.6 Hz, H-2′), 3.75 (m, 1H, H-6), 3.80 (m, 2H, H-7), 3.81 (s, 3H, OC*H*$_3$), 3.85 (dd, 1H, *J* = 11.0 and 7.8 Hz, H-4), 4.00 (ddd, 1H, *J* = 11.9, 4.5 and 3.2 Hz, H-3′), 4.16 (ddq, 1H, *J* = 11.9, 6.3 and 2.2 Hz, H-5′), 4.30 (dd, 1H, *J* = 9.1 and 5.2 Hz, H-3), 4.65 (d, 1H, *J* = 5.2 Hz, H-2), 5.00 (d, 1H, *J* = 1.6 Hz, H-1′).

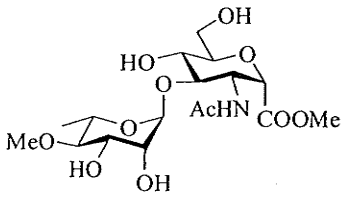
^{13}C NMR (D_2O): δ20.29, 22.32, 34.80, 50.47, 53.27, 60.47, 65.48, 66.19, 68.29, 68.68, 77.94, 78.00, 101.56, 171.53, 174.64.

Compound **244**: $[\alpha]_D$ −43.8 (*c* 0.37, H_2O).

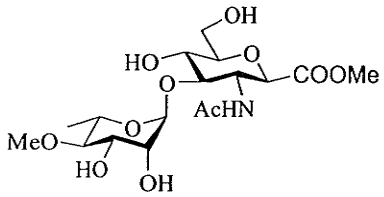
^{1}H NMR (D_2O): δ1.17 (d, 3H, *J* = 6.3 Hz, H-6′), 1.56 (q, 1H, *J* = 11.9 Hz, H-4′), 1.74 (ddd, 1H, *J* = 11.9, 2.3 and 0.7 Hz, H-4′), 2.03 (s, 3H, C*H*$_3$C=O), 3.52 (ddd, 1H, *J* = 9.8, 5.3 and 1.9 Hz, H-6), 3.54 (t, 1H, *J* = 9.8 Hz, H-5), 3.65 (dd, 1H, *J* = 5.3 and 1.5 Hz, H-2′), 3.68 (dd, 1H, *J* = 10.6 and 9.8 Hz, H-4), 3.75(s, 3H, OC*H*$_3$), 3.76 (dd, 1H, *J* = 12.7 and 5.3 Hz, H-7), 3.91 (dd, 1H, *J* = 12.7 and 1.9 Hz, H-7), 4.00 (ddd, 1H, *J* = 11.9, 5.3 and 0.7 Hz, H-3′), 4.01 (t, 1H, *J* = 10.6 Hz, H-3), 4.09 (d, 1H, *J* = 10.6 Hz, H-2), 4.22 (ddq, 1H, *J* = 11.9, 6.3 and 2.3 Hz, H-5′), 4.94 (d, 1H, *J* = 1.5 Hz, H-1′).

^{13}C NMR (D_2O): δ20.26 (C-6′), 22.22 (CH_3C=O), 34.77 (C-4′), 53.35 (C-3), 53.64 (OCH_3), 61.14 (C-7), 65.46 (C-3′), 66.13 (C-5′), 68.66 (C-5), 68.74 (C-2′), 76.45 (C-2), 80.10 (C-6), 81.92 (C-4), 102.41 (C-1′), 171.25 (*C*=O), 174.77 (*C*=O).

4′-*O*-methyl-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester (243) and **4′-*O*-methyl-α-L-rhamnopyranosyl-(1→4)- 3-acetamido-2,6-anhydro-3-deoxy-D-*glycero*-D-*gulo*-heptonic acid methyl ester (245)**



243



245

Compounds **243** (28% yield) and **245** (15% yield) were obtained as amorphous white solids from the reaction of **240** according to general procedure D above.

Compound **243**: $[\alpha]_D$ −1.0 (*c* 1.23, H_2O).

^{1}H NMR (D_2O): δ1.27 (d, 3H, *J* = 6.3 Hz, H-6′), 2.04 (s, 3H, CH_3C=O), 3.18 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 3.54 (s, 3H, OCH_3), 3.59 (dd, 1H, *J* = 8.0 and 8.0 Hz, H-5), 3.67-3.73 (m, 1H, H-6), 3.81 (s, 3H, OCH_3), 3.77-3.95 (m, 6H, H-7, 4, 5′, 3′, 2′), 4.29 (dd, 1H, *J* = 9.3 and 5.3 Hz, H-3), 4.64 (d, 1H, *J* = 5.3 Hz, H-2), 4.94 (d, 1H, *J* = 1.6 Hz, H-1′).

^{13}C NMR (D_2O): δ17.09, 22.32, 50.60, 53.28, 60.44, 60.55, 68.26, 68.40, 70.33, 71.13, 72.71, 77.98, 78.20, 82.64, 100.79, 171.49, 174.63.

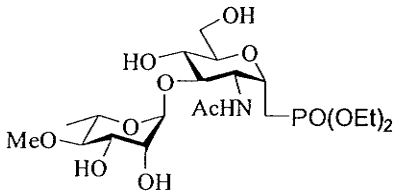
Compound **245**: $[\alpha]_D$ −40.0 (*c* 0.35, CH_2Cl_2).

^{1}H NMR (D_2O): δ1.26 (d, 3H, *J* = 6.3 Hz, H-6′), 2.02 (s, 3H, CH_3C=O), 3.17 (dd, 1H, *J* = 7.8 and 7.8 Hz, H-4′), 3.53 (s, 3H, OCH_3), 3.48-3.57 (m, 2H, H-5, 3′), 3.67 (dd, 1H, *J* = 10.6 and 10.0 Hz, H-4), 3.71-3.81 (m, 3H, H-7, 6, 2′), 3.93 (dd, 1H, *J* = 12.5 and

1.9 Hz, H-7), 3.99 (dd, 1H, J = 10.0 and 6.3 Hz, H-5′), 4.00 (dd, 1H, J = 10.6 and 10.6 Hz, H-3), 4.09 (d, 1H, J = 10.6 Hz, H-2), 4.87 (d, 1H, J = 1.4 Hz, H-1′).

^{13}C NMR (D_2O): δ17.04, 22.22, 53.37, 53.64, 60.37, 61.12, 68.33, 68.54, 70.27, 71.18, 76.41, 80.17, 81.89, 82.60, 101.52, 171.21, 174.76.

4′-*O*-methyl--α-L-rhamnopyranosyl-(1→3)-diethyl *C*-(2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate (249)



249

Compound **249** was obtained as colorless thick oil (89% yield) from the reaction of **248** according to general procedure D above.

$[\alpha]_D$ +11.0 (*c* 0.20, CH_2Cl_2).

^{1}H NMR (D_2O): δ1.27 (d, 3H, J = 6.3 Hz, H-6′), 1.20-1.40 (m, 6H, 2 × CH_3CH$_2$), 2.04 (s, 3H, CH_3C=O), 2.11 (ddd, 1H, J = 19.4, 16.0 and 3.5 Hz, PCH_2), 2.45 (ddd, 1H, J = 16.0, 16.0 and 11.0 Hz, PCH_2), 3.18 (dd, 1H, J = 9.5 and 9.5 Hz, H-4′), 3.54 (s, 3H, OCH_3), 3.64 (dd, 1H, J = 7.9 and 7.9 Hz, H-4), 3.66 (dd, 1H, J = 9.4 and 7.9 Hz, H-3), 3.71 (ddd, 1H, J = 7.9, 4.0 and 2.5 Hz, H-5), 3.77 (dd, 1H, J = 11.9 and 2.5 Hz, H-6), 3.81 (dd, 1H, J = 9.5 and 3.0 Hz, H-3′), 3.84 (dd, 1H, J = 3.0 and 1.5 Hz, H-2′), 3.88 (dd, 1H, J = 11.9 and 4.0 Hz, H-6), 3.92 (dq, 1H, J = 9.5 and 6.3 Hz, H-5′), 4.10-4.20 (m, 4H, CH$_3$CH_2), 4.33-4.43 (m, 1H, H-1), 4.89 (d, 1H, J = 1.5 Hz, H-1′).

^{13}C NMR (D_2O): δ15.95 (*C*H$_3$CH$_2$), 16.03 (*C*H$_3$CH$_2$), 17.14 (C-6′), 22.33 (*C*H$_3$C=O), 23.51 (d, J = 142.6 Hz, P*C*H$_2$), 52.25 (d, J = 13.5 Hz, C-2), 60.32 (C-6), 60.43 (O*C*H$_3$), 63.90 (d, J = 6.7 Hz, CH$_3$*C*H$_2$), 64.05 (d, J = 6.7 Hz, CH$_3$*C*H$_2$), 68.22 (C-4), 68.47 (C-5′), 68.60 (d, J = 5.2 Hz, C-1), 70.32 (C-3′), 71.15 (C-2′), 74.87 (C-3), 77.97 (C-5), 82.64 (C-4′), 100.75 (C-1′), 174.55 (CH$_3$*C*=O).

^{31}P NMR (D_2O): δ 32.33.

Anal. Calcd for $C_{20}H_{38}NO_{12}$: C, 46.60; H, 7.43; N, 2.72; Found: C, 46.26; H, 7.52; N, 2.80.

References

List of References

1. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Tuberculosis*; Shinnick, T. M., Editor; Springer: Berlin, 1996; pp 1-27.

2. Daffe, M.; Draper, P. *Adv. Microbial Physi.* **1998**, *39*, 131-203

3. Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734-6743.

4. Chatterjee, D.; Bozic, C. M.; McNeil, M.; Brennan, P. J. *J. Biol. Chem.* **1991**, *266*, 9652-9660.

5. Rastogi, N.; Goh, K. S.; David, H. L. *Antimicrob. Agents Chemother* **1990**, *34*, 759-764.

6. Takayama, K.; Armstrong, E. L.; Kunugi, K. A.; Kilbum, J. O. *Antimicrob. Agents Chemother* **1979**, *16*, 240-242.

7. Maddry, J. A.; Suling, W. J.; Reynolds, R. C. *Res. Microbiol.* **1996**, *147*, 106-112.

8. Heym, B.; Philip, W.; Cole, S. T. *Tuberculosis* **1996**, 49-69.

9. Ritter, T. K.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 3508-3533.

10. Zhang, Y.; Heym, B.; Allen, B.; Young, D.; Coles, S. *Nature* **1992**, *358*, 591-593.

11. Pathak, A. K.; Pathak, V.; Maddry, J.; Suling, W. J.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *9*, 3145-3151.

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

13. Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *9*, 3129-3143.

14. Leysen, D. C.; Haemers, A.; Blanchaert, L. V. A. I.; Bollaert, W.; Schoenmaekers, K.; Pattyn, S. R. *Pharm. Ed. Sci.* **1987**, *42*, 823-831.

15. Brennan, P. J.; Besra, G. S. *Biochem. Soc. Trans.* **1997**, *25*(1), 188-194.

16. McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1990**, *265*, 18200-18206.

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

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

19. Zechel, D.; Withers, S. G. *Comprehensive Natural Product Chemistry*; Poulter, C. D., Editor; Elservier Science B. V.: Amsterdam, 1999; pp 279-314.

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

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

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

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

24. Kishi, Y. *Pure Appl. Chem* **1993**, *65*, 771-778.

25. Casero, F.; Cipolla, L.; Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *J. Org. Chem.* **1996**, *61*, 3428-3432.

26. Schafer, A.; Thiem, J. *J. Org. Chem.* **2000**, *65*, 24-29.

27. Gaurat, O.; Xie, J.; Valery, J.-M. *Tetrahedron Lett.* **2000**, *41*, 1187-1189.

28. Norris, A. J.; Toyokuni, T. *J. Carbohydr. Chem.* **1999** , *18*(9), 1097-1105.

29. Cipolla, L.; Ferla, B. L.; Panza, L.; Nicotra, F. *J. Carbohydr. Chem.* **1998**, *17*(7), 1003-1013.

30. Bouix, C.; Bisseret, P.; Eustache, J. *Tetrahedron Lett.* **1998**, *39*, 825-828.

31. Schramm, V. L. *Annu. Rev. Biochem.* **1998**, *67*, 693-720.

32. Juaristi, E.; Cuevas, G. *The Anomeric Effect*; CRC Press, Inc.: London, 1995; pp 8-9.

33. Luengo, J. I.; Gleason, J. G. *Tetrahedron Lett.* **1992** , *33*, 6911-6914.

34. Broxterman, H. J. G.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1988**, *29*, 4893-4896.

35. Grugier, J.; Xie, J.; Duarte, I.; Valery, J.-M. *J. Org. Chem.* **2000**, *65*, 979-984.

36. Lehle, L.; Tanner, W. *FEBS Lett.* **1976**, *71*, 167-170.

37. Schmidt, R. R.; Frische, K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1747-1750.

38. Muller, B.; Schaub, C.; Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2893-2897.

39. Schaub, C.; Muller, B.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 1745-1758.

40. Timmers, C. M.; Dekker, M.; Buijsman, R. C.; van der Marel, G. A.; Ethell, B.;

Anderson, G.; Burchell, B.; Mulder, G. J.; Boom, J. H. *Bioorg. Med. Chem.* **1997**, *7*, 1501-1506.

41. Noort, D.; van Straten, G. J. P. H.; van der Marel, G. A.; Bossuyt, X.; Blanckaet, N.; Mulder, G. J.; van Boom, J. H. *Bioorg. Med. Chem.* **1992**, *6*, 583-588.

42. Heskamp, B. M.; Veeneman, G. H.; van der Marel, G. A.; van Boeckel, C. A. A.; van Boom, J. H. *Tetrahedron* **1995**, *51*, 8397-8406.

43. Pathak, A. K.; Pathak, V.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **2001**, *42*, 979-982.

44. McGurk, P.; Chang, G. X.; Lowary, T. L.; McNeil, M.; Field, R. A. *Tetrahedron Lett.* **2001**, *42*, 2231-2234.

45. Pathak, A. K.; Pathak, V.; Suling, W. J.; Gurcha, S. S.; Morehouse, C. B.; Besra, G. S.; Maddry, J. A.; Reynolds, R. C. *Bioorg. Med. Chem.* **2002**, *10*, 923-928.

46. Besra, G. S. ; Moorehouse, C. B.; Rittner, C. M.; Waechter, C. J.; Brennan, P. J. *J. Biol. Chem.* **1997**, *272*, 18460-18466.

47. Brown, J. R. ; Field, R. A.; Barker, A.; Guy, M.; Grewal, R.; Khoo, K.-H.; Brennan, P. J.; Besra, G. S.; Chatterjee, D. *Bioorg. Med. Chem.* **2001**, *9*, 815-824.

48. Pathak, R.; Shaw, A. K.; Bhaduri, A. P.; Chandrasekhar, K. V. G.; Srivastava, A.; Srivastava, K. K.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. S.; Arora, S.; Sinha, S. *Bioorg. Med. Chem.* **2001**, *10*, 1695-1702.

49. Pathak, R.; Pant, C. S.; Shaw, A. K.; Bhaduri, A. P.; Gaikwad, A. N.; Sinha, S.; Srivastava, A.; Srivastava, K. K.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. *Bioorg. Med. Chem.* **2002**, *10*, 3187-3196.

50. Du, Y.; Linhardt, R. *Tetrahedron* **1998**, *54*, 9913-9959.

51. Wong, M. F.; Weiss, K. L.; Curley, R. W. *J. Carbohydr. Chem.* **1996**, *15*, 763-768.

52. Leteux, C.; Veyrieres, A. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2647-2655.

53. Gervay, J.; Hadd, M. J. *J. Org. Chem.* **1997**, *62*, 6961-6967.

54. Allevi, P.; Anastasia, M.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Chem. Soc., Chem. Commun.* **1987**, 101-102.

55. Bombard, S.; Maillet, M.; Capman, M.-L. *Carbohydr. Res.* **1995**, *275*, 433-440.

56. Marron, T. G.; Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9037-9040.

57. Uchiyama, T.; Vassilev, V. P.; Kajimoto, T.; Wong, W.; Huang, H.; Lin, C.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5395-5396.

58. Uchiyama, T.; Woltering, T. J.; Wong, W.; Lin, C.-C.; Kajimoto, T.; Takebayashi, M.; Weitz-Schmidt, G.; Asakura, T.; Noda, M.; Wong, C.-H. *Bioorg. Med. Chem.* **1996**, *4*, 1149-1165.

59. Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9033-9036.

60. Minehan, T. G.; Kishi, Y. *Tetrahedron Lett.* **1997**, *38*, 6815-6818.

61. Streicher, H.; Geyer, A.; Schmidt, R. R. *Chem. Eur. J.* **1996**, 2, 502-510.

62. Hosomi, A.; Sakata, Y.; Sakurai, H. *Carbohydr. Res.* **1987**, *171*, 223-232.

63. Allevi, P.; Anastasia, M.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Chem. Soc., Chem. Commun.* **1987**, 1245.

64. Bennek, J. A.; Grey, G. R. *J. Org. Chem.* **1987**, *52*, 892-897.

65. Hosomi, A.; Sakata, Y.; Sakurai, H. *Tetrahedron Lett.* **1984**, *25*, 2383-2386.

66. Keck, G. E.; Enholm, E. J.; Kachensky, D. F. *Tetrahedron Lett.* **1984**, *25*, 1867-1870.

67. Williams, R. M.; Stewart, A. O. *Tetrahedron Lett.* **1983** , *27*, 2715-2716.

68. Stewart, A. O.; Williams, R. M. *J. Am. Chem. Soc.* **1985**, *107*, 4289-4296.

69. Hildbrand, S.; Laser, A.; Parel, S. P.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 5499-5511.

70. Gudmundsson, K. S.; Drach, J. C.; Townsend, L. B. *J. Org. Chem.* **1997**, *62*, 3453-3459.

71. Streicher, H.; Reiner, M.; Schmidt, R. R. *J. Carbohydr. Chem.* **1997**, *16*, 277-298.

72. Csuk, R.; Schaade, M.; Krieger, C. *Tetrahedron* **1996**, *52*, 6397-6408.

73. Toshima, K.; Miyamoto, N.; Matsuo, G.; Nakata, M.; Matsumura, S. *J. Chem. Soc., Chem. Commun.* **1996**, 1379-1380.

74. Seeberger, P. H.; Bilodeau, M. T.; Danishefsky, S. J. *Aldrichimica Acta* **1997**, *30*, 75-92.

75. Du, Y.; Kong, F. *J. Carbohydr. Chem.* **1995**, *14*, 341-352.

76. Bellosta, V. ; Czernecki, S. *J. Chem. Soc., Chem. Commun.* **1989**, 199-200.

77. Leeuwenburgh, M. A.; Timmers, C. M.; van der Marel, G. A.; van Boom, J. H.; Mallet, J.-M.; Sinay, P. G. *Tetrahedron Lett.* **1997**, *38*, 6251-6254.

78. Hoffmann, M.; Kessler, H. *Tetrahedron Lett.* **1994**, *35*, 6067-6070.

79. Eisele, T.; Ishida, H.; Hummel, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1995**, 2113-2121.

80. Schmidt, R. R.; Preuss, R.; Betz, R. *Tetrahedron Lett.* **1987**, *28*, 6591-6594.

81. Bearder, J. R.; Dewis, M. L.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 227-233.

82. Frey, O.; Hoffmann, M.; Wittmann, V.; Kessler, H. *Helv. Chim. Acta* **1994**, *77*, 2060-2065.

83. Frey, O.; Hoffmann, M.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2026-2028.

84. von Roedern, E. G.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156-10167.

85. Wittman, V.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1091-1093.

86. Smith, M. B.; March J. *Advanced Organic Chemistry: Reactions, Mechanisms and Structure (5th Ed.)*; John Wiley & Sons, Inc.: 2001, pp 329-331.

87. Hoffmann, M. ; Burkhart, F.; Hessler, G.; Kessler, H. *Helv. Chim. Acta* **1996**, *79*, 1519-1532.

88. Burkhart, F.; Hoffmann, M.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1191-1192.

89. Harenbrock, M.; Matzeit, A.; Schafer, H. J. *Liebigs Ann. Chem.* **1996**, 55-62.

90. Ponten, F.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 7463-7466.

91. Roe, B. A.; Boojamra, C. G.; Griggs, J. L.; Bertozzi, C. R. *J. Org. Chem.***1996**, *61*, 6442-6445.

92. Molander, G. A.; Harris, C. R. *Chem. Rev.* **1996**, *96*, 307-338.

93. Mazeas, D.; Skrydstrup, T.; Doumeix, O.; Brau, J.-M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1383-1386.

94. de Pouilly, P.; Chenede, A.; Mallet, J.-M.; Sinay, P. *Bull Soc. Chim. Fr.* **1993**, *130*, 256-265.

95. Mazeas, D.; Skrydstrup, T.; Beau, J.-M. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*,

909-912.

96. Hung, S.-C.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2671-2674.

97. Dawe, R. D.; Fraser-Reid, B. *J. Org. Chem.* **1984**, *49*, 522-528.

98. Hsieh, H.-P. ; McLaughlin, L. W. *J. Org. Chem.* **1995**, *60*, 5356-5359.

99. Xie, J. *Recent Research Development in Organic Chemistry* **1999**, *3(pt. 2)*, 505-523.

100. Werner, R. M.; Williams, L. M.; Davis, J. T. *Tetrahedron Lett.* **1998**, *39*, 9135-9138.

101. Xie, J.; Molina, A.; Czernecki, S. *J. Carbohydr. Chem.* **1999**, *18*, 481-498.

102. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 51-53.

103. Cui, J.; Horton, D. *Carbohydr. Res.* **1998**, *309*, 319-330.

104. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 76.

105. Cipolla, L.; Lay, L.; Nicotra, F. *J. Org. Chem.* **1997**, *62*, 6678-6681.

106. Cipolla, L.; Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1993-1995.

107. Jimenez-Barbero, J.; Espinosa, J. F.; Asensio, J. L.; Canada, F. J.; Poveda, A. *Adv. Carbohydr. Chem. Biochem.* **2001**, *56*, 235-284.

108. Lemieux, R. U.; Pavia, A. A.; Martin, J. C. *Can. J. Chem.* **1987**, *65*, 213-223.

109. Smith, A. B.; Rivero, R. A.; Hale, K. J.; Vaccaro, H. A. *J. Am. Chem. Soc.* **1991**, *113*, 2092-2112.

110. Lemieux, R. U.; Pavia, A. A.; Martin, J. C.; Watanabe, K. A. *Can. J. Chem.* **1969**, *47*, 4427-4439.

111. Goekjian, P. G.; Wu, T.-C. K. Y. *Eur. J. Org. Chem.* **1991**, *56*, 6412-6422.

112. Wu, T.-C.; Goekjian, P. G.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4819-4823.

113. Smith, M. B.; March J. *Advanced Organic Chemistry: Reactions, Mechanisms and Structure (5th Ed.)*; John Wiley & Sons, Inc.: 2001; pp 474.

114. Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155-224.

115. All the pKa values are obtained by comparison with the pKa values of related compounds from the Evans pKa table (http://daecr1.harvard.edu/pdf/evans_pKa_table.pdf).

116. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 103-154.

117. Schmidt, R. R. *Pure Appl. Chem* **1989**, *61*, 1257-1270.

118. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503-1531.

119. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 19-24.

120. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 41-46.

121. Boons, G.-J.; Hale, K. J. *Organic Synthesis With Carbohydrates*; Sheffield Academic Press Ltd: England, 2000; pp 82-84.

122. Hanessian, S.; Lou, B. *Chem. Rev.* **2000**, *100*, 4443-4463.

123. Jung, K.-H. ; Muller, M.; Schmidt, R. R. *Chem. Rev.* **2000**, *100*, 4423-4442.

124. Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465-4493.

125. Collins, P.; Ferrier, R. *Monosaccharides*; John Wiley & Sons: 1996; pp 60-94.

126. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry PartA: Structure and Mechanism (3rd Ed.)*; Plenum Press: New York and London, 1990; pp 664-668.

127. Koenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957-981.

128. Bommer, R.; Kinzy, R.; Schmidt, R. R. *Liebigs Ann. Chem.* **1991**, 425-433.

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

130. Garegg, P. J.; Helland, A. C. *J. Carbohydr. Chem.* **1993**, *12*, 105-117.

131. Eliel, E. L.; Nader, F. W. *J. Am. Chem. Soc.* **1970**, *92*, 584-590.

132. Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: Berlin, 1983; pp 78-136.

133. Kirby, A. J. *Acc. Chem. Res.* **1984**, *17*, 305-311.

134. Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167-1195.

135. Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992**, *114*, 1087-1088.

136. Barresi, F. ; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376-9377.

137. Bedault, G. M.; Dutton, G. G. S. *Carbohydr. Res.* **1974**, *37*, 309-319.

138. Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506-4507.

139. Lemieux, R. U.; Hayimi, J. L. *Can. J. Chem.* **1965**, *43*, 2162-2173.

140. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.

141. Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244-1251.

142. Dondoni, A.; Marra, A.; Pasti, C. *Tetrahedron: Asymmetry* **2000**, *11*, 305-317.

143. Konrad, S.; Thomas, H.; Athanassiors, G. EP0385287, September 5, 1990.

144. Macher, I. *Carbohydr. Res.* **1987**, *162*, 79-84.

145. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry PartA: Structure and Mechanism (3rd Ed.)*; Plenum Press: New York and London, 1990; pp 397-407.

146. Giannis, A.; Munster, P.; Sandhoff, K.; Steglich, W. *Tetrahedron* **1988**, *44*, 7177-7180.

147. Costantino, V.; Imperatore, C.; Fattorusso, E.; Mangoni, A. *Tetrahedron Lett.* **2001**, *42*, 8185-8187.

148. Erdik, E. *Organozinc Reagents in Organic Synthesis*; CRC Press, Inc.: London, 1996; pp 18-69.

149. Zhang, X.; Qiu, W.; Burton, D. J. *Tetrahedron Lett.* **1999**, *40*, 2681-2684.

150. Boersma, J. *Comprehensive Organometallic Chemistry*; Wilkinson, G.; Stone, F. G. A.; Abel, E. W., Editor; Pergamon: New York, 1982; Vol. 2, pp 823-862.

151. Maryanoff, B.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863-927.

152. Boschetti, A.; Nicotra, F.; Panza, L.; Russo, G. *J. Org. Chem.* **1988**, *53*, 4181-4185.

153. Giannis, A.; Sandhoff, K. *Carbohydr. Res.* **1987**, *171*, 201-210.

154. Petrusova, M.; BeMiller, J. N.; Petrus, L. *Tetrahedron Lett.* **1996**, *37*, 2341-2344.

155. Taskinen, E. *Tetrahedron* **1993**, *49*, 11389-11394.

156. Taskinen, E. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1824-1834.

157. Kessler, H.; Hoffmann, M. *Tetrahedron Lett.* **1994**, *35*, 6067-6070.

158. Burkhart, F.; Kessler, H. *Tetrahedron Lett.* **1998**, *39*, 255-256.

159. Lehn, J.-M. ; Wipff, G. *J. Am. Chem. Soc.* **1976**, *98*, 7498-7505.

160. Bernardi, F.; Csizmadia, I. G.; Mangini, A.; Schlegel, H. B.; Whangbo, M.-H.; Wolfe, S. *J. Am. Chem. Soc.* **1975**, *97*, 2209-2218.

161. Plesnicar, B. *Oxidation in Organic Chemistry (Part C)*; Trahanovsky, Editor; Academic Press, New York, 1978; pp 211-253.

162. Cohen, T.; Bhupathy, M. *Acc. Chem. Res.* **1989**, *22*, 152-161.

163. Cohen, T.; Lin, M.-T. *J. Am. Chem. Soc.* **1984**, *106*, 1130-1131.

164. Wittman, V.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1091-1093.

165. Petrusova, M.; BeMiller, J. N.; Krihova, A.; Petrus, L. *Carbohydr. Res.* **1996**, *295*, 57-67.

166. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574-1585.

167. Pravdie, N.; Fletcher, H. G. *Carbohydr. Res.* **1971**, *19*, 353-364.

168. Ayadi, E.; Czernecki, S.; Xie, J. *J. Carbohydr. Chem.* **1996**, *15*, 191-199.

169. Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1967**, *89*, 2416.

170. Nishiyama, Y.; Tujuno, T.; Yamano, T.; Hayashishita, M.; Itoh, K. *Chem. Lett.* **1997**, 165-167.

171. Dondoni, A.; Mariotti, G.; Marra, A.; Massi, A. *Synthesis* **2001**, 2129-2137.

172. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry Part B: Reactions and Synthesis (3rd Ed.)*; Plenum Press: New York, 1990; pp 219-282.

173. Emde, H.; Domsch, D.; Feger, H.; Frick, U.; Gotz, A.; Hergott, H. H.; Hofmann, K.; Kober, W.; Krageloh, K.; Oesterle, T.; Steppan, W.; West, W.; Chen, G. S. *Synthesis* **1982**, 1-26.

174. Giese, B.; Bartmann, D. *Tetrahedron Lett.* **1985**, *26*, 1197-1200.

175. Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11-15.

176. Silverstein, R. M.; Webster, F. X. *Spectrometric Identification of Organic*

Compounds (6th Ed.); John Wiley and Sons, Inc.: New York, 1998; pp 185-187.

177. Smith, M. B. *Organic Synthesis (2nd Ed.)*; McGraw-Hill: New York, 2002; pp 369-393.

178. Motherwell, W. B.; Tozer, M. J.; Ross, B. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1437-1439.

179. Pasetto, P.; Chen, X.; Drain, C. M.; Franck, R. W. *J. Chem. Soc., Chem. Commun.* **2001**, 81-82.

180. Streicher, H.; Reiner, M.; Schmidt, R. R. *J. Carbohydr. Chem.* **1997**, *16*, 277-298.

181. Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863-927.

182. Boutagy, J.; Thomas, R. *Chem. Rev.* **1974**, *74*, 87-99.

183. Vidal, S.; Vidil, C.; Morere, A.; Garcia, M.; Montero, J.-L. *Eur. J. Org. Chem.* **2000**, 3433-3437.

184. Narkunan, K.; Nagarajan, M. *J. Org. Chem.* **1994**, *59*, 6386-6390.

185. Yamanoi, T.; Akiyama, T.; Ishida, E.; Hiroyuki, A.; Amemiya, M.; Inazu, T. *Chem. Lett.* **1989**, 335.

186. Altenbach, H. J.; Holzapfel, W. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 67-68.

187. Hanessian, S.; Rancourt, G.; Guindon, Y. *Can. J. Chem.* **1978**, *56*, 1843-1846.

188. Li, Z.; Racha, S.; Dan, L.; Ei-Subbagh, H.; Abushanab, E. *J. Org. Chem.* **1993**, *58*, 5779-5783.

189. Alexakis, A.; Vrancken, E.; Mangeney, P.; Chemla, F. *J. Chem. Soc., Chem. Commun.* **2000**, 3352-3353.

190. Lambert, J. B.; Emblidge, R. W.; Zhao, Y. *J. Org. Chem.* **1994**, *59*, 5397-5403.

191. Roe, B. A.; Boojamra, C. G.; Griggs, J. L.; Bertozzi, C. R. *J. Org. Chem.* **1996**, *61*, 6442-6445.

192. Horton, D. *Organic Syntheses* **1966**, *46*, 1-5.

193. Curran, D. P.; Porter, N. A.; Giese, B. *Stereochemistry of Radical Reactions*; VCH Verlagsgesellschaft mbH: Weinheim, 1996; pp 1-22.

194. Giese, B. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 969-980.

195. Giese, B.; Dupuis, J. *Tetrahedron Lett.* **1984**, *25*, 1349-1352.

196. Curran, D. P.; van Elburg, P. A.; Giese, B.; Gilges, S. *Tetrahedron Lett.* **1990**, *31*, 2861-2864.

197. Azuhata, T.; Okamoto, Y. *Synthesis* **1983**, 916-917.

198. Walker, B. J. *Organophosphorus Chemistry*; Perguin Books Ltd: England, 1972; pp 37 and 97.

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

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

201. Lindberg, B.; Erbing, B. *Acta Chem. Scand.* **1976**, *B 30*, 611-612.

202. Hultin, P. G.; Buffie, R. M. *Carbohydr. Res.* **1999**, *322*, 14-25.

203. Haines, A. H. *Adv. Carbohydr. Chem. Biochem.*Academic Press: 1976; pp 11-109.

204. Buck, K. W.; Foster, A. B.; Perry, A. R.; Webber, J. M. *J. Chem. Soc.* **1963**, 4171-4177.

205. Tejima, S.; Fletcher Jr., H. G. *J. Org. Chem.* **1963**, *28*, 2999-3004.

206. Postema, M. H. D. *Tetrahedron* **1992**, *48*(40), 8545-8599.

207. Chatt, J. *Chem. Rev.* **1951**, *48*, 7-43.

208. Bhattacharya, A. K.; Thyagarajan, G. *Chem. Rev.* **1981**, *81*, 415-430.

209. Mukaiyama, T.; Murakami, M. *Synthesis* **1987**, 1043-1054.

210. Normant, J. F.; Alexakis, A.; Ghribi, A.; Mangeney, P. *Tetrahedron* **1989**, *45*, 507-516.

211. Westera, G.; Blomerg, C.; Bickelhaupt, F. *J. Organometallic Chem.* **1974**, *82*, 291-299.

212. Stichler-Bonaparte, J.; Vasella, A. *Helv. Chim. Acta* **2000**, *84*, 2355-2367.

213. Lafont, D.; Boullanger, P.; Cadas, O.; Descotes, G. *Synthesis* **1989**, 191-194.

214. Stick, R. V. *Carbohydrates - The Sweet Molecules of Life*; Academic Press: San Diego, 2001; pp 93-99.

215. Tailler, D.; Jacquinet, J.-C.; Noirot, A.-M.; Beau, J.-M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3163-3164.

216. Borodkin, V. S.; Ferguson M. A. J.; Nikolaev, A. V. *Tetrahedron Lett.* **2001**, *42*,

5305-5308.

217. Herzig, J. *Carbohydr. Res.* **1986**, *153*, 162-167.

218. Oscarson, S. *Carbohydrates in Chemistry and Biology* **2000**, *1*, 91-116.

219. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334.

220. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734-753.

221. Tabeur, C.; Mallet, J.-M.; Bono, F.; Herbert, J.-M.; Petitou, M.; Sinay, P. *Bioorg. Med. Chem.* **1999**, *7*, 2003-2012.

222. Tvaroska, I.; Bleha, T. *Adv. Carbohydr. Chem. Biochem.***1989**, 47, 45-123.

223. Mikusova, K.; Mikus, M.; Besra, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.* **1996**, *271*(13), 7820-7828.

224. Darrow, J. W.; Drueckhammer, D. G. *J. Org. Chem.* **1994**, *59*, 2976-2985.

225. Harvey, T. C.; Siamind, C.; Weiler, L.; Withers, S. G. *J. Org. Chem.* **1997**, *62*, 6722-6725.

226. Hanessian, S.; Rogel, O. *J. Org. Chem.* **2000**, *65*, 2667-2674.

227. Beltrame, P.; Gelli, G.; Loi, A. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1001-1003.

228. Mallory, R. A.; Rovinski, S.; Kohen, F.; Scheer, I. *J. Chem. Soc.* **1967**, *32*, 1417-1422.

229. Coutrot, P.; Savignac, P. *Synthesis* **1978**, 36-38.

230. Coutrot, P.; Youssefi-Tabrizi, M.; Grison, C. *J. Organometal. Chem.* **1986**, *316*, 13-18.

231. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry PartA: Structure and Mechanism (3rd Ed.)*; Plenum Press: New York and London, 1990; pp 359-361.

232. Erdik, E. *Organozinc Reagents in Organic Synthesis*; CRC Press: New York, 1996; pp 108-206.

233. Perrin, D. D.; Armaego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1988.

234. Holmquist, L. *Acta Chem. Scand.* **1970**, *24*, 173-178.

235. Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1977**, *53*, 67-84.

236. Warren, C. D.; Shaban, M. A. E.; Jeanloz, R. W. *Carbohydr. Res.* **1977**, *59*, 427-448.

237. Chen, S.-H. ; Huang S.; Gao, Q.; Golik, J.; Farina, V. *J. Org. Chem.* **1994**, *59*, 1475-1484.

238. Dondoni, A. ; Scherrmann, M.-C. *J. Org. Chem.* **1994**, *59*, 6404-6412.

239. Zurabyan, S. E.; Antonenko, T. S.; Khorlin, Y. *Carbohydrate Polymers* **1970**, *15*, 21-27.

240. Rollin, P.; Sinay, P. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2513-2517.

241. Zurabyan, S. E.; Volosyuk, T. P.; Khorlin, A. J. *Carbohydr. Res.* **1969**, *9*, 215-220.

242. Cipolla, L.; Ferla, B. L.; Lay, L.; Peri, F.; Nicotra, F. *Tetrahedron: Asymmetry* **2000**, *11*, 295-303.

243. Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51-65.

244. Zuurmond, H. M.; Veeneman, G. H.; van der Marel, G. A.; van Boom, J. H. *Carbohydr. Res.* **1993**, *241*, 153-164.

245. Gross, P. H.; Jeanloz, R. W. *J. Org. Chem.* **1967**, *32*, 2759-2763.

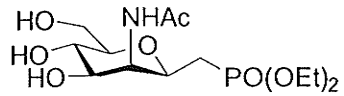
246. Kuhn, R.; Baer, H. H.; Seeliger, A. *Liebigs Ann. Chem.* **1958**, *611*, 236-249.

247. Dasgupta, F.; Garegg, P. J. *Synthesis* **1988**, 626-628.

248. Wen, X.; Crick, D. C.; Brennan, P. J.; Hultin, P. G. *Bioorg. Med. Chem.* **2003**, *11*, 3579-3587.

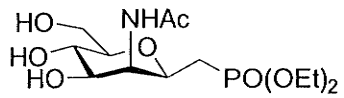
Appendix: ^{1}H, ^{13}C NMR Spectra

SpinWorks 2.0: 1D proton spectrum



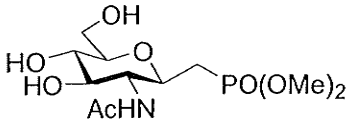
49

SpinWorks 2.0: C13CPD CD3CN u hultin 1



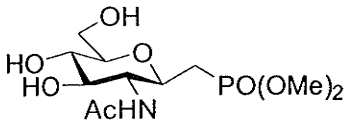
49

SpinWorks 2.0: PROTON D2O u hultin 1

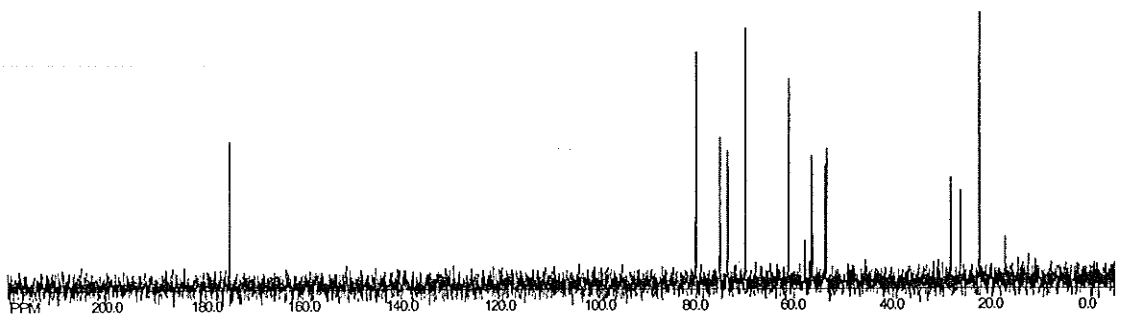


50

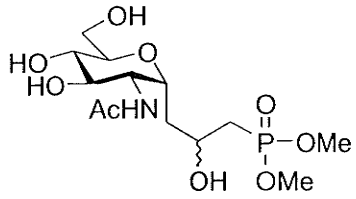
SpinWorks 2.0: C13CPD D2O u hultin 1



50

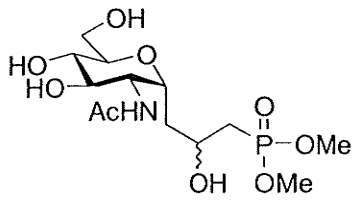


SpinWorks 2.0: PROTON D2O u hutin 2

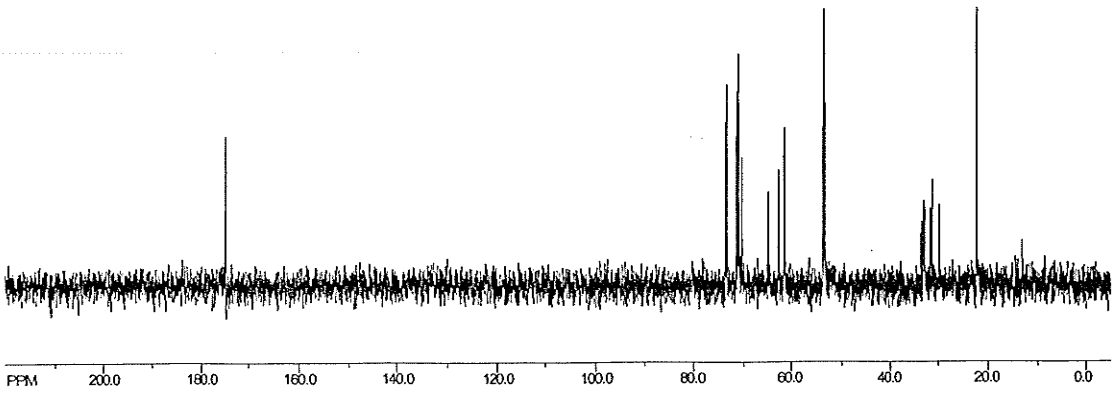


51

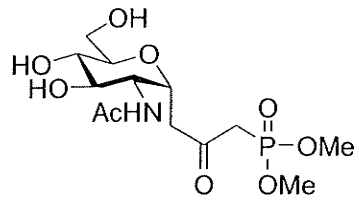
SpinWorks 2.0: C13CPD D2O u hutin 2



51

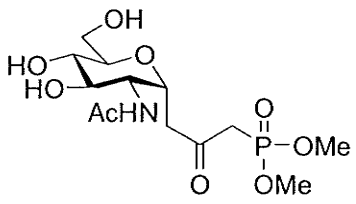


SpinWorks 2.0: PROTON MeOH u hut5n 1



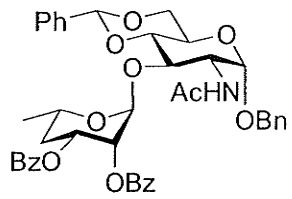
52

SpinWorks 2.0: C13CPD MeOH u hut5n 1

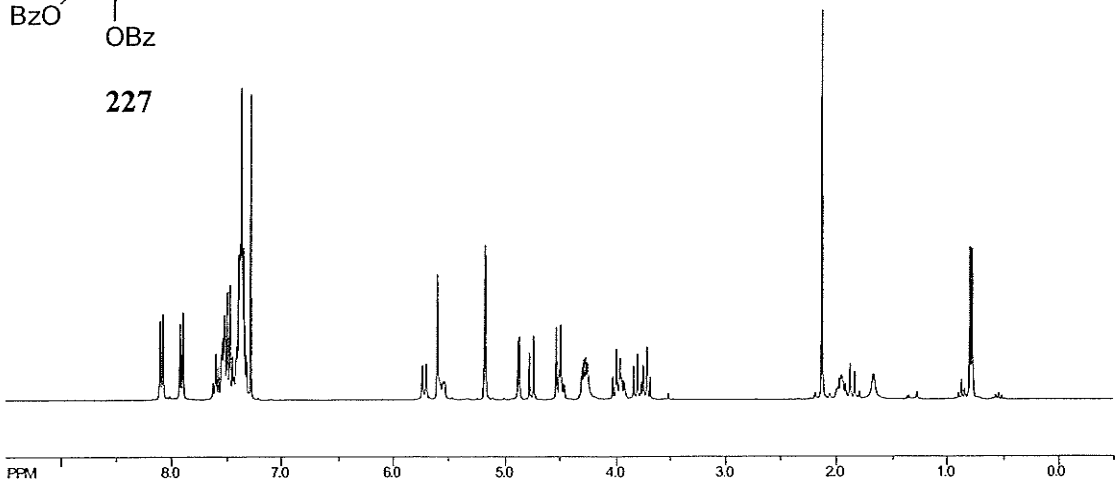


52

SpinWorks 2.0: PROTON CDCl3 u hultin 1



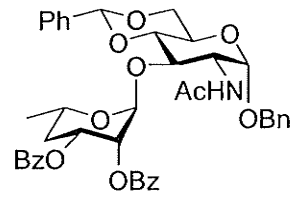
227



file: C:\Documents and Settings\Weilan Zhang\My Documents\Xenghu's Doc\NW NMR\uw-493-5.fid exp: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.567182 ppm = 0.094169 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130000 MHz
processed size: 32768 complex points
LB: 0.300 GB: 0.0000

SpinWorks 2.0: C13CPD CDCl3 u hultin 1

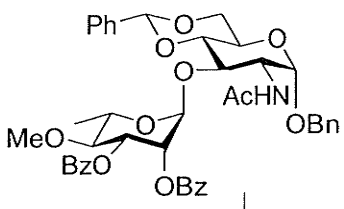


227

file: C:\Documents and Settings\Weilan Zhang\My Documents\Xenghu's Doc\NW NMR\uw-493-5.fid exp: <zgpg30>
transmitter freq.: 75.476310 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514832 ppm = 0.287069 Hz/pt
number of scans: 828

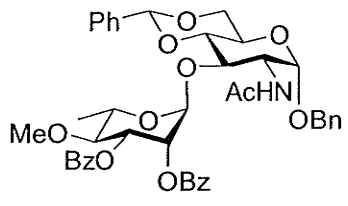
freq. of 0 ppm: 75.457740 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.0: PROTON CDCl3 u hultin 1



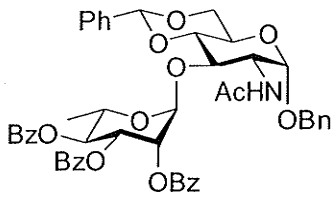
228

SpinWorks 2.0: C13CPD CDCl3 u hultin 1

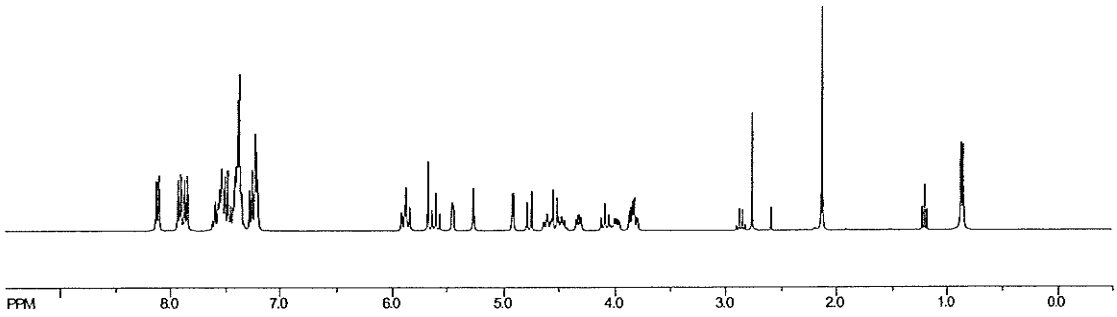


228

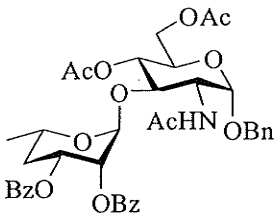
SpinWorks 2.0: proton



229

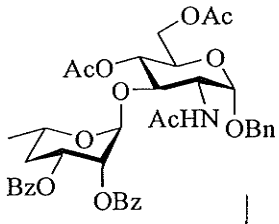


SpinWorks 2.0: PROTON CDCl3 u hutin 1



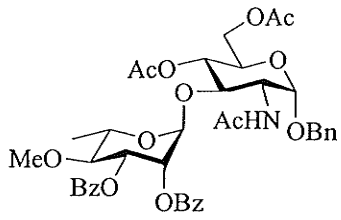
230

SpinWorks 2.0: C13CPD CDCl3 u hutin 1



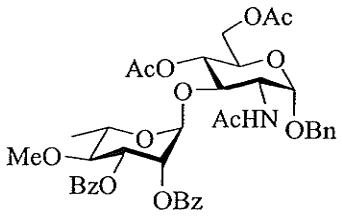
230

SpinWorks 2.0: PROTON CDCl3 u hultin 1



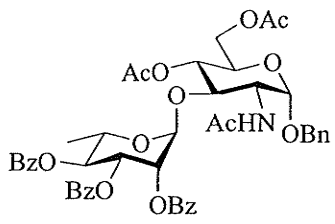
231

SpinWorks 2.0: C13CPD CDCl3 u hultin 1



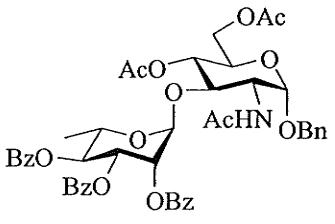
231

SpinWorks 2.0: PROTON CDCl3 u hultin 1



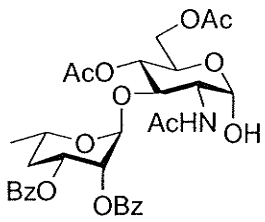
232

SpinWorks 2.0: C13CPD CDCl3 u hultin 1

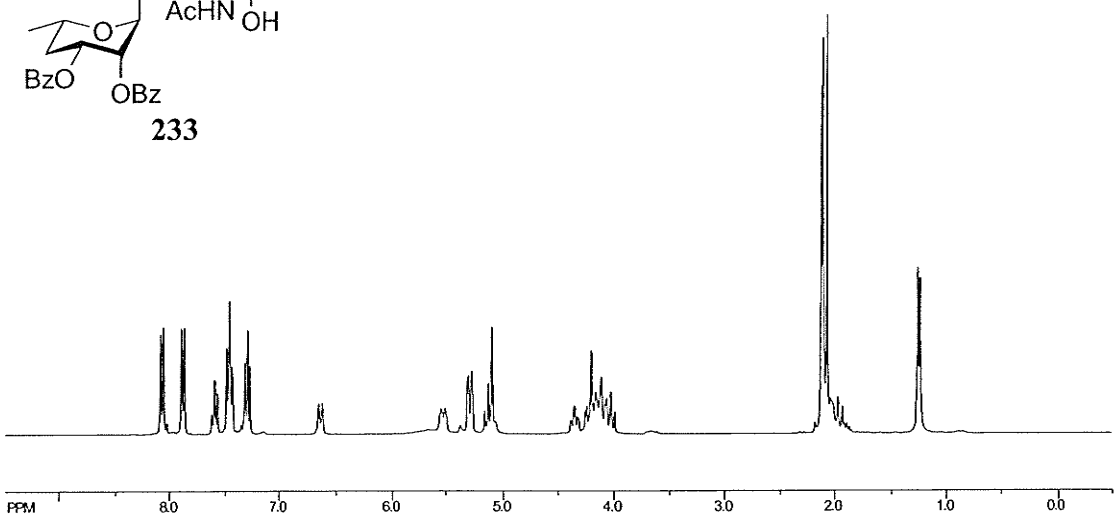


232

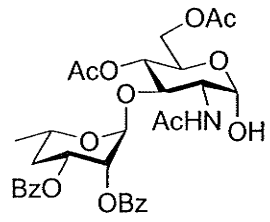
SpinWorks 2.0: PROTON CDCl3 u hultin 2



233

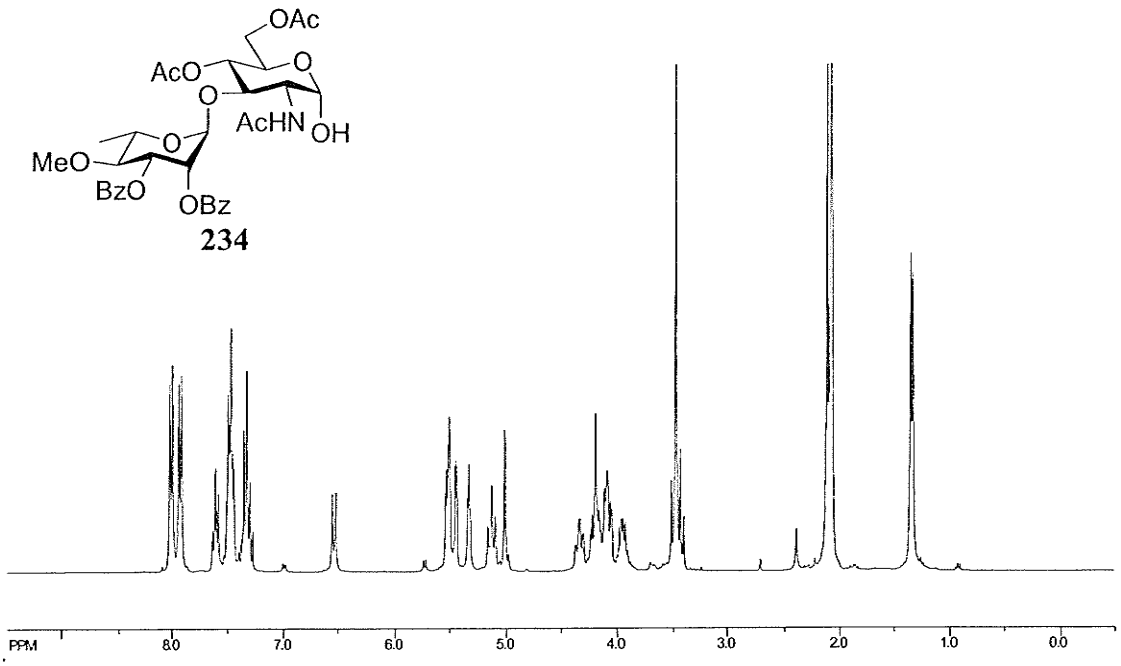


SpinWorks 2.0: C13CPD CDCl3 u hultin 1



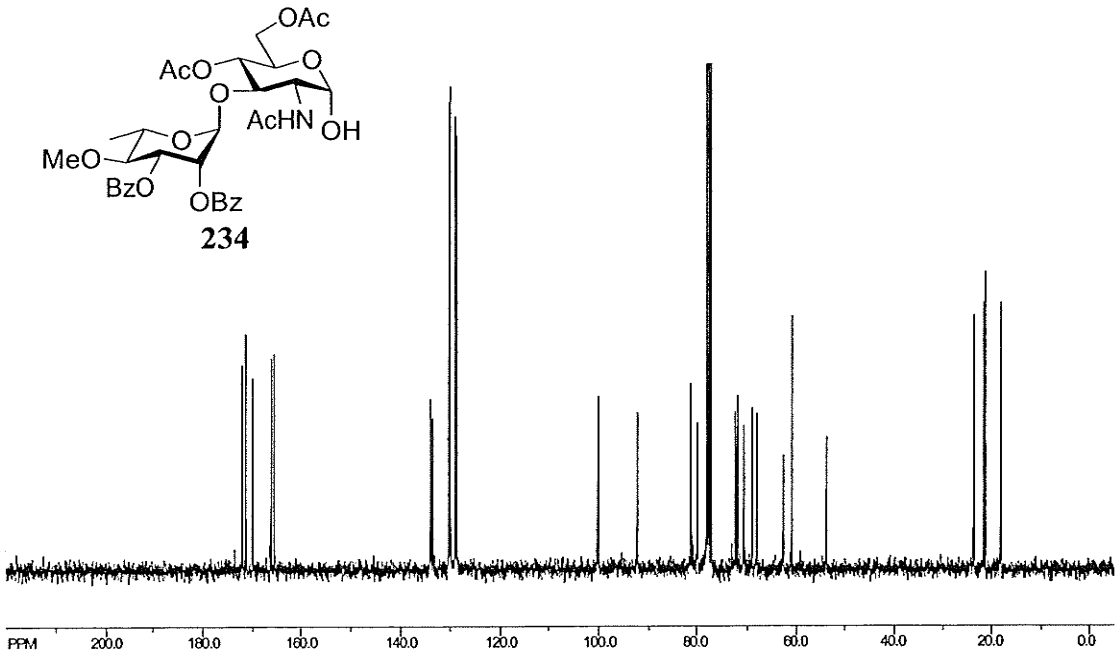
233

SpinWorks 2.0: PROTON CDCl3 u hultin 1

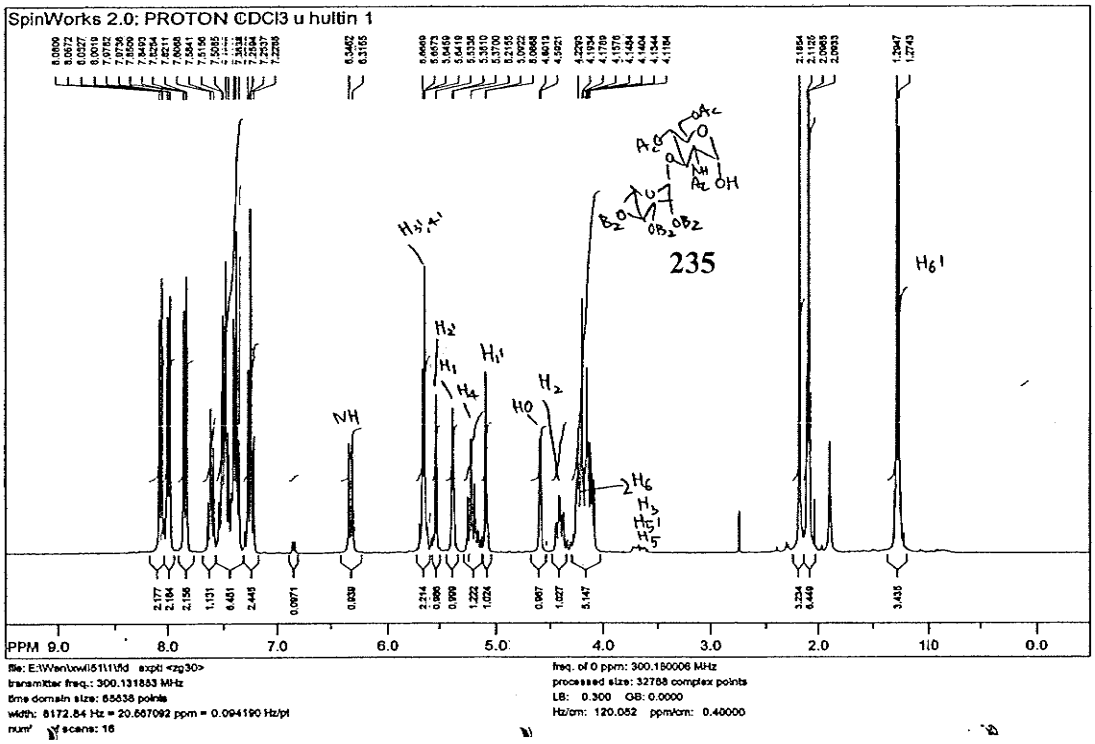


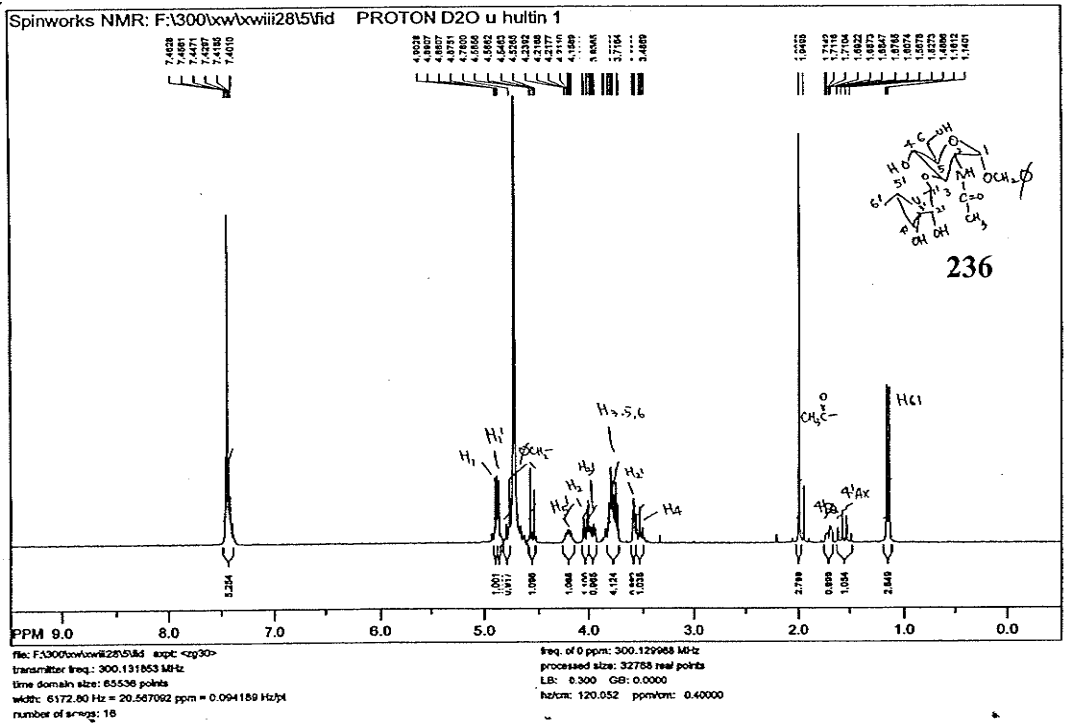
234

SpinWorks 2.0: C13CPD CDCl3 u hultin 1

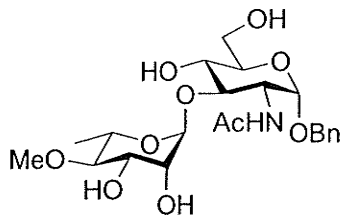


234


SpinWorks 2.0: PROTON CDCl3 u hultin 1
235
NH
H6'
PPM 9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

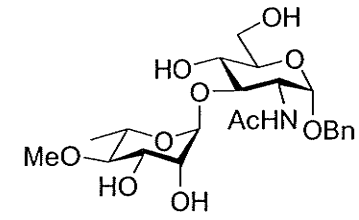

Spinworks NMR: F:\300\xw\xwiii28\5\fid PROTON D2O u hultin 1
236
H6'
PPM 9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

SpinWorks 2.0: PROTON D2O u hultin 1

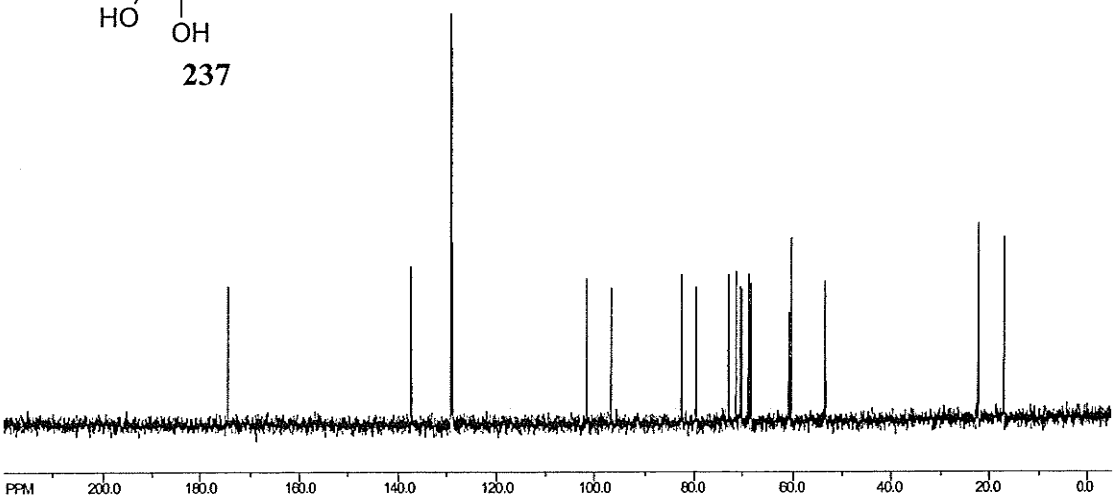


237

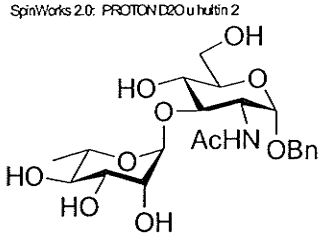
SpinWorks 2.0: C13CPD D2O u hultin 1



237

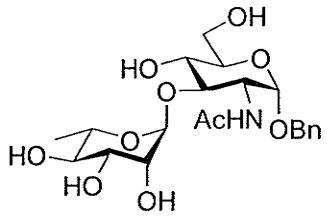


SpinWorks 2.0: PROTON D2O u hultin 2



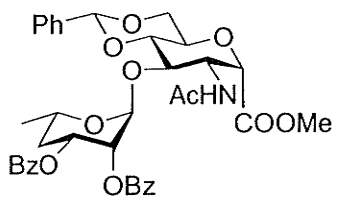
238

SpinWorks 2.0: C13CPD D2O u hultin 2

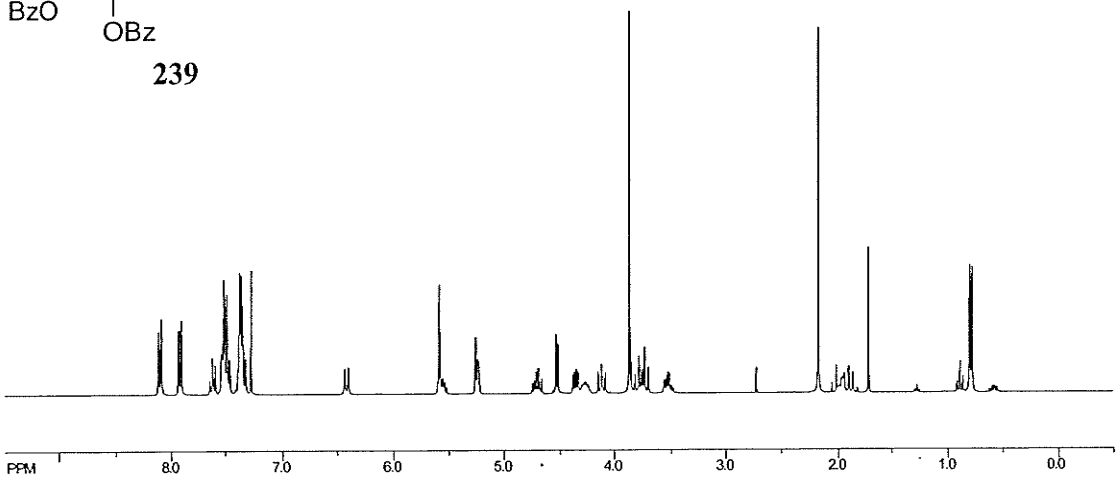


238

SpinWorks 2.0: PROTON CDCl3 u hultin 1



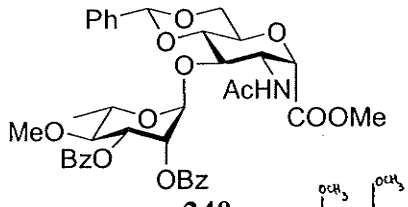
239



file: C:\Documents and Settings\Weilan Zheng\My Documents\Xenghui's Doc\WW NMR\wiii18f1.fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.557092 ppm = 0.094189 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130008 MHz
processed size: 32768 complex points
LB: 0.300 GB: 0.0000

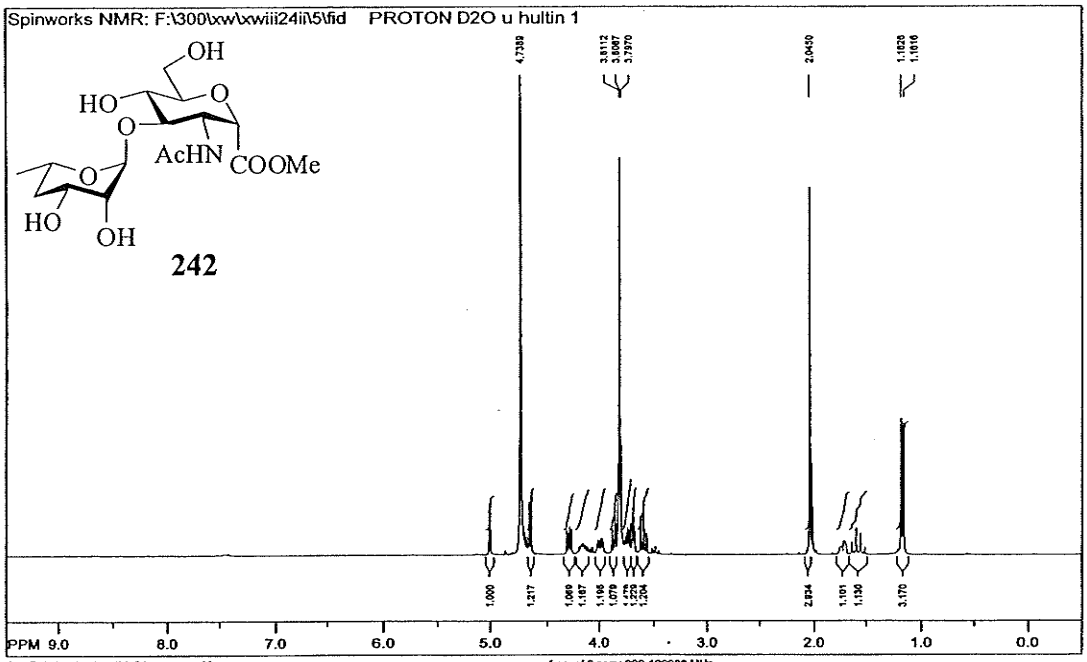
Spinworks NMR: F:\300\xw\xwiii17\1\fid PROTON CDCl3 u hultin 1



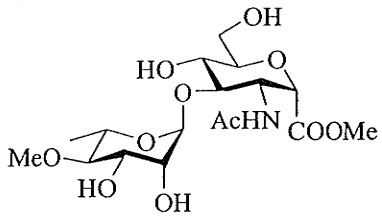
240

file: F:\300\xw\xw\iii17\1\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.557092 ppm = 0.094189 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130008 MHz
processed size: 32768 real points
LB: 0.300 GB: 0.0000
hz/cm: 120.052 ppm/cm: 0.40000


Spinworks NMR: F:\300\xw\xwiii24ii\5\fid PROTON D2O u hultin 1
OH
HO
O
O
AcHN
COOMe
HO
OH
242
4.7389
3.8112
3.8087
3.7970
2.0450
1.1628
1.1616
1.000
1.217
1.069
1.187
1.198
1.078
1.478
1.253
1.284
2.824
1.101
1.150
3.170
PPM 9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
file: F:\300\xw\xwiii24ii\5\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.567092 ppm = 0.094189 Hz/pt
number of scans: 16
freq. of 0 ppm: 300.129968 MHz
processed size: 32768 real points
LB: 0.300 GB: 0.0000
hz/cm: 120.052 ppm/cm: 0.40000

SpinWorks 2.0: PROTON D2O u hultin 2

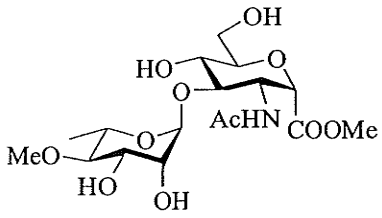


243

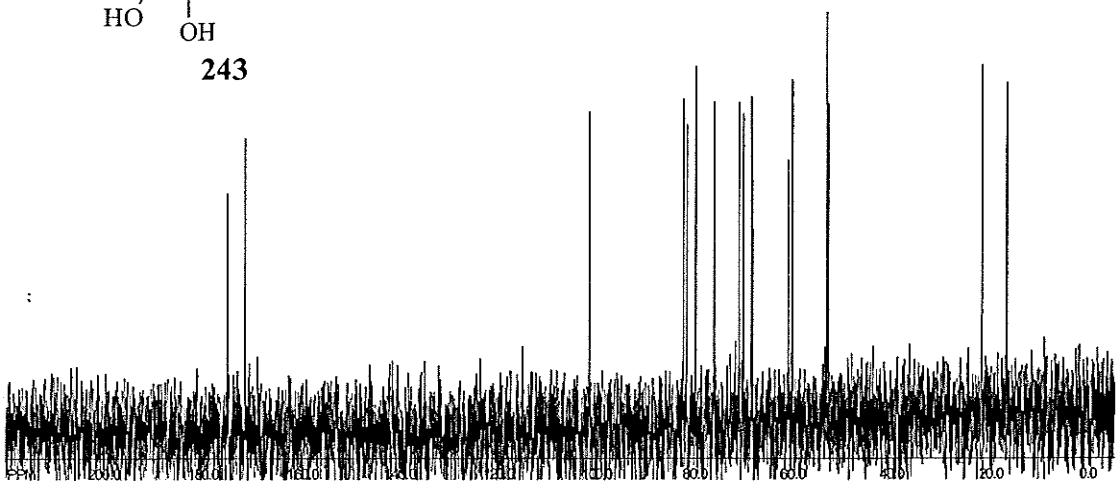
file: C:\Documents and Settings\Weilan Zhang\My Documents\Xenghu's Doc\WW NMR\ww8258.1fid exp: <zg30>
transmitter freq.: 300.131953 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.557302 ppm = 0.094189 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130000 MHz
processed size: 32768 complex points
LB: 0.300 GB: 0.0000

SpinWorks 2.0: C13CPD D2O u hultin 1



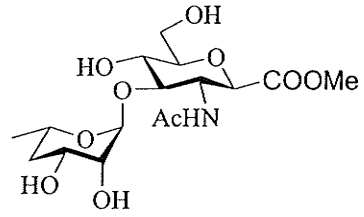
243



file: C:\Documents and Settings\Weilan Zhang\My Documents\Xenghu's Doc\WW NMR\ww8258.5fid exp: <zgpg30>
transmitter freq.: 75.475310 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514800 ppm = 0.287359 Hz/pt
number of scans: 2517

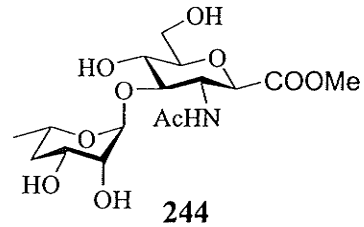
freq. of 0 ppm: 75.457719 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.0: PROTON D2O u hultin 2

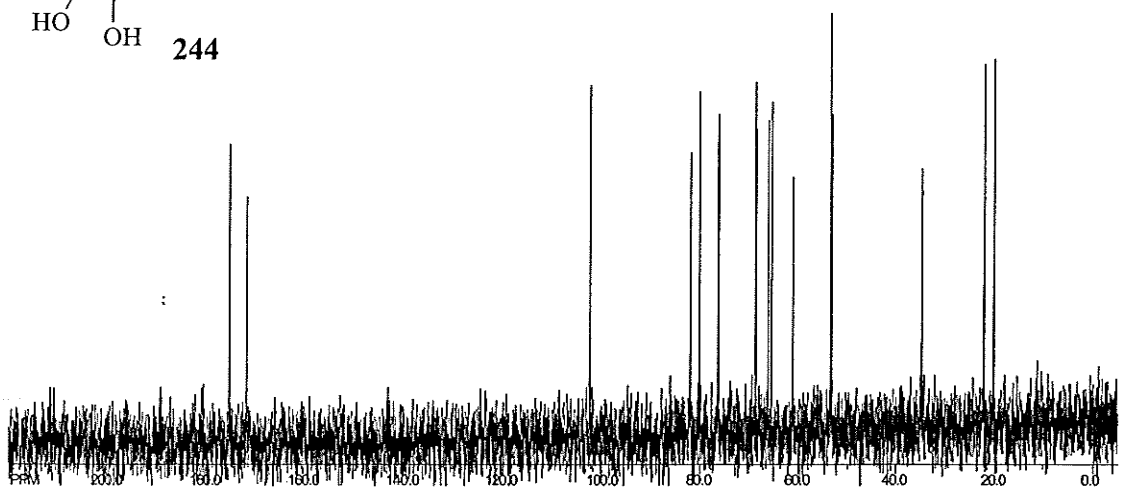


244

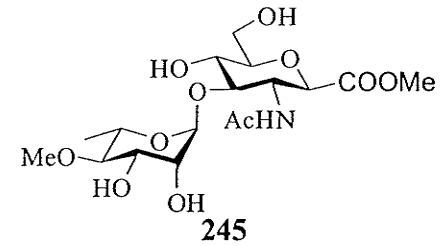
SpinWorks 2.0: C13CPD D2O u hultin 2



244



SpinWorks 2.0: PROTON D2O u hutin 2

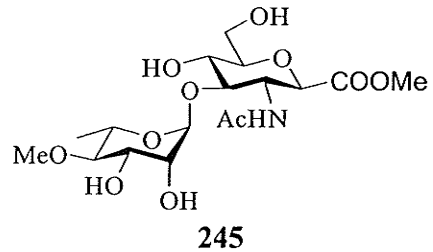


245

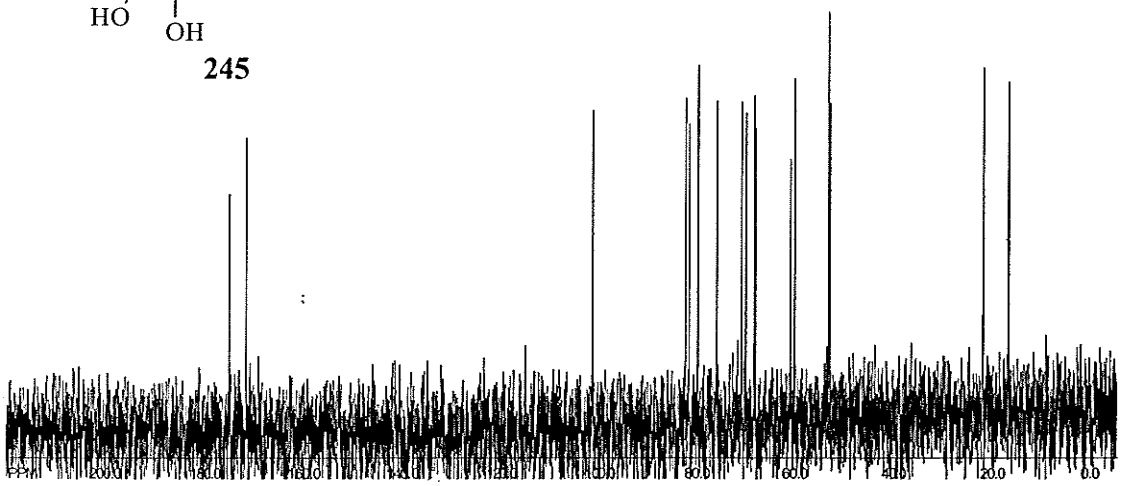
file: C:\Documents and Settings\Weilian Zhang\My Documents\Xenghu's Doc\WW NMR\ww8239.fid expt: <zg30>
transmitter freq.: 300.131253 MHz
time domain size: 65536 points
width: 6172.80 Hz = 20.557032 ppm = 0.094169 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130000 MHz
processed size: 32768 complex points
LB: 0.300 GB: 0.0000

SpinWorks 2.0: C13CPD D2O u hutin 1



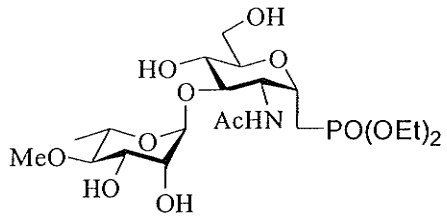
245



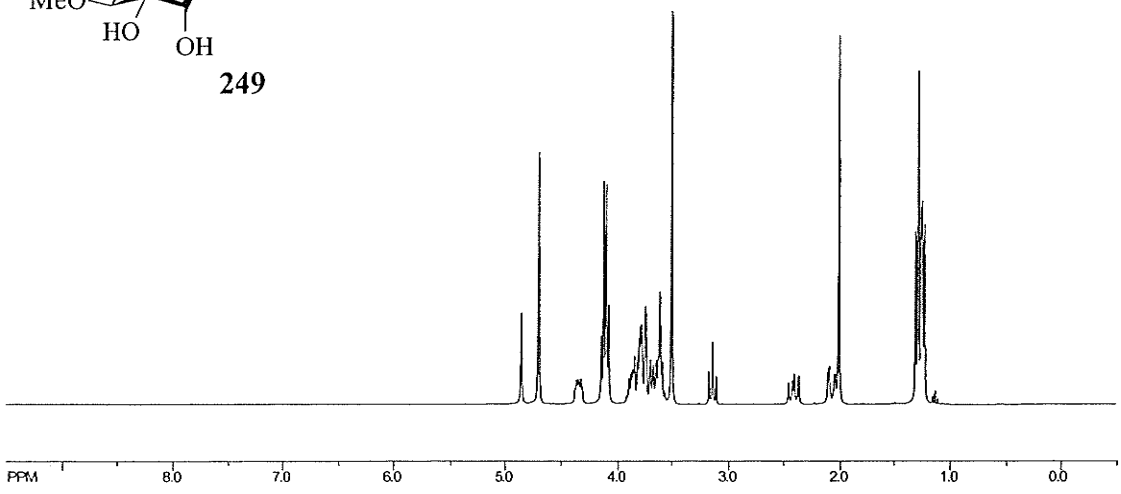
file: C:\Documents and Settings\Weilian Zhang\My Documents\Xenghu's Doc\WW NMR\ww8239.fid expt: <zgpg30>
transmitter freq.: 75.479330 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.51802 ppm = 0.287369 Hz/pt
number of scans: 2617

freq. of 0 ppm: 75.467769 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

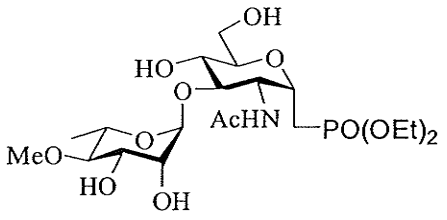
SpinWorks 2.0: PROP31DEC D2O u hultin 1



249



SpinWorks 2.0: C13CPD D2O u hultin 1



249