A NOVEL CLASS OF GLYCOSIDASE INHIBITORS RELATED TO SALACINOL

by

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ABSTRACT

This thesis focuses on the design and synthesis of analogues of salacinol as potential glycosidase inhibitors, together with the investigation of their enzyme inhibitory activities.

Salacinol is a naturally occurring sulfonium ion with an internal sulfate counterion, and is one of the active principles in the aqueous extracts of *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of diabetes. The syntheses of two novel amino acids, nitrogen analogues of salacinol, containing a carboxylate inner salt are described, along with the X-ray crystal structure of one of these analogues in the active site of *Drosophila melanogaster* Golgi mannosidase II (dGMII). The compound derived from 1,4-dideoxy-1,4-imino-D-arabinitol inhibits dGMII, one of the critical enzymes in the glycoprotein processing pathway, with an IC_{50} of 0.3 ± 0.01 mM. Inhibition of GMII has been identified as a target for control of metastatic cancer. The X-ray crystal structure of the complex of this compound with dGMII provides insight into the requirements for an effective inhibitor. The same compound inhibits recombinant human maltase glucoamylase (MGA), one of the key intestinal enzymes involved in the breakdown of glucose oligosaccharides in the small intestine, and a target for the treatment of Type 2 diabetes, with a K_i value of 21 ± 1 μM.

The syntheses of two sulfonium compounds, analogues of salacinol containing a carboxylate inner salt, are also described. The compound derived from 1,4-anhydro-4-

thio-D-arabinitol inhibits recombinant human maltase glucoamylase (MGA) with a K_i value of 10 ± 1 μM.

The binding of the nanomolar inhibitor swainsonine to *Drosophila melanogaster* Golgi α-mannosidase II (dGMII) involves a large contribution of interactions between the six-membered ring of the inhibitor and the hydrophobic pocket within the enzyme active site. Spiro aza- and thia-heterocycles and a spiro-analogue of salacinol were designed with the expectation that the hydrocarbon portions would make hydrophobic contributions to binding. The former sets of compounds were synthesized successfully but the salacinol analogue proved to be elusive. The stereochemistry of the final compounds was determined by means of 1D-NOESY experiments. Unfortunately, the aza- and thia-heterocycles were not effective inhibitors of Golgi α-mannosidase II or human maltase glucoamylase.

Keywords:

Glycosidase inhibitors, salacinol, analogues, carboxylate counterions, epoxide, spiroheterocycle.

Subject terms:

Glycosidase inhibitors, salacinol, synthesis, enzymatic activity.

DEDICATION

This thesis is dedicated to my parents with gratitude for all their support and encouragement.

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LIST OF ABBREVIATIONS

Ac	acetyl
AcOH	acetic acid
aq	aqueous
Ar	aromatic
Arg	arginine
Asp	aspartic acid
B	boat
Bn	benzyl
br	broad
c	concentration
C	chair
Calcd	calculated
Cbz	carbobenzyloxy
d	doublet
DBU	diaza(1,3)bicyclo[5.4.0]undecane
dd	doublet of doublet
ddd	doublet of doublet of doublet
dm	decimetre
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide

dGMII	*Drosophila melanogaster* Golgi α-mannosidase II
DMNJ	deoxymannojirimycin
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
DSS	2,2-dimethyl-2-silapentane-5-sulfonate
E	envelope
eq	equatorial
equiv	equivalent
ER	endoplasmic reticulum
EtOH	ethanol
EtOAc	ethyl acetate
g	gram
Glc	glucose
Glc*N*Ac	*N*-acetylglucosamine
GMII	Golgi α-mannosidase II
h	hour
H	half chair
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HRMS	high resolution mass spectrometry
IC_{50}	concentration of an inhibitor required for 50% inhibition of its target
J	coupling constant in Hz
K_M	Michaelis constant
K_i	inhibition constant
LDA	lithium diisopropylamide

Lit.	literature
m	multiplet
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
Man	mannose
Me	methyl
MeOH	methanol
MES	4-morpholinoethanesulfonic acid
MGA	maltase glucoamylase
mp	melting point
MPD	methylpentane diol
ms	milliseconds
µl	microliter
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
PDB	Protein Data Bank
Ph	phenyl
Phe	phenylalanine
PPDol	dolichol pyrophosphate
psi	$lb/inch^2$
Py	pyridine
RMS	root mean square
RMSD	root mean square deviation
RNA	ribonucleic acid
s	singlet
T	twist
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Trp	tryptophan

TS	transition state
TsCl	tosyl chloride
Tyr	tyrosine

THESIS OVERVIEW

In **Chapter 1**, an introduction to glycosidases, their mechanism of action, and some examples of naturally occurring and synthetic inhibitors of glycosidases is presented.

In **Chapter 2**, the manuscript (Chen, W.; Kuntz, D. A.; Hamlet, T.; Sim, L.; Rose, D. R.; Pinto, B. M. *Bioorg. Med. Chem.* **2006**, *14*, 8332-8340) is presented. It describes the synthesis of two novel amino acids, nitrogen analogues of the naturally occurring glycosidase inhibitor, salacinol, containing a carboxylate inner salt, along with the crystal structure of one of these analogues in the active site of *Drosophila melanogaster* Golgi mannosidase II (dGMII).

In **Chapter 3**, the manuscript (Chen, W.; Sim, L.; Rose, D. R.; Pinto, B. M. *Carbohydr. Res.* **2007**, *342*, 1661-1667) is presented. It describes the synthesis of analogues of salacinol containing a carboxylate inner salt as potential glycosidase inhibitors. It was thought that sulfonium salts carrying a permanent positive charge would mimic the oxacarbenium-like transition state for the enzyme-catalyzed hydrolysis reaction.

In **Chapter 4**, the manuscript (Chen, W.; Pinto, B. M. *Carbohydr. Res.* **2007**, *342*, 2163-2172) is presented. It describes the synthesis of aza- and thia-spiroheterocycles and the attempted synthesis of spiro-sulfonium compounds related to salacinol.

In **Chapter 5**, the general conclusions resulting from the work in this thesis are presented.

CHAPTER 1: INTRODUCTION

1.1 General Introduction to Carbohydrates

Carbohydrates are the most abundant family of natural products in nature, in fact around two-thirds of the carbon in the biosphere exists as carbohydrates.[1,2] They are a major source of metabolic energy, for example starch in plants and glycogen in animals. The significance of carbohydrates in various biological processes such as cellular interactions, viral binding to host cells, cell differentiation, cancer metastasis, and the folding and turnover of glycoproteins is becoming increasely apparent.[1,2] Glycobiology, which is the study of the structure/function relationships of carbohydrate molecules and the role they play in biological systems, has become one of the major fields of modern biotechnology. In biological systems, the majority of carbohydrates present in cells are attached to proteins or lipids although carbohydrates can be present without being attached to other molecules.[2,3] The attached carbohydrate is often referred to as an oligosaccharide. The highly branched and large number of different linkage types of oligosaccharides allows glycoconjugates to display a further level of structural and functional diversity compared with linear proteins and nucleic acids or with lipids.

A glycoprotein is a biomolecule composed of a protein and a carbohydrate (an oligosaccharide or monosaccharide).[1-3] A carbohydrate can be covalently attached to the protein either via the OH group of a serine or threonine (*O*-glycosylated) or through the side-chain amide NH_2 of an asparagine (*N*-glycosylated). The carbohydrate portions of these glycoconjugates are referred to as *O*- and *N*-linked glycans, respectively. The carbohydrate portion of a glycoprotein is usually a small sugar of no more than 8 to 10 individual monosaccharide units. Monosaccharides commonly found in eukaryotic glycoproteins include glucose, *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine,

mannose, fucose, xylose and *N*-acetylneuraminic acid (sialic acid). It has been estimated that fifty percent of cellular proteins are glycoproteins,[2,3] making glycosylation one of the most common post-translational modification of proteins. The carbohydrate component of glycoproteins can assist in protein folding, make proteins resistant to proteolysis, affect protein physical properties such as solubility, viscosity and freezing point, as well as help proteins to orient correctly in a membrane and make them recognizable to another biochemical or cell.

Glycolipids are a class of compounds having solubility properties of a lipid and containing one or more molecules of a covalently attached sugar.[1-3] Their roles are to provide energy and also to serve as markers for cellular recognition. Glycolipids are present in the plasma membranes of all eukaryotic cells, most often localized at the outer leaflet of the membrane, with their hydrophilic sugar chain protruding on the surface where it acts as a recognition site for specific chemicals, helps to maintain the stability of the membrane and attaches cells to one another to form tissues. Glycolipids built on ceramide are known as glycosphingolipids, because ceramide is formed by attachment of a fatty acid via an amide linkage to the long-chain amino alcohol sphingosine (Figure 1.1). There are two main subgroups of glycosphingolipid that can be differentiated by whether the first sugar attached to sphingosine is galactose or glucose. Galactosphingolipid is enriched in brain tissue and is a major component of the myelin sheaths around nerves. Glucosphingolipid is present in the cell membranes of many cell types and is abundant in serum. Glycosphingolipids carry blood group antigens and define tumor-specific or developmental antigens. In addition, they serve as receptors for

many microorganisms and toxins, as well as modulators of cell surface receptors that mediate cell growth and as mediators of cell adhesion.

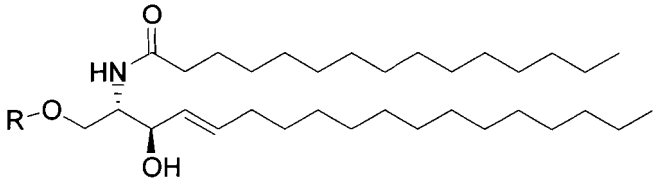


Figure 1.1 Structure of ceramide (R = H) and glycosphingolipid (R = sugar).

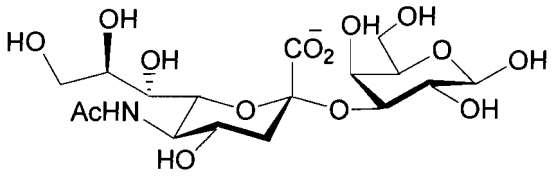
1.2 Glycosidases

1.2.1 Introduction

In contrast to proteins, the oligosaccharide portions of glycoproteins are assembled without a template through a series of individually catalysed reactions. The sugar structures are not encoded directly in the DNA sequences but are determined by transcription and translation of genes to generate glycosyltransferase that in turn control synthesis of the glycan portions of glycoconjugates.[1]

Glycosyl hydrolases (glycosidases) and glycosyl transferases are important enzymes that are responsible for the hydrolysis and formation, respectively, of glycosidic bonds. Both glycosidases and glycosyltransferases act specifically and catalyze hydrolysis of glycosidic linkages involving a particular sugar. For example, an exo galactosidase cleaves only galactose (Gal) residues from the non-reducing end of an oligosaccharide. Their specificity is also for particular linkages of a sugar. For example,

some sialidases can only hydrolyze α-2→3 linkages of a sialic acid residue (NeuAc) (Figure 1.2).[1]



NeuAcα2-3Gal

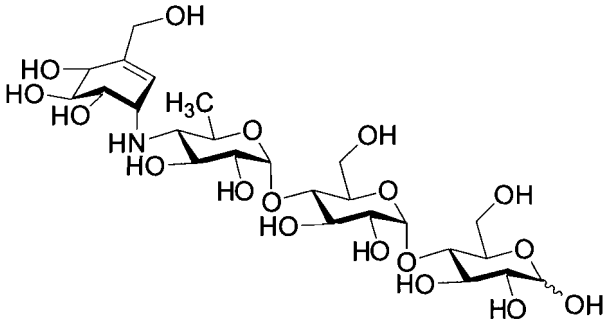
Figure 1.2 Structure of NeuAcα2→3Gal

The fundamental roles of glycosidases and glycosyl transferases include the control of metabolism, e.g. the breakdown and reassembly of oligosaccharides, the processing of various oligosaccharide-containing proteins and lipids, as well as the formation of cell walls and other barrier structures.[2,4]

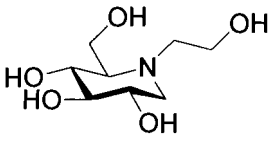
1.2.2 Digestive Glycosidases

Pancreatic α–amylase is a glycosidase that mediates the hydrolysis of complex starches into oligosaccharides within the small intestine. Membrane-bound intestinal α–glucosidases further hydrolyze these oligosaccharides to glucose. Inhibition of intestinal α–glucosidases can be used to treat diabetes through the lowering of blood glucose levels. Idiopathic diabetes (arising spontaneously or from an obscure or unknown cause) is divided into two main types: insulin-dependent and non-insulin-dependent. Insulin secretion from β-cells of pancreas is principally regulated by plasma glucose levels. Actions of insulin-insulin receptor interactions and activation of the kinase cascade lead

to altered activities of glycogen phosphorylase and glycogen synthase to maintain low blood glucose levels. In non-insulin-dependent diabetes mellitus (type 2 diabetes), insulin secretion can be normal but the cells are not very sensitive to the insulin. Many of the vascular consequences of insulin resistance are due to the persistent hyperglycemia seen in type 2 diabetes. Carbohydrate analogues, such as acarbose (**1.1**) and miglitol (**1.2**) (Figure 1.3) reversibly inhibit the function of pancreatic α–amylase and membrane-bound intestinal α–glucosidases.[5,6] The consequence of this inhibition is a reduction in digestion and the consequent absorption of glucose into the systemic circulation.



Acarbose (1.1)



Miglitol (1.2)

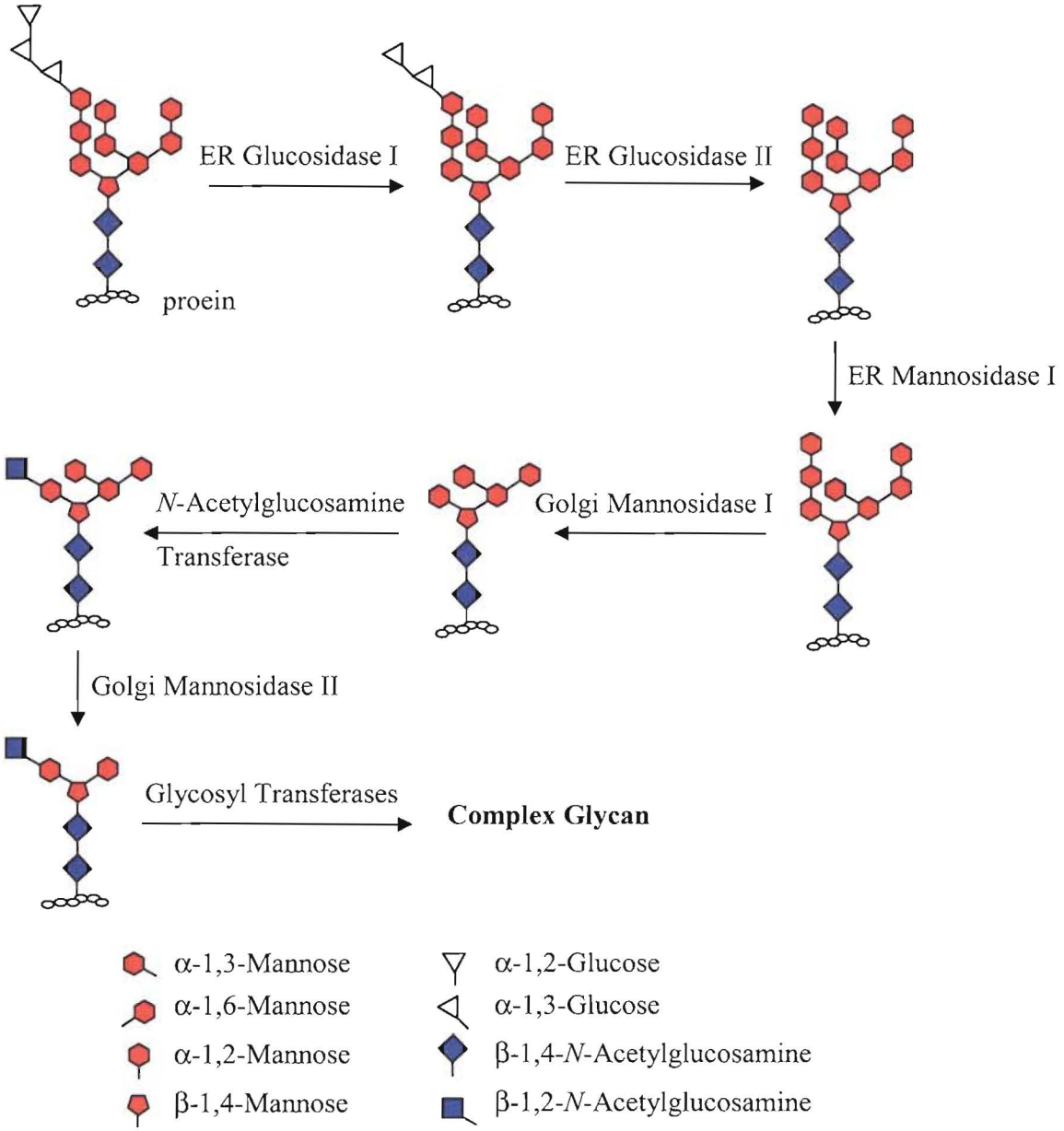
Figure 1.3 Structures of acarbose (**1.1**) and miglitol (**1.2**)

1.2.3 *N*-Linked Oligosaccharide Processing

Both glycosyltransferases and glycosidases are also involved in processing of the oligosaccharides on a glycoprotein. The *N*-linked biosynthetic pathway, which involves the formation and maturation of the oligosaccharides attached to an asparagine side chain of proteins, is necessary for the proper processing of proteins formed in the endoplasmic reticulum (ER) and Golgi apparatus.[1-3]

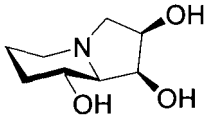
The maturation process is initiated from dolichol pyrophosphate (PPDol) in the ER where a series of glycosyltransferase enzymes converts PPDol to a lipid linked glycan precursor, $Glc_3Man_9GlcNAc_2PPDol$ (where Glc is glucose, Man is mannose, and Glc*N*Ac is *N*-acetylglucosamine). This precursor is then transferred to specific asparagine residues in the growing polypeptide chain by oligosaccharyl transferases in the ER. Carbohydrate units attached to the asparagine have a common inner-core structure.

The processing of complex *N*-linked glycoproteins occurs initially in the ER and later in the Golgi apparatus by the participation of a large number of enzymes, as shown in Scheme 1.1. The trimming process starts with the action of glucosidases I and II, which removes the three terminal glucose residues from $Glc_3Man_9GlcNAc_2$. A collection of processing mannosidases in the ER and the Golgi complex then cleave the four α-1→2-Man residues to yield $Man_5GlcNAc_2$. The Golgi Glc*N*Ac transferase, which initiates branching of complex oligosaccharides, then adds Glc*N*Ac, while Golgi mannosidase II removes one α-1→3- and one α-1→6-linked mannose residues, yielding $GlcNAcMan_3GlcNAc_2$. This oligosaccharide product is extended by branching Glc*N*Ac transferases to give bi-, tri-, and tetra-antennary structures. Finally, the distal sugars such as galactose, *N*-acetylgalactosamine, fucose, and sialic acid residues are added by the action of the corresponding glycosyl transferases to yield the wide variety of structures found in mature glycoproteins.



Scheme 1.1 Processing reactions in *N*-linked glycan biosynthesis.

Interference with the trimming process via glucosidase and/or mannosidase inhibitors blocks the maturation of oligosaccharides to complex structures.[7] The display of immature or "aberrant" structures has implications for the propagation of tumors. The tumor cells exhibit very complex carbohydrate[2,3] structures and it is believed that these structures provide signal stimuli for rapid proliferation and metastasis of tumor cells. Since the tumor and normal cells have different rates of cell growth, a glycosidase inhibitor can be used to inhibit the assembly of complex oligosaccharide structures. For example, swainsonine (**1.3**) (Figure 1.4), a plant-derived alkaloid is a Golgi α-mannosidase II inhibitor and it interferes with the *N*-glycosylation pathway in glycoprotein biosynthesis. Treatment with swainsonine (**1.3**) has led to a significant reduction of tumor mass in human patients with advanced malignancies, and is a promising drug therapy for patients suffering from breast, liver, lung cancer, and other malignancies.[8-10] The potential to inhibit cancer growth and metastasis by interfering with the expression of complex carbohydrate structures, suggests an alternative therapeutic strategy for the treatment of cancer.



Swainsonine (1.3)

Figure 1.4 Structure of swainsonine (**1.3**).

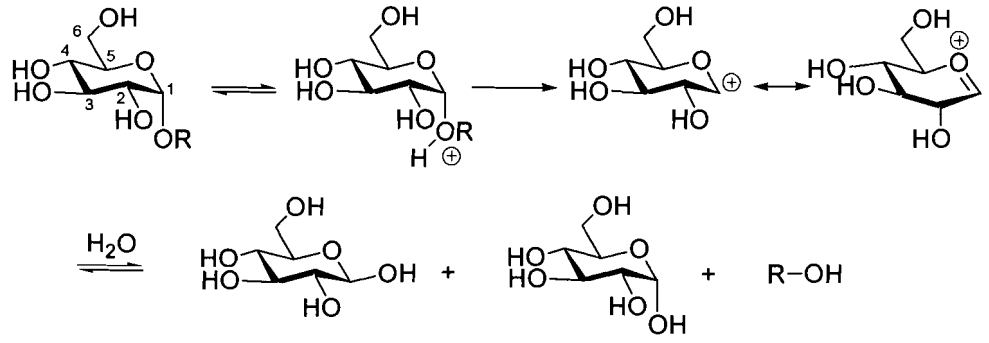
1.2.4 Glycosidase Mechanism

Much research has been devoted to the discovery of new glycosidase inhibitors given the important roles these enzymes play in many disease states. A guiding principle for the design of potent glycosidase inhibitors is mimicking the transition state of the enzyme-catalyzed reaction. The rationale behind this principle is the proposal by Linus Pauling, namely, that enzymes bind to transition states more tightly than to the ground states.[11] The tight binding of the transition state greatly lowers its energy relative to the uncatalyzed reaction. The transition state can be stabilized so much that the theoretical limit for the dissociation constant of a transition state analogue can be up to 10^{-20} M.[12] In order to design transition state analogues of glycosidases, one should analyze the mechanism of action of these enzymes.

The general accepted mechanism for acid-catalyzed glycosidic bond cleavage in solution involves the protonation of the exocyclic oxygen atom at the anomeric center C(1) to give a protonated glycoside (Scheme 1.2). Subsequent rate-limiting cleavage of the C(1) - O(1) bond leads to an oxacarbenium ion intermediate, which then is attacked by a nucleophilic water molecule to form the products. The transition state is believed to quite closely resemble the intermediate oxacarbenium ion. This is supported by evidence of various degrees of sp^2 character of the anomeric carbon atom in the transition state.[13]

It has been shown that the vast majority of enzyme-catalyzed hydrolysis of glycosides occur via a nucleophilic substitution at the anomeric carbon. This substitution can take place with either retention or inversion of the anomeric configuration, corresponding to a "retaining glycosidase" or "inverting glycosidase", respectively. The sugar substrate can be a five-membered (furanose) or a six-membered (pyranose) ring, so

that we have "furanoside hydrolase" and "pyranoside hydrolase".[4] Koshland first proposed the mechanisms for the retaining and inverting glycosidases in 1953.[14] Since then, there is no evidence against this proposed mechanism.

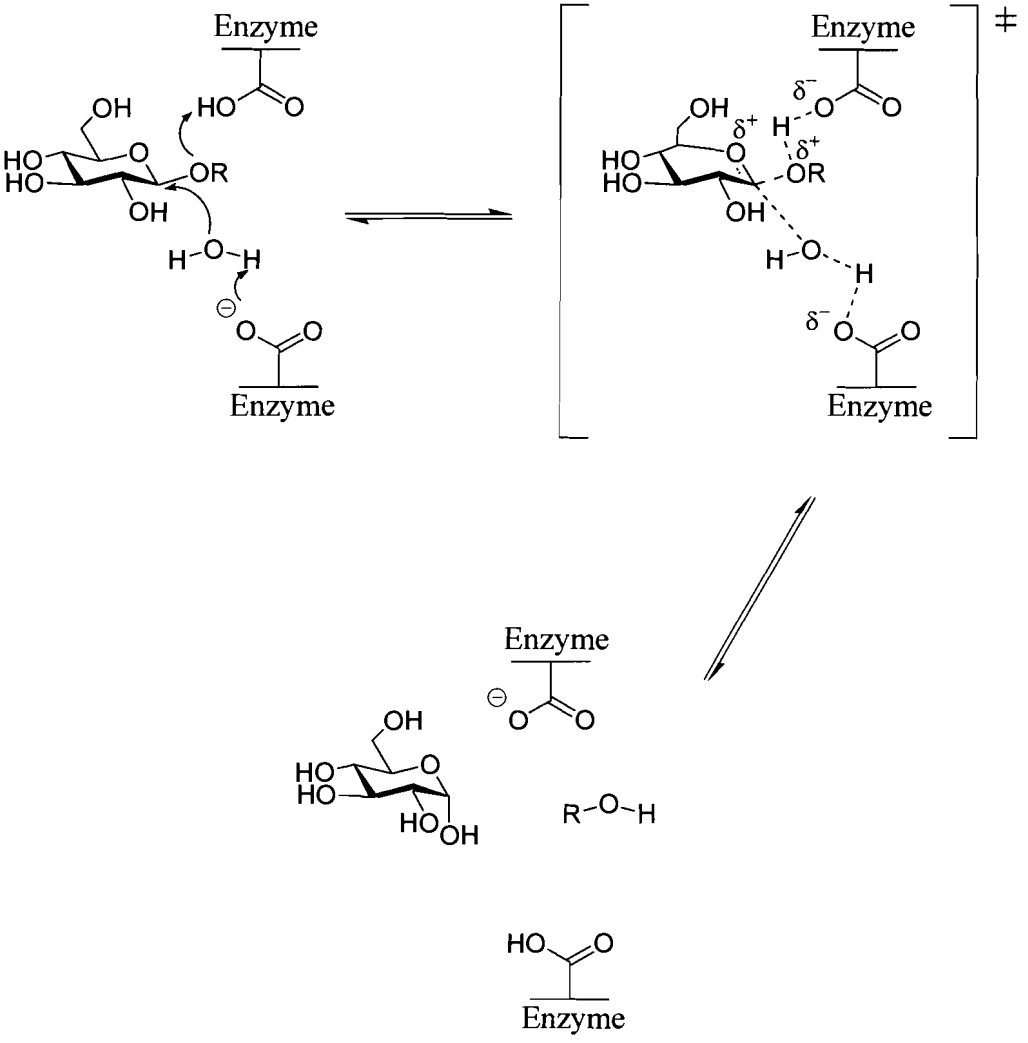


Scheme 1.2 Acid-catalyzed hydrolysis of a glycosidic bond.

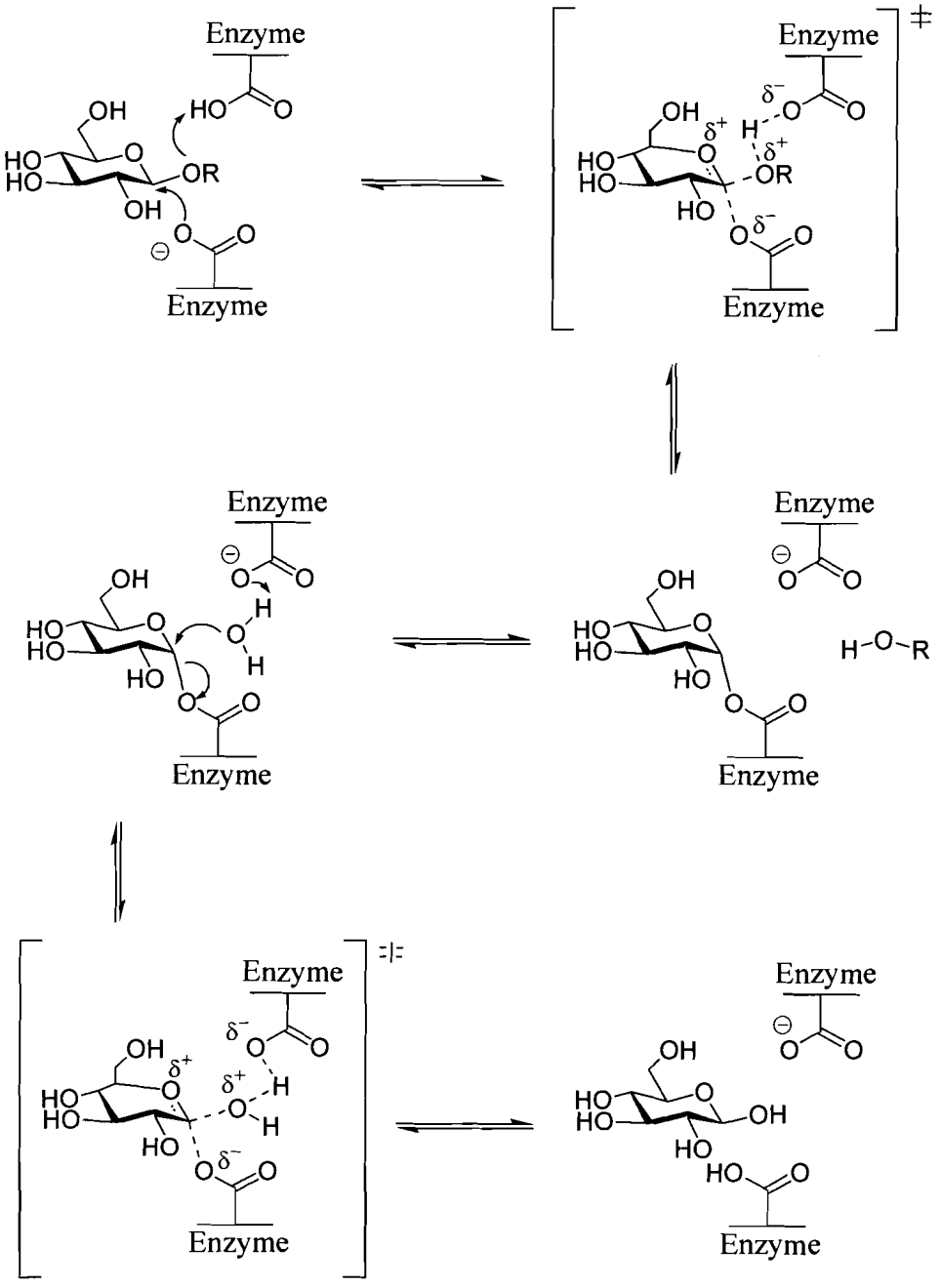
In inverting glycosidases the hydrolysis reaction takes place via a single displacement chemical step (Scheme 1.3). The two carboxyl groups, within the glycosidase active site, are some 10.5 Å apart allowing for the substrate and a water molecule to bind between them. One carboxyl group serves as a general acid, protonating the aglycon while the other acts as a general base, directing a water molecule to attack the anomeric carbon.[15-17] The transition states of the reactions catalyzed by inverting glycosidases are likely to resemble those of non-enzymatic glycoside hydrolysis. Glycosyl cation intermediates are often drawn for enzymatic process by analogy with the reaction in free solution. However, in solvents less polar than water, glycosyl cation intermediates are too unstable to exist.[18]

In contrast, the carboxyl groups in retaining glycosidases are 5.5 Å apart, consistent with a double displacement mechanism involving a covalent glycosyl-enzyme intermediate (Scheme 1.4).[15-17] In the first step, one of the carboxylic acid residues acts as a general acid, protonating the aglycon, whereas the second carboxylate acts as a nucleophile, forming the glycosyl-enzyme intermediate. In the second step, the carboxylate acts as a general base deprotonating the nucleophilic water molecule which attacks the anomeric carbon and displaces the carboxylate leaving group. Thus, this mechanism has two oxacarbenium ion-like transition states. Direct trapping of the covalent glycosyl-enzyme intermediates has been achieved in several different ways.[15-17,19]

Complementary to the mechanism of β-retaining glycosidases, the mechanism of α-retaining glycosidases involves a β-linked intermediate. The catalytic nucleophile carboxylate of retaining glycosidases is located close to the α–face of the pyranoside ring of the substrate in the β–glycosidases, and to the β face in the α–glycosidases. It was proposed[19] that, in the β–glycosidases, the interaction between the nucleophile carboxyl oxygens and the anomeric carbon as well as the 2-hydroxyl of the substrate will favor development of a positive charge at the anomeric center (Figure 1.5, b). However, for the α-glycosidases, the endocyclic oxygen of the substrate has been proposed to possess a greater share of the positive charge (Figure 1.5, a).[19]



Scheme 1.3 Proposed inverting mechanism of glycosidases (β-glucosidase is used as example)



Scheme 1.4 Proposed retaining mechanism of glycosidases (β-glucosidase is used as example).

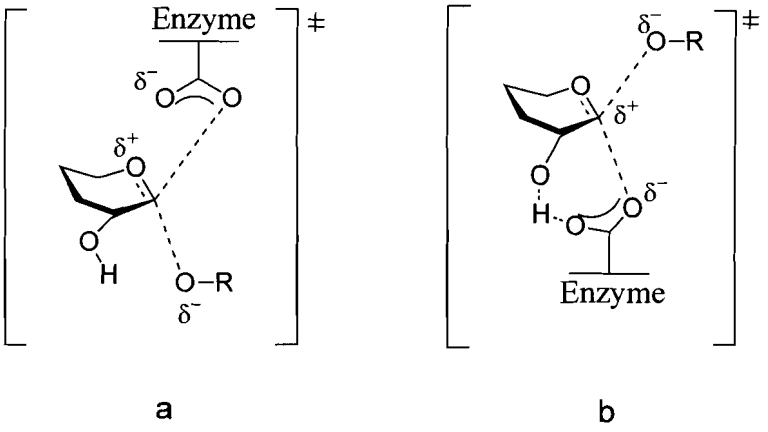


Figure 1.5 Postulated transition states for the enzyme-catalyzed hydrolysis of a glycosidic bond.

Pauling had emphasized the importance of "shape" when he proposed that potent inhibitors should mimic the transition state both electronically and structurally.[20] The stereoelectronic requirement for planarity of an oxacarbenium ion is unambiguous. In the case of a furanosyl cation, the ring probably possesses an envelope conformation in which the C-1, C-2, C-4, and O-4 atoms adopt a coplanar arrangement, such as the conformations of a ribofuranosyl cation, ^{3}E or E_3 (Figure 1.6).[4] In the case of a pyranosyl cation it probably has a half-chair or a classical boat conformation which enables the C-1, C-2, O-5, and C-5 to be coplanar, such as the conformations of a glucopyranosyl cation, $^{4}H_3$, $^{3}H_4$, $^{2,5}B$ or $B_{2,5}$ (Figure 1.6).

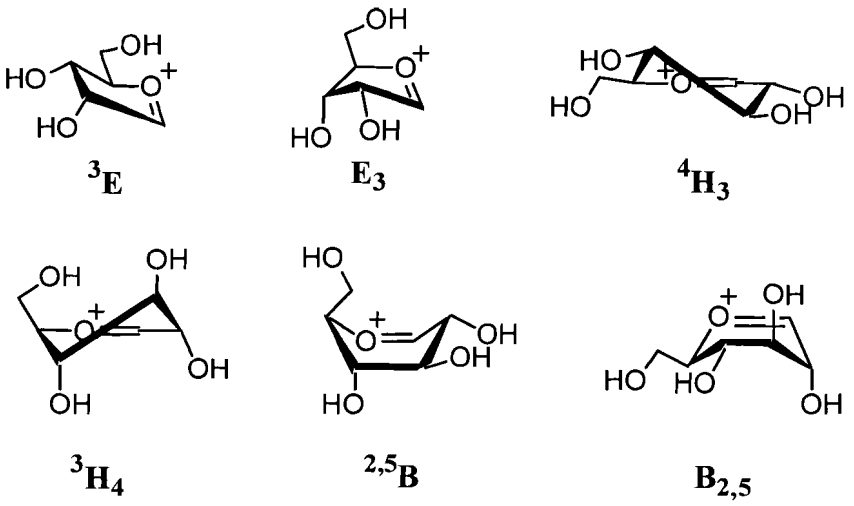


Figure 1.6 Proposed conformations of oxacarbenium ions.

1.3 Glycosidase Inhibitors

Glycosidase inhibitors have been the subject of great interest as they are important tools for studying glycosidase mechanisms and many have potential in therapeutic applications.[5,6,3,2] Based on the understanding of the glycosidase mechanism, a sp^2-hybridized anomeric center, a positive charge between the ring oxygen and anomeric carbon, a half chair/boat conformation of the TS, and a proper hydroxyl group configuration are proposed to be important characteristics of a good inhibitor.[21-23] However, the relative importance of two features of the TS, which are the shape and the charge, has remained the subject of much debate.[20,24-26]

1.3.1 Inhibitors that Mimic a Positively Charged Exocyclic Oxygen

The first step in the glycosidase-mediated hydrolysis of glycosides involves the protonation of the exocyclic oxygen of glycosidic bond; thus, an early transition state may have a substantial build up of positive charge on this atom (Figure 1.7, A).[23] Many

compounds with a nitrogen in place of the exocyclic oxygen, like acarbose (**1.1**), trehazoline (**1.4**), and bicyclo-[4.1.0]heptane derivative (**1.5**), are considered as mimics of the conjugate acid of the glycoside (Figure 1.3 and Figure 1.8).[23] Acarbose (**1.1**) is a potent α-glucosidase inhibitor.[5] Trehazoline (**1.4**) exhibits powerful specific inhibition of various trehalases and is expected to have potential as an insecticide or fungicide.[27] Compound **1.5** strongly inhibits yeast α-glucosidase.[28] Single-crystal X-ray diffraction showed that the bicyclic compound **1.5** adopts a half chair conformation, because of the fused cyclopropane ring.

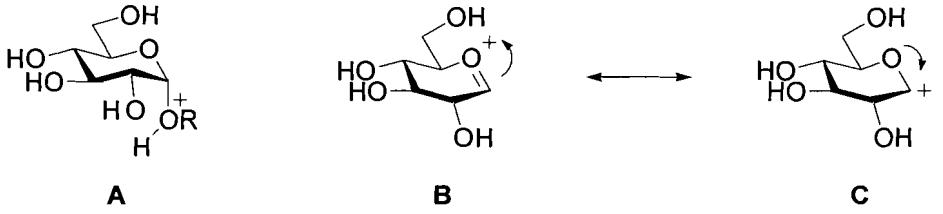


Figure 1.7 Postulated charge build up on different atoms during glycosidase-mediated hydrolysis reactions.

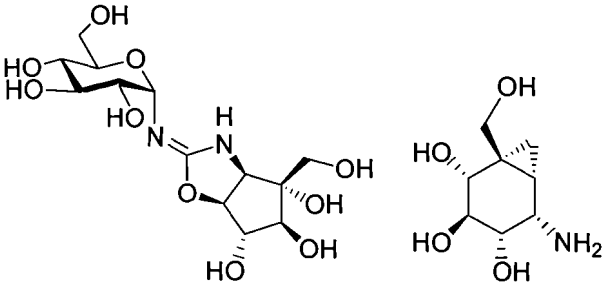


Figure 1.8 Structures of compounds **1.4** and **1.5**.

In our group, 5-thio-D-mannopyranosylamine (**1.6**) and 5-thio-D-mannopyranosylamidinium bromide (**1.7**) were synthesized as potential glycosidase inhibitors (Figure 1.9).[29] Both these compounds were found to bind in a boat (^{1,4}B) conformation in the Golgi α-mannosidase II active site.

The aminocyclopentane mannostatin A (**1.8**), isolated from the soil microorganism *Streptoverticillus*, is a potent inhibitor of class II α-mannosidases.[30] It had been considered as an inhibitor that mimics the positively charged exocyclic oxygen. However, in the cocrystal structure with *Drosophila melanogaster* Golgi α-mannosidase II (dGMII), it adopts a $^{2}T_{1}$ twist envelope conformation and mimics the covalent enzyme-mannosyl intermediate, indicating that potent inhibitors of glycosidase do not have to mimic an oxacarbenium ion-like transition state.[31] Potent inhibition alone is not a good measure of transition state analogy and arguably, the most rigorous approach to establishing transition state analogy has been to use free energy correlations between inhibitor binding and transition state stabilization.[23]

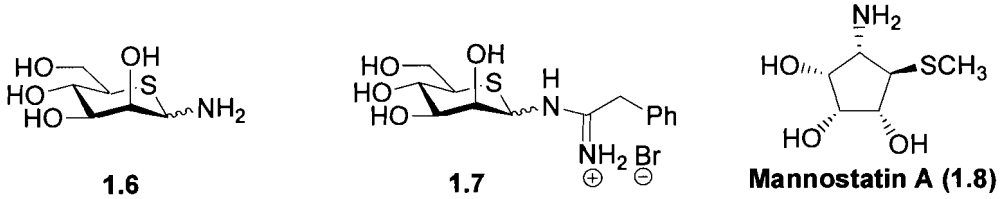
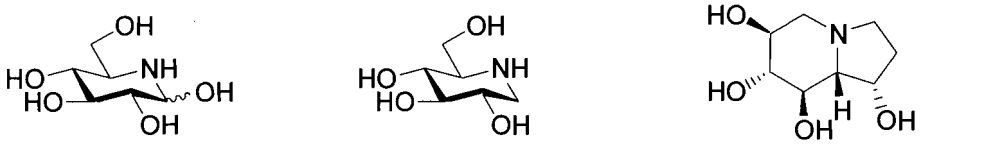


Figure 1.9 Structures of compounds **1.6 – 1.8**.

1.3.2 Inhibitors that Mimic a Positively Charged Endocyclic Oxygen

1.3.2.1 Nitrogen Containing Analogues

There are also inhibitors that can mimic the charge that is built up on the endocyclic oxygen atom (Figure 1.7, B). Many naturally occurring glycosidase inhibitors such as nojirimycin (**1.9**), deoxynojirimycin (DNJ **1.10**) and castanospermine (**1.11**) (Figure 1.10), generally known as iminoalditols or aza-sugars, are examples of compounds that mimic the charge built up on the endocyclic oxygen atom.[23] The nitrogen atom of the inhibitors is believed to be protonated in the active site to generate a positive charge which interacts with the negatively charged catalytic nucleophile or the carboxylate derived from deprotonation of the catalytic acid.[22] These inhibitors mimic the chair conformation of the ground state instead of the half-chair conformation of the TS. A linear free energy relationship analysis of the resemblance of DNJ (**1.10**) with the transition state of β-glucosidase hydrolysis showed that DNJ (**1.10**) was not a TS analogue.[22]



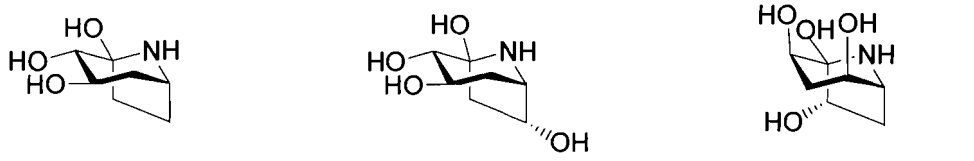
Nojirimycin (1.9) **Deoxynojirimycin (1.10)** **Castanospermine (1.11)**

Figure 1.10 Structures of compounds **1.9** – **1.11**.

Nojirimycin (**1.9**), isolated from the microorganism *Streptomyces*, was discovered as the first glucose analogue with the nitrogen atom in place of the endocyclic oxygen and was shown to be a potent inhibitor of both α- and β-glucosidases.[32] Stable

deoxynojirimycin (**1.10**), obtained by the reduction of nojirimycin (**1.9**) or isolated from the roots of mulberry trees, was found to have excellent α-glucosidase inhibitory activity *in vitro*.[22,33] The DNJ derivative, miglitol (**1.2**), can be almost completely absorbed in the intestinal tract and hence shows better *in vivo* activity than DNJ (**1.10**). Castanospermine (**1.11**), isolated from the plant *Castanosperum australe*, is an example of a naturally occurring indolizidine alkaloid which inhibits β-glucosidases.[34]

Nortropanes (8-azabicyclo[3.2.1]octanes) also contain a five- and a six-membered ring system but have different ring connectivity than indolizidines. Calystegines[35] have a nortropane ring system, with two to four hydroxyl groups in various positions, stereochemistries, and aminoketal functionality. Many calystegines show potent glycosidase inhibitory activity, particularly of glucosidases and galactosidases, and are interesting lead compounds for pharmaceutical research.[22] Calystegine A_3 (**1.12**), calystegine B_1 (**1.13**) and calystegine B_5 (**1.14**) (Figure 1.11) are examples of this class of compounds.



Calystegine A_3 (1.12) **Calystegine B_1 (1.13)** **Calystegine B_5 (1.14)**

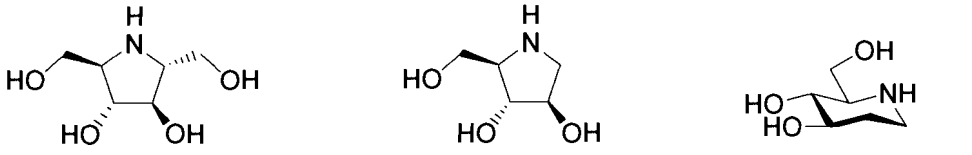
Figure 1.11 Structures of calystegine analogues **1.12** – **1.14**.

Swainsonine (**1.3**) was isolated from the plant *Swainsona canescens* and found to be a nM inhibitor of Golgi α-mannosidase II.[36] The inhibitory activity of swainsonine has

been attributed to its highly complementary shape with the enzyme active site and the structural resemblance of its flattened five-membered ring with the transition state.[37]

2,5-Dideoxy-2,5-imino-D-mannitol (DMDP, **1.15**) (Figure 1.12) was first isolated in 1976 from the leaves of the legume *Derris elliptica* and was later shown to be a powerful β-glucosidase inhibitor.[38] Another example of a pyrrolidine is 1,4-dideoxy-1,4-imino-D-arabinitol [D-ABI, **(1.16)**, Figure 1.12] which was first found in the fruits of *Angylocalyx boutiqueanus*,[39] and it is a potent α-glucosidase inhibitor.[40] A possible way to suppress hepatic glucose production and lower the blood glucose level in type II diabetes patients may be by inhibiting the hepatic glycogen phosporylase.[41] D-ABI was found to be a potent inhibitor of hepatic glycogen phosphorylase.[42]

The natural product fagomine (**1.17**) was also found to be a weak inhibitor of glycogen phosphorylase with an IC_{50} value of 200 μM.[43]



DMDP (1.15) **D-ABI (1.16)** **Fagomine (1.17)**

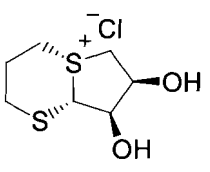
Figure 1.12 Structures of compounds **1.15 – 1.17**.

1.3.2.2 Sulfonium Salts

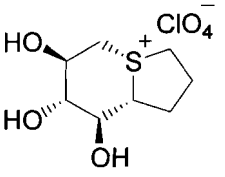
The nitrogen atom in aminosugars (section 1.3.2.1) is known to be protonated at physiological pH, thus providing the stabilizing electrostatic interactions between the inhibitor and the carboxylate residues in the enzyme active site. An alternative way of

achieving such a charged species would be to synthesize compounds that bear a permanent positive charge at the nitrogen position, for example, sulfonium salts. Species with positively charged sulfur atoms are known to be quite stable, as opposed to the highly unstable oxonium ions.

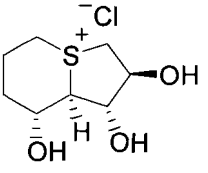
The syntheses of bicyclic sulfonium salts to serve as glycosidase inhibitors have precedent in the work of Siriwardena and co-workers.[44-46] Most recently, they have reported the synthesis of compound **1.18** (Figure 1.13) and have shown that it is not only a potent inhibitor of several mannosidases, but that it also exhibits greater selectivity than does swainsonine (**1.3**).[45] This concept of exchanging a nitrogen atom with a sulfonium-ion was also exploited by our group. We designed and successfully synthesized the sulfonium-ion analogue (**1.19**, Figure 1.13) of castanospermine (**1.11**),[47,48] in which the bridge nitrogen atom is replaced by a sulfonium ion. Compound **1.19** was shown to be a mM inhibitor (K_i = 1.32 mM) of glycoamylase G2. The synthesis of the sulfonium derivative (**1.20**) of swainsonine (**1.3**) has also been reported by our group.[49]



1.18



1.19

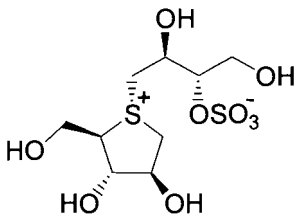


1.20

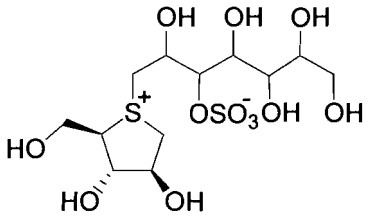
Figure 1.13 Structures of compounds **1.18 – 1.20.**

In addition, the discovery of a new class of glycosidase inhibitors, namely salacinol (**1.21**) and kotalanol (**1.22**) from *Salacia reticulata* (known as kothalahimbutu in Singhalese), with intriguing inner-salt sulfonium sulfate structures[50-52] has led to

significant synthetic efforts to prepare sulfonium salts with potential glycosidase inhibitory activities.[53-67] The 1,4-anhydro-4-thio-D-arabinitol moiety with a positive charge at the sulfur atom is postulated to bind to glycosidase enzymes by mimicry of the shape and charge of the oxacarbenium-ion transition state in the glycosidase-mediated hydrolysis reaction.[68] The inhibitory activities of salacinol (**1.21**, Figure 1.14) against sucrase and maltase are nearly equivalent to acarbose (**1.1**), an α-glucosidase inhibitor which is clinically used for the treatment of diabetes. The inhibitory activity of salacinol (**1.21**) against isomaltase is greater than that of acarbose (**1.1**).[51] Several analogues of salacinol and compounds related to salacinol have been synthesized as potential glycosidase inhibitors.[69,70]



Salacinol (1.21)



Kotalanol (1.22)

Figure 1.14 Structures of salacinol **1.21** and kotalanol **1.22**.

1.3.3 Inhibitors that Mimic a Positive Charge in the Anomeric Position and Inhibitors that Mimic a Positive Charge in Several Other Locations

The synthetic alkaloid isofagomine (**1.23**, Figure 1.15) was first designed to mimic the intermediate carbocation or oxacarbenium ion resonance form **C** (Figure 1.7, C) by incorporating a nitrogen atom at the anomeric centre.[71,72] Compound **1.23** is a potent inhibitor of β-glucosidase, while α-glucosidase inhibition is moderate.[71,72] While

β-galactosidases and β-glucuronidases were all strongly inhibited by isofagomines **1.24**[73,74] and **1.25**,[75,76] respectively, the α-galactosidases and α-glucuronidases, on the other hand, were generally more powerfully inhibited by the 1-deoxynojirimycin analogues, **1.26** and **1.27**, respectively. As mentioned in the section 1.2.4, it was postulated that a greater positive charge is developed at the anomeric center of the transition state for β-glycosidases while the endocyclic oxygen has a greater share of the positive charge for the α-glycosidases.[19] The ring nitrogen atom of deoxynojirimycin (**1.10**) is well positioned to form a hydrogen-bonded ion pair with the anionic catalytic nucleophile of α-, but not of β-glucosidases.[19] The reverse is true for isofagomine (**1.23**). Thus, the isofagomines (**1.23**, **1.24** and **1.25**), which arguably mimic the charge development on the anomeric carbon, are potent β–glycosidase inhibitors but very modest inhibitors of α-glycosidases.

Based on the observation that the position of the nitrogen atom in the ring greatly influences whether α- or β-glycosidases are strongly inhibited, 1-azafagomine (**1.28**), which has a nitrogen atom in place of both the endocyclic oxygen and anomeric carbon atoms, was designed and synthesized.[77,78] This compound indeed turned out to be a potent inhibitor of both the α- or β-glycosidases.[79]

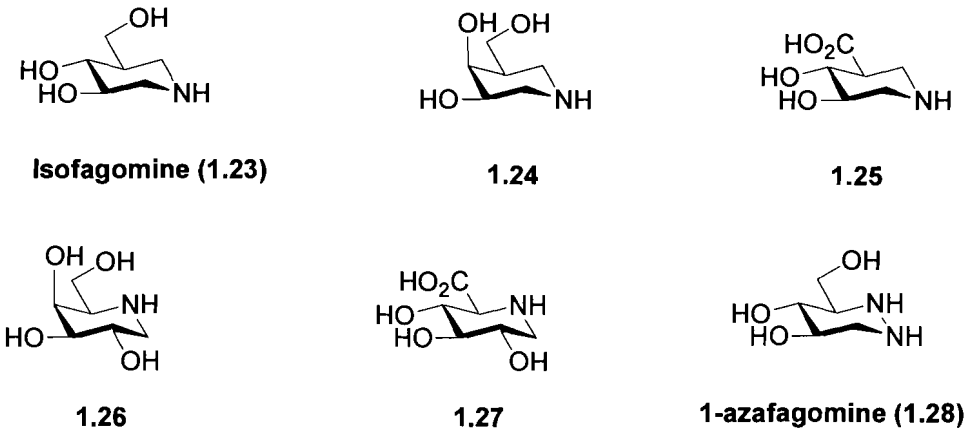
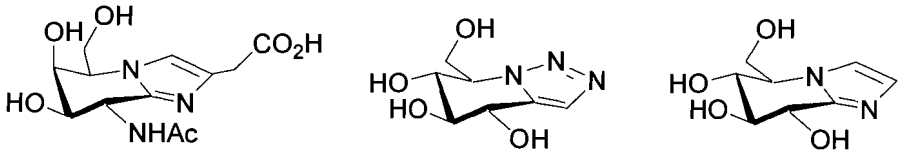


Figure 1.15 Structures of compounds **1.23 – 1.28**.

Nagstatin (**1.29**, Figure 1.16), an inhibitor of *N*-acetyl-β-D-glucosaminidase (NAG-ase), was discovered in the fermentation broth of *Streptomyces amakusaensis* MG846-fF3 and its inhibitory activity is believed to be mainly associated with the imidazole ring.[80,81] The six-membered rings of the synthetic inhibitors, gluco-configured 1,2,3-triazole (**1.30**) and imidazole (**1.31**), possess half-chair conformations as a result of their fusion with the aromatic ring, mimicking the shape of the transition state.[23,82,83] However, 1,2,3-triazole (**1.30**) was a significantly weaker inhibitor of almond β-glucosidase than imidazole (**1.31**). This was rationalized by Heightman and Vasella who proposed that β-glycosidases protonate their substrate within the mean plane of the pyranose ring rather than from above (Figure 1.17).[23,82,83] A heteroatom in place of the glycosidic oxygen atom is required for strong inhibition. The imidazole (**1.31**, pK_a ca. 6) is likely to be protonated so that the positions which mimic both the endo- and exocyclic oxygens of the transition state are positively charged and therefore mimics charges at these two positions.[23]



Nagstatin (1.29) **1.30** **1.31**

Figure 1.16 Structures of compounds **1.29 – 1.31**.

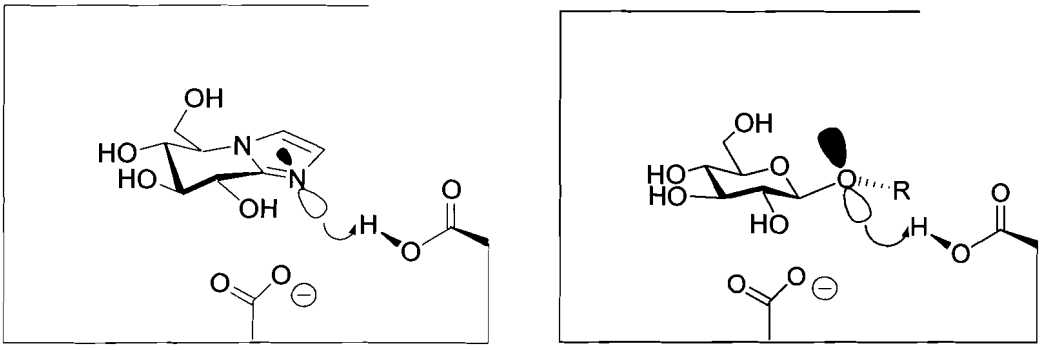


Figure 1.17 β-Glycosidases protonate their substrate within the mean plane of pyranose ring rather than from above.

Amidine analogues of D-glucose, which are the first analogues mimicking both the partial positive charge on the endo- as well as on the exocyclic oxygen, and the flattened conformation of the transition state, were synthesised by the Ganem group in 1990.[84,85] The amidine (**1.32**, Figure 1.18) was found to inhibit the β-glucosidase (sweet almond) relatively strongly. However, its inhibitory activity is not specific for this

enzyme as it can also inhibit the α-mannosidase (jack bean) and β-galactosidase (bovine liver). Therefore, it was called a broad spectrum inhibitor. Its derivatives, amidrazone (**1.33**) and amidoxime (**1.34**), are also broad spectrum inhibitors of glycosidases.[26,86] Glycosidases are substrate specific and so are transiton-state analogues. Thus, it was assumed that the flattened chair conformation and the sp^2 hybridization of the anomeric carbon of the transition state are more important than the positive charge for inhibition. In order to obtain better selectivity, amidine and amidoxime derivatives **1.35** and **1.36** with various substitutents at the exocyclic nitrogen were synthesized to mimic the aglycon in the natural substrate.[87,88] They were found to improve the selectivity.

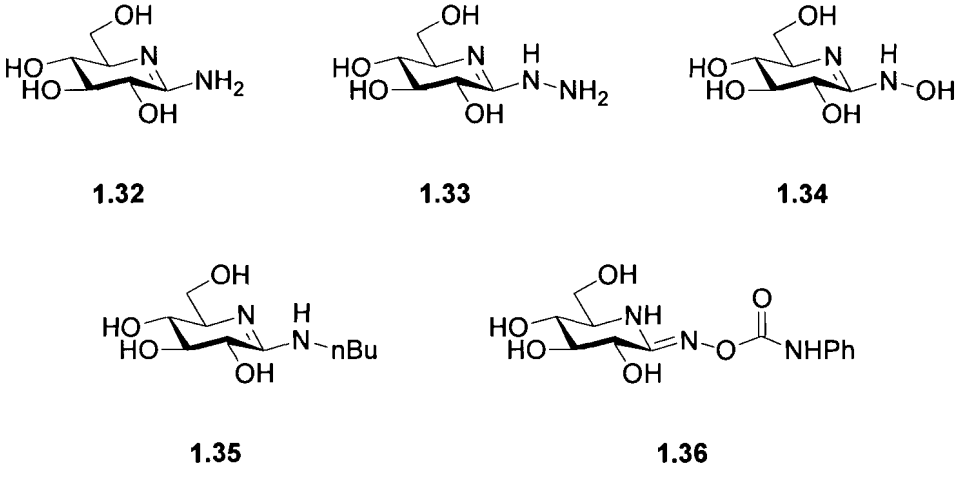
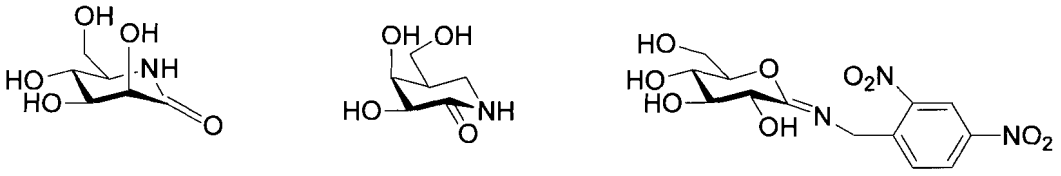


Figure 1.18 Structures of compounds **1.32 – 1.36**

1.3.4 Inhibitors that Do Not Mimic a Positive Charge

Neutral lactam-type compound **1.37** (Figure 1.19) was also found to be a good inhibitor of both the α- and β-mannosidases.[89] It has a sp^2-hybridized anomeric carbon and a $B_{2,5}$ conformation so that it may geometrically resemble the transition state of glycoside hydrolysis. The lactam **1.38** has a nitrogen atom at its anomeric position

similar to the isofagomine **1.23** and the carbonyl group at the 2-position.[90] It is a strong inhibitor with K_i value of 18 nM against β-galactosidase. The aldonolactone oxime derivative **1.39** has a very low basicity and is regarded as a neutral inhibitor. It was found to inhibit α-glucosidase in the low micromolar range.[91]



1.37 **1.38** **1.39**

Figure 1.19 Structures of compounds **1.37 – 1.39**.

Glycotetrazoles cannot be expected to be fully protonated in the active site.[23,92] The glucotetrazole (**1.40**, Figure 1.20) and mannotetrazole (**1.41**) were found to have a half-chair conformation.[83] Compound **1.40** inhibited β-glucosidase and β-galactosidase with a K_i of about 1.5 μM.[93] It has been shown that about one-half of the binding energy is contributed from the charge-dipole interaction between the catalytic nucleophile and the tetrazole (Figure 1.21). The other half came from protonation of the glycosidic nitrogen within the mean plane of the sugar by the catalytic acid.[94]

1.40 **1.41**

Figure 1.20 Structures of compounds **1.40** and **1.41**.

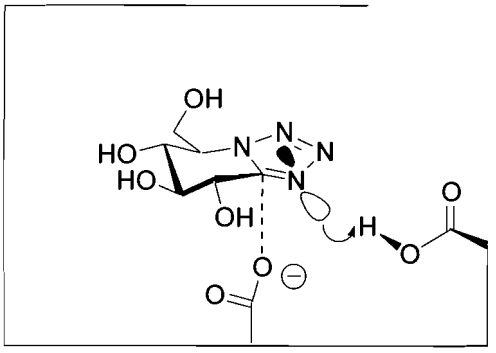
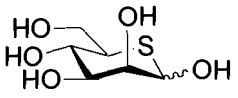


Figure 1.21 Charge-dipole interaction between the catalytic nucleophile and the tetrazole

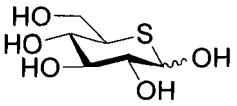
1.3.4.1 Thiosugars

Thiosugars, containing a sulfur atom in place of the ring oxygen atom or in the interglycosidic linkage, have been well studied; some of these compounds have shown inhibitory activities against various enzymes. The differences in biological activities between thiosugars and their oxygen analogues depend on geometric, conformational, flexibility, and electronic differences.[95-97] Thiosugars have become very important targets due to their potential as glycosidase inhibitors.

The first naturally occurring thiosugar, 5-thio-D-mannose (**1.42**, Figure 1.22), was isolated in 1987 from the marine sponge *Clathria pyramida*.[98] It has shown antibacterial activities against both Gram positive and Gram negative bacteria. The synthetic thiosugar, 5-thio-D-glucopyranose (**1.43**),[99] was found to inhibit D-glucose-stimulated insulin release.[100]



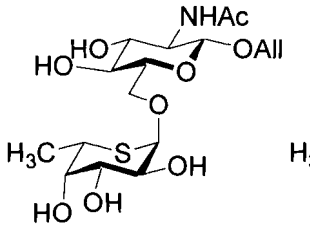
1.42



1.43

Figure 1.22 Structures of compounds **1.42** and **1.43**.

Four 5-thio-L-fucose-containing disaccharides (**1.44** – **1.47**, Figure 1.23) were synthesized by Izumi *et al.*[101] Only the α(1→2)-linked disaccharide **1.47** shows inhibitory activity (K_i = 0.21 mM) against *Bacillus* α-L-fucosidase. In contrast, all of the disaccharides show inhibitory activity (K_i = 30-91 μM) against the bovine epididymis α-L-fucosidase.



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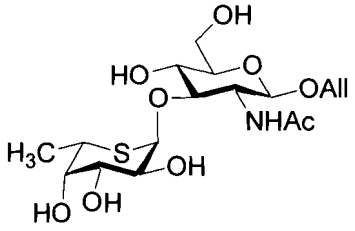

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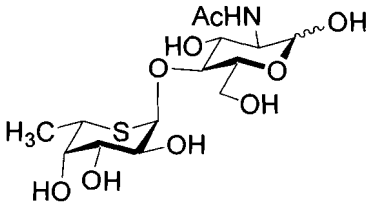

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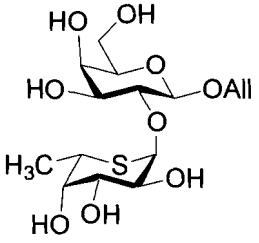
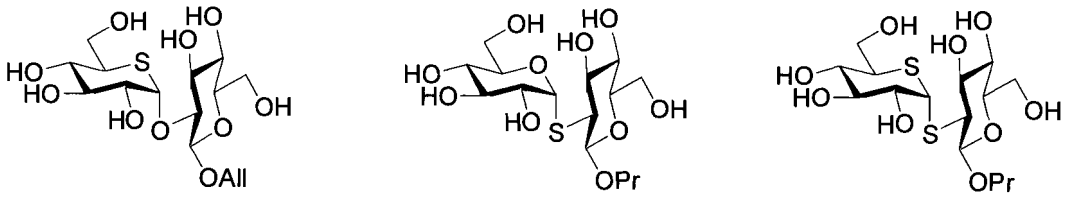


1.47

Figure 1.23 Structures of compounds **1.44** – **1.47**.

Kojibioside analogues **1.48 - 1.50** (Figure 1.24) were synthesized in our laboratory.[102-104] Compound **1.48** was found to be a poor inhibitor of glucosidase II but a competitive inhibitor of glucosidase I (K_i = 2.0 mM).[105] In contrast, compound **1.49** was determined to be a moderate inhibitor of glucosidase II (K_i = 1.0 mM), but showed poor activity against glucosidase I.[105]



1.48 **1.49** **1.50**

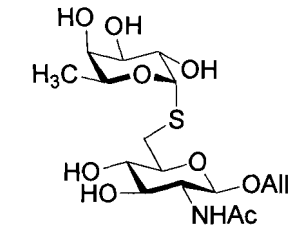
Figure 1.24 Structures of compounds **1.48 – 1.50**.

Hashimoto *et al.* synthesized a series of α-L-fucopyranosyl disaccharides (**1.51 – 1.54**, Figure 1.25) for the purpose of characterizing α-L-fucosidases.[106] Compound **1.53** was found to be a competitive inhibitor of the kidney α-L-fucosidase, with a K_i value of 0.65 mM.

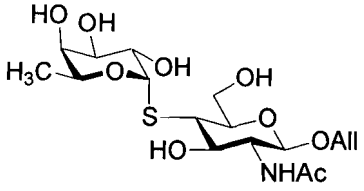
The disaccharide analogues of α-D-Man-(1→2)-α-D-Man-OMe (**1.55–1.57**, Figure 1.26), containing sulfur in the ring and/or interglycosidic linkage, were synthesized in our laboratory as potential inhibitors of the processing mannosidase Class I enzymes.[107]

1.4 The Aim of This Research Work

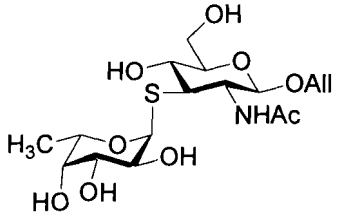
In this work, some analogues of the naturally occuring glycosidase inhibitor, salacinol (**1.21**), were designed in order to probe structure-activity relationships and search for new inhibitors. Some of these desired target compounds were successfully synthesized and tested against certain enzymes.



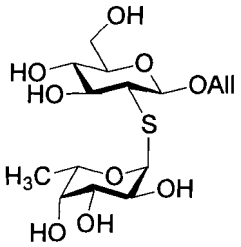
1.51



1.52

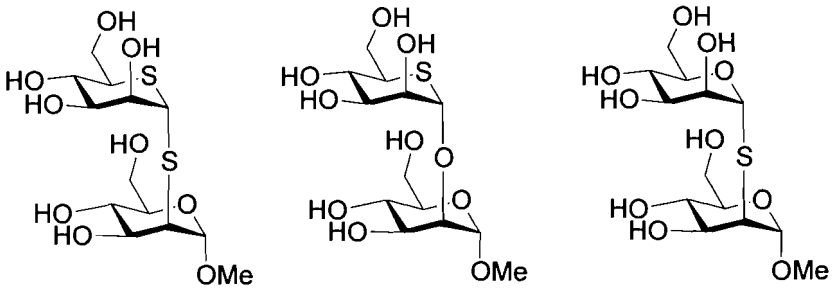


1.53



1.54

Figure 1.25 Structures of compounds **1.51** – **1.54**.



1.55 **1.56** **1.57**

Figure 1.26 Structures of compounds **1.55 – 1.57**.

CHAPTER 2:
SYNTHESIS, ENZYMATIC ACTIVITY AND X-RAY CRYSTALLOGRAPHY OF AN UNUSUAL CLASS OF AMINO ACIDS: NITROGEN ANALOGUES RELATED TO SALACINOL CONTAINING A CARBOXYLATE INNER SALT

2.1 Introduction

Glycosidases are involved in several important biological processes, such as digestion, the biosynthesis of glycoproteins, and the catabolism of glycoconjugates.[3,2] Since glycosidase inhibitors have shown antiviral, insect antifeedant, antidiabetic, and anticancer effects, as well as immune modulatory properties, they have attracted considerable attention. The transition-state structure in the enzyme-mediated hydrolysis of glycosides is believed to resemble an oxacarbenium ion intermediate with a distorted conformation.[4,21,23] Thus, mimicking this distorted, positively charged species is one factor that should lead to an effective inhibitor of glycosidase enzymes.

Many alkaloid sugar mimics with a nitrogen in the ring have been isolated from plants and microorganisms and inhibit various glycosidases.[108,109,73] 1-Deoxynojirimycin (**1.10**, Figure 2.1), which is a D-glucose analogue with an NH group in place of the ring oxygen atom, has been shown to inhibit intestinal α-glucosidases and pancreatic α-amylase both *in vitro* and *in vivo*, as well as α-glucosidases I and II involved in *N*-linked oligosaccharide processing.[22] Two *N*-alkylated analogues of deoxynojirimycin, namely miglitol (**1.2**) and *N*-butyldeoxynojirimycin (**2.1**), are currently in use as drugs for the treatment of Type II diabetes and Gaucher's disease, respectively. Both drugs act by inhibition of glucosidase enzymes. 1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1) (**1.16**), which was first isolated from the fruits of the legume *Angylocalyx boutiquenus*, was found to be a potent inhibitor of hepatic glycogen phosphorylase.[42] Its synthetic L-enantiomer (L-AB1) (**2.2**) is a powerful inhibitor of mammalian α-D-glucosidases.[110,111] The naturally occurring glycosidase inhibitor acarbose (**1.1**, Figure 2.2),[112] which contains a nitrogen atom in one of the linkages between the sugar and pseudosugar units,

is the highest-affinity carbohydrate analogue for a binding protein and has also been used for the treatment of type-2 diabetes.[5,6] It is generally believed that this strong binding originates from electrostatic interactions of the positively charged, protonated nitrogen atom with carboxylate residues in the enzyme active-site.[22]

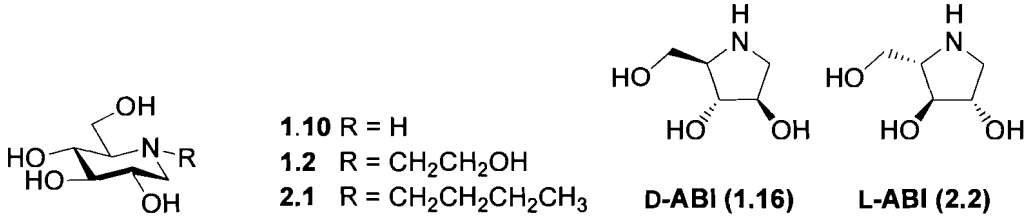


Figure 2.1 Structures of 1-deoxynojirimycin (**1.10**), miglitol (**1.2**), *N*-butyldeoxynojirimycin (**2.1**), D-AB1 (**1.16**) and L-AB1 (**2.2**)

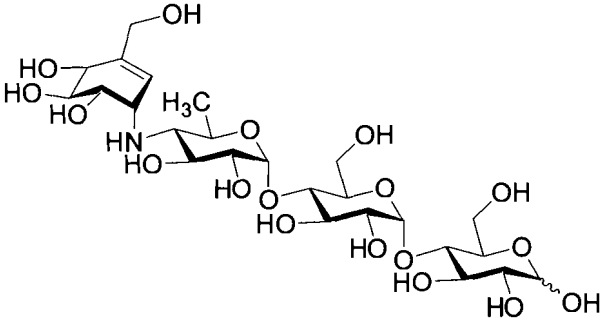
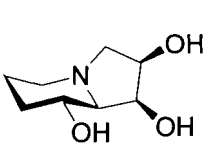
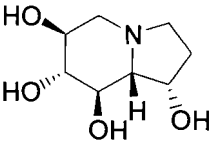


Figure 2.2 Structure of acarbose (**1.1**)

A similar mode of action has been suggested for the naturally occurring indolizidine alkaloids swainsonine (**1.3**, Figure 2.3) and castanospermine (**1.11**).



Swainsonine (1.3)



Castanospermine (1.11)

Figure 2.3 Structures of swainsonine **(1.3)** and castanospermine **(1.11)**

Sulfonium-ion mimics of the oxacarbenium ion contain a permanent positive charge, which could make strong interactions with the active-site carboxylate residues. The most interesting glycosidase inhibitors in the form of cyclic sulfonium ions are perhaps the naturally occurring compounds salacinol (**1.21**, Figure 2.4) and kotalanol (**1.22**).[51,50] The α-glucosidase inhibitory activity of salacinol was confirmed to be as strong as that of acarbose, which is used clinically.[33] The zwitterionic structure of salacinol (**1.21**) and kotalanol (**1.22**) is unique in that a negative charge is positioned at the sulfate group and the positive charge is centered at the sulfur atom.[51] Molecular dynamics simulations have shown that enzyme charge distribution plays an important role in guiding charged ligands to the active site of *Torpedo californica* acetylcholinesterase.[113] Zhou *et al.* have shown that the electrostatic potential within the active site can be used to predict the electrostatic rate-enhancement for acetylcholinesterase-substrate binding.[114] Of note, zwitterionic inhibitors have been predicted to bind to a neuraminidase enzyme more effectively than the singly charged, anionic species.[115] Thus, the zwitterionic salacinol should be quite different from conventional glycosidase inhibitors which mimic just the positive charge of the transition-state. Structural modification of salacinol represents a promising approach in the search for new glycosidase inhibitors. One strategy is to replace the sulfur atom in

salacinol with a nitrogen atom, and we have reported the synthesis of nitrogen analogues (**2.3** and **2.4**, Figure 2.5) of salacinol and their evaluation as glycosidase inhibitors.[116] Compound **2.3** also selectively inhibits the lysosomal α-glucosidases.[117]

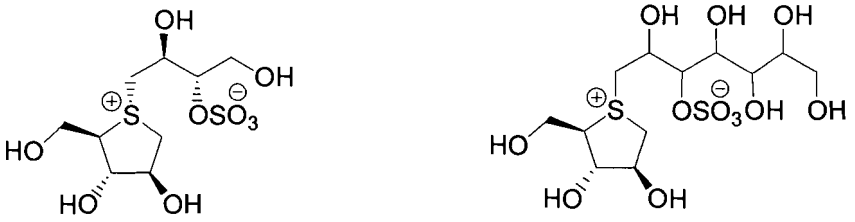


Figure 2.4 Structures of salacinol **(1.21)** and kotalanol **(1.22)**.

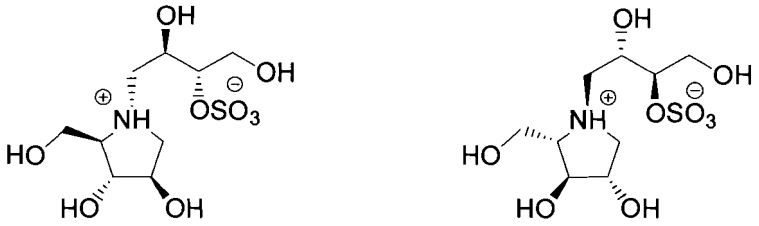
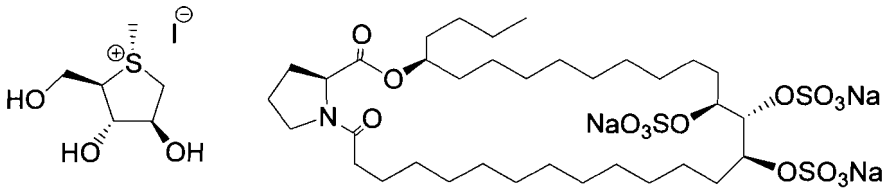


Figure 2.5 Structures of compounds **2.3** and **2.4**.

The fact that salacinol has greater inhibitory activity and specificity against α-glucosidases than the methyl sulfonium ion (**2.5**, Figure 2.6) indicates that the sulfate group is important.[118] Yuasa *et al.*[119] reported that docking of salacinol into the binding site of glucoamylase indicated close contacts between the sulfate ion with Arg305. Crystallographic analysis of the interactions of *Drosophila melanogaster* Golgi α-mannosidase II (dGMII) with salacinol and its analogues shows that the sulfate group

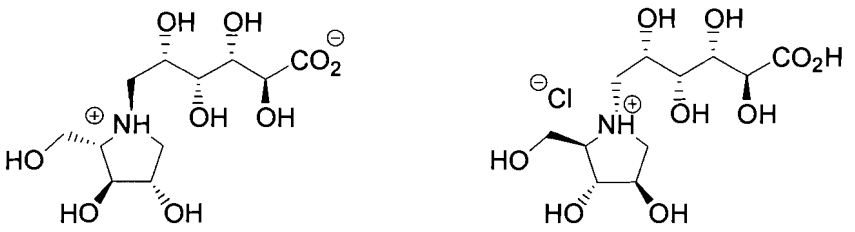
does interact with residues in the enzyme active site.[68] Compound **2.6**, isolated from a marine sponge in Japan, was also reported to be a strong inhibitor of α-glucosidase.[120] The sulfate groups in compound **2.6** may play a role similar to that proposed for the sulfate group of salacinol.[119]



2.5 **2.6**

Figure 2.6 Structures of compounds **2.5** and **2.6**.

An intriguing question is whether the corresponding carboxylate analogues of salacinol will act as inhibitors of glucosidases. We now report the synthesis of novel amino acids that are nitrogen analogues (**2.7** and **2.8**) of salacinol containing a carboxylate inner salt (Figure 2.7).



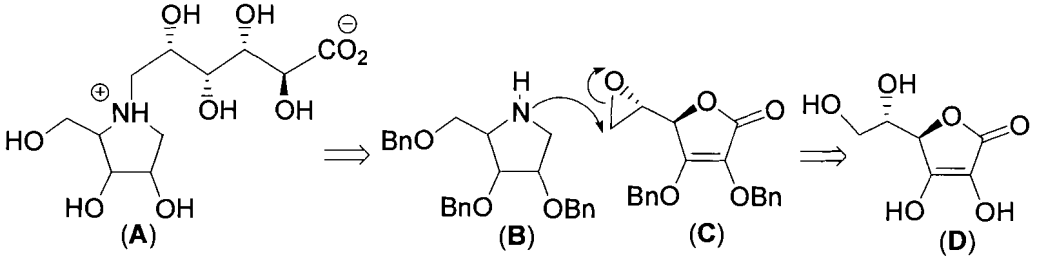
2.7 **2.8**

Figure 2.7 Structures of compounds **2.7** and **2.8**.

2.2 Results and Discussion

2.2.1 Synthesis

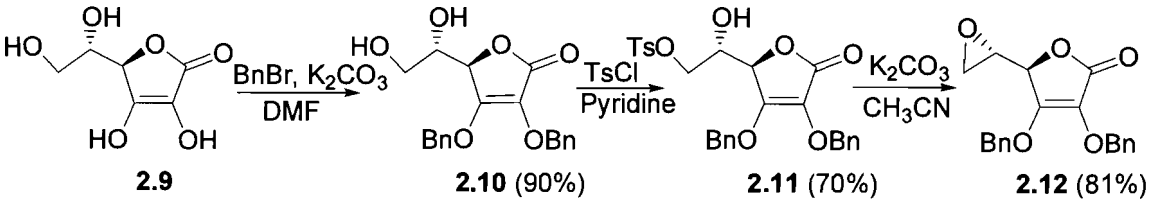
Retrosynthetic analysis indicated that amino acids **A** could be obtained by alkylation of the iminoarabinitols **B** at the nitrogen atom (Scheme 2.1). The alkylating agent could be an epoxide **C**, whereby regioselective attack of the amine at the least hindered primary center should afford the desired amino acids.[121] The epoxide **C** could be synthesized from inexpensive vitamin C (**D**).



Scheme 2.1 Retrosynthetic analysis of compounds **2.7** and **2.8**.

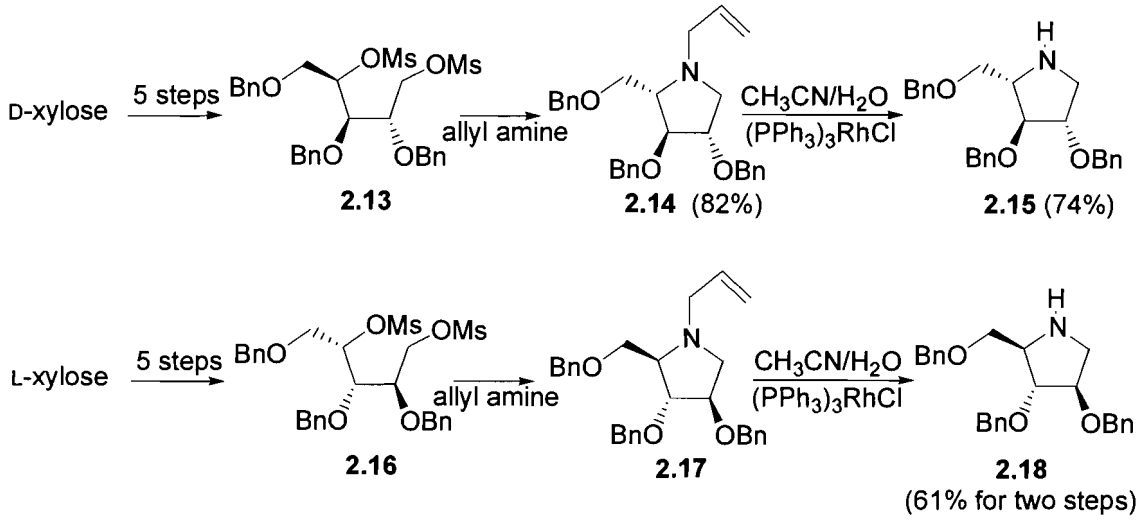
The epoxide **2.12** was synthesized using a simplified procedure of Raic-Malic *et al.* (Scheme 2.2).[122] The iminoarabinitols **2.15** and **2.18** were synthesized from D-xylose

and L-xylose, respectively, following a similar strategy that was used previously in our laboratory (Scheme 2.3).[123-125]



2.9 **2.10** (90%) **2.11** (70%) **2.12** (81%)

Scheme 2.2 Synthesis of compound **2.12**.



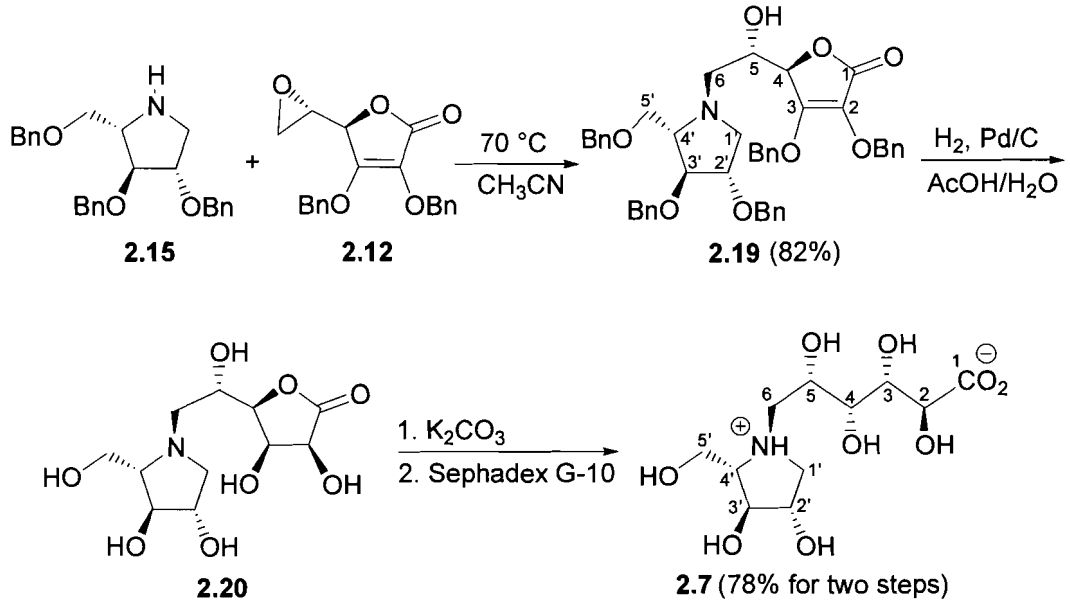
2.13 **2.14** (82%) **2.15** (74%)

2.16 **2.17** **2.18** (61% for two steps)

Scheme 2.3 Synthesis of compound **2.15** and **2.18**.

Coupling of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-L-arabinitol **2.15** with the benzyl-protected L-ascorbic acid epoxide **2.12**[122] in dry acetonitrile at 70 °C gave the protected compound **2.19** in 82% yield (Scheme 2.4). No side products were obtained. Debenzylation of the coupled product **2.19** by hydrogenolysis and subsequent stereoselective catalytic reduction of the C4'-C5' double bond of the L-ascorbic acid

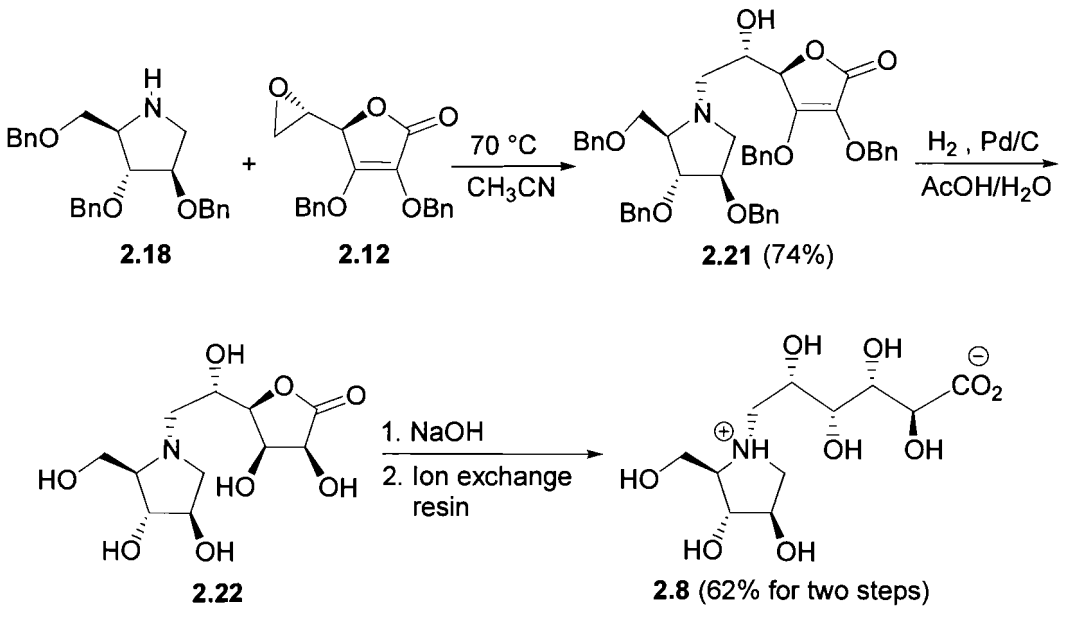
moiety, using a widely employed procedure,[126-128] afforded compound **2.20**. Catalytic hydrogenation of the L-ascorbic acid was reported to proceed with complete diastereoselection.[126,127] The reduction of the double bond in **2.19** was monitored by MALDI-TOF mass spectrometry as a hydrogen chloride salt. Even though a high pressure of hydrogen was used, the reduction was complete only after 4 days. Without further purification, the crude compound **2.20** was treated with aqueous potassium carbonate. After hydrolysis of the lactone ring in compound **2.20** and neutralization of potassium carbonate with acid, the resulting inorganic salts were removed using Sephadex G-10 chromatography to yield compound **2.7**. The overall yield for the two steps was 78%. The structure of the zwitterion **2.7** was confirmed by MALDI-TOF mass spectrometry, microanalysis data, as well as ^{1}H and ^{13}C NMR spectroscopy.



Scheme 2.4 Synthesis of compound **2.7**.

Compound **2.8**, the diastereomer of compound **2.7**, was similarly obtained by reaction of the amine **2.18** with the epoxide **2.12** to produce the protected compound **2.21** in 74% yield (Scheme 2.5). Deprotection, stereoselective catalytic reduction, as well as hydrolysis, and exchange of Na^+ ion with excess cation exchange resin gave compound **2.8** in 62% yield. In this case, the compound was obtained as a chloride salt, as confirmed by MALDI-TOF mass spectrometry, microanalysis data, as well as ^1H and ^{13}C NMR spectroscopy.

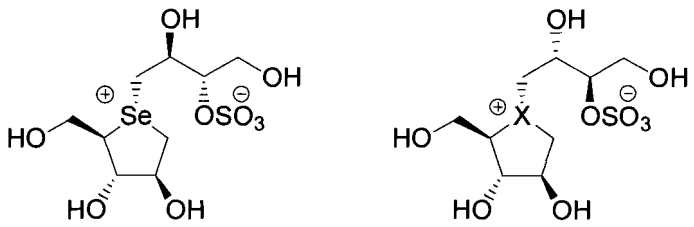
NMR spectra were performed on samples of compounds **2.7** and **2.8** in deuterated water, made basic with small amounts of sodium deuteroxide to give the corresponding amines, to ensure the peaks were more defined. By analogy with previous work done with related compounds in our laboratory,[57,116,124] we believe that the broadening of the peaks in the absence of base is due to chemical exchange between the ammonium salts and the corresponding tertiary amines, a process that is in the intermediate-exchange regime on the NMR time scale. In the presence of base, only the rapidly inverting tertiary amine is present and a conformationally averaged NMR spectrum in the fast-exchange regime is observed.



Scheme 2.5 Synthesis of compound **2.8**.

2.2.2 Enzyme Inhibitory Activity

The enzyme inhibitory activity of the amino acids **2.7** and **2.8** was measured. Compound **2.8** inhibits recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of glucose into glucose itself, with a K_i value of 21 μM. Salacinol itself has a K_i value of 0.2 μM. Compound **2.8** is also active against *Drosophila melanogaster* Golgi α-mannosidase II (dGMII) with an IC_{50} of 0.3 mM. This is a significant improvement (25-fold) over the inhibition measured for other salacinol analogues which all inhibited dGMII with an IC_{50} of approximately 7.5 mM (**1.21**, **2.3**, **2.23-2.25**) (Figure 2.4, 2.5 and 2.8).[68] Compound **2.7** is not active on either enzyme.



2.23 **2.24 X = S** **2.25 X = Se**

Figure 2.8 Structures of compounds **2.23 – 2.25**.

2.2.3 X-ray Crystallography

The crystal structure of compound **2.8** bound in the active site of dGMII was solved by Dr. D. A. Kuntz. Statistics for data collection and refinement are presented below (Table 2.1). The electron density of the bound compound **2.8** is also shown (Figure 2.9). Close interactions (with a distance of less than 3.2 Å) are highlighted (Figure 2.10) and the corresponding distances in comparison with the nanomolar inhibitor swainsonine are detailed (Table 2.2). Similar to the other salacinol analogs (and in contrast to most other inhibitors bound to dGMII) only a single hydroxyl group (OH2) interacts with the active site zinc atom. Also, as seen in other salacinol analogues, Tryptophan95 stacks on top of the ring portion of **2.8** and the ring hydroxyl groups form hydrogen bonds with aspartate residues (D92, D204, and D472 with OH1 and D472 with OH2) as well as tyrosine (Y727 OH with OH2). The C6 OH forms hydrogen bonds with the carbonyl oxygen of R876 as well as a bound water molecule. The residue D204 makes a hydrogen bond with the nitrogen atom of compound **2.8**. In contrast to D-arabinitol analogues (**1.21**, **2.3**, **2.8**, **2.23-2.25**), whose cyclic parts fit the active site of dGMII, L-arabinitol analogue **2.7** is not recognized by the enzyme.

Table 2.1 Statistics for data collection and refinement

PDB code/HET symbol	**2FYV**/Cmpd **2.8**
Space Group	$P2_12_12_1$
Cell dimensions (Å)	68.81X108.64X137.38
Data Collection (values in parenthesis represent high resolution shell)	
Resolution (Å)	30-1.90 (1.95-1.90)
Unique Reflections/Redundancy	81152/5.7 (5388/2.7)
I/sigma I	12.4 (2.5)
% Completeness	99.6 (95.4)
R merge	0.092 (0.41)
Wilson B ($Å^2$)	13.3
Structure Refinement	
R_{workt}/R_{free} (reflections for R_{free})	0.162/0.212 (1848)
Amino Acids/Alternate Conformers	1044/10
Water Molecules/HeteroAtoms	1048/49
rmsd bonds (Å)/rmsd angles (°)	0.2/1.9
Average B Factors ($Å^2$)	
Overall	16.8
Protein Main Chain/Side Chain	14.5/16.4
Water	26.7
Inhibitor(range)	25.6(10-44)
Zn/MPD/PO_4	10.8/21.4/42.9

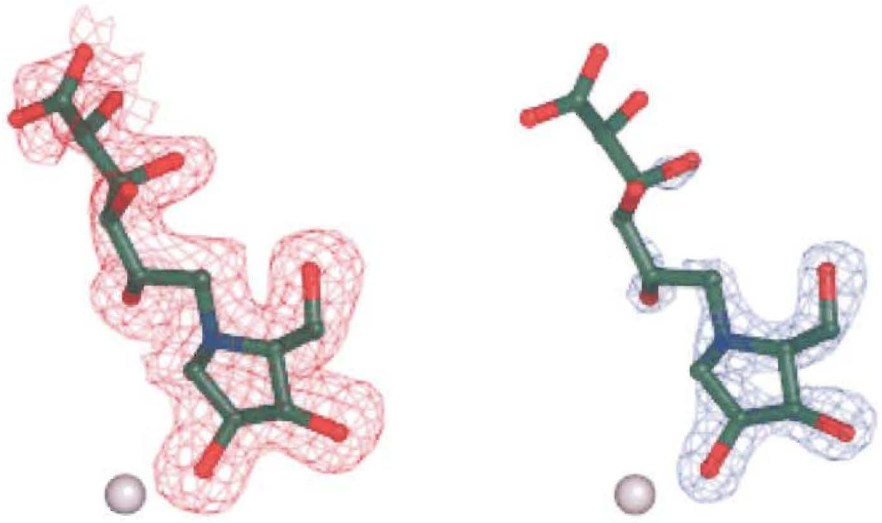


Figure 2.9 Stereoview of compound **2.8** in the active site of *Drosophila melanogaster Golgi* mannosidase II and its surrounding electron density. The electron density was determined as a simulated annealing omit map (Fo-Fc) and is contoured at 2 sigma (red) or 5 sigma (blue). The active site zinc ion is shown in grey.

It is in the acyclic chain of compound **2.8** that the interactions of the inhibitor exhibit the most significant differences from the other salacinol analogues, and it is these novel interactions which may account for the increased potency of compound **2.8** in comparison to its parent compound **2.3**. The hydroxyl groups of this chain form extensive contacts with both side-chains and water molecules in the active site. The electron density in this region is more ill-defined than that of the ring region and this indicates that there is mobility of the chain. Flexibility of this region is also reflected in the temperature (B) factors which are a measure of atomic mobility. B-factors in the ring region are in the range of 10-15 $Å^2$ (the zinc bound OH1 is below 10 $Å^2$) while those in the tail region approach 44 $Å^2$.

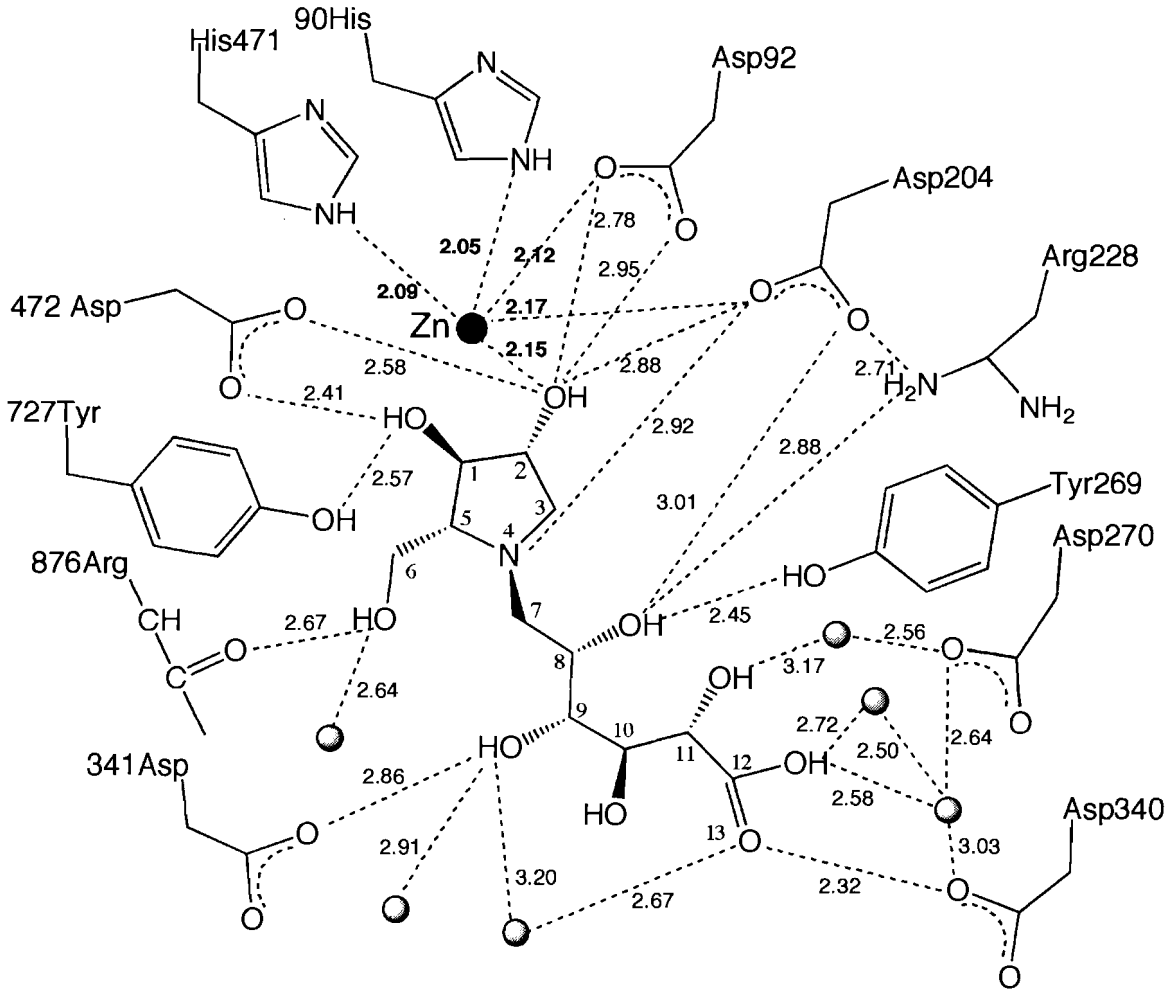


Figure 2.10 Interactions of compound **2.8** with *Drosophila* GMII. Only interactions less than 3.2 Å are indicated. The zinc ion in the active site is depicted as a black ball and water molecules are shown as grey spheres. Distances are given in Angstrom units. Numbering of the inhibitor is as it occurs in the PDB file.

Hydrogen bonds in the acyclic region occur between O8 and the catalytic nucleophile D204, as well as R228 and Y269. O9 interacts with the acid-base catalyst residue D341 and two water molecules. O11 hydrogen bonds to one water while O12 makes close contacts with two water molecules. The carbonyl O13 interacts with D340

and 2 water molecules, one of which is shared with O9 and the other shared with O12, D340 and D270.

Table 2.2 Comparison of interatomic distances of swainsonine (**1.3**) and compound **2.8** in *Drosophila melanogaster* Golgi mannosidase II

	Swainsonine		**Compound 2.8**	
Zinc Interactions				
Protein Atom	**Inhibitor Atom**	**Distance(Å)**		**Distance(Å)**
H90 NE2		2.1		2.05
D92 OD1		2.24		2.12
D204 OD2		2.17		2.17
H471 NE2		2.09		2.09
	OH-1	2.2	OH-2	2.15
	OH-2	2.13	----	
Protein-Ligand Interactions				
Protein Atom	**Inhibitor Atom**	**Distance (Å)**	**Inhibitor Atom**	**Distance (Å)**
A) IN COMMON WITH SWAINSONINE				
H90 NE2	OH-2	2.97	----	
D92 OD1	OH-1	3.04	OH-2	2.78
D92 OD2	OH-2	2.54	OH-2	2.95
D204 OD2	OH-1	2.83	OH-2	2.88
	OH-2	2.97	-----	
	N-4	2.75	N-4	2.92
H471 NE2	OH-1	3.12	----	
D472 OD1	OH-8	2.56	OH-1	2.52
D472 OD2	OH-1	2.59	OH-2	2.55
Y727 OH	OH-8	2.64	OH-1	2.57
B) NOT SHARED WITH SWAINSONINE				
R228 NH2			OH-8	2.88
Y269 OH			OH-8	2.45
D340 OD1			O13	2.32
D341 OD2			OH-9	2.86
R876 O			OH-6	2.67
WATERS			OH-6	2.64
			OH-9	2.91, 3.20
			OH-11	3.17
			OH-12	2.58, 2.72
			O-13	2.67

In Figure 2.11, an overlay of **2.8** (this work) and swainsonine (**1.3**) (from PDB 1HWW) bound in the crystal structure of dGMII is shown. Although the reason for the potency of swainsonine has not been clearly determined, it is one of the best inhibitors of dGMII, with an IC_{50} value in the range of 20-40 nM, and is believed to closely mimic the oxacarbenium ion which occurs on the reaction pathway. The position of the nitrogen moiety, which is designed to serve as the mimic of the positive charge on the oxacarbenium ion, is almost identical in the two bound structures. However, in contrast with swainsonine, only a single hydroxyl group of compound **2.8** is in contact with the active site zinc ion. As well, the orientation of the second hydroxyl group, which forms hydrogen bonds with D472 and Y727, differs in the two structures, and it is possible that this geometry is not ideal for forming strong interactions. While the position of the head group of compound **2.8** is comparable to the other salacinol analogues[68] the region of space occupied by the acyclic tail region is quite different. In Figure 2.11, overlays of compound **2.8** bound in the crystal structure of dGMII with bound ghavamiol **2.3** (Fig. 2.11, B) or the diastereomer of salacinol **2.24** (Fig. 2.11, C) solved previously (PDB 1TQU and 1TQT[68]) are shown. In both cases the position of the sulfate group is quite different from the carboxyl group, and the space through which the aliphatic chain passes is also quite different. Interestingly, the region of the active site through which the aliphatic chain of compound **2.8** passes is very comparable to that of a recently solved benzyl-mannostatin A:dGMII complex.[31] The overlay of the two complexes (Fig 2.11D) shows them to intertwine, although the nature of the interactions formed by the two tail moieties is different. The benzyl tail reduced the potency of the mannostatin to which it was attached[129] while in the present case the carboxylate tail greatly increased the

inhibitory activity of the salacinol head group. Given the much better inhibitory properties of the mannostatin derivatives (nM) in comparison to the salacinol derivatives (mM) it is possible that attachment of a similar carboxylate tail to a mannostatin head group would lead to a very effective mannosidase inhibitor.

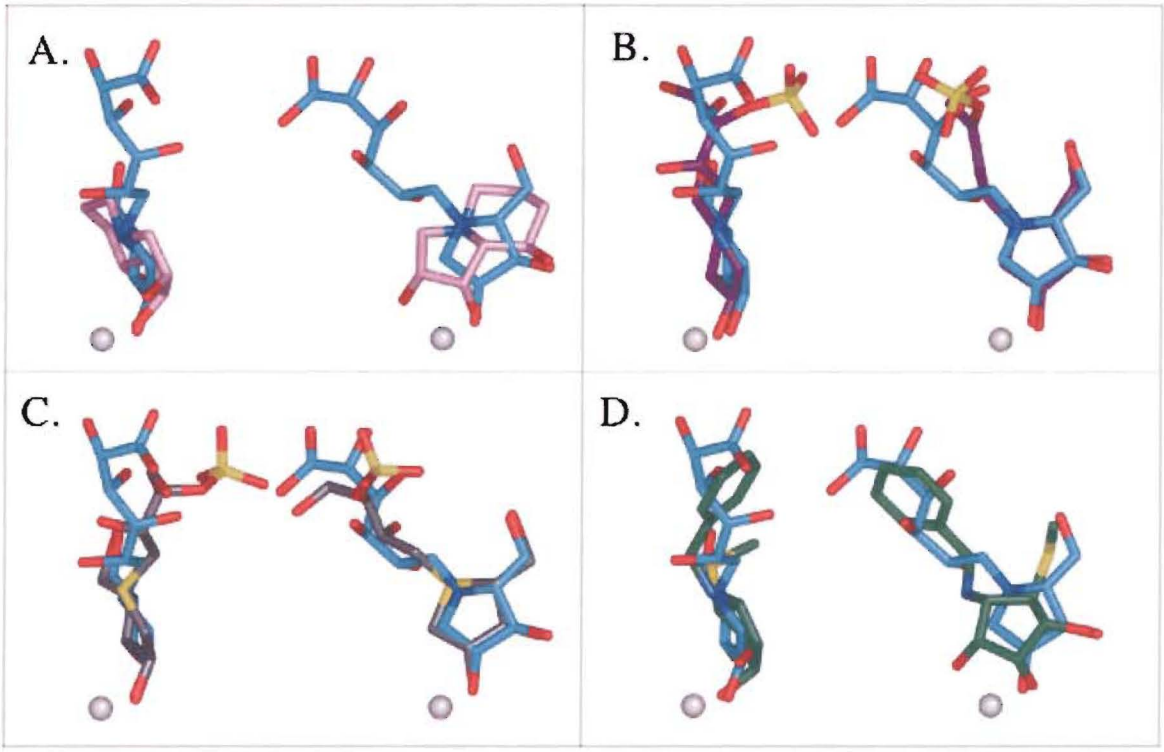


Figure 2.11 Overlay of compounds bound in the active site of dGMII. Compound **2.8** (cyan) is overlayed with A. Swainsonine (pink, PDB 1HWW) B. Ghavamiol **2.3** (magenta, PDB 1TQU) C. Salacinol diastereomer **2.24** (grey, PDB 1TQT) or D. *N*-Benzyl mannostatin (green, PDB 2F7P).

2.3 Experimental Section

2.3.1 General

Optical rotations were measured at 23 °C on an Autopol II automatic polarimeter. Analytical thin-layer chromatography (TLC) was performed on aluminum plates

precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4, and heated. Compounds were purified by flash chromatography on Kieselgel 60 (230-400 mesh). ^1H and ^{13}C NMR spectra were recorded on the following: Bruker AMX-400 NMR spectrometer at 400.13 MHz, Bruker AMX-600 NMR spectrometer at 600.13 MHz, and Varian INOVA 500 NMR spectrometer at 499.97 MHz. Chemical shifts are given in ppm downfield from TMS for those spectra measured in $CDCl_3$ and CD_3OD and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. Assignments were fully supported by two-dimensional $^1H,^1H$ (COSY) and $^1H,^{13}C$ (HMQC) experiments using standard Bruker or Varian pulse programs. Processing of the spectra was performed with standard UXNMR and WINNMR software (Bruker) or MestReC software (Varian). MALDI mass spectra were obtained on a PerSeptive Biosystems, Voyager DE time-of-flight spectrometer for samples dispersed in a 2,5-dihydroxybenzoic acid matrix. High resolution mass spectra were obtained by the electrospray ionization (ESI) technique, using a TOF mass spectrometer at 10000 RP.

2.3.2 **Enzyme Activity Assays** (Performed by Lyann Sim and Dr. D. A. Kuntz)

Measurement of dGMII inhibition was carried out as outlined previously.[68] Analysis of recombinant MGA inhibition and determination of the kinetic constants for competitive inhibition have been described.[68] Briefly, analysis of MGA inhibition was performed using maltose as the substrate and measuring the release of glucose. Reactions were carried out in 100mM MES buffer pH 6.5 at 37°C. The reaction was stopped by

boiling for 3 min. 20 μL aliquots were taken and added to 100 μL of glucose oxidase assay reagent (Sigma) in a 96-well plate. Reactions were developed for 1 hour and absorbance was measured at 450 nm to determine the amount of glucose produced by MGA activity in the reaction. All reactions were performed in triplicate and absorbance measurements were averaged to give a final result.

2.3.3 **Enzyme Kinetic** (Performed by Lyann Sim)

Kinetic parameters of recombinant MGA were determined using the glucose oxidase assay to follow the production of glucose upon addition of enzyme (15 nM) at increasing maltose concentrations (from 2.5 mM to 30 mM) with a reaction time of 15 minutes. The K_i value was determined by measuring the rate of maltose hydrolysis by MGA at varying inhibitor concentrations. Data were plotted in Lineweaver-Burk plots (1/rate vs. 1/[substrate]) and the K_i value was determined by the equation $K_i = K_m[I]/(V_{max})s- K_m$, where "s" is the slope of the line. The K_i reported was an average of the K_i values obtained from each of the different inhibitor concentrations.

2.3.4 **Structure Determination of dGMII-2.8 Complex** (Performed by Dr. D. A. Kuntz)

Preparation of dGMII crystals soaked with compound **2.8** was carried out essentially as described previously.[68] In this case however, the crystals were first washed with reservoir buffer containing phosphate instead of Tris, to reduce any effects of Tris binding in the active site. The crystals were soaked for 24 hours with a 2 mM solution of compound **2.8** in phosphate containing reservoir buffer. The crystals were passed through phosphate buffered cryo-solutions containing 1mM inhibitor prior to rapid freezing in a liquid nitrogen stream. X-ray diffraction data was collected at 100 K with a

Bruker X8 Proteum system consisting of a CCD detector and a Bruker Microstar rotating anode generator. Data were integrated and scaled using the Proteum suite of programs (Bruker AXS, Madison WI). Structure solution and refinement were carried out using the programs CNS[130] and O[131] as previously described.[68] Diagrams were rendered in Pymol (http://pymol.sourceforge.net).

2.3.5 Synthesis

***N*-Allyl-2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-L-arabinitol (2.14), *N*-allyl-2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (2.15), 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-L-arabinitol (2.17)** and **2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (2.18)** were synthesized according to the original procedures given in the literature.[123-125]

6-((2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-imino-L-arabinitol)-4-*N*-yl)-2,3-di-*O*-benzyl-6-deoxy-L-ascorbic acid (2.19).

A mixture of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-L-arabinitol **2.15** (404 mg, 1.00 mmol) and 5,6-anhydro-2,3-di-*O*-benzyl-L-ascorbic acid **2.12** (340 mg, 1.00 mmol) were dissolved in dry CH_3CN (5 mL). The mixture was stirred in a round bottom flask in an oil-bath (70 °C) overnight. The solvent was removed under reduced pressure and the product was purified by column chromatography (hexanes-EtOAc, 3:1) to afford compound **2.19** (612 mg, 82%) as a yellow oil. $[\alpha]_D$ +23 (*c* 0.6, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.11-7.30 (25H, m, Ar), 5.12 and 5.04 (2H, 2d, $J_{A,B}$ = 11.8 Hz, C=C-OCH_2Ph), 5.01 and 4.98 (2H, 2d, $J_{A,B}$ = 11.3 Hz, C=C-OCH_2Ph), 4.57 (1H, d, $J_{4,5}$ = 1.5 Hz, H-4), 4.44 and 4.42 (2H, 2d, $J_{A,B}$ = 6.3 Hz, CH_2Ph), 4.39 and 4.36 (2H, 2d, $J_{A,B}$ = 9.4

Hz, CH_2Ph), 4.35 and 4.33 (2H, 2d, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 3.89 (1H, ddd, $J_{1'a,2'}$ = 1.6 Hz, $J_{1'b,2'}$ = 5.5 Hz, $J_{2',3'}$ = 1.8 Hz, H-2'), 3.84 (1H, ddd, $J_{5,6a}$ = 8.0 Hz, $J_{5,6b}$ = 6.0 Hz, H-5), 3.76 (1H, dd, $J_{3',4'}$ = 3.9 Hz, H-3'), 3.46 (1H, dd, $J_{4',5'a}$ = 5.9 Hz, $J_{5'a,5'b}$ = 9.8 Hz, H-5'a), 3.43 (1H, dd, $J_{4',5'b}$ = 5.9 Hz, H-5'b), 3.09 (1H, dd, $J_{1'a,1'b}$ = 10.5 Hz, H-1'a), 2.88 (1H, dd, $J_{6a,6b,}$ = 13.0 Hz, H-6a), 2.84 (1H, dt, H-4'), 2.77 (1H, dd, H-1'b), 2.73 (1H, dd, H-6b). ^{13}C NMR ($CDCl_3$): δ 169.5 (C-1), 157.4 (C-3), 137.8, 137.7, 137.7, 135.8, 135.3 (5C$_{ipso}$), 128.7-127.2 (25C, Ar), 120.6 (C-2), 84.5 (C-3'), 81.6 (C-2'), 75.5 (C-4), 73.6 and 72.9 (2C=C-O*C*H$_2$Ph), 72.8, 71.0, 70.7 (3*C*H$_2$Ph), 70.3 (C-5'), 68.9 (C-4'), 66.9 (C-5), 58.0 (C-1'), 56.69 (C-6). MALDI-TOF MS: *m/e* 763.85 (M^+ + Na), 742.075 (M^+ + H). Anal. calcd. For $C_{46}H_{47}O_8N$: C 74.43, H 6.38, N 1.89; found: C 74.20, H 6.35, N 2.14.

6-((1,4-Dideoxy-1,4-imino-L-arabinitol)-4-*N*-ammonium)-6-deoxy-L-gulonate (2.7).

The protected compound **2.19** (300 mg, 0.400 mmol) was dissolved in AcOH-H_2O (4:1, 6 mL) and stirred with Pd/C (30 mg) under H_2 (70 psi). After 4 days, the reaction mixture was filtered through cotton, which was subsequently washed with H_2O. The combined filtrates were concentrated under vacuum. Concentrated hydrochloric acid (1 mL) was added and the mixture was concentrated by high vacuum. The obtained solid was dissolved in 1 mL H_2O, pH was adjusted to 9.0 by adding solid K_2CO_3 and the mixture was stirred for 3 h. The solution was neutralized with dilute hydrochloric acid and then concentrated. The residue was purified by Sephadex G-10 chromatography column to give compound **2.7** as a colorless oil (104 mg, 78%). IR (neat) 3209, 1610, 1412, 1073, 765 cm^{-1}. $[\alpha]_D$ +10 (*c* 0.1, H_2O). ^{1}H NMR (D_2O, pH = 12.0): δ 4.03 (1H, d, $J_{2,3}$ = 4.5 Hz, H-2), 3.98 (1H, brd, H-2'), 3.83 (1H, d, H-3), 3.79 (2H, brd, H-3', H-5), 3.64 (1H, m, H-4), 3.61 (2H, m, 2xH-5'), 2.99 (1H, d, $J_{1'a,1'b}$ = 11.1 Hz, H-1'a), 2.91 (1H,

dd, $J_{6a,6b}$ = 12.8 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a), 2.73 (1H, dd, $J_{1'b,2'}$ = 5.0 Hz, H-1'b), 2.47 (1H, m, H-4'), 2.43 (1H, dd, $J_{5,6b}$ = 7.1 Hz, H-6b). ^{13}C NMR (D_2O, pH = 12.0): δ 179.1 (C-1), 76.5 (C-3'), 73.3 (C-2'), 71.4 (C-2), 70.2 (C-3), 69.8 (C-4'), 69.3 (C-4), 68.3 (C-5), 58.4 (C-5'), 57.4 (C-1'), 54.9 (C-6). MALDI-TOF MS: *m/e* 334.47 (M^+ + Na), 312.43 (M^+ + H). Anal. calcd. For $C_{11}H_{21}O_9N$: C 42.44, H 6.80, N 4.50; found: C 42.19, H 6.66, N 4.36.

6-((2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol)-4-*N*-yl)-2,3-di-*O*-benzyl -6-deoxy-L-ascorbic acid (2.21).

A mixture of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol **2.18** (444 mg, 1.10 mmol) and 5,6-anhydro-2,3-di-*O*-benzyl-L-ascorbic acid **2.12** (374 mg, 1.10 mmol) were dissolved in dry CH_3CN (5 mL). The mixture was stirred in a round bottom flask in an oil-bath (70 °C) overnight. The solvent was removed under reduced pressure, and column chromatography (hexanes-EtOAc, 3:1) of the crude product gave compound **2.21** (605 mg, 74%) as a yellow oil. $[\alpha]_D$ +6 (*c* 0.7, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.13-7.30 (25H, m, Ar), 5.22 and 5.04 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C=C-OCH_2Ph), 5.11 and 5.05 (2H, 2d, $J_{A,B}$ = 11.3 Hz, C=C-OCH_2Ph), 4.51 (1H, brd, H-4), 4.50-4.40 (6H, m, 3CH_2Ph), 3.96 (1H, dt, $J_{1'a,2'}$ = 1.7 Hz, $J_{1'b,2'}$ = 5.5 Hz, H-2'), 3.88 (1H, dd, $J_{2',3'}$ = 1.8 Hz , $J_{3',4'}$ = 4.4 Hz, H-3'), 3.87 (1H, m, H-5), 3.53 (1H, dd, $J_{4',5'a}$ = 5.3 Hz, $J_{5'a,5'b}$ = 9.8 Hz, H-5'a), 3.51 (1H, dd, $J_{4',5'b}$ = 5.6 Hz, H-5'b), 3.23 (1H, d, $J_{1'a,1'b}$ = 10.4 Hz, H-1'a), 3.17 (1H, dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 10.9 Hz, H-6a), 2.88 (1H, q, H-4'), 2.64 (1H, dd, H-1'b), 2.52 (1H, dd, $J_{5,6b}$ = 3.5 Hz, H-6b). ^{13}C NMR ($CDCl_3$): δ 169.9 (C-1), 157.4 (C-3), 138.2, 138.2, 138.1, 136.3, 135.8 (5C_{ipso}), 128.9-127.6 (25C, Ar), 121.2 (C-2), 84.8 (C-3'), 82.0 (C-2'), 75.9 (C-4), 74.2 and 73.6 (2C=C-OCH_2Ph), 73.4, 71.7, 71.3 (3CH_2Ph), 70.2 (C-5'), 69.1 (C-

4'), 66.2 (C-5), 57.4 (C-1'), 57.1 (C-6). MALDI-TOF MS: *m/e* 764.03 (M^+ + Na), 741.95 (M^+ + H). Anal. calcd. For $C_{46}H_{47}O_8N$: C 74.43, H 6.38, N 1.89; found: C 74.27, H 6.39, N 2.03.

6-((1,4-Dideoxy-1,4-imino-D-arabinitol)-4-*N*-ammonium)-6-deoxy-L-gulonic acid hydro-chloride (2.8).

The protected compound **2.21** (600 mg, 0.800 mmol) was dissolved in AcOH-H_2O (4:1, 10 mL) and stirred with Pd/C (50 mg) under H_2 (70 psi). After 4 days, the reaction mixture was filtered through cotton, which was subsequently washed with H_2O. The combined filtrates were concentrated under vacuum. Concentrated hydrochloric acid (2 mL) was added and the mixture was concentrated by high vacuum. The obtained solid was dissolved in 2 mL H_2O and pH was adjusted to 9.0 by adding solid NaOH. The solution was stirred for 3 h. The solution was neutralized with dilute hydrochloric acid. Na^+ ion was removed with excess Amberlite IR-120-P (H^+ form) and the resin was removed by filtration. The aqueous solution was concentrated to give compound **2.8** as a colorless oil (165 mg, 62%). IR (neat) 3416, 1600, 1450, 1074, 770 cm^{-1}. $[\alpha]_D$ +2 (*c* 0.5, H_2O). ^1H NMR (D_2O, pH = 12.0): δ 3.94 (1H, d, $J_{2,3}$ = 4.8 Hz, H-2), 3.89 (1H, m, H-2'), 3.75–3.71 (3H, m, H-3, H-3', H-5), 3.57-3.52 (2H, m, 2H-5'), 3.50 (1H, m, H-4), 2.90 (1H, dd, $J_{1'a,1'b}$ = 10.4 Hz, $J_{1'a,2'}$ = 1.1 Hz, H-1'a), 2.76 (1H, dd, $J_{6a,6b}$ = 13.0 Hz, $J_{5,6a}$ = 9.4 Hz, H-6a), 2.57 (1H, dd, $J_{1'b,2'}$ = 5.6 Hz, H-1'b), 2.36 (1H, m, H-6b), 2.33 (1H, m, H-4'); ^{13}C NMR (D_2O, pH = 12.0): δ 179.0 (C-1), 79.2 (C-3'), 75.9 (C-2'), 74.1 (C-2), 72.7 (C-3), 72.3 (C-4', C-4), 70.0 (C-5), 60.7 (C-5'), 58.99 (C-1'), 57.5 (C-6). MALDI-TOF MS: *m/e* 334.52 (M^+ + Na), 312.32 (M^+ + H). Anal. calcd. For $C_{11}H_{22}O_9NCl$: C 38.00, H 6.37, N 4.03; found: C 38.15, H 6.45, N 3.86.

CHAPTER 3:
SYNTHESIS OF ANALOGUES OF SALACINOL CONTAINING A CARBOXYLATE INNER SALT AND THEIR INHIBITORY ACTIVITIES AGAINST HUMAN MALTASE GLUCOAMYLASE

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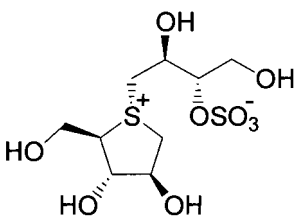
Carbohydr. Res. **2007**, *342*, 1661-1667.

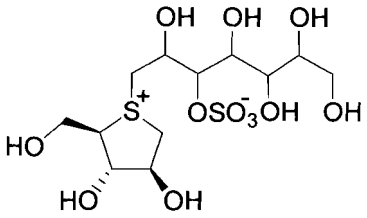
3.1 Introduction

Glycosidases are fundamentally involved in the control of several important biological phenomena such as the breakdown of oligosaccharides, cell-cell adhesion, cell growth, inflammatory processes, fertilization, and bacterial, viral and parasitic infections.[3,2,82] Since glycoside cleavage is a biologically widespread process, glycosidase inhibitors have many potential applications. The transition-state structure in the enzyme-mediated hydrolysis of glycosides is believed to resemble an oxacarbenium ion with a distorted conformation. Thus, mimicking this distorted, positively charged species is one factor that could lead to a strong inhibitor of glycosidase enzymes.[4,21,23]

The α-glucosidase inhibitors salacinol **1.21** and kotalanol **1.22** (Figure 3.1) have been isolated from *Salacia reticulata*,[51,55] and *S. oblonga* and *S. chinensis*,[52] traditionally used in the Ayurvedic system of India and Sri Lanka for the treatment of diabetes.[132] The sulfonium-ion structure of these compounds has stimulated different groups to carry out the synthesis of salacinol,[53,54] and other carbohydrate-based cyclic sulfonium compounds,[45,47,49,62,63,116,121,131,132] such as **2.5**[118] and **3.1** (Figure 3.2),[133] as a new class of glycosidase inhibitor. Our group has reported heteroanalogues of salacinol having nitrogen[116] or selenium[134] instead of sulfur, namely ghavamiol **2.3** and blintol **2.23**, respectively (Figure 3.2). We reasoned that the interaction of a permanent positive charge with active-site carboxylate residues would make a dominant contribution to the interaction energy.

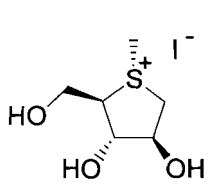


Salacinol (1.21)

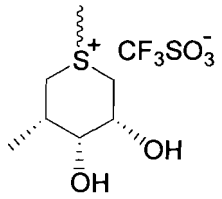


Kotalanol (1.22)

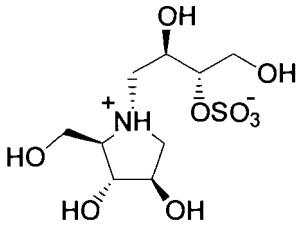
Figure 3.1 Structures of compounds salacinol (**1.21**) and kotalanol (**1.22**).



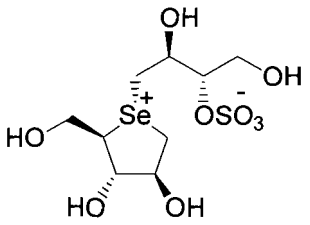
2.5



3.1



2.3



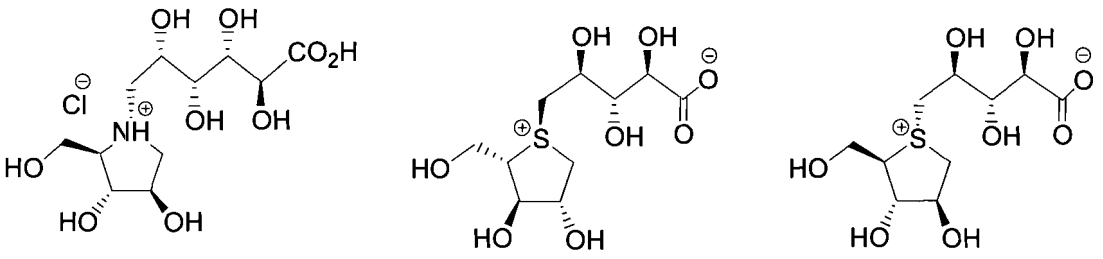
2.22

Figure 3.2 Structures of the cyclic sulfonium compounds **2.5** and **3.1**, ghavamiol (**2.3**), and blintol (**2.23**).

The zwitterionic structure of salacinol **1.21** and kotalanol **1.22** is unique in that the ring sulfonium ion is stabilized by an internal sulfate counterion.[51] Structural modification of salacinol represents a promising approach in the search for new glycosidase inhibitors. The fact that salacinol (**1.21**) has greater inhibitory activity and

specificity against α-glucosidases than the methyl sulfonium ion (**2.5**) indicates that the hydroxyl groups on the acyclic chain and/or the sulfate group are advantageous.[118] Yuasa *et al.*[119] reported that docking of salacinol (**1.21**) into the binding site of glucoamylase indicated close contacts between the sulfate ion with Arg305. Crystallographic analysis of the interactions of *Drosophila melanogaster* Golgi α-mannosidase II with salacinol (**1.21**) and its analogues also shows that the sulfate group interacts with residues in the enzyme active site.[68] However, a recent study reported that compounds lacking the sulfate group are also active.[135]

An intriguing question is whether the corresponding carboxylate analogues of salacinol will act as inhibitors of glucosidases. We have recently described a novel class of amino acids patterned after salacinol (**1.21**) that consist of an iminoarabinitol alkylated with a polyhydroxylated chain containing a carboxylate residue **2.8** (Figure 3.3).[65] This compound was found to be an inhibitor of recombinant human maltase glucoamylase, with a K_i value of 21 μM. In addition, this compound was also active against *Drosophila melanogaster* Golgi mannosidase II (dGMII), with an IC_{50} of 0.3 mM.[65] We now report a synthetic route to the corresponding sulfonium ion-inner carboxylates **3.2** and **3.3**. These compounds also serve as the first representatives of a new class of hitherto undescribed molecules.

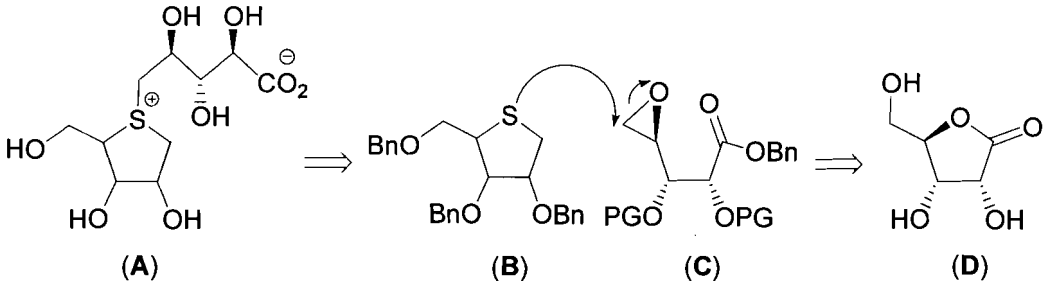


2.8 **3.2** **3.3**

Figure 3.3 Structures of the compounds **2.8**, **3.2** and **3.3**.

3.2 Results and Discussion

Retrosynthetic analysis indicated that the carboxylate analogues **A** of salacinol could be obtained by alkylation of the thioarabinitols **B** at the sulfur atom (Scheme 3.1). The alkylating agent could be an epoxide **C**, whereby regioselective attack of the sulfur at the least hindered primary center should afford the desired sulfonium ions.[136] The epoxide **C** could be synthesized, in turn, from D-ribonolactone **D**.

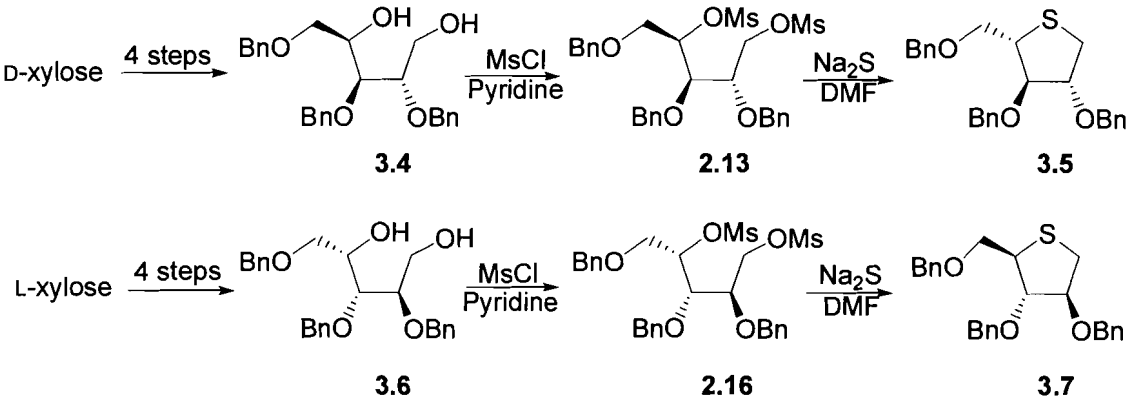


(A) **(B)** **(C)** **(D)**

Scheme 3.1 Retrosynthetic analysis of compounds **3.2** and **3.3**.

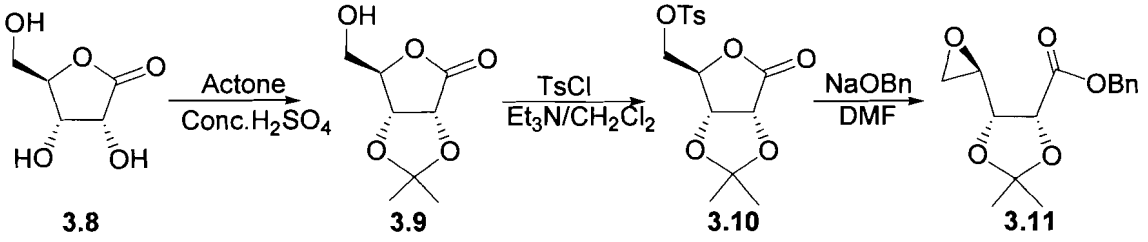
The thioarabinitol **3.5** was synthesized from D-xylose following the same strategy that was used by Satoh *et al.* (Scheme 3.2).[137] Thus, treatment of the diol **3.4** with

methanesulfonyl chloride in pyridine afforded the dimesylate **2.13** in 88% yield. Treatment of **2.13** with sodium sulfide in DMF produced compound **3.5** in 95% yield. Starting from L-xylose, the enantiomer **3.7**[138] was synthesized in an analogous fashion.



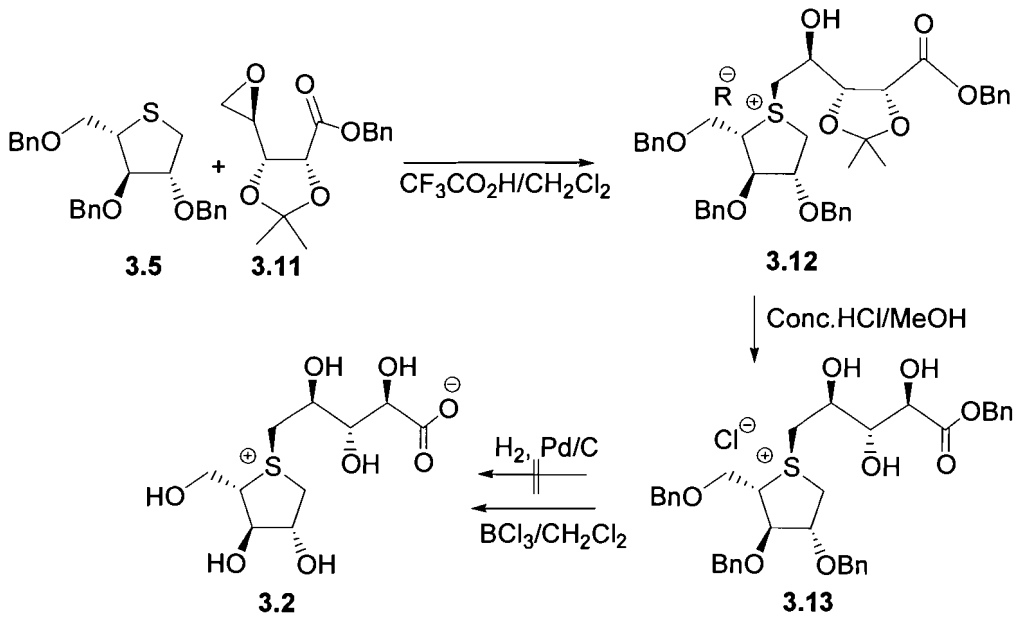
Scheme 3.2 Synthesis of compounds **3.5** and **3.7**.

With compound **3.5** in hand, we turned our attention to the synthesis of the epoxide **3.11** (Scheme 3.3). D-Ribonolactone **3.8** was converted to 2,3-*O*-isopropylidene-D-ribonolactone **3.9**[139] that was then tosylated to give compound **3.10** in 88% yield. Treatment of compound **3.10** with sodium benzylate afforded the desired epoxide **3.11**[140] in 86% yield.



Scheme 3.3 Synthesis of compound **3.11**.

The coupling reaction was examined next. Regioselective ring opening of the epoxide **3.11** by the nucleophilic attack of the sulfur atom in the thioether **3.5** occurred rapidly in a mixture of CF_3COOH and CH_2Cl_2 to give compound **3.12** in 58% yield (Scheme 3.4). The unreacted starting materials were recovered, and no other polar compound was observed on TLC. Even after purification of compound **3.12** by flash chromatography, the ^1H and ^{13}C NMR spectra showed some extra peaks which could not be ascribed to the diastereomer. We concluded that these peaks resulted either from an inseparable impurity or by the presence of a similar compound with another external negative counterion. The compound was therefore processed as follows for the purpose of characterization. Counterion exchange and deprotection of the hydroxyl groups on the side chain were achieved in a mixture of concentrated hydrochloric acid and methanol, the resulting compound **3.13** being purified by flash chromatography. Hydrogenolysis of the coupled compound **3.13** over Pd/C catalyst did not go as planned to give compound **3.2** (Scheme 3.4) because of poisoning of the catalyst. Debenzylation of the protected compound **3.13** was therefore accomplished by treatment with boron trichloride[49] in CH_2Cl_2, affording compound **3.2** in 50% yield. No other product was obtained. The stereochemistry at the sulfur atom was determined by means of a 1D-NOE experiment.

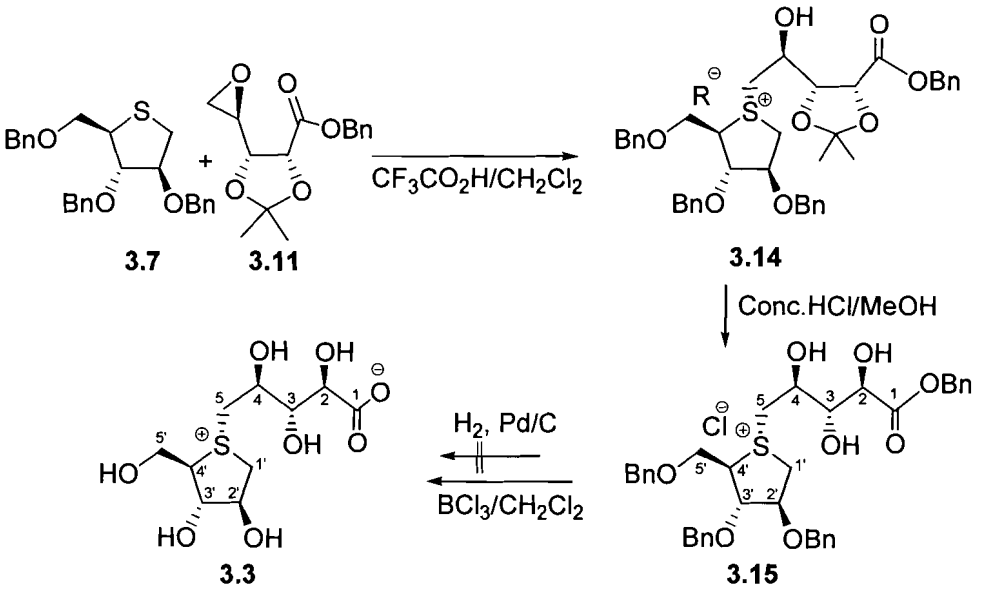


Scheme 3.4 Synthesis of compound **3.2**.

Compound **3.3**, the diastereomer of **3.2**, was similarly obtained by reaction of the thioether **3.7** with the epoxide **3.11** to produce the protected compound **3.14** in 66% yield (Scheme 3.5). Some of starting materials was recovered. No other polar compound was observed on TLC. As with compound **3.12**, the ^{1}H and ^{13}C NMR spectra showed that no diastereomer formed. Counterion exchange and deprotection of the hydroxyl groups on the side chain gave compound **3.15** in 75% yield. The stereochemistry at the sulfur atom was determined by means of a 1D-NOE experiment. Treatment of compound **3.15** with boron trichloride in CH_2Cl_2 afforded compound **3.3** in 52% yield. No other product was obtained.

Finally, we comment on the inhibitory activities (performed by Lyann Sim) of the compounds synthesized in this study against recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of

glucose into glucose itself. Only compound **3.3**, with the D-arabinitol configuration in the heterocyclic ring displayed by salacinol, was active, with a K_i value of 10 ± 1 μM. Salacinol itself has a K_i value of 0.19 ± 0.02 μM.[141]



Scheme 3.5 Synthesis of compound **3.3**.

3.3 Experimental Section

3.3.1 Synthesis

2, 3-*O*-Isopropylidene-D-ribonolactone (3.9)[139]

To a suspension of D-ribonolactone **3.8** (10.0 g, 68.0 mmol) in acetone (200 mL) was added concentrated sulfuric acid (4 mL) dropwise while the solution was cooled in an ice bath. The starting material dissolved in 5 min. The mixture was stirred for 12 h at room temperature. Ammonia gas was passed through the ice-cooled solution. The resulting white solid was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (1:3 hexanes-

EtOAc) to afford **3.9** as a white solid (10.6 g, 80%): mp 134-137 °C; lit. mp 135-138 °C.[139]

2, 3-*O*-Isopropylidene-5-*O*-*p*-toluenesulfonyl-D-ribonolactone (3.10)[140]

To a solution of compound **3.9** (1.00 g, 5.30 mmol) in CH_2Cl_2 (25 mL) was added Et_3N (0.8 mL, 5.8 mmol). After 5 min, tosyl chloride (1.20 g, 6.36 mmol) was added in portions at 0 °C and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was poured into water (40 mL) and CH_2Cl_2 (30 mL). The organic phase was dried (Na_2SO_4) and concentrated on a rotary evaporator. The product was purified by flash chromatography (3:1 hexanes-EtOAc) to afford compound **3.10** as a white solid (1.6 g, 88%): mp 111-114 °C; lit. mp 117-118 °C.[140] H NMR ($CDCl_3$): δ 7.77-7.37 (4H, 2d, $J_{A,B}$ = 8.2 Hz, Ar), 4.77 (1H, d, $J_{2,3}$ = 5.6 Hz, H-2), 4.75 (1H, d, H-3), 4.68 (1H, dd, H-4), 4.34 (1H, dd, $J_{4,5b}$ = 1.9 Hz, $J_{5a,5b}$ = 11.2 Hz, H-5b), 4.18 (1H, dd, $J_{4,5a}$ = 2.5 Hz, H-5a), 2.47 (3H, s, CH_3Ph), 1.46 and 1.39 (6H, 2s, $C(CH_3)_2$).

4, 5-Anhydro-2, 3-*O*-isopropylidene-D-ribonic acid benzyl ester (3.11)[140]

A solution of sodium benzylate prepared from benzyl alcohol (710 mg, 6.57 mmol) and NaH (60.0 mg, 1.50 mmol) in DMF (7.1 mL) was added to compound **3.10** (0.500 g, 1.46 mmol) in DMF (1.1 mL) at 0 °C. The reaction mixture was stirred for 1 h and the solvent was removed under high vacuum. The white solid was filtered and the filtrate was concentrated. The crude product was purified by column chromatography (5:1 hexanes-EtOAc) to afford **3.11** as a colorless oil (350 mg, 86%). ^{1}H NMR (CD_2Cl_2): δ 7.42-7.37 (5H, m, Ar), 5.22 and 5.17 (2H, 2d, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.80 (1H, d, $J_{2,3}$

= 6.92 Hz, H-2), 4.11 (1H, dd, H-3), 2.93 (1H, ddd, $J_{3,4}$ = 6.19 Hz, $J_{4,5a}$ = 3.90 Hz, $J_{4,5b}$ = 2.55 Hz, H-4), 2.64 (1H, dd, $J_{5a,5b}$ = 5.21 Hz, H-5b), 2.61 (1H, dd, H-5a), 1.58 and 1.39 (6H, 2s, $C(CH_3)_2$).

Benzyl-5-(2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-episulfoniumylidene-L-arabinitol)-5-deoxy-D-ribonate chloride (3.13)

A mixture of compound **3.11** (130 mg, 0.470 mmol) and compound **3.5** (196 mg, 0.470 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and CF_3CO_2H (53.0 mg, 0.470 mmol) was added. The mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and column chromatography (EtOAc-MeOH-H_2O, 40:1:1) of the crude product gave compound **3.12** as a colorless oil (190 mg, 58%) that was reacted as follows. MALDI-TOF MS: *m/e* 699.42 (M^+). Without further purification, compound **3.12** (190 mg, 0.270 mmol) was dissolved in a mixture of concentrated HCl (1 mL) and methanol (40 mL). The mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and column chromatography (8:1 CH_2Cl_2-MeOH) of the crude product gave **3.13** as a colorless oil (128 mg, 71%). $[\alpha]_D$ +20 (*c* 1.0, CH_2Cl_2). ^1H NMR (CD_2Cl_2): δ 7.43-7.35 (20H, m, Ar), 5.07 (2H, 2d, $J_{A,B}$ = 11.1 Hz, CO_2CH_2Ph), 4.51 – 4.31 (6H, m, 3xCH_2Ph), 4.49 (1H, m, H-2'), 4.45 (1H, m, H-4), 4.42 (1H, m, H-2), 4.21 (1H, m, H-3'), 4.18 (1H, m, H-4'), 4.13 (1H, m, H-1'b), 4.07 (1H, m, H-3), 4.06 (1H, m, H-5b), 3.96 (1H, m, H-1'a), 3.32 (1H, br, H-5a), 3.28 (1H, m, H-5'b), 3.23 (1H, m, H-5'a). ^{13}C NMR (CD_2Cl_2): δ 173.5 (C-1), 139.1, 138.3, 138.3, 137.8 ($4C_{ipso}$), 130.5-129.5 (20C, Ar), 84.8 (C-3'), 84.8 (C-2'), 77.1 (C-3), 75.3, 74.1, 73.8 (3xOCH_2Ph), 75.0 (C-2), 69.0 (C-5'), 68.8 (C-4), 68.5 (CO_2CH_2Ph), 67.0 (C-4'), 53.7 (C-

5), 49.5 (C-1'). MALDI-TOF MS: *m/e* 659.20 (M^+). Anal. Calcd. for $C_{38}H_{43}ClO_8S$: C 65.65, H 6.23. Found: C 65.62, H 6.40.

5-(1,4-Dideoxy-1,4-episulfoniumylidene-L-arabinitol)-5-deoxy-D-ribonate inner salt (3.2)

Compound **3.13** (100 mg, 0.140 mmol) was dissolved in CH_2Cl_2 (5 mL). BCl_3 was passed through the solution for 2 min at -78 °C. The solution was stirred at -78 °C for 1 h. Air was passed through the reaction flask until no white gas formed. H_2O was added slowly to quench the reaction. The resulting mixture was concentrated under reduced pressure. Column chromatography (7:3:1 EtOAc-MeOH-H_2O and then pure H_2O) of the crude product gave **3.2** as a colorless oil (21.0 mg, 50%). IR (neat) 3372, 1614, 1395, 1064, 620 cm^{-1}. $[\alpha]_D$ +29 (*c* 0.2, H_2O). ^1H NMR (D_2O): δ 4.68 (1H, dt, $J_{1',2'}$ = 3.7 Hz, H-2'), 4.40 (1H, t, $J_{2',3'} = J_{3',4'}$ = 3.3 Hz, H-3'), 4.22 (1H, b, H-4), 4.14 (1H, d, $J_{2,3}$ = 2.7 Hz, H-2), 4.08 (1H, ddd, H-4'), 4.03 (1H, dd, $J_{4',5'b}$ = 5.2 Hz, $J_{5'a,5'b}$ = 12.3 Hz, H-5'b), 3.95 (1H, b, H-3), 3.91 (1H, dd, $J_{4',5'a}$ = 8.1 Hz, H-5'a), 3.82 (2H, m, H-4) , 3.78 (2H, d, H-1'). ^{13}C NMR (D_2O): δ 179.8 (C-1), 79.8 (C-3'), 79.0 (C-2'), 76.8 (C-3), 75.5 (C-2), 71.9 (C-4'), 69.5 (C-4), 61.3 (C-5'), 51.8 (C-5), 49.1 (C-1'). MALDI-TOF MS: *m/e* 321.35 (M^+ + Na), 299.48 (M^+ + H). HRMS: (M+H) calcd. for $C_{10}H_{19}O_8S$, 299.0801; Found: 299.0801.

Benzyl-5-(2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-episulfoniumylidene-D-arabinitol)-5-deoxy-D-ribonate triflate (3.15)

A mixture of compound **3.11** (277 mg, 1.00 mmol) and compound **3.7** (417 mg, 1.00 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and CF_3CO_2H (113 mg, 1.00 mmol) was added. The mixture was stirred at room temperature for 3 h and the solvent was removed under reduced pressure. Column chromatography (EtOAc-MeOH-H_2O, 40:1:1) of the crude product gave compound **3.14** as a colorless oil (460 mg, 66%) that was reacted as follows. MALDI-TOF MS: *m/e* 699.46 (M^+). Without further purification, compound **3.14** (200 mg, 0.230 mmol) was dissolved in a mixture of concentrated HCl (1 mL) and methanol (40 mL). The mixture was stirred at rt for 6 h. The solvent was removed under reduced pressure. The resulting chloride salt was stirred with silver triflate (117 mg, 0.460 mmol) in CH_2Cl_2 (2 mL) for 1h. The mixture was concentrated and column chromatography (8:1 CH_2Cl_2-MeOH) of the crude product gave compound **3.15** as a colorless oil (142 mg, 75%). $[\alpha]_D$ +15 (*c* 0.9, CH_2Cl_2). ^{1}H NMR (CD_3OD): δ 7.40-7.20 (20H, m, Ar), 5.17 (2H, s, CO_2CH_2Ph), 4.67–4.44 (6H, m, $3OCH_2Ph$), 4.63 (1H, d, H-2'), 4.43 (1H, s, H-3'), 4.35 (1H, d, $J_{2,3}$ = 3.5 Hz, H-2), 4.30 (1H, dd, $J_{4',5'b}$ = 6.7 Hz, $J_{4',5'a}$ = 9.6 Hz, H-4'), 4.24 (1H, ddd, $J_{4,5a}$ = 7.2 Hz, $J_{4,5b}$ = 3.4 Hz, H-4), 4.02 (1H, d, $J_{1'a,1'b}$ = 13.1 Hz, H-1'b), 3.96 (1H, dd, $J_{3,4}$ = 6.3 Hz, H-3), 3.84 (1H, dd, $J_{5a,5b}$ = 13.0 Hz, H-5b), 3.80 (1H, dd, H-5'b), 3.79 (1H, dd, $J_{1'a,2'}$ = 3.1 Hz, H-1'a), 3.73 (1H, dd, H-5a), 3.70 (1H, dd, $J_{5'a,5'b}$ = 10.0 Hz, H-5'a). ^{13}C NMR (CD_3OD): δ 173.4 (C-1), 138.5-138.0 ($4C_{ipso}$), 129.9-129.1 (20C, Ar), 84.4 (C-2'), 84.1 (C-3'), 76.3 (C-3), 74.4 (C-2), 73.6, 73.3, 73.1 ($3xOCH_2Ph$), 68.5 (C-4), 68.0 (C-5'), 67.8 (CO_2CH_2Ph), 67.6 (C-4'), 51.8 (C-5), 49.8 (C-1'). MALDI-TOF MS: *m/e* 659.25 (M^+). Anal. Calcd. for $C_{39}H_{43}F_3O_{11}S_2$: C 57.91, H 5.36. Found: C 57.55, H 5.49.

5-(1,4-Dideoxy-1,4-episulfoniumylidene-D-arabinitol)-5-deoxy-D-ribonate inner salt (3.3)

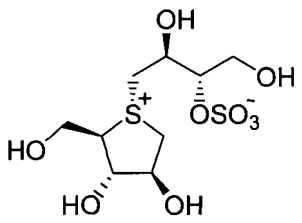
Compound **3.15** (150 mg, 0.210 mmol) was dissolved in CH_2Cl_2 (10 mL). Boron trichloride was passed through the solution for 2 min at -78 °C. The solution was stirred at -78 °C for 1 h. Air was passed through the reaction flask until no white gas formed. Water was added slowly to quench the reaction. The resulting mixture was concentrated under reduced pressure. Column chromatography (7:3:1 EtOAc-MeOH-H_2O and then pure H_2O) of the crude product gave **3.3** as a colorless oil (33.0 mg, 52%). IR (neat) 3364, 1599, 1407, 1070, 617 cm^{-1}. $[\alpha]_D$ -12 (*c* 0.6, H_2O). ^1H NMR (D_2O): δ4.57 (1H, dt, $J_{1',2'}$ = 3.8 Hz, H-2'), 4.26 (1H, dd, $J_{2',3'}$ = 3.5 Hz, H-3'), 4.09 (1H, ddd, $J_{4,5a}$ = 8.8 Hz, $J_{4,5b}$ = 3.1 Hz, $J_{3,4}$ = 5.5 Hz, H-4), 4.01 (1H, d, $J_{2,3}$ = 3.7 Hz, H-2), 3.96 (1H, dd, $J_{4',5'b}$ = 4.7 Hz, $J_{5'a,5'b}$ = 12.1 Hz, H-5'b), 3.89 (1H, ddd, $J_{3',4'}$ = 3.2 Hz, $J_{4',5'a}$ = 8.1 Hz, H-4'), 3.86 (1H, dd, H-3), 3.77 (1H, m, H-5'a), 3.75 (1H, dd, $J_{5a,5b}$ = 13.4 Hz, H-5b), 3.72 (2H, d, H-1'), 3.64 (1H, dd, H-5a). ^{13}C NMR (D_2O): δ 179.4 (C-1), 79.3 (C-3'), 78.6 (C-2'), 76.5 (C-3), 75.1 (C-2), 71.5 (C-4'), 69.1 (C-4), 61.0 (C-5'), 51.5 (C-5), 49.6 (C-1'). HRMS: (M+H) calcd. for $C_{10}H_{19}O_8S$, 299.0801; found: 299.0795.

CHAPTER 4:
SYNTHESIS OF AZA- AND THIA-SPIROHETEROCYCLES AND ATTEMPTED SYNTHESIS OF SPIRO SULFONIUM COMPOUNDS RELATED TO SALACINOL

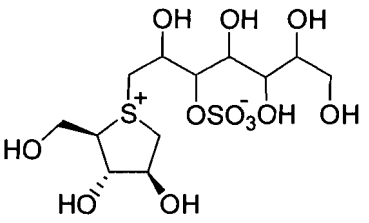
4.1 Introduction

Glycosidases belong to a group of hydrolytic enzymes that are involved in a variety of biologically widespread process. Their inhibition can have profound effects on quality control, maturation, transport, and secretion of glycoproteins and can alter cell–cell or cell–virus recognition processes.[3,2,82] The transition-state structure in the enzyme-mediated hydrolysis of glycosides is believed to resemble an oxacarbenium ion with a distorted conformation. Thus, mimicking this distorted, positively charged species is one factor that could lead to a strong inhibitor of glycosidase enzymes.[4,21,23]

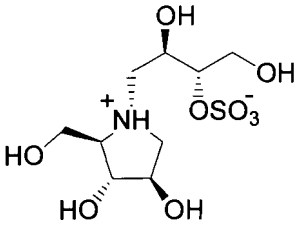
The α-glucosidase inhibitors salacinol **1.21** and kotalanol **1.22** (Figure 4.1) have been isolated from *Salacia reticulata*,[51,55] and *S. oblonga* and *S. chinensis*,[52] traditionally used in the Ayurvedic system of India and Sri Lanka for the treatment of diabetes.[132] Our group has reported the syntheses of compounds **2.8**[142] and **3.3**[143] (Figure 4.1) related to salacinol that contain a carboxylate inner salt and heteroanalogues of salacinol containing nitrogen[116] or selenium[134] instead of sulfur, namely ghavamiol **2.3** and blintol **2.23** (Figure 4.1), respectively. Compounds **2.8**, **3.3** and **2.23** inhibit recombinant human maltase glucoamylase (MGA),[141] one of the key intestinal enzymes involved in the breakdown of glucose oligosaccharides in the small intestine. We reasoned that the interaction of a permanent positive charge with active-site carboxylate residues would make a dominant contribution to the interaction energy.



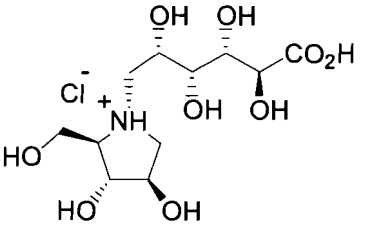
Salacinol (1.21)



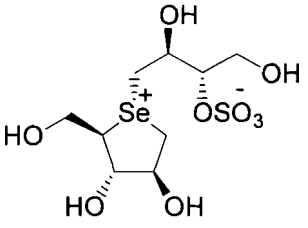
Kotalanol (1.22)



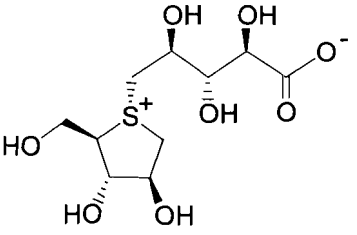
2.3



2.8



2.23



3.3

Figure 4.1 Structures of salacinol (**1.21**), kotalanol (**1.22**), ghavamiol (**2.3**), compound **2.8**, blintol (**2.23**) and compound **3.3**

In addition, compounds **1.21**, **2.3**, **2.8** and **2.23** were found to inhibit *Drosophila* Golgi α-mannosidase II (dGMII), a key enzyme involved in *N*-glycan processing and thus a target in the development of anti-cancer therapies, with IC_{50} values in the low mM range.[65,68] The structure of dGMII in complex with the inhibitor swainsonine (**1.3**) (IC_{50} 20 nM, Figure 4.2 and Figure 4.3) has been published,[37] although the reason for the potency of swainsonine has not been clearly determined. The binding of the inhibitor

involves a large contribution of hydrophobic interactions with aromatic residues Trp95, Phe206 and Tyr727. The plane of the six-membered ring is nearly parallel to Phe206 and approximately at 90° to Tyr727. Therefore, we now present the synthesis of the aza spiroheterocycle, **4.1** (Figure 4.2), the hydrocarbon portion of which is expected to interact with the hydrophobic pocket of Tyr727, Phe206 and Trp415.

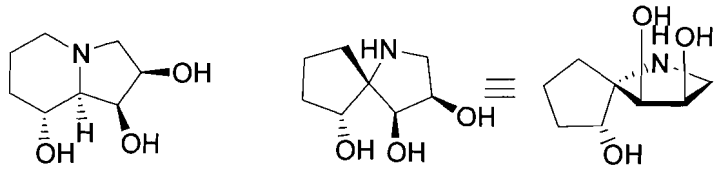


Figure 4.2 Structures of swainsonine (**1.3**) and aza spiroheterocycle (**4.1**)

Overlay of compounds bound in the active site of dGMII, obtained from X-ray crystallography, indicated that the position of the head groups of compounds **2.3**, **2.8** and the diastereomer of salacinol (**2.24**) is similar (Figure 4.4 and Figure 4.5).[65] The positions of the nitrogen atoms and the positively charged sulfur atom, which are designed to mimic the positive charge on the oxacarbenium ion, are almost identical. However, it is the thiomethyl group of a nanomolar inhibitor *N*-benzyl mannostatin (**4.2**) (Figure 4.4 and Figure 4.5) and the hydrophobic hydrocarbon moiety of swainsonine (**1.3**) that occupy the hydrophobic region of the active site through which the hydrophilic C-5 hydroxyl groups of salacinol analogues pass. The thiomethyl group, which is critical for high affinity, is believed to make important nonpolar interactions with aromatic rings in the active site of dGMII.[31] Accordingly, we also present the attempted synthesis of spiro

sulfonium compounds **4.3** and **4.4** (Figure 4.6) related to salacinol but with more nonpolar moieties than salacinol analogues themselves.

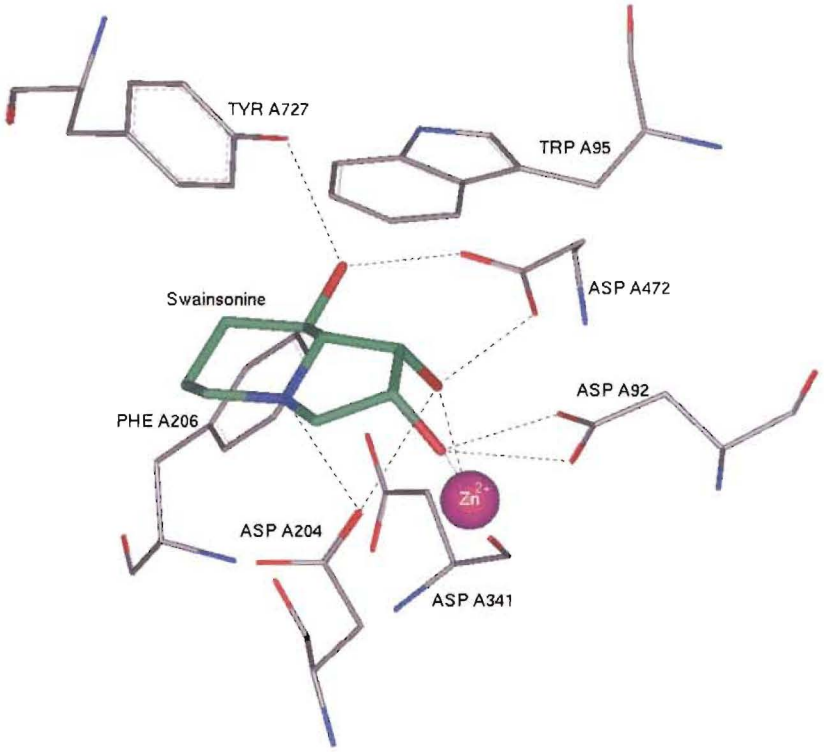
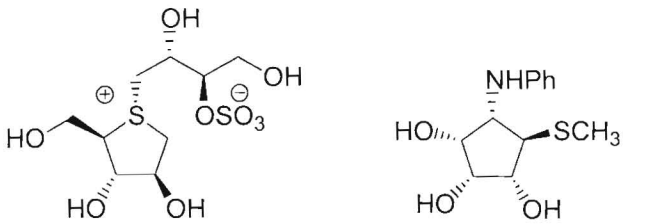


Figure 4.3 Structure of swainsonine (**1.3**) bound in the active site of dGMII (PDB 1HWW).



2.24 *N*-benzyl mannostatin (**4.2**)

Figure 4.4 Structures of the diastereomer of salacinol, **2.24**, and *N*-benzyl mannostatin **4.2**

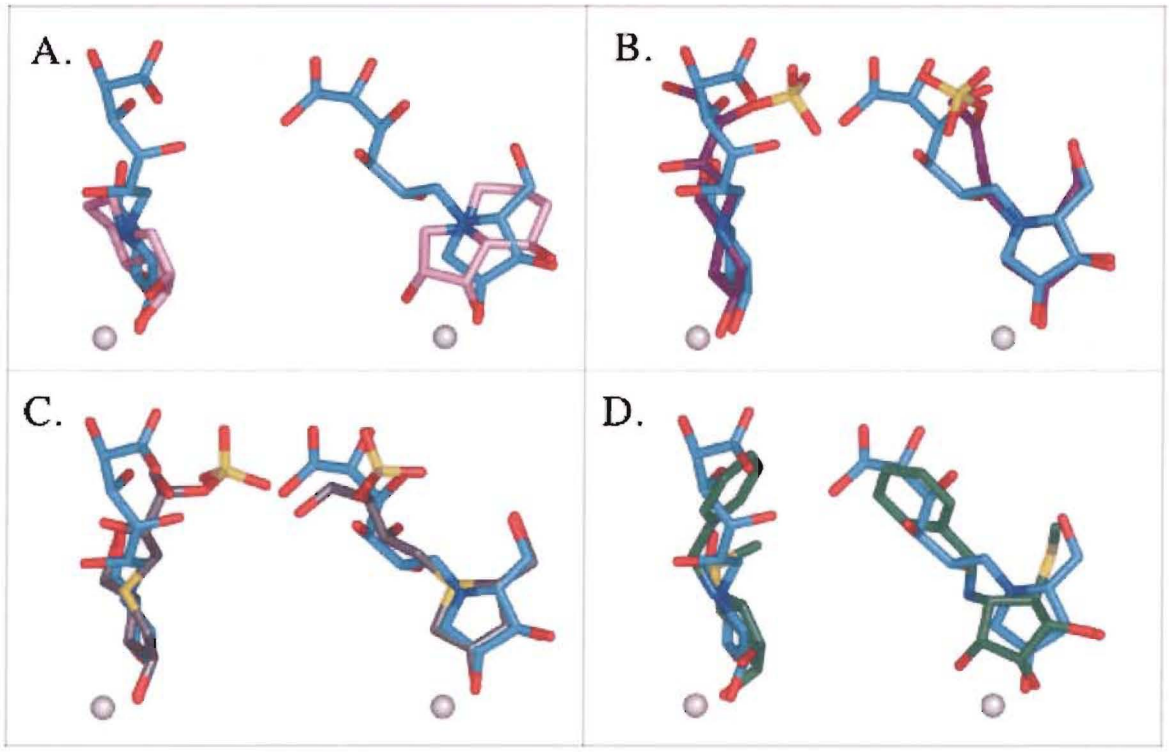
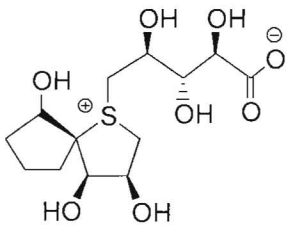
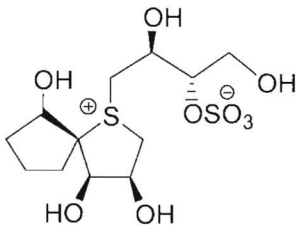


Figure 4.5 Overlay of compounds bound in the active site of dGMII. Compound **2.8** (cyan) is overlayed with A. Swainsonine (pink, PDB 1HWW) B. Ghavamiol **2.3** (magenta, PDB 1TQU) C. Salacinol Diastereomer **2.24** (grey, PDB 1TQT) or D. *N*-Benzyl mannostatin (green, PDB 2F7P).



4.3



4.4

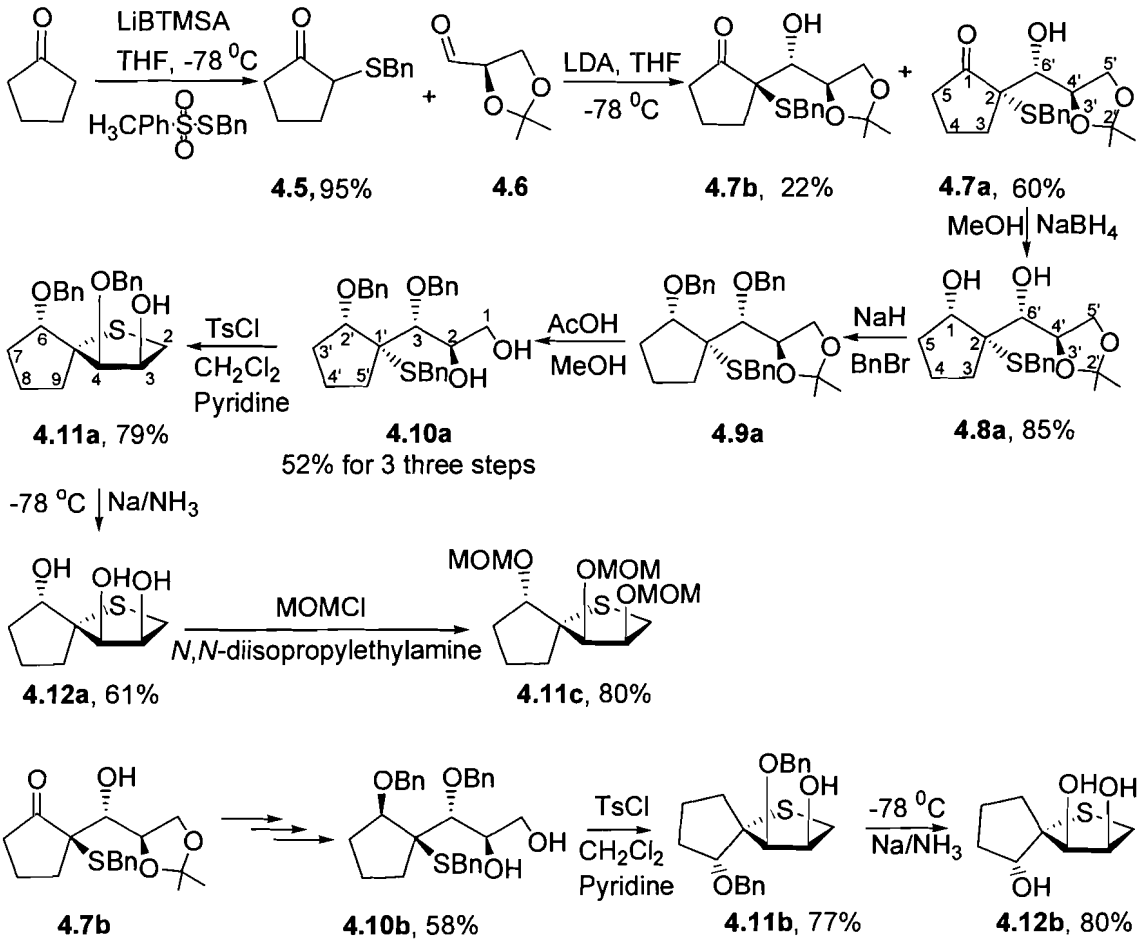
Figure 4.6 Structures of the spiro sulfonium compounds **4.3** and **4.4**

4.2 Results and Discussion

Paquette *et al.*[144-148] have synthesized a number of 4'-spiroalkylated nucleosides to impose conformational constraints that might have importance in modulation of the sugar-phospate DNA backbone, and ultimately the secondary structure of DNA as well as base recognition.[149] Our synthesis takes advantage of the stereochemistry of (*R*)-isopropylidene glyceraldehyde and provides an alternative synthetic route to 4'-spiroalkylated compounds.

2-Benzylsulfanylcyclopentanone **4.5** was prepared in one step from the enolate of cyclopentanone and the thiolating reagent *S*-benzyl 4-methylbenzenethiosulfonate (Scheme 4.1). A lithium-mediated aldol reaction of compound **4.5** with (*R*)-isopropylidene glyceraldehyde **4.6**[150] gave two products **4.7a** and **4.7b** in a ratio of 3:1 that were separated by chromatography. No other diastereomer was obtained. The reaction is believed to go through a six-membered ring transition state with the enolate generated toward the α-substituent (Figure 4.7). Both products are predicted to arise by application of the Felkin-Anh model for asymmetric induction, assuming the alkoxyl group to be the "large" group (Figure 4.7).[151] The major product **4.7a** was reduced selectively by $NaBH_4$ to generate compound **4.8a**, suggesting that the β-hydroxyl group helps to deliver hydride from the *si* face of the ketone. The hydroxyl groups of compound **4.8a** were benzylated and the isopropylidene protecting group was removed. Treatment of compound **4.10a** with tosyl chloride afforded the spiro compound **4.11a**, presumably via a regioselective tosylation reaction of the primary alcohol, followed by the formation of a benzyl sulfonium-ion intermediate. The hydroxyl groups of compound **4.11a** were deprotected by Birch reduction to furnish the thia spiroheterocycle **4.12a**. In a similar

way, the spiro compound **4.12b** was synthesized (Scheme 4.1). Compound **4.11a** was also transformed into its methoxymethyl derivative **4.11c** for future use (Scheme 4.1).



Scheme 4.1 Synthesis of compounds **4.12a**, **4.11c** and **4.12b**.

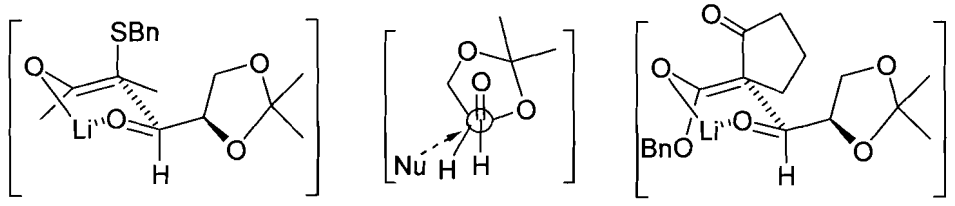
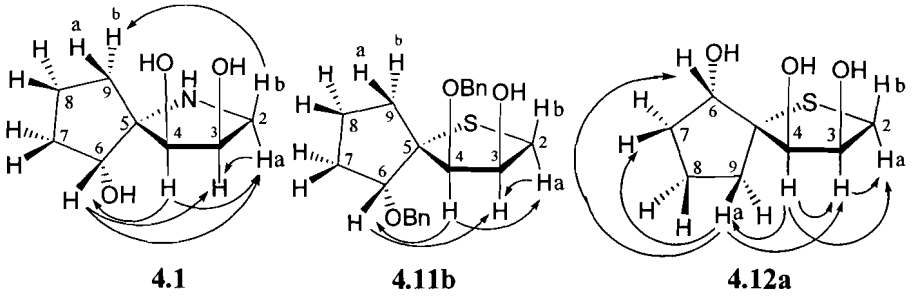


Figure 4.7 Putative transition states for the aldol reactions

The stereochemistry of compounds **4.12a** and **4.11b** was confirmed on the basis of NOE studies (Figure 4.8). For example, the *syn* nature of H-2a, H-3, H-4 and H-9a in compound **4.12a** was made evident by the strong correlations observed between H-9a/H-4 and H-9a/H-3. A strong NOE with H-9a/H-6 was also observed, indicating the *syn* relationship of these two protons.

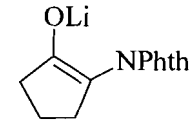


4.1 **4.11b** **4.12a**

Figure 4.8 Observed NOE correlations for compounds **4.1**, **4.11b** and **4.12a**

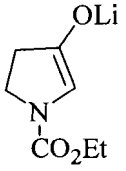
The synthesis of the aza spiroheterocycle was examined next. Unlike the enolate of the α-thio ketone that formed toward the α-substituent predominantly, the enolates of the α-amino ketones **4.13** and **4.14** (Figure 4.9) were not readily accessible by conventional enolization techniques.[152] Thus, the β-ketoester **4.15** was employed as a functional equivalent of compound **4.13** to react with the aldehyde **4.6** (Scheme 4.2). We reasoned that a Curtius rearrangement[153] would convert the carboxylate to an amine-derived functional group. Thus, aldol condensation of compound **4.15** with compound **4.9** gave, after acylation of the hydroxyl group, the four possible diastereomers **4.16a-d** in a ratio of 6:5:1:36 (based on weight and NMR spectroscopic analysis). No other products were obtained. The major product was presumably obtained through a six-membered ring transition state and the stereochemical course of this aldol reaction corresponds to that

predicted by the Felkin-Anh model (Figure 4.7).[151] The optically pure compound **4.16d** was isolated by silica chromatography from the mixture of its diastereomers **4.16a-c** which were inseparable. The ketone **4.16d** was reduced stereoselectively to the alcohol **4.17** which was subsequently acylated. The compound **4.17** was converted to the benzyl carbamate **4.18** by a Curtius rearrangement reaction (Scheme 4.3).[153] Deprotection of compound **4.18** and conversion of the resulting alcohol **4.19** to the tosylate **4.20** involved the utilization of standard procedures. The amine in compound **4.20** was released and subsequently treated, without purification, with DBU to produce the desired spirocycles **4.21a** and **4.21b** as a 1:1 inseparable mixture that was subsequently deacylated to furnish the target compound **4.1**. As with compound **4.11b**, the steterochemistry at C-4, C-5 and C-6 in **4.1** was confirmed on the basis of NOE studies (Figure 4.8) by the strong correlations observed between H-6/H-4 and H-6/H-3. A strong NOE between H-3/H-2a indicated a *syn* relationship of these two protons; a weak NOE was also observed between H-3 and H-2b, as usually observed in pyrrolidine derivatives.[154]



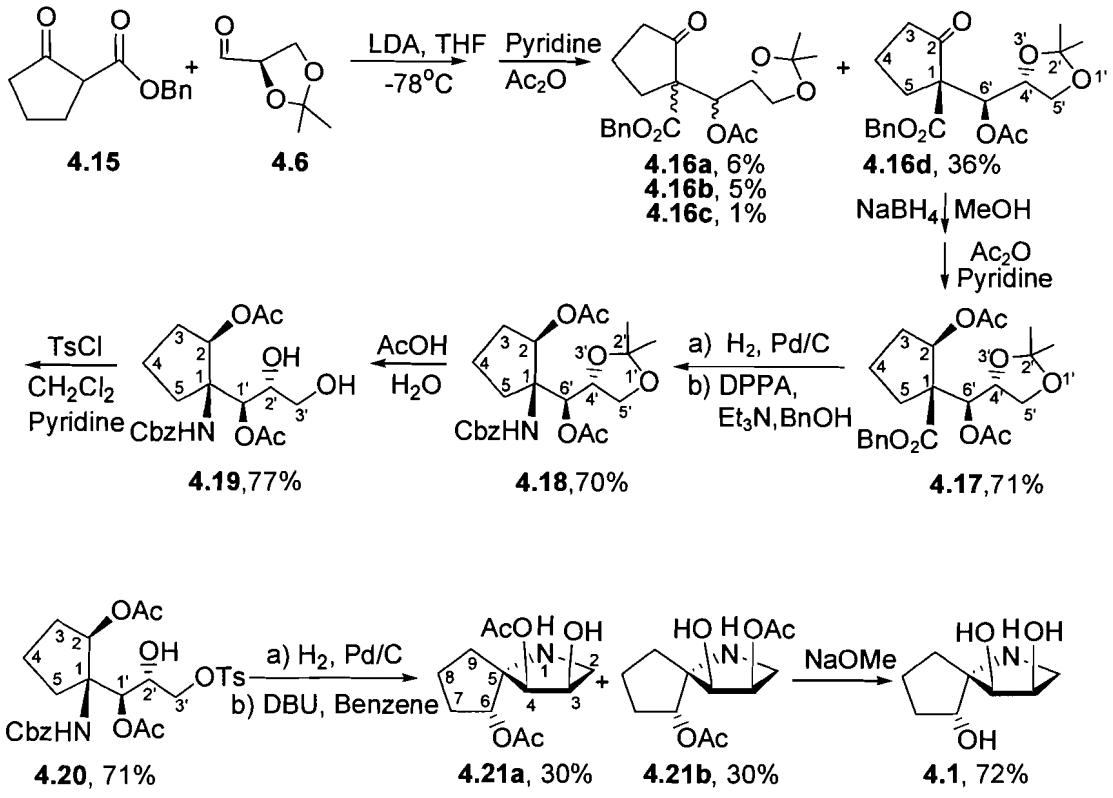
Phth = Phthaloyl

4.13

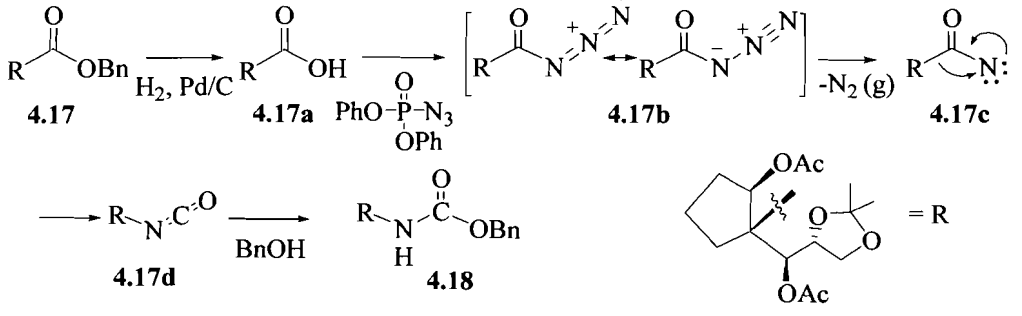


4.14

Figure 4.9 Structures of enolates **4.13** and **4.14**



Scheme 4.2 Synthesis of compound **4.1**.

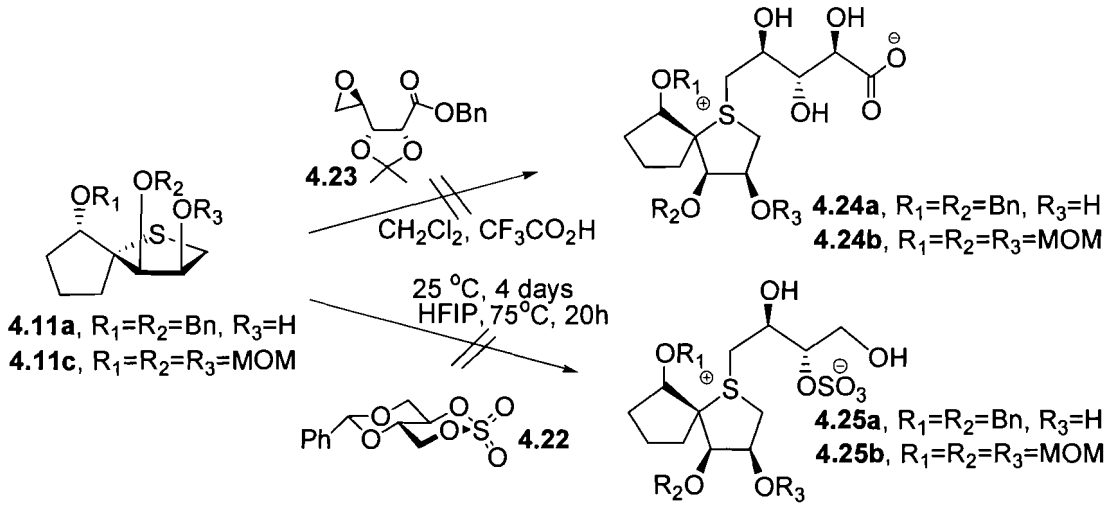


Scheme 4.3 Curtius rearrangement reaction.

The synthesis of analogues of salacinol (**1.21**) was examined next. However, despite many attempts under a variety of conditions, the coupling reactions of the

compounds **4.11a** or **4.11c** with the cyclic sulfate **4.22** or the epoxide **4.23** failed to yield the protected precursors of analogues of salacinol (**1.21**), **4.24a/4.24b** or **4.25a/4.25b** (Scheme 4.4).

Compounds **4.1**, **4.12a**, and **4.12b** were tested for inhibition of human maltase glucoamylase, a critical intestinal enzyme involved in the breakdown of maltooligosaccharides to glucose, as well as for inhibition of Golgi mannosidase II. These compounds did not show effective inhibition of either enzyme, presumably because of poor fit in the respective active sites.



Scheme 4.4 Attempted synthesis of compounds **4.24a**, **4.24b**, **4.25a** and **4.25b**.

4.3 Experimental Section

4.3.1 Synthesis

(2*R*)-2-Benzylsulfanyl-2-[(*R*)-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-hydroxymethyl]-cyclopentanone (4.7b) and (2*S*)-2-benzylsulfanyl-2-[(*R*)-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-hydroxymethyl]-cyclopentanone (4.7a)

To a solution of diisopropylamine (0.7 mL, 5.2 mmol) in dry THF (13 mL) was added *n*-BuLi (2.6 mL, 2.0 M in hexane, 5.20 mmol) at 0 °C. After 10 min, the solution was cooled to -78 °C, and 2-benzylsulfanylcyclopentanone (0.960 g, 4.65 mmol) was added over 3 min. After the mixture had been stirred for 2 h at -78 °C, isopropylidene-D-glyceraldehyde (0.604 g, 4.65 mmol) was slowly added and the the mixture was stirred for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution, the mixture was extracted with ether, and the ether layer was concentrated. The crude product was purified by column chromatography (hexanes-EtOAc, 2:1) to afford compound **4.7a** (0.930 g, 60%) and compound **4.7b** (0.310 g, 22%) as colorless oils. Compound **4.7a** $[\alpha]_D$ -11 (*c* 0.02, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.34-7.22 (5H, m, Ar), 4.19 (1H, dd, $J_{4',5'a}$ = 6.4 Hz, $J_{5'a,5'b}$ = 8.5 Hz, H-5'a), 4.13 (1H, ddd, $J_{4',5'b}$ = 5.0 Hz, H-4'), 3.97 (1H, dd, H-5'b), 3.91 (1H, d, $J_{A,B}$ = 12.2 Hz, SCH_2Ph), 3.79 (1H, d, $J_{4',6'}$ = 8.1 Hz, H-6' C*H*OH), 3.68 (1H, d, SCH_2Ph), 2.58 (1H, dd, $J_{4a,5a}$ = 8.3 Hz, $J_{5a,5b}$ = 18.7 Hz, H-5a), 2.35 (1H, dd, $J_{4b,5b}$ = 9.1 Hz, H-5b), 2.67 (1H, m, H-3a), 2.06 (1H, m, H-4a), 1.95 (1H, m, H-4b), 1.80 (1H, dd, $J_{3a,3b}$ = 14.1 Hz, $J_{3a,4b}$ = 6.8 Hz, H-3b), 1.34 (3H, s, CCH_3), 1.34 (3H, s, CCH_3). ^{13}C NMR ($CDCl_3$) δ: 211.8 (*C*=O), 137.2 (C_{ipso}), 129.3-127.6 (5C, Ar), 109.9 (*C*$(CH_3)_2$), 75.7 (C-4'), 73.3 (C-6'), 68.4 (C-5'), 60.4 (C-2), 36.8 (C-5), 33.6 (S*C*H_2Ph), 31.3 (C-3), 26.0, 25.5 (2*C*H_3), 18.0 (C-4). Anal. Calcd. for $C_{18}H_{24}O_4S$: C

64.26, H 7.19. Found: C 64.38, H 7.09. **4.7b** $[\alpha]_D$ +53 (*c* 0.03, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.34-7.22 (5H, m, Ar), 4.56 (1H, ddd, $J_{4',5'a}$ = 6.4 Hz, $J_{4',5'b}$ = 8.2 Hz, $J_{4',6'}$ = 2.6 Hz, H-4'), 4.45 (1H, d, H-6' C*H*OH), 3.90 (2H, m, H-5'a and H-5'b), 3.79 (1H, d, $J_{A,B}$ = 12.3 Hz, SCH_2Ph), 3.66 (1H, d, SCH_2Ph), 2.63 (1H, dd, $J_{4a,5a}$ = 8.3 Hz, $J_{5a,5b}$ = 18.8 Hz, H-5a), 2.41 (1H, m, H-3a), 2.20 (1H, m, H-5b), 2.08 (1H, m, H-4a), 1.94 (1H, m, H-4b), 1.50 (1H, dd, $J_{3a,3b}$ = 13.4 Hz, $J_{3a,4a}$ = 6.6 Hz, H-3b), 1.41 (3H, s, CCH_3), 1.35 (3H, s, CCH_3). ^{13}C NMR ($CDCl_3$) δ: 211.7 (*C*=O), 136.9 (C_{ipso}), 129.3-127.5 (5C, Ar), 108.3 ($C(CH_3)_2$), 76.0 (C-4'), 67.8 (C-6'), 63.7 (C-5'), 57.7 (C-2), 36.1 (C-5), 33.6 (S*C*H_2PH), 29.2 (C-3), 26.6, 25.9 (2*C*H_3), 18.2 (C-4). Anal. Calcd. for $C_{18}H_{24}O_4S$: C 64.26, H 7.19. Found: C 64.58, H 6.86.

(1*S*,2*R*)-2-Benzylsulfanyl-2-[(*R*)-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-hydroxymeth yl]-cyclopentanol (4.8a)

To a solution of **4.7a** (200 mg, 0.595 mmol) in dry methanol (20 mL) was added $NaBH_4$ (10.0 mg, 1.19 mmol) slowly at room temperature. When TLC showed the reaction to be complete, the solvent was removed. The residue was extracted with saturated aqueous NH_4Cl solution and CH_2Cl_2. The organic layer was concentrated to give compound **4.8a** as a colorless oil (170 mg, 85%). $[\alpha]_D$ -8 (*c* 0.02, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.39-7.22 (5H, m, Ar), 4.29 (1H, dt, $J_{1,OH}$ = 4.9 Hz, $J_{1,5}$ = 8.1 Hz, H-1), 4.25 (1H, m, H-4'), 4.23 (1H, m, H-5'a), 4.09 (1H, m, H-5'b), 3.99, 3.92 (2H, d, $J_{A,B}$ = 12.1 Hz, 2SCH_2Ph), 3.67 (1H, dd, $J_{4',6'}$ = 6.4 Hz, $J_{6',OH}$ = 5.9 Hz, H-6' C*H*OH), 1.98 (3H, m, H-5a, H-5b and H-3a), 1.78 (2H, m, H-3b and H-4b), 1.63 (1H, m, H-4a), 1.42 (3H, s, CCH_3), 1.37 (3H, s, CCH_3). ^{13}C NMR ($CDCl_3$) δ: 137.1 (C_{ipso}), 128.9-127.1 (5C, Ar), 109.1

($C(CH_3)_2$), 81.3 (C-1), 73.3 (C-6'), 76.6 (C-4'), 67.4 (C-5'), 64.2 (C-2), 33.5 (SCH_2Ph), 32.0 (C-3), 30.6 (C-5), 26.6, 25.4 ($2CH_3$), 19.3 (C-4). Anal. Calcd. for $C_{18}H_{26}O_4S$: C 63.87, H 7.74. Found: C 63.58, H 7.92.

(2*R*,3*R*)-3-Benzyloxy-3-[(1*R*,2*S*)-2-benzyloxy-1-benzylsulfanylcyclopentyl]-propane-1,2-diol (4.10a) and (2*R*,3*R*)-3-benzyloxy-3-[(1*S*,2*R*)-2-benzyloxy-1-benzylsulfanylcyclopentyl]-propane-1,2-diol (4.10b)

To a solution of compound **4.7a** (200 mg, 0.595 mmol) in dry methanol (20 mL) was added $NaBH_4$ (10.0 mg, 1.19 mmol) slowly at room temperature. When TLC showed the reaction to be complete, the solvent was removed. The residue was extracted with saturated aqueous NH_4Cl solution and CH_2Cl_2. The organic layer was concentrated to give a colorless oil. The resulting compound **4.8a** was dissolved in dry DMF (5 mL), NaH (55.0 mg, 1.19 mmol) was added slowly at 0°C, and the solution was stirred for 3 h. BnBr (0.2 mL, 1.2 mmol) was added dropwise and the solution was stirred at room temperature for 2 h. The reaction was quenched with methanol (5 mL) and the mixture was concentrated. The residue was extracted with CH_2Cl_2 and brine, and the organic layer was concentrated to give a colorless oil. The resulting compound **4.9a** was dissolved in 80% acetic acid (20 mL) and refluxed for 1 h, after which the solvent was removed. The crude product was purified by column chromatography (hexanes-EtOAc, 2:1) to afford compound **4.10a** as a colorless oil (0.147 g, 52%). Compound **4.10a** $[\alpha]_D$ -4 (*c* 0.04, acetone). ^1H NMR ($CDCl_3$) δ: 7.41-7.26 (15H, m, Ar), 4.76 (1H, d, $J_{A,B}$ = 11.0 Hz, OCH_2Ph), 4.58 (1H, d, $J_{A,B}$ = 11.6 Hz, OCH_2Ph), 4.56 (1H, d, OCH_2Ph), 4.48 (1H, d, OCH_2Ph), 4.29 (1H, t, $J_{2',3'}$ = 7.7 Hz, H-2'), 4.13 (1H, m, H-2), 3.84 (1H, d, $J_{A,B}$ = 11.8

Hz, SCH_2Ph), 3.81 (1H, dd, $J_{1a,1b}$ = 11.5 Hz, $J_{1a,2}$ = 3.0 Hz, H-1a), 3.76 (1H, d, SCH_2Ph), 3.77 (1H, dd, $J_{1b,2}$ = 3.8 Hz, H-1b), 3.70 (1H, d, $J_{2,3}$ = 7.9 Hz, H-3), 2.16-1.87 (5H, m, H-3'a, H-5'a, H-3'b, H-4'a, H-5'b) , 1.60 (1H, m, H-4'b). ^{13}C NMR ($CDCl_3$) δ: 138.2, 138.1, 137.7 (3C$_{ipso}$), 129.6-127.5 (15C, Ar), 81.8 (C-2'), 80.0 (C-3), 75.5 (O*C*H$_2$Ph), 73.7 (C-2), 72.0 (O*C*H$_2$Ph), 64.13 (C-1'), 62.8 (C-1), 33.0 (S*C*H$_2$Ph), 33.0 (C-3'), 29.5 (C-5'), 20.5 (C-4'). Anal. Calcd. for $C_{29}H_{34}O_4S$: C 72.77, H 7.16. Found: C 72.59, H 7.39. Compound **4.10b** was synthesized from compound **4.7b** similarly in three steps (58%). Compound **4.10b** $[\alpha]_D$ -3 (*c* 0.02, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.40-7.20 (15H, m, Ar), 4.75 (1H, d, $J_{A,B}$ = 11.1 Hz, OCH_2Ph), 4.64 (1H, d, $J_{A,B}$ = 11.3 Hz, OCH_2Ph), 4.59 (1H, d, OCH_2Ph), 4.39 (1H, d, OCH_2Ph), 4.26 (1H, m, H-2), 4.14 (1H, d, $J_{A,B}$ = 11.7 Hz, SCH_2Ph), 4.13 (1H, t, $J_{2',3'}$ = 8.9 Hz, H-2'), 3.91 (1H, d, SCH_2Ph), 3.87 (2H, m, 2H-1), 3.73 (1H, d, $J_{2,3}$ = 7.9 Hz, H-3), 2.17 (2H, m, 2H-3'), 2.07 (1H, m, H-5'a), 2.00 (1H, m, H-4'a), 1.95 (1H, m, H-5'b), 1.63 (1H, m, H-4'b). ^{13}C NMR ($CDCl_3$) δ: 138.6, 138.5, 137.4 (3C$_{ipso}$), 129.4-127.2 (15C, Ar), 85.1 (C-2' and C-3), 75.6 (O*C*H$_2$Ph), 73.4 (C-2), 72.1 (O*C*H$_2$Ph), 64.1 (C-1'), 63.8 (C-1), 34.0 (S*C*H$_2$Ph), 32.5 (C-5'), 28.5 (C-3'), 19.0 (C-4'). Anal. Calcd. for $C_{29}H_{34}O_4S$: C 72.77, H 7.16. Found: C 72.43, H 6.84.

(3*R*,4*S*,5*R*,6*S*)-4,6-Bis-benzyloxy-1-thia-spiro[4.4]nonan-3-ol (4.11a) and **(3*R*,4*S*,5*S*,6*R*)-4,6-bis-benzyloxy-1-thia-spiro[4.4]nonan-3-ol (4.11b)**

To a solution of compound **4.10a** (0.900 g, 1.88 mmol) in CH_2Cl_2 (40 mL) and pyridine (2 mL) was added TsCl (0.360 g, 1.88 mmol). The mixture was stirred for 4 days. TLC showed that the reaction was complete. The organic solution was extracted with brine, the organic layer was dried over Na_2SO_4, and concentrated. The crude product

was purified by column chromatography (hexanes-EtOAc, 4:1) to afford compound **4.11a** as a colorless oil (0.55 g, 79%). **4.11a** $[\alpha]_D$ -4 (*c* 0.02, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.42-7.22 (10H, m, Ar), 4.81 (1H, d, $J_{A,B}$ = 11.0 Hz, OCH_2Ph), 4.71 (1H, d, $J_{A,B}$ = 11.9 Hz, OCH_2Ph), 4.55 (1H, d, OCH_2Ph), 4.49 (1H, m, H-3), 4.45 (1H, d, OCH_2Ph), 4.16 (1H, dd, $J_{6,7a}$ = 9.5 Hz, $J_{6,7b}$ = 7.4 Hz, H-6), 3.76 (1H, d, $J_{3,4}$ = 3.9 Hz, H-4), 3.04 (1H, dd, $J_{2a,2b}$ = 11.6 Hz, $J_{2a,3}$ = 4.5 Hz, H-2a), 2.84 (1H, d, H-2b), 2.10 (1H, m, H-9a), 1.96 (1H, m, H-7a), 1.79 (1H, m, H-9b), 1.71 (1H, m, H-8a), 1.63 (1H, m, H-7b), 1.59 (1H, m, H-8b). ^{13}C NMR ($CDCl_3$) δ: 138.1, 137.1 (2C$_{ipso}$), 128.4-126.9 (10C, Ar), 84.6 (C-4), 78.3 (C-6), 72.2 (O*C*H$_2$Ph), 71.8 (O*C*H$_2$Ph), 71.2 (C-3), 65.3 (C-5), 37.1 (C-9), 35.4 (C-2), 27.1 (C-7), 19.1 (C-8). Anal. Calcd. for $C_{22}H_{26}O_3S$: C 71.32, H 7.07. Found: C 71.21, H 7.22. Compound **4.11b** was synthesized from compound **4.10b** by a similar procedure (77%). Compound **4.11b** $[\alpha]_D$ -97 (*c* 0.01, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.42-7.22 (10H, m, Ar), 4.63 (1H, d, $J_{A,B}$ = 12.3 Hz, OCH_2Ph), 4.45 (1H, d, $J_{A,B}$ = 11.5 Hz, OCH_2Ph), 4.44 (1H, m, H-3), 4.43 (1H, d, OCH_2Ph), 4.34 (1H, d, OCH_2Ph), 3.76 (1H, d, $J_{3,4}$ = 4.1 Hz, H-4), 3.65 (1H, t, $J_{6,7}$ = 8.0 Hz, H-6), 3.04 (1H, dd, $J_{2a,2b}$ = 11.6 Hz, $J_{2a,3}$ = 5.3 Hz, H-2a), 2.88 (1H, dd, $J_{2b,3}$ = 5.2 Hz, H-2b), 2.15 (1H, m, H-9a), 1.94 (2H, m, H-7a and H-9b), 1.82 (1H, m, H-8a), 1.59 (1H, m, H-8b), 1.52 (1H, m, H-7b). ^{13}C NMR ($CDCl_3$) δ: 138.6, 137.7 (2C$_{ipso}$), 128.8-127.9 (10C, Ar), 84.8 (C-4), 81.7 (C-6), 73.9 (O*C*H$_2$Ph), 72.3 (O*C*H$_2$Ph), 71.9 (C-3), 65.9 (C-5), 33.7 (C-2), 33.2 (C-9), 28.5 (C-7), 19.7 (C-8). Anal. Calcd. for $C_{22}H_{26}O_3S$: C 71.32, H 7.07. Found: C 71.60, H 7.30.

(3*R*,4*S*,5*R*,6*S*)-1-Thia-spiro[4.4]nonane-3,4,6-triol (4.12a) and (3*R*,4*S*,5*S*,6*R*)-1-thia-spiro[4.4]nonane-3,4,6-triol (4.12b)

NH_3 (10 mL) was condensed into a two-neck round bottom flask at -78°C, and lithium (14.2 mg, 2.04 mmol) was added slowly. To the resulting blue solution, compound **4.11a** (190 mg, 0.510 mmol) in dry ether (5 mL) was added dropwise. The mixture was stirred for 0.5 h and then allowed to warm to RT to allow NH_3 to evaporate. At -78 °C, the reaction was quenched by addition of MeOH (10 mL). The mixture was concentrated and the crude product was purified by column chromatography (CH_2Cl_2-methanol, 10:1) to afford compound **4.12a** as a colorless oil (61 mg, 61%). Compound **4.12a** $[\alpha]_D$ -16 (*c* 0.06, methanol). ^{1}H NMR (CD_3OD) δ: 4.25 (1H, ddd, H-3), 4.20 (1H, t, $J_{6,7}$ = 7.1 Hz, H-6), 3.86 (1H, d, $J_{3,4}$ = 3.8 Hz, H-4), 2.97 (1H, dd, $J_{2a,2b}$ = 11.0 Hz, $J_{2a,3}$ = 5.7 Hz, H-2a), 2.70 (1H, dd, $J_{2b,3}$ = 4.4 Hz, H-2b), 2.02 (1H, m, H-9a), 1.91 (1H, m, H-7a), 1.86 (1H, m, H-9b), 1.65 (1H, m, H-8a), 1.59 (1H, m, H-7b), 1.56 (1H, m, H-8b). ^{13}C NMR (CD_3OD) δ: 78.8 (C-4), 74.4 (C-3), 72.4 (C-6), 66.7 (C-5), 37.2 (C-9), 33.0 (C-2), 30.9 (C-7), 19.5 (C-8). Anal. Calcd. for $C_8H_{14}O_3S$: C 50.50, H 7.42. Found: C 50.84, H 7.56. Compound **4.12b** was obtained from compound **4.12b** by a similar procedure (80%). Compound **4.12b** $[\alpha]_D$ -77 (*c* 0.01, methanol). ^{1}H NMR (CD_3OD) δ: 4.36 (1H, ddd, $J_{2a,3}$ = 6.5 Hz, $J_{2b,3}$ = 7.6 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 3.74 (1H, t, $J_{6,7}$ = 6.7 Hz, H-6), 3.73 (1H, d, H-4), 2.87 (1H, dd, $J_{2a,2b}$ = 10.2 Hz, H-2a), 2.83 (1H, dd, H-2b), 2.26 (1H, m, H-9a), 1.95 (1H, m, H-7a), 1.85 (1H, m, H-9b), 1.65 (1H, m, H-8a), 1.56 (1H, m, H-8b), 1.52 (1H, m, H-7b). ^{13}C NMR (CD_3OD) δ: 78.2 (C-4), 76.9 (C-6), 74.9 (C-3), 68.1 (C-5), 32.5 (C-9 and C-2), 32.0 (C-7), 19.2 (C-8). Anal. Calcd. for $C_8H_{14}O_3S$: C 50.50, H 7.42. Found: C 50.39, H 7.29.

(1*R*)-1-[(*S*)-Acetoxy-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-methyl]-2-oxocyclopentane-carboxylic acid benzyl ester (4.16d) and its diastereomers (4.16a-c)

To a solution of diisopropylamine (0.7 mL, 4.9 mmol) in dry THF (12 mL) at 0 °C was added *n*-BuLi (2.4 mL, 4.8 mmol, 2M in pentane). After 10 min, the solution was cooled to -78 °C, and 2-oxocyclopentanecarboxylic acid benzyl ester (0.900 g, 4.13 mmol) was added over 3 min. After the solution had been stirred for 2 h at -78 °C, 2,3-*O*-isopropylidene-D-glyceraldehyde (0.536 g, 4.13 mmol) was added. The mixture was stirred for 30 min and then quenched with saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted with ether twice. The organic layer was concentrated, the residue was redissolved in CH_2Cl_2 (5 mL), and Ac_2O (0.840 g, 8.26 mmol) and pyridine (0.650 g, 8.26 mmol) were added to the solution, and the mixture was stirred overnight. The reaction mixture was concentrated and the crude product was purified by column chromatography (hexanes-ethyl acetate, 3:1) to afford a mixture of compounds **4.16a, 4.16b** and **4.16c** (0.190 g, 6%, 5%, 1%) and **4.16d** (0.570 g, 36%) as colorless oils. **4.16d** $[\alpha]_D$ -18 (*c* 0.03, methanol). ^{1}H NMR ($CDCl_3$) δ: 7.38-7.28 (5H, m, Ar), 5.82 (1H, d, $J_{4',6'}$ = 6.7 Hz, H-6' AcOC*H*), 5.20 (1H, d, $J_{A,B}$ = 12.6 Hz, OCH_2Ph), 5.04 (1H, d, OCH_2Ph), 3.97 (2H, m, H-4' and H-5'a), 3.86 (1H, m, H-5'b), 2.74 (1H, m, H-5a), 2.36 (1H, m, H-3a), 2.18 (2H, m, H-3b and H-5b), 2.02 (2H, m, 2H-4), 1.97 (3H, S, OCCH_3), 1.25, 1.26 (6H, S, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ: 210.8 (CH_2*C*OC), 169.3, 166.7 (2*C*O_2), 135.5 (C_{ipso}), 128.7, 128.4, 127.9 (5C, Ar), 109.5 (*C*(CH_3)$_2$), 75.7 (C-4'), 74.3 (C-6'), 67.6 (OCH_2Ph), 67.5 (C-5'), 64.1 (C-1), 38.4 (C-3), 28.2 (C-5), 26.0, 25.5 (C(*C*H_3)$_2$), 20.8 (CO*C*H_3), 19.9 (C-4). Anal. Calcd. for $C_{21}H_{26}O_7$: C 64.60, H 6.71. Found: C 64.35, H 6.75. **4.16a-c** Anal. Calcd. for $C_{21}H_{26}O_7$: C 64.60, H 6.71. Found: C 64.29, H 6.85.

(1*S*,2*R*)-2-Acetoxy-1-[(*S*)-acetoxy-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]cyclo -pentane carboxylic acid benzyl ester (4.17)

To a solution of **4.16d** (0.670 g, 1.72 mmol) in methanol (50 mL) was added $NaBH_4$ (130 mg, 3.44 mmol) slowly. When TLC showed that the reduction was complete, methanol was removed. The residue was extracted with CH_2Cl_2 and saturated aqueous NH_4Cl solution. The organic layer was concentrated. CH_2Cl_2 (10 mL) and Ac_2O (0.350 g, 3.44 mmol) were added, and the resulting mixture was stirred overnight. The mixture was concentrated and the crude product was purified by column chromatography (hexanes-ethyl acetate, 3:1) to afford compound **4.17** as a colorless oil (0.530 g, 71%). Compound **4.17** $[\alpha]_D$ +5 (*c* 0.06, methanol). ^1H NMR ($CDCl_3$) δ: 7.38-7.31 (5H, m, Ar), 5.53 (1H, d, $J_{4',6'}$ = 6.1 Hz, H-6'), 5.45 (1H, t, $J_{2,3}$ = 5.2 Hz, H-2), 5.18, 5.08 (2H, d, $J_{A,B}$ = 12.2 Hz, OCH_2Ph), 4.10 (1H, ddd, H-4'), 3.88 (1H, dd, $J_{4',5'a}$ = 6.39 Hz, $J_{5'a,5'b}$ = 8.20 Hz, H-5'a), 3.69 (1H, dd, $J_{4',5'b}$ = 7.32 Hz, H-5'b), 2.39 (1H, m, H-5a), 1.94 (2H, m, H-5b and H-3a), 1.77 (1H, m, H-4a), 1.63 (1H, m, H-3b), 1.55 (1H, m, H-4b). ^{13}C NMR ($CDCl_3$) δ: 172.3, 170.2, 169.8 (3*C*=O), 135.5 (C_{ipso}), 128.8-128.4 (5C, Ar), 109.3 ($C(CH_3)_2$), 79.0 (C-2), 75.4 (C-4'), 74.1 (C-6'), 67.4 (CH_2Ph), 66.5 (C-5'), 59.4 (C-1), 32.4 (C-5), 30.2 (C-3), 26.3, 26.3 (C(CH_3)$_2$), 21.3 (C-4), 21.2, 21.0 (2xCOCH_3). Anal. Calcd. for $C_{23}H_{30}O_8$: C 63.58, H 6.96. Found: C 63.42, H 7.04.

[(1*S*,2*R*)-2-Acetoxy-1-[(*S*)-acetoxy-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-methyl] cyclo- pentyl]-carbamic acid benzyl ester (4.18)

A mixture of **4.17** (200 mg, 0.460 mmol) and Pd/C (10 mg) in methanol (10 mL) was stirred at room temperature under an atmosphere of H_2 for 10 h. The catalyst was filtered and the solvent was removed. The residue was dissolved in toluene (10 mL) and Et_3N (48.0 mg, 0.460 mmol) was added, followed by the addition of DPPA (308 mg,

0.460 mmol). After the mixture had been refluxed for 2 h, BnOH (50.0 mg, 0.460 mmol) was added. The mixture was refluxed for 12 h. The solvent was removed and the crude product was purified by column chromatography (hexanes-ethyl acetate, 3:1) to afford compound **4.18** as a colorless oil (140 mg, 70%). **4.18** $[\alpha]_D$ +58 (*c* 0.01, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.38-7.30 (5H, m, Ar), 5.48 (1H, m, H-2), 5.40 (1H, d, $J_{4',6'}$ = 6.0 Hz, H-6'), 5.06 (2H, S, OCH_2Ph), 4.26 (1H, dt, $J_{4',5'}$ = 6.47 Hz, H-4'), 3.97 (1H, dd, $J_{5'a,5'b}$ = 8.10 Hz, H-5'a), 3.79 (1H, dd, H-5'b), 2.18 (2H, m, H-3a and H-5a), 2.07 (7H, m, H-3b and $2COCH_3$), 1.78 (1H, m, H-4a), 1.68 (2H, m, H-5b and H-4b), 1.31, 1.30 (6H, S, $C(CH_3)_2$). ^{13}C NMR ($CDCl_3$) δ: 170.4, 169.9 ($2xOCOCH_3$), 154.8 ($NHCO_2Bn$), 136.5 (C_{ipso}), 128.7, 128.4, 128.3 (5C, Ar), 109.3 ($C(CH_3)_2$), 80.0 (C-1), 74.9 (C-4'), 74.1 (C-6'), 67.3 (OCH_2Ph), 66.7 (C-2), 66.4 (C-5'), 33.6 (C-3), 31.8 (C-5), 26.3, 25.4 ($C(CH_3)_2$), 21.4, 21.1 ($2COCH_3$), 20.3 (C-4). Anal. Calcd. for $C_{23}H_{31}NO_8$: C 61.46, H 6.95, N 3.12. Found: C 61.13, H 7.25, N 3.33.

[(1*S*,2*R*)-2-Acetoxy-1-[(1*S*,2*R*)-1-acetoxy-2,3-dihydroxypropyl]cyclopentyl]-carbamic acid benzyl ester (4.19)

A mixure of **4.18** (1.70 g, 3.78 mmol) in 80% acetic acid (50 mL) was refluxed for 10 min. The solution was concentrated under high vacuum. The crude product was purified by column chromatography (hexanes-ethyl acetate, 1:1) to afford compound **4.19** as a colorless oil (1.20 g, 77%). **4.19** $[\alpha]_D$ +41 (*c* 0.12, acetone). ^{1}H NMR ($CDCl_3$) δ: 7.41-7.32 (5H, m, Ar), 5.48 (1H, d, $J_{1',2'}$ = 9.5 Hz, H-1'), 5.10, 5.07 (2H, d, $J_{A,B}$ = 12.3 Hz, OCH_2Ph), 4.94 (1H, m, H-2), 3.67 (1H, ddd, $J_{2',3'a}$ = 2.89 Hz, $J_{2',3'b}$ = 5.99 Hz, H-2'), 3.54 (1H, dd, $J_{3'a,3'b}$ = 11.6 Hz, H-3'a), 3.43 (1H, dd, H-3'b), 2.18 (1H, m, H-5a), 2.08 (6H, S, $2COCH_3$), 2.03 (1H, m, H- 3a), 1.77 (3H, m, H-3b, H-5b and H-4a), 1.70 (1H, m, H-4b).

^{13}C NMR ($CDCl_3$) δ: 173.0, 171.0 (2x$OCOCH_3$), 156.2 ($NHCO_2Bn$), 136.1 (C_{ipso}), 128.7, 128.4, 128.1 (5C, Ar), 77.9 (C-2), 74.1 (C-1'), 70.5 (C-2'), 68.3 (C-1), 67.5 (CH_2Ph), 63.8 (C-3'), 36.0 (C-5), 32.0 (C-3), 21.4, 21.3 (2x$COCH_3$), 19.9 (C-4). Anal. Calcd. for $C_{20}H_{27}NO_8$: C 58.67, H 6.65, N 3.42. Found: C 58.85, H 6.71, N 3.46.

[(1*S*,2*R*)-2-Acetoxy-1-[(1*S*,2*R*)-1-acetoxy-2-hydroxy-3-(toluene-4-sulfonyloxy)propyl] cyclopentyl]-carbamic acid benzyl ester (4.20)

To a solution of **4.19** (200 mg, 0.489 mmol) in pyridine-CH_2Cl_2 (10 mL, 20:1) was added TsCl (94.0 mg, 0.489 mmol). The mixture was stirred for 4 days. When TLC showed the reaction to be complete, the mixture was extracted with saturated aqueous NH_4Cl solution three times. The organic layer was concentrated and the crude product was purified by column chromatography (hexanes-ethyl acetate, 1:1) to afford compound **4.20** as a colorless oil (190 mg, 71%). **4.20** $[\alpha]_D$ +21 (*c* 0.01, methanol). ^1H NMR ($CDCl_3$) δ: 7.78 (2H, d, 2Ar), 7.35 (5H, m, Ar), 5.26 (1H, d, $J_{1',2'}$ = 9.0 Hz, H-1'), 5.10, 5.06 (2H, d, $J_{A,B}$ = 12.1 Hz, OCH_2Ph), 4.78 (1H, m, H-1), 4.00 (1H, dd, $J_{2',3'a}$ = 2.8 Hz, $J_{3'a,3'b}$ = 9.8 Hz, H-3'a), 3.92 (1H, dd, $J_{2',3'b}$ = 5.34 Hz, H-3'b), 3.89 (1H, ddd, H-2'), 2.45 (3H, S, $PhCH_3$), 2.15 (2H, m, H-3a and H-5a), 2.05, 2.08 (6H, S, $2COCH_3$), 1.78 (3H, m, H-3b, H-5b and H-4a), 1.65 (1H, m, H-4b). ^{13}C NMR ($CDCl_3$) δ: 170.3, 170.1 ($2OCOCH_3$), 156.4 ($NHCO_2Bn$), 145.1 ($C_{ipso}(SO_3Ph)$), 136.0 ($C_{ipso}(CH_2Ph)$), 132.7 ($C_{ipso}(PhCH_3)$), 130.0-128.2 (9C, Ar), 77.3 (C-2), 73.70 (C-1'), 71.1 (C-2'), 68.74 (C-1), 68.7 (CH_2Ph), 67.4 (C-3'), 35.9 (C-3), 31.8 (C-5), 21.8 ($PhCH_3$), 21.2, 21.0 ($2COCH_3$), 19.2 (C-4). Anal. Calcd. for $C_{27}H_{33}NO_{10}S$: C 57.54, H 5.90, N 2.49. Found: C 57.41, H 6.07, N 2.65.

(3*S*,4*R*,5*R*,6*R*)-3,6-diacetoxy-1-aza-spiro[4.4]nonan-4-ol (4.21a) and (3*S*,4*R*,5*S*,6*R*)-4,6-diacetoxy-1-aza-spiro[4.4]nonan-3-ol (4.21b)

A mixture of **4.20** (100 mg, 0.177 mmol) and Pd/C (10 mg) in acetic acid (5 mL) was stirred under an atmosphere of H_2 for 10 h. When TLC showed the reaction to be complete, the catalyst was filtered and the solvent was removed under high vacuum. Benzene (10 mL) and DBU (54.0 mg, 0.354 mmol) were added and the mixture was refluxed for 3 h. The mixture was concentrated and the crude product was purified by column chromatography (hexanes-ethyl acetate, 1:1) to afford a 1:1 mixture of compounds **4.21a** and **4.21b** as a white solid (27.0 mg, 60%). **4.21a** ^{1}H NMR (CD_3OD) δ: 5.09 (1H, ddd, $J_{2a,3}$ = 6.2 Hz, $J_{2b,3}$ = 4.4 Hz, $J_{3,4}$ = 5.3 Hz, H-3), 4.86 (1H, m, H-6), 4.2 (1H, d, H-4), 3.10 (1H, dd, $J_{2a,2b}$ = 12.6 Hz, H-2a), 2.90 (1H, dd, H-2b), 2.20 (1H, m, H-7a), 2.18 (1H, m, H-9a), 2.07, 2.05 (2COCH_3), 1.73(1H, m, H-8a), 1.72 (1H, m, H-7b), 1.65 (1H, m, H-8b), 1.62 (1H, m, H-9b). ^{13}C NMR (CD_3OD) δ: 171.3, 171.0 (2xO$COCH_3$), 80.7 (C-6), 75.2 (C-3), 73.2 (C-5), 72.2 (C-4), 47.8 (C-2), 31.5 (C-7), 30.3 (C-9), 20.1 (C-8), 19.9, 19.7 (2xCOCH_3). **4.21b** ^{1}H NMR (CD_3OD) δ: 5.10 (1H, d, $J_{3,4}$ = 5.1 Hz, H-4), 4.86 (1H, m, H-6), 4.30 (1H, ddd, H-3), 3.08 (1H, dd, $J_{2a,2b}$ = 11.8 Hz, $J_{2a,3}$ = 6.0 Hz, H-2a), 2.84 (1H, dd, $J_{2b,3}$ = 5.3 Hz, H-2b), 2.34 (1H, m, H-9a), 2.12 (COCH_3), 2.08 (1H, m, H-7a), 2.00 (COCH_3) 1.73(1H, m, H-8a), 1.65 (1H, m, H-8b), 1.64(1H, m, H-7b), 1.5(1H, m, H-9b). ^{13}C NMR (CD_3OD) δ: 171.2, 170.9 (2xO$COCH_3$), 80.6 (C-6), 75.3 (C-4), 72.5 (C-5), 70.9 (C-3), 50.2 (C-2), 31.6 (C-7), 30.6 (C-9), 20.1 (C-8), 19.9, 19.6 (2xCOCH_3). Anal. Calcd. for $C_{12}H_{19}NO_5$: C 56.02, H 7.44, N 5.44. Found: C 55.78, H 7.65, N 5.76.

(3*S*,4*R*,5*S*,6*R*)-1-Aza-spiro[4.4]nonan-3,4,6-triol (4.1)

A mixture of compounds **4.21a** and **4.21b** (25.7 mg, 0.100 mmol) was dissolved in dry MeOH (2 mL) and 1M MeONa (2 mL) was added. The mixture was stirred for 1h at RT and the solvent was removed. The residue was purified by column chromatography (CH_2Cl_2-methanol, 1:1) to afford **4.1** as a colorless oil (13.0 mg, 72%). $[\alpha]_D$ -4.5 (*c* 0.02, methanol). ^{1}H NMR (CD_3OD) δ: 4.22 (1H, ddd, H-3), 4.12 (1H, d, $J_{3,4}$ = 5.2 Hz, H-4), 3.77 (1H, t, $J_{6,7}$ = 5.2 Hz, H-6), 3.02 (1H, dd, $J_{2a,2b}$ = 11.9 Hz, $J_{2a,3}$ = 6.2 Hz, H-2a), 2.77 (1H, dd, $J_{2b,3}$ = 4.9 Hz, H-2b), 2.19 (1H, ddd, $J_{8a,9a}$ = 7.2 Hz, $J_{8b,9a}$ = 9.4 Hz, $J_{9a,9b}$ = 13.5 Hz, H-9a), 2.04 (1H, m, H-7a), 1.75 (1H, m, H-8a), 1.63 (2H, m, H-7b and H-8b), 1.49 (1H, ddd, $J_{8a,9b}$ = 5.0 Hz, $J_{8b,9b}$ = 8.5 Hz, H-9b). ^{13}C NMR (CD_3OD) δ: 77.9 (C-6), 74.6 (C-5), 72.7 (C-4), 72.3 (C-3), 50.1 (C-2), 32.5 (C-7), 30.3 (C-9), 19.6 (C-8). HRMS Calcd. for $C_8H_{16}NO_3$ (M+H): 174.1125. Found: 174.1120.

3,4,6-Tris-methoxymethoxy-1-thia-spiro[4.4]nonane (4.11c)

To a solution of compound **4.12a** (30.0 mg, 0.158 mmol) and *N*,*N*-diisopropylethylamine (0.4 mL, 2.42 mmol) in DMF (1 mL) was added chloromethyl methyl ether (75.0 mg,0.316 mmol) at room temperature. The mixture was stirred for 18h and the solvent was removed. The residue was purified by column chromatography (hexanes-ethyl acetate, 5:1) to afford compound **4.11c** as a colorless oil (40.0 mg, 80%). **4.11c** $[\alpha]_D$ -18 (*c* 0.06, methanol). ^{1}H NMR ($CDCl_3$) δ: 4.95 (1H, d, $J_{A,B}$ = 7.1 Hz, OCH_2O), 4.79 (1H, d, $J_{A,B}$ = 6.8 Hz, OCH_2O), 4.70 (1H, d, OCH_2O), 4.69 (1H, d, $J_{A,B}$ = 6.7 Hz, OCH_2O), 4.65 (1H, d, OCH_2O), 4.64 (1H, d, OCH_2O), 4.35 (1H, ddd, $J_{2a,3}$ = 7.2 Hz, $J_{2b,3}$ = 9.8 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 4.28 (1H, d, H-4), 3.43 (3H, s, CH_3), 3.43 (3H, s, CH_3), 3.38 (3H, s, CH_3), 2.98 (1H, dd, $J_{2a,2b}$ = 10.0 Hz, H-2a), 2.95 (1H, dd, H-2b), 2.10

(1H, m, H-8a), 1.89 (3H, m, H-7a, H-7b, H-9a), 1.80 (1H, m, H-8b), 1.68 (1H, m, H-9b). ^{13}C NMR ($CDCl_3$) δ: 97.4, 96.1, 95.9 (3xO*C*H_2O), 82.0 (C-6), 81.9 (C-4), 80.4 (C-3), 64.9 (C-5), 56.7, 55.9, 55.7 (3*C*H_3), 40.8 (C-8), 30.3 (C-2), 30.1 (C-7), 21.8 (C-9). HRMS Calcd. for: 322.1452. Found: 322.1442.

CHAPTER 5:
GENERAL CONCLUSIONS

5.1 Analogues of Salacinol Containing a Carboxylate Inner Salt

Amino acids, **2.7** and **2.8**, patterned after salacinol (**1.21**), which consist of an iminoarabinitol alkylated with a polyhydroxylated chain containing a carboxylate residue, were successfully synthesized. The synthetic strategy relied on the nucleophilic attack of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino L- or D-arabinitol at the least hindered carbon of 5,6-anhydro-2,3-di-*O*-benzyl-L-ascorbic acid to yield coupled adducts. Screening of compounds **2.7** and **2.8** against dGMII showed that only compound **2.8** was active, with an IC_{50} of 0.3 mM. This is a significant improvement (25-fold) over the inhibition measured for salacinol (**1.21**) which inhibited dGMII with an IC_{50} of approximately 7.5 mM. We noted from the X-ray structure of compound **2.8** bound in the active site of dGMII that it is in the acyclic chain of **2.8** that the interactions of the inhibitor exhibit the most significant differences from salacinol (**1.21**). It is these novel interactions which may account for the increased potency of compound **2.8** in comparison to its parent compound **1.21**, and might be exploited in future inhibitor design. Compound **2.8** also inhibits recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of glucose into glucose itself, with a K_i value of 21 μM. Salacinol (**1.21**) itself has a K_i value of 0.2 μM.

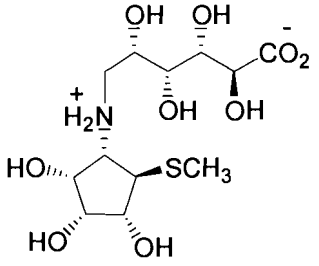
Sulfonium ions **3.2** and **3.3**, analogues of salacinol containing a carboxylate inner salt, were successfully synthesized. The synthetic strategy relied on the nucleophilic attack of 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio- D- or L-arabinitol at the least hindered carbon of 4,5-anhydro-2,3-*O*-isopropylidene-D-ribonic acid benzyl ester to yield coupled adducts. Screening of compounds **3.2** and **3.3** against recombinant human maltase glucoamylase showed that only compound **3.3** was active, with a K_i value of 10 ± 1 μM.

5.2 Aza- and Thia-spiroheterocycles

The syntheses of aza- and thia- spiroheterocycles (**4.1**, **4.12a** and **4.12b**) were successfully achieved. We expected that the hydrocarbon portions of these compounds would make hydrophobic contributions to binding with dGMII as with the six-membered ring of the nanomolar inhibitor swainsonine (**1.3**). The stereochemistry of these compounds was determined by means of 1D-NOESY experiments. Screening of **4.1**, **4.12a** and **4.12b** against dGMII and MGA showed that they are not effective inhibitors, suggesting that these compounds do not fit optimally in the enzyme active sites. The synthesis of spiro sulfonium compounds (**4.3** and **4.4**) related to salacinol (**1.21**) was attempted. The coupling reactions of compounds **4.11a** or **4.11c** with the cyclic sulfate **4.22** or the epoxide **4.23** failed to yield the protected precursors of target compounds, **4.24a/4.24b** or **4.25a/4.25b**.

5.3 Future Work

As suggested in section **2.2.3**, we have designed target compound **5.1** (Figure 5.1) in which a carboxylate side chain is attached to a mannostatin head group. This compound would be a very effective mannosidase inhibitor.



5.1

Figure 5.1 Structure of compound **5.1**.

REFERENCES

1. Taylor, M. E.; Drickamer, K. *Introduction to Glycobiology*, 2nd ed.; Oxford University Press: NY, 2006.

2. Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: NY, 1999.

3. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683-720.

4. Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171-1202.

5. Holman, R. R.; Cull, C. A.; Turner, R. C. *Diabetes Care* **1999**, *22*, 960-964.

6. Jacob, G. S. *Curr. Opin. Struct. Biol.* **1995**, *5*, 605-611.

7. Elbein, A. D. *FASEB J.* **1991**, *5*, 3055-3063.

8. Mohla, S.; White, S.; Grzegorzewski, K.; Nielsen, D.; Dunston, G.; Dickson, L.; Cha, J. K.; Asseffa, A.; Olden, K. *Anticancer Res.* **1990**, *10*, 1515-1522.

9. Goss, P. E.; Baptiste, J.; Fernandes, B.; Baker, M.; Dennis, J. W. *Cancer Res.* **1994**, *54*, 1450-1457.

10. Goss, P. E.; Reid, C. L.; Bailey, D.; Dennis, J. W. *Clin. Cancer Res.* **1997**, *3*, 1077-1086.

11. Pauling, L. *Nature* **1948**, *161*, 707-709.

12. Wolfenden, R.; Snider, M. J. *Acc. Chem. Res.* **2001**, *34*, 938-945.

13. Bennet, A. J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 7287-7294.

14. Koshland, D. E. *Biol. Rev.* **1953**, *28*, 416-436.

15. McCarter, J. D.; Withers, S. G. *Curr. Opin. Struct. Biol.* **1994**, *4*, 885-892.

16. Davies, G.; Henrissat, B. *Structure* **1995**, *3*, 853-859.

17. Wang, Q. P.; Graham, R. W.; Trimbur, D.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 11594-11595.

18. Sinnott, M. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 2026-2032.

19. Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11-18.

20. Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375-1377.

21. Vasella, A.; Davies, G. J.; Bohm, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 619-629.

22. Stutz, A. E. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Wiley-VCH, Weinheim: NY, 1999.

23. Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. *Chem. Rev.* **2002**, *102*, 515-553.

24. Wolfende. R. *Acc. Chem. Res.* **1972**, *5*, 10-18.

25. Kajimoto, T.; Liu, K. K. C.; Pederson, R. L.; Zhong, Z. Y.; Ichikawa, Y.; Porco, J. A.; Wong, C. H. *J. Am. Chem. Soc.* **1991**, *113*, 6187-6196.

26. Ganem, B.; Papandreou, G. *J. Am. Chem. Soc.* **1991**, *113*, 8984-8985.

27. Ando, O.; Satake, H.; Itoi, K.; Sato, A.; Nakajima, M.; Takahashi, S.; Haruyama, H.; Ohkuma, Y.; Kinoshita, T.; Enokita, R. *J. Antibiot.* **1991**, *44*, 1165-1168.

28. Tanaka, K. S. E.; Winters, G. C.; Batchelor, R. J.; Einstein, F. W. B.; Bennet, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 998-999.

29. Kavlekar, L. M.; Kuntz, D. A.; Wen, X.; Johnston, B. D.; Svensson, B.; Rose, D. R.; Pinto, B. M. *Tetrahedron: Asymmetry* **2005**, *16*, 1035-1046.

30. Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1989**, *42*, 883-889.

31. Kawatkar, S. P.; Kuntz, D. A.; Woods, R. J.; Rose, D. R.; Boons, G. J. *J. Am. Chem. Soc.* **2006**, *128*, 8310-8319.

32. Inouye, S.; Tsuruoka, T.; Niida, T. *J. Antibiot.* **1966**, *19*, 288-292.

33. Asano, N. *Glycobiology* **2003**, *13*, 93-104.

34. Hohenschutz, L. D.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.; Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981**, *20*, 811-814.

35. Tepfer, D.; Goldmann, A.; Pamboukdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Denarie, J.; Rosenberg, C. *J. Bacteriol.* **1988**, *170*, 1153-1161.

36. Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Aust. J. Chem.* **1979**, *32*, 2257-2264.

37. van den Elsen, J. M. H.; Kuntz, D. A.; Rose, D. R. *EMBO J.* **2001**, *20*, 3008-3017.

38. Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, *15*, 747-749.

39. Nash, R. J.; Bell, E. A.; Williams, J. M. *Phytochemistry* **1985**, *24*, 1620-1622.

40. Fleet, G. W. J.; Smith, P. W. *Tetrahedron* **1986**, *42*, 5685-5692.

41. Martin, J. L.; Veluraja, K.; Ross, K.; Johnson, L. N.; Fleet, G. W. J.; Ramsden, N. G.; Bruce, I.; Orchard, M. G.; Oikonomakos, N. G.; Papageorgiou, A. C.; Leonidas, D. D.; Tsitoura, H. S. *Biochemistry* **1991**, *30*, 10101-10116.

42. Fosgerau, K.; Westergaard, N.; Quistorff, B.; Grunnet, N.; Kristiansen, M.; Lundgren, K. *Arch. Biochem. Biophys.* **2000**, *380*, 274-284.

43. Jakobsen, P.; Lundbeck, J. M.; Kristiansen, M.; Breinholt, J.; Demuth, H.; Pawlas, J.; Candela, M. P. T.; Andersen, B.; Westergaard, N.; Lundgren, K.; Asano, N. *Bioorg. Med. Chem.* **2001**, *9*, 733-744.

44. Siriwardena, A. H.; Chiaroni, A.; Riche, C.; Eldaher, S.; Winchester, B.; Grierson, D. S. *J. Chem. Soc., Chem. Commun.* **1992**, 1531-1533.

45. Siriwardena, A.; Strachan, H.; El-Daher, S.; Way, G.; Winchester, B.; Glushka, J.; Moremen, K.; Boons, G. J. *ChemBioChem* **2005**, *6*, 845-848.

46. Gonzalez-Outeirino, J.; Glushka, J.; Siriwardena, A.; Woods, R. J. *J. Am. Chem. Soc.* **2004**, *126*, 6866-6867.

47. Svansson, L.; Johnston, B. D.; Gu, J. H.; Patrick, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2000**, *122*, 10769-10775.

48. Johnson, M. A.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2003**, *125*, 5663-5670.

49. Kumar, N. S.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 1262-1264.

50. Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339-1340.

51. Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997**, *38*, 8367-8370.

52. Matsuda, H.; Murakami, T.; Yashiro, K.; Yamahara, J.; Yoshikawa, M. *Chem. Pharm. Bull.* **1999**, *47*, 1725-1729.

53. Yuasa, H.; Takada, J.; Hashimoto, H. *Tetrahedron Lett.* **2000**, *41*, 6615-6618.

54. Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001**, *66*, 2312-2317.

55. Yoshikawa, M.; Morikawa, T.; Matsuda, H.; Tanabe, G.; Muraoka, O. *Bioorg. Med. Chem.* **2002**, *10*, 1547-1554.

56. Ghavami, A.; Sadalapure, K. S.; Johnston, B. D.; Lobera, M.; Snider, B. B.; Pinto, B. M. *Synlett* **2003**, 1259-1262.

57. Szczepina, M. G.; Johnston, B. D.; Yuan, Y.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 12458-12469.

58. Gallienne, E.; Benazza, M.; Demailly, G.; Bolte, J.; Lemaire, M. *Tetrahedron* **2005**, *61*, 4557-4568.

59. Kumar, N. S.; Pinto, B. M. *Carbohydr. Res.* **2005**, *340*, 2612-2619.

60. Nasi, R.; Pinto, B. M. *Carbohydr. Res.* **2006**, *341*, 2305-2311.

61. Choubdar, N.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 4671-4674.

62. Liu, H.; Sim, L.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 3007-3013.

63. Johnston, B. D.; Jensen, H. H.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 1111-1118.

64. Muraoka, O.; Yoshikai, K.; Takahashi, H.; Minematsu, T.; Lu, G. X.; Tanabe, G.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2006**, *14*, 500-509.

65. Chen, W.; Kuntz, D. A.; Hamlet, T.; Sim, L.; Rose, D. R.; Pinto, B. M. *Bioorg. Med. Chem.* **2006**, *14*, 8332-8340.

66. Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926-3937.

67. Nasi, R.; Sim, L.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2007**, *5*, 180-186.

68. Kuntz, D. A.; Ghavami, A.; Johnston, B. D.; Pinto, B. M.; Rose, D. R. *Tetrahedron: Asymmetry* **2005**, *16*, 25-32.

69. Mohan, S.; Pinto, B. M. *Carbohydr. Res.* **2007**, *342*, 1551-1580.

70. Liu, H.; Nasi, R.; Jayakanthan, K.; Sim, L.; Heipel, H.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2007**, *72*, 6562-6572.

71. Jespersen, T. M.; Bols, M.; Sierks, M. R.; Skrydstrup, T. *Tetrahedron* **1994**, *50*, 13449-13460.

72. Jespersen, T. M.; Dong, W. L.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed.* **1994**, *33*, 1778-1779.

73. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319-384.

74. Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett.* **1995**, *36*, 4585-4586.

75. Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007-3018.

76. Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 2707-2708.

77. Liang, X. F.; Bols, M. *J. Org. Chem.* **1999**, *64*, 8485-8488.

78. Ernholt, B. V.; Thomsen, I. B.; Jensen, K. B.; Bols, M. *Synlett* **1999**, 701-704.

79. Ernholt, B. V.; Thomsen, I. B.; Lohse, A.; Plesner, I. W.; Jensen, K. B.; Hazell, R. G.; Liang, X. F.; Jakobsen, A.; Bols, M. *Chem. Eur. J.* **2000**, *6*, 278-287.

80. Aoyagi, T.; Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1404-1408.

81. Aoyama, T.; Naganawa, H.; Suda, H.; Uotani, K.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1557-1558.

82. de Melo, E. B.; Gomes, A. D.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277-10302.

83. Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750-770.

84. Tong, M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137-6139.

85. Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340-347.

86. Pan, Y. T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. *J. Biol. Chem.* **1992**, *267*, 8313-8318.

87. Hoos, R.; Naughton, A. B.; Thiel, W.; Vasella, A.; Weber, W.; Rupitz, K.; Withers, S. G. *Helv. Chim. Acta* **1993**, *76*, 2666-2686.

88. Legler, G.; Finken, M. T. *Carbohydr. Res.* **1996**, *292*, 103-115.

89. Nishimura, Y.; Adachi, H.; Satoh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 4871-4882.

90. Sohoel, H.; Liang, X. F.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1584-1585.

91. Therisod, M.; Therisod, H.; Lubineau, A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2055-2058.

92. Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *J. Antibiot.* **1995**, *48*, 286-288.

93. Ermert, P.; Vasella, A.; Weber, M.; Rupitz, K.; Withers, S. G. *Carbohydr. Res.* **1993**, *250*, 113-128.

94. Heightman, T. D.; Vasella, A.; Tsitsanou, K. E.; Zographos, S. E.; Skamnaki, V. T.; Oikonomakos, N. G. *Helv. Chim. Acta* **1998**, *81*, 853-864.

95. Witczak, Z. J. *Current Medicinal Chemistry* **1999**, *6*, 165-178.

96. Witczak, Z. J.; Boryczewski, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3265-3268.

97. Witczak, Z. J.; Chen, H.; Kaplon, P. *Tetrahedron: Asymmetry* **2000**, *11*, 519-532.

98. Capon, R. J.; Macleod, J. K. *J. Chem. Soc., Chem. Commun* **1987**, 1200-1201.

99. Feather, M. S.; Whistler, R. L. *Tetrahedron Lett.* **1962**, 667-668.

100. Hellman, B.; Lernmark, A.; Sehlin, J.; Taljedal, I. B.; Whistler, R. L. *Biochem. Pharmacol.* **1973**, *22*, 29-35.

101. Izumi, M.; Tsuruta, O.; Harayama, S.; Hashimoto, H. *J. Org. Chem.* **1997**, *62*, 992-998.

102. Andrews, J. S.; Johnston, B. D.; Pinto, B. M. *Carbohydr. Res.* **1998**, *310*, 27-33.

103. Andrews, J. S.; Pinto, B. M. *Carbohydr. Res.* **1995**, *270*, 51-62.

104. Mehta, S.; Jordan, K. L.; Weimar, T.; Kreis, U. C.; Batchelor, R. J.; Einstein, F. W. B.; Pinto, B. M. *Tetrahedron: Asymmetry* **1994**, *5*, 2367-2396.

105. Andrews, J. S., Ph.D. Thesis, Simon Fraser Unviersity, Burnaby, BC, Canada, **1995**.

106. Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron: Asymmetry* **1994**, *5*, 2351-2366.

107. Johnston, B. D.; Pinto, B. M. *Carbohydr. Res.* **1998**, *310*, 17-25.

108. Elbein, A. D. *Annu. Rev. Biochem.* **1987**, *56*, 497-534.

109. Fellows, L. E.; Kite, G. C.; Nash, R. J.; Simmonds, M. S. J.; Scofield, A. M. In *Plant Nitrogen Metabolism;* Poulton, J. E.; Romero, J. T.; Conn, E. E. Ed.; Plenum: New York, 1989; pp. 395-427.

110. Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127-3130.

111. Scofield, A. M.; Fellows, L. E.; Nash, R. J.; Fleet, G. W. J. *Life Sci.* **1986**, *39*, 645-650.

112. Bock, K.; Sigurskjold, B. W. *Stud. Nat. Prod. Chem.* **1990**, *7*, 29-86.

113. Antosiewicz, J.; Wlodek, S. T.; McCammon, J. A. *Biopolymers* **1996**, *39*, 85-94.

114. Zhou, H. X.; Briggs, J. M.; McCammon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 13069-13070.

115. Bonnet, P.; Bryce, R. A. *Protein Science* **2004**, *13*, 946-957.

116. Ghavami, A.; Johnston, B. D.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2001**, *123*, 6268-6271.

117. Li, Y. J.; Scott, C. R.; Chamoles, N. A.; Ghavami, A.; Pinto, B. M.; Turecek, F.; Gelb, M. H. *Clinical Chemistry* **2004**, *50*, 1785-1796.

118. Yuasa, H.; Takada, J.; Hashimoto, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1137-1139.

119. Yuasa, H.; Saotome, C.; Kanie, O. *Trends in Glycoscience and Glycotechnology* **2002**, *14*, 231-251.

120. Nakao, Y.; Maki, T.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *Tetrahedron* **2000**, *56*, 8977-8987.

121. Efange, S. M. N.; Michelson, R. H.; Dutta, A. K.; Parsons, S. M. *J. Med. Chem.* **1991**, *34*, 2638-2643.

122. Raic-Malic, S.; Svedruzic, D.; Gazivoda, T.; Marunovic, A.; Hergold-Brundic, A.; Nagl, A.; Balzarini, J.; De Clercq, E.; Mintas, M. *J. Med. Chem.* **2000**, *43*, 4806-4811.

123. Ghavami, A.; Johnston, B. D.; Maddess, M. D.; Chinapoo, S. M.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *Can. J. Chem.* **2002**, *80*, 937-942.

124. Veerapen, N.; Yuan, Y.; Sanders, D. A. R.; Pinto, B. M. *Carbohydr. Res.* **2004**, *339*, 2205-2217.

125. Overkleeft, H. S.; Vanwiltenburg, J.; Pandit, U. K. *Tetrahedron* **1994**, *50*, 4215-4224.

126. Czarnocki, Z.; Mieczkowski, J. B.; Ziolkowski, M. *Tetrahedron*: *Asymmetry* **1996**, *7*, 2711-2720.

127. Andrews, G. C.; Crawford, T. C.; Bacon, B. E. *J. Org. Chem.* **1981**, *46*, 2976-2977.

128. Joseph, C. C.; Regeling, H.; Zwanenburg, B.; Chittenden, G. J. F. *Tetrahedron* **2002**, *58*, 6907-6911.

129. Li, B.; Kawatkar, S. P.; George, S.; Strachan, H.; Woods, R. J.; Siriwardena, A.; Moremen, K. W.; Boons, G. J. *ChemBioChem* **2004**, *5*, 1220-1227.

130. Brunger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr., Sect. D: Bio. Crystallogr.* **1998**, *54*, 905-921.

131. Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. *Acta Crystallogr., Sect. A* **1991**, *47*, 110-119.

132. Matsuda, H.; Morikawa, T.; Yoshikawa, M. *Pure Appl. Chem.* **2002**, *74*, 1301-1308.

133. Ulgar, V.; Fernandez-Bolanos, J. G.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1242-1246.

134. Johnston, B. D.; Ghavami, A.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2002**, *124*, 8245-8250.

135. Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926-3937.

136. Cimetiere, B.; Jacob, L.; Julia, M. *Bull. Soc. Chim. Fr.* **1991**, 926-938.

137. Satoh, H.; Yoshimura, Y.; Sakata, S.; Miura, S.; Machida, H.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 989-992.

138. Yoshimura, Y.; Watanabe, M.; Satoh, H.; Ashida, N.; Ijichi, K.; Sakata, S.; Machida, H.; Matsuda, A. *J. Med. Chem.* **1997**, *40*, 2177-2183.

139. Bennis, K.; Calinaud, P.; Gelas, J.; Ghobsi, M. *Carbohydr. Res.* **1994**, *264*, 33-44.

140. Rao, B. V.; Lahiri, S. *J. Carbohydr. Chem.* **1996**, *15*, 975-984.

141. Rossi, E. J.; Sim, L.; Kuntz, D. A.; Hahn, D.; Johnston, B. D.; Ghavami, A.; Szczepina, M. G.; Kumar, N. S.; Sterchi, E. E.; Nichols, B. L.; Pinto, B. M.; Rose, D. R. *FEBS J.* **2006**, *273*, 2673-2683.

142. Chen, W.; Kuntz, D. A.; Hamlet, T.; Sim, L.; Rose, D. R.; Pinto, B. M. *Bioorg. Med. Chem.* **2006**, *14*, 8332-8340.

143. Chen, W.; Sim, L.; Rose, D. R.; Pinto, B. M. *Carbohydr. Res.* **2007**, 342, 1661-1667.

144. Paquette, L. A.; Dong, S. Z. *J. Org. Chem.* **2005**, *70*, 5655-5664.

145. Paquette, L. A.; Fabris, F.; Gallou, F.; Dong, S. Z. *J. Org. Chem.* **2003**, *68*, 8625-8634.

146. Paquette, L. A.; Kahane, A. L.; Seekamp, C. K. *J. Org. Chem.* **2004**, *69*, 5555-5562.

147. Paquette, L. A.; Owen, D. R.; Bibart, R. T.; Seekamp, C. K. *Org. Lett.* **2001**, *3*, 4043-4045.

148. Dong, S. Z.; Paquette, L. A. *J. Org. Chem.* **2005**, *70*, 1580-1596.

149. Paquette, L. A. *Aust. J. Chem.* **2004**, *57*, 7-17.

150. Sugisaki, C. H.; Ruland, Y.; Baltas, M. *Eur. J. Org. Chem.* **2003**, 672-688.

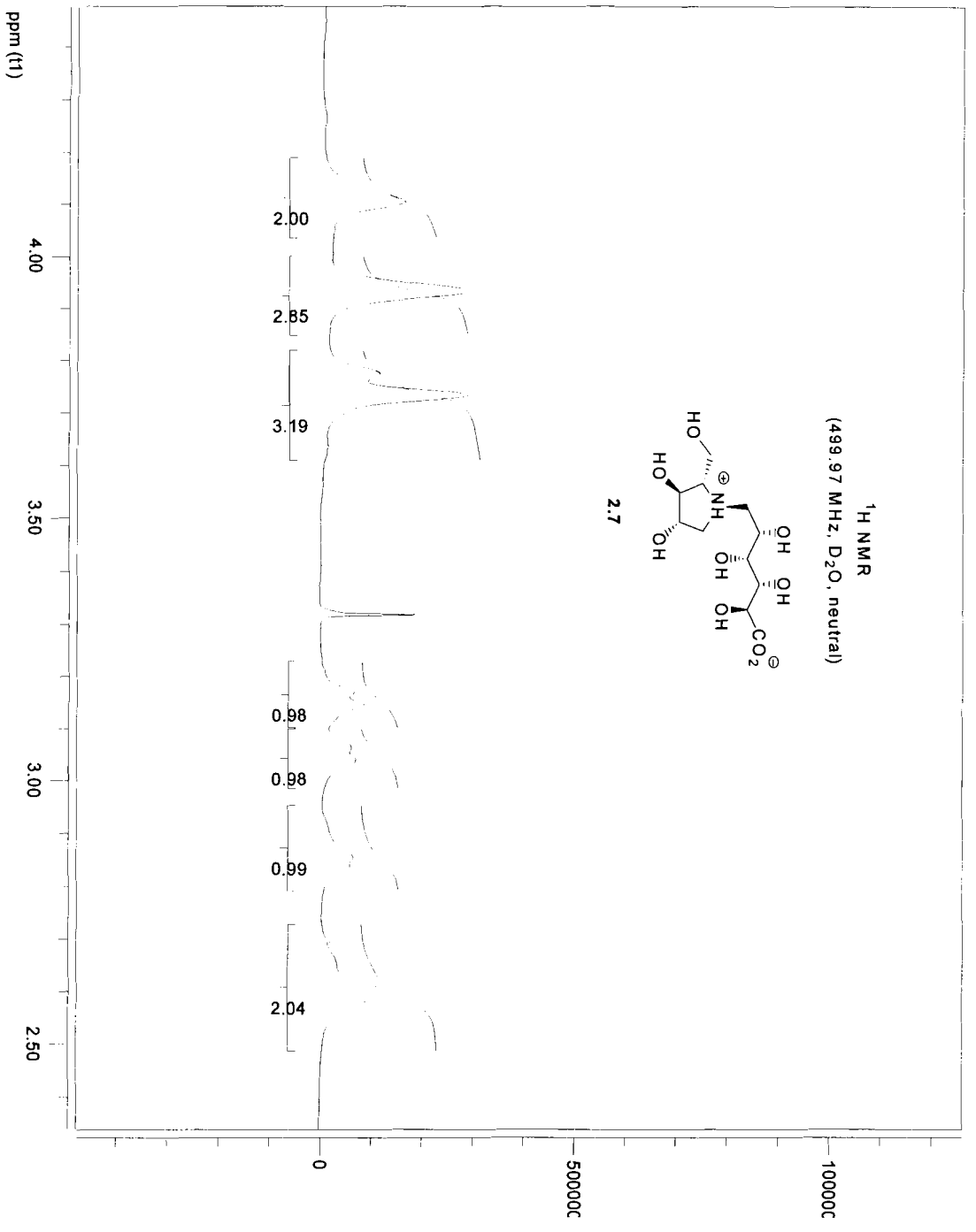
151. Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pirrung, M. C.; White, C. T.; Vanderveer, D. *J. Org. Chem.* **1980**, *45*, 3846-3856.

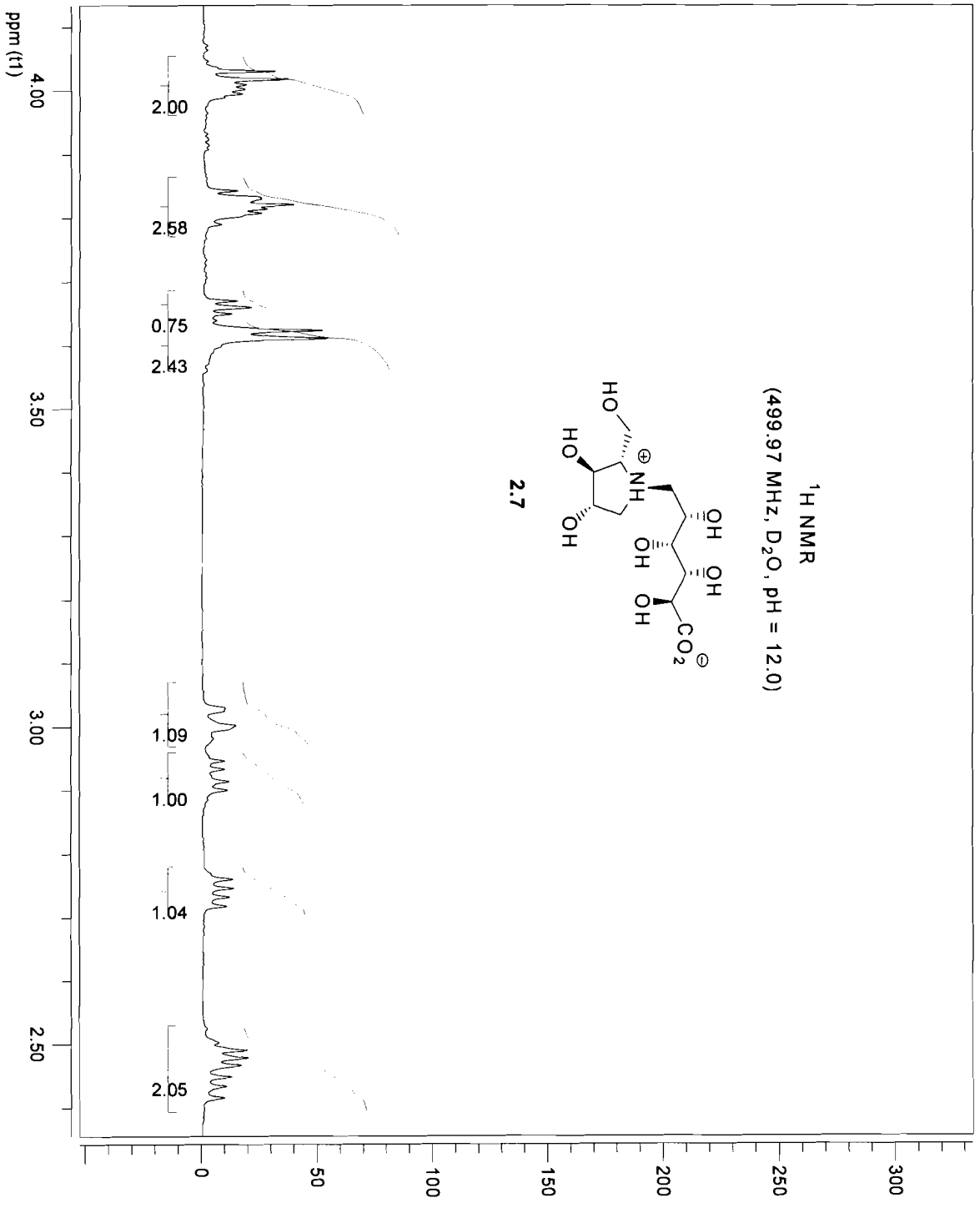
152. Garst, M. E.; Bonfiglio, J. N.; Grudoski, D. A.; Marks, J. *Tetrahedron Lett.* **1978**, 2671-2674.

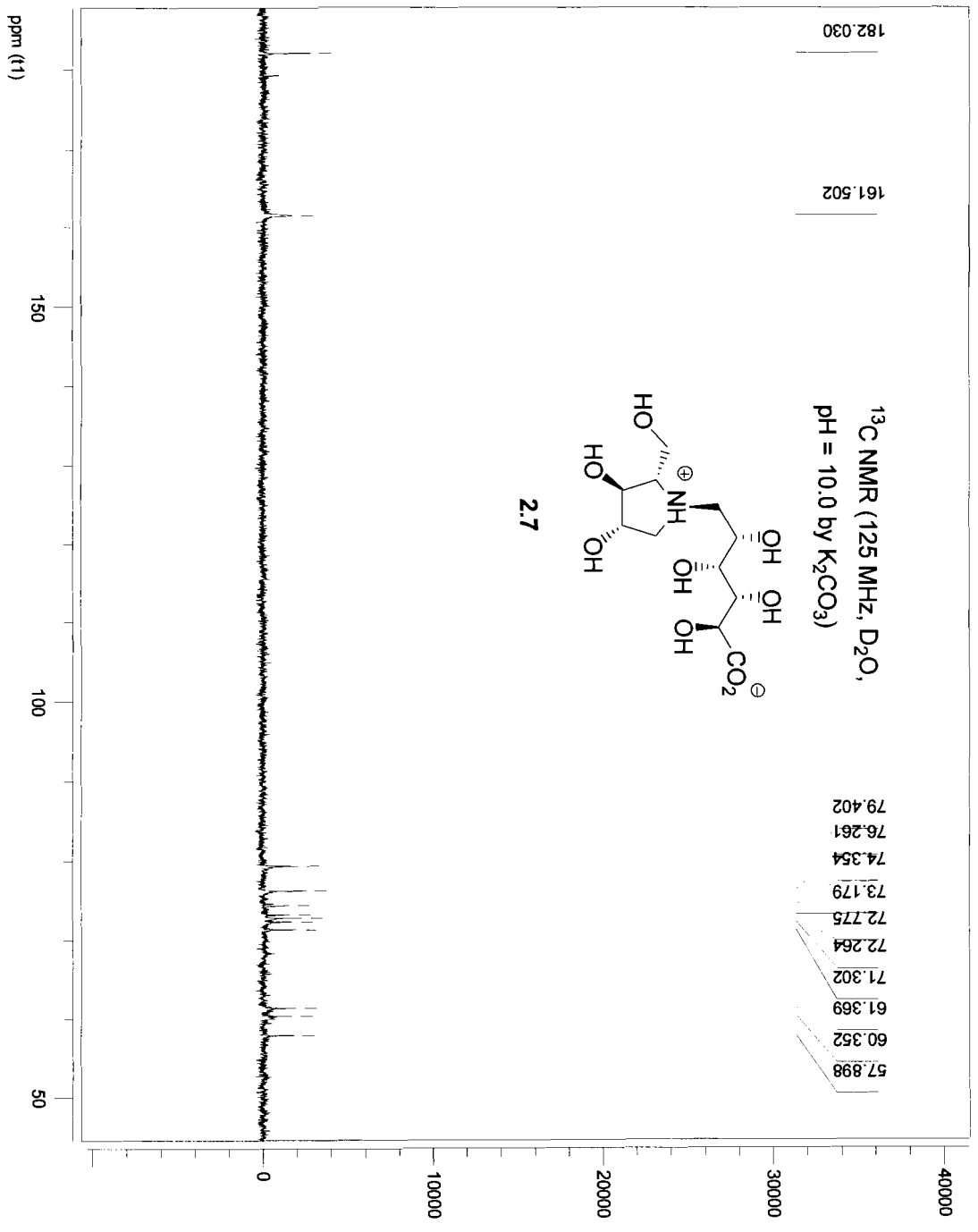
153. Dake, G. *Tetrahedron* **2006**, *62*, 3467-3492.

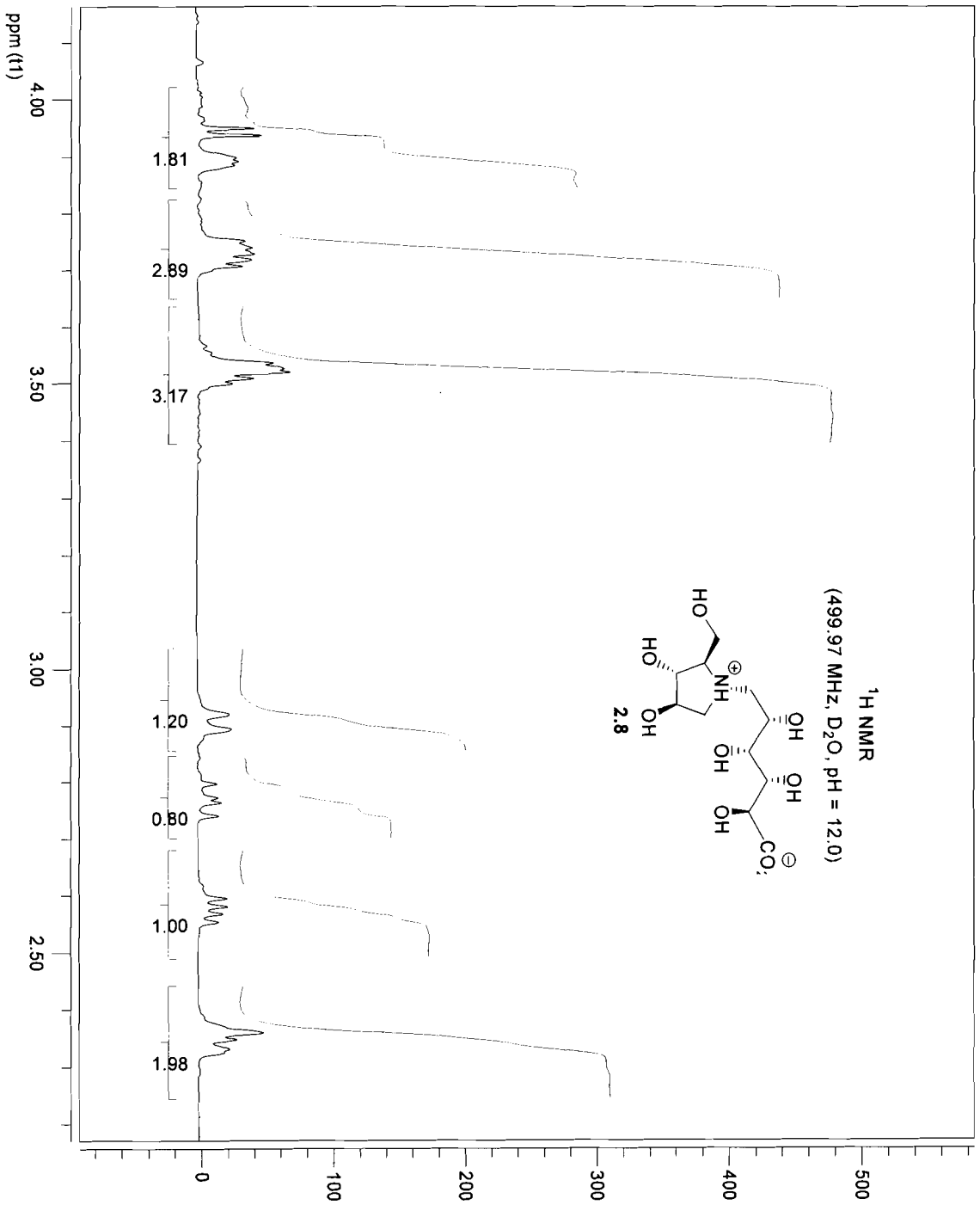
154. Kim, J. H.; Curtis-Long, M. J.; Seo, W. D.; Ryu, Y. B.; Yang, M. S.; Park, K. H. *J. Org. Chem.* **2005**, *70*, 4082-4087.

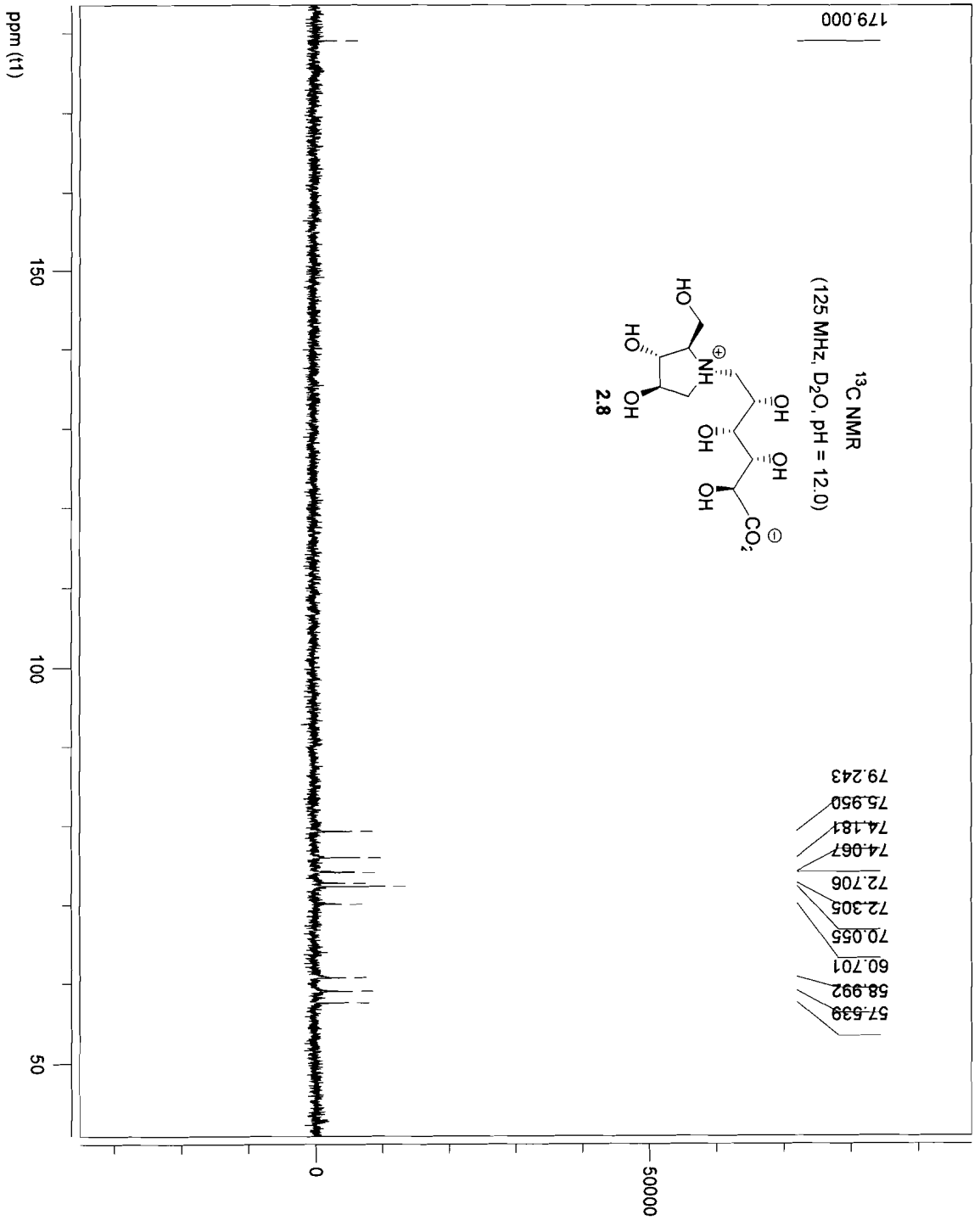
APPENDIX: NMR SPECTRA FOR FINAL PRODUCTS

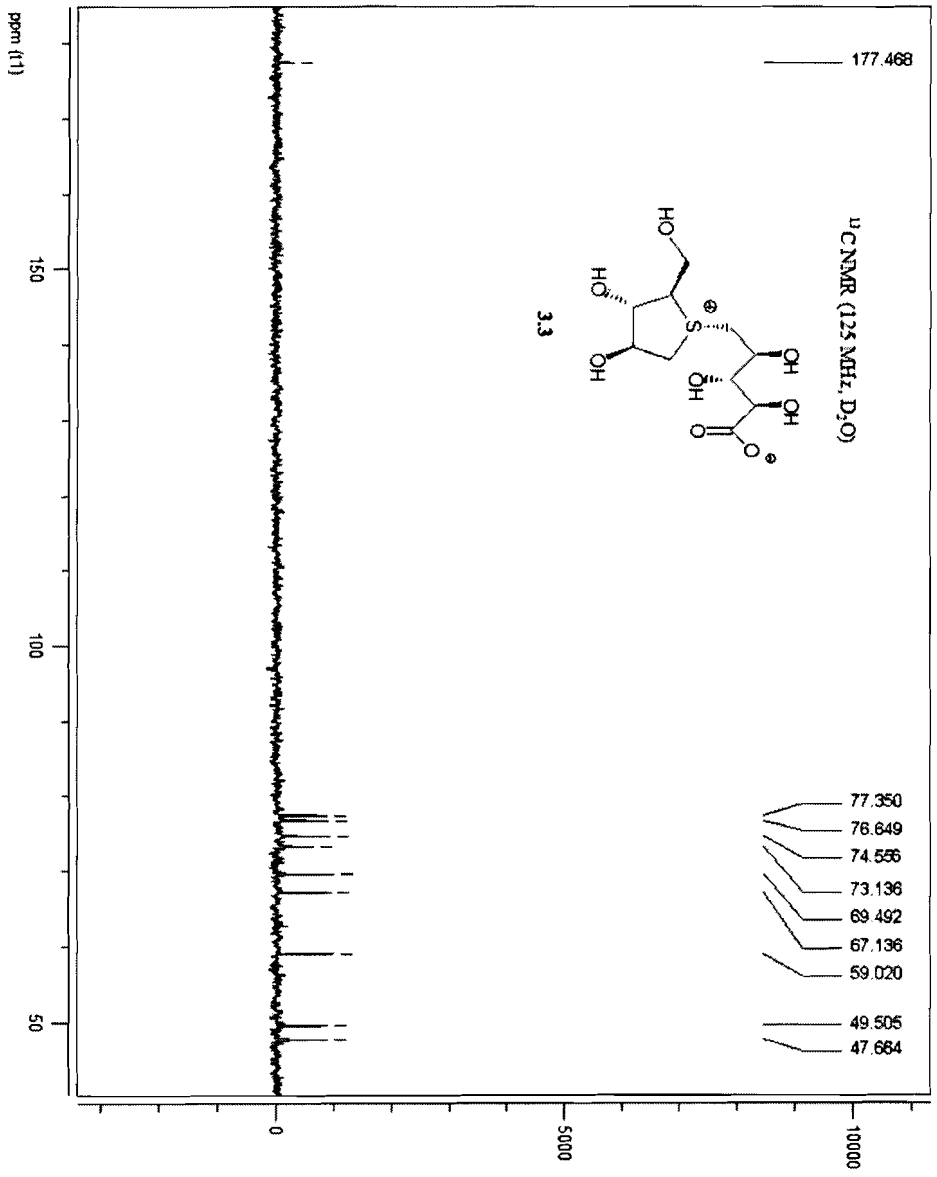

^{1}H NMR
(499.97 MHz, D_2O, neutral)
2.7
2.00
2.85
3.19
0.98
0.98
0.99
2.04
ppm (t1)
4.00
3.50
3.00
2.50
0
50000
100000

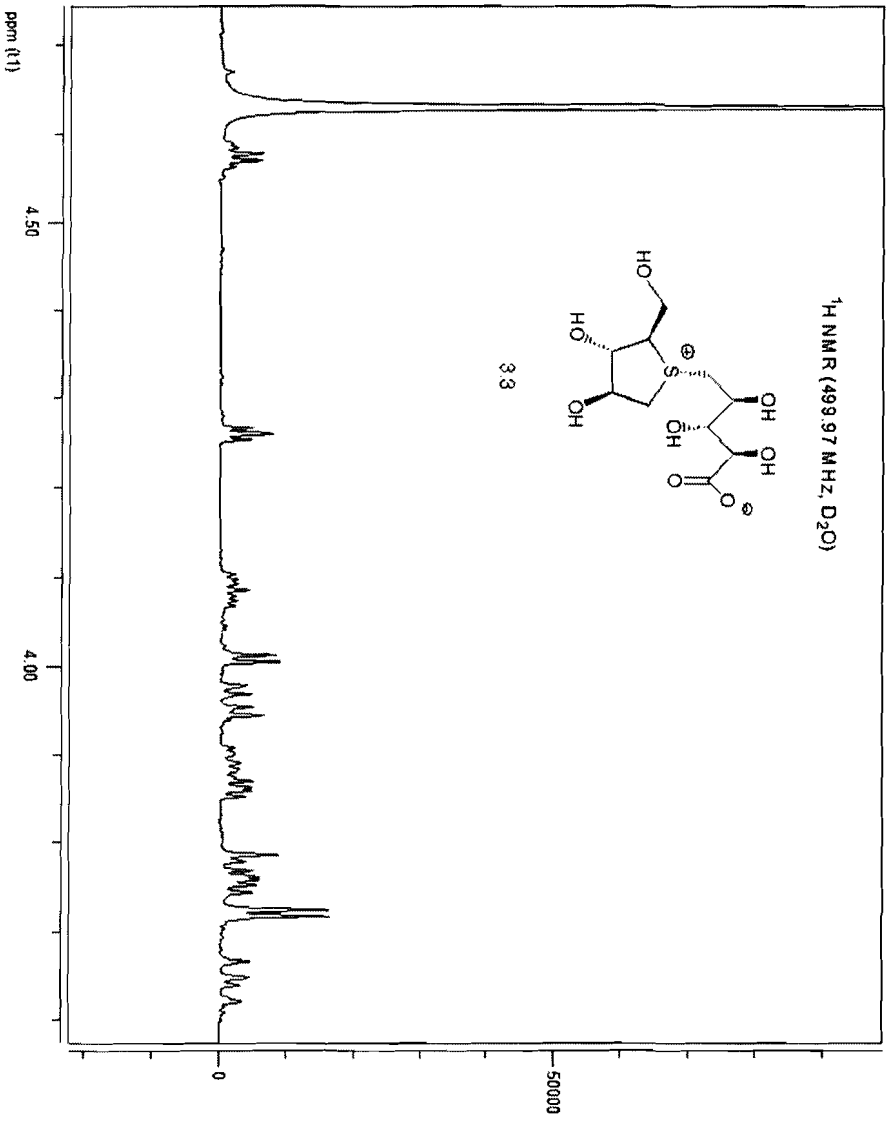

^{1}H NMR
(499.97 MHz, D_2O, pH = 12.0)
2.7
ppm (t1)
4.00
3.50
3.00
2.50
2.00
2.58
0.75
2.43
1.09
1.00
1.04
2.05
0
50
100
150
200
250
300

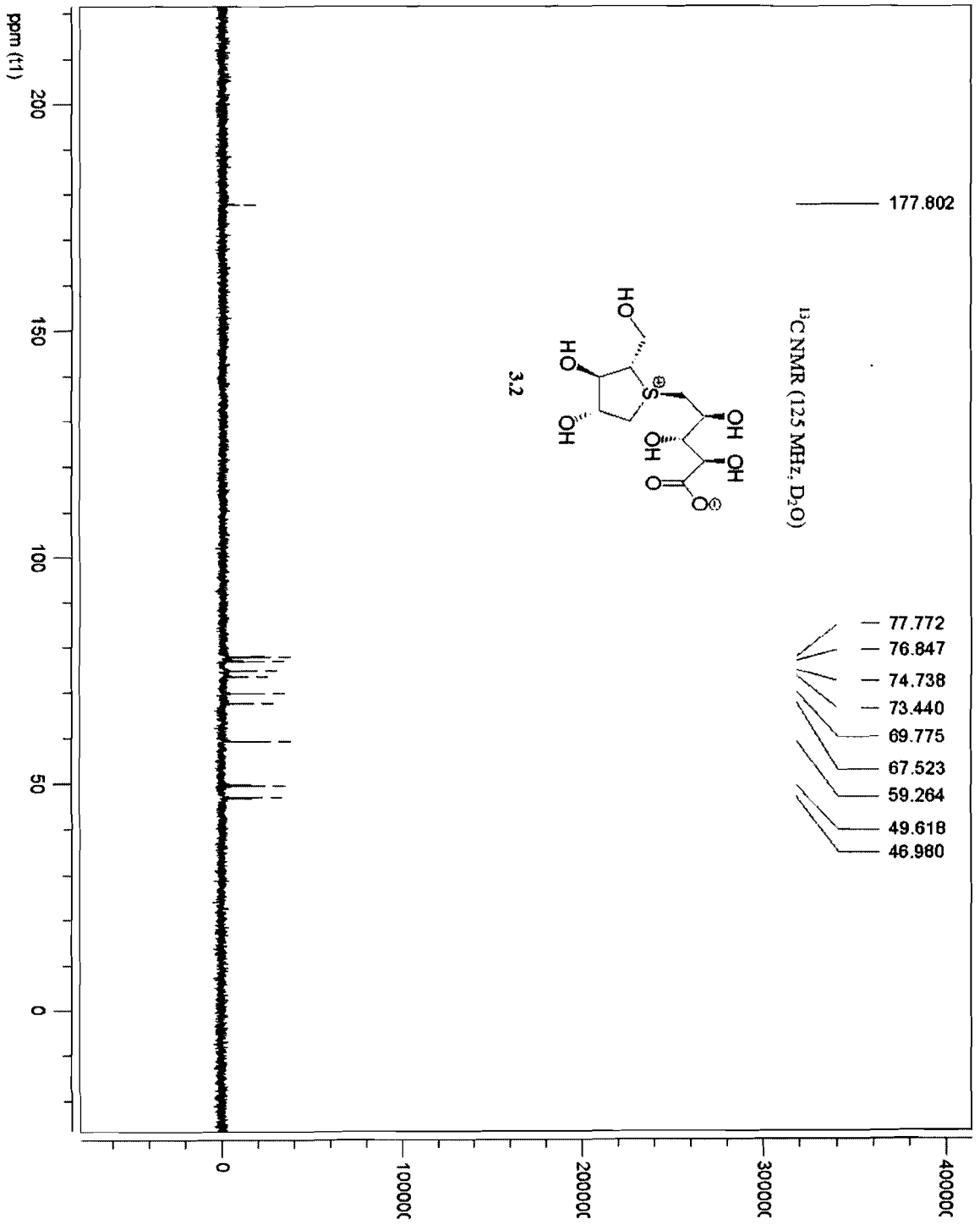

^{13}C NMR (125 MHz, D_2O, pH = 10.0 by K_2CO_3)
2.7
182.030
161.502
79.402
76.261
74.354
73.179
72.775
72.264
71.302
61.369
60.352
57.898
ppm (t1)
150
100
50
0
10000
20000
30000
40000

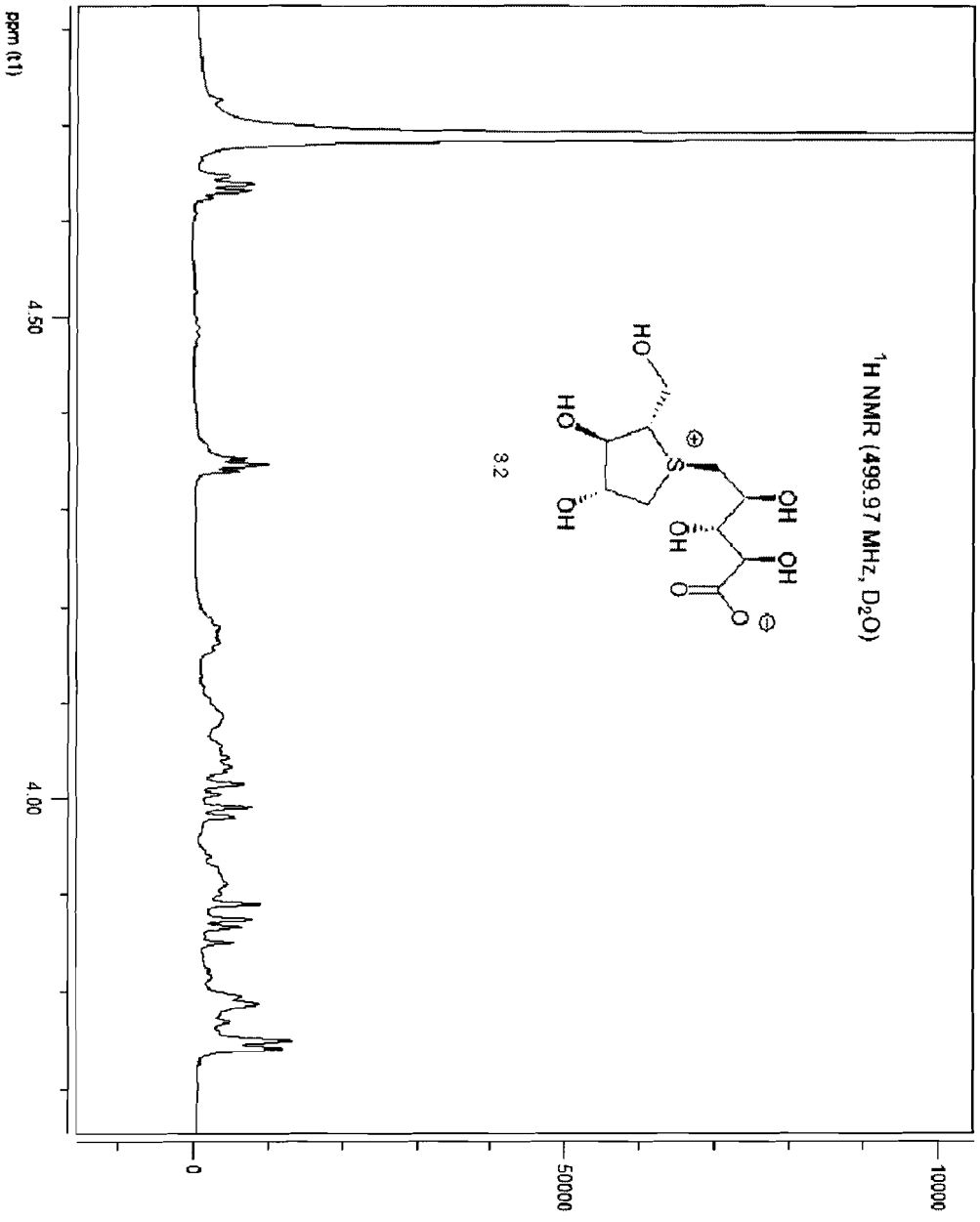

ppm (f1)
4.00
3.50
3.00
2.50
1.81
2.89
3.17
1.20
0.80
1.00
1.98
0
100
200
300
400
500
^{1}H NMR
(499.97 MHz, D_2O, pH = 12.0)
2.8

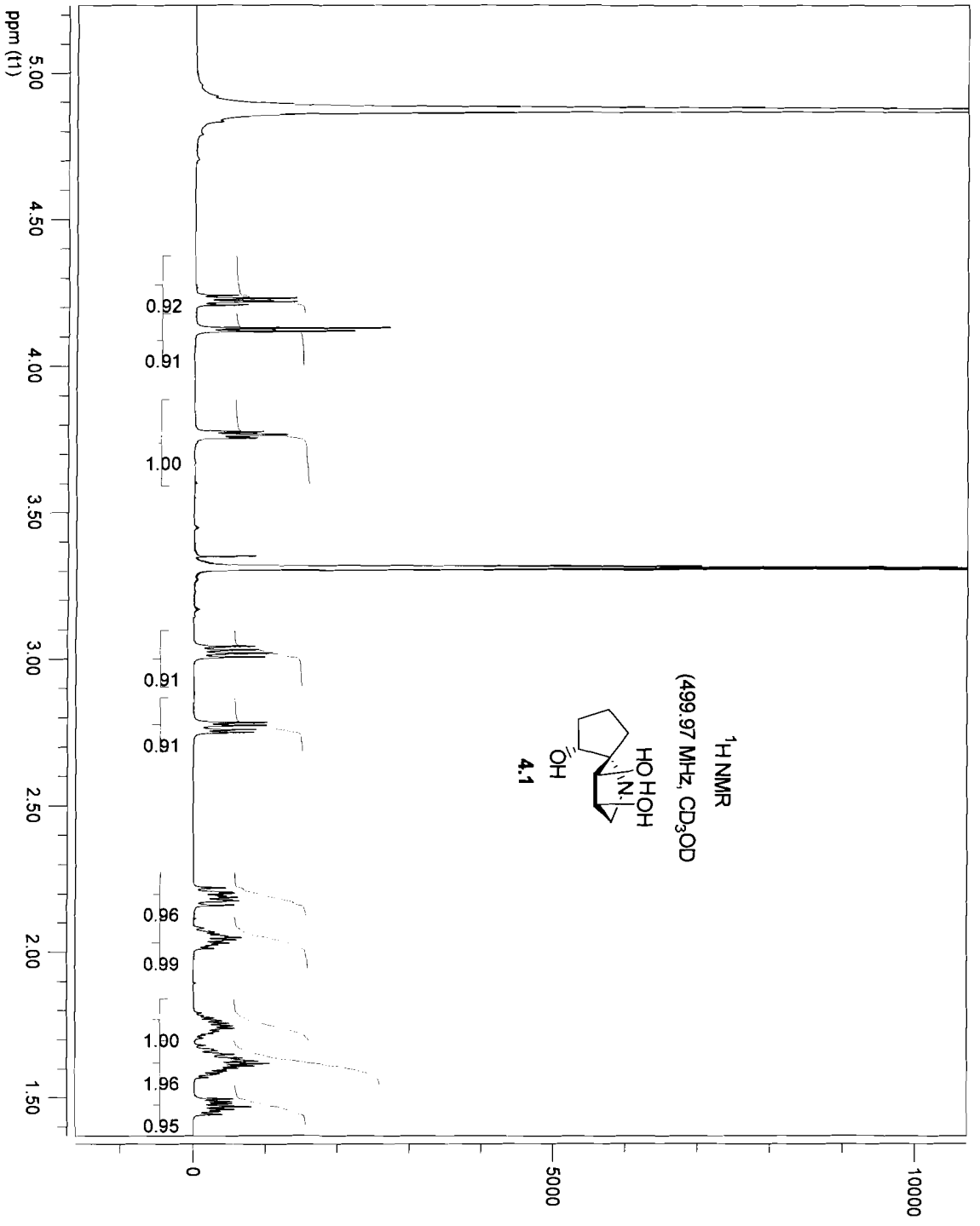

179.000
^{13}C NMR
(125 MHz, D_2O, pH = 12.0)
2.8
57.539
58.992
60.701
70.055
72.305
72.706
74.067
74.181
75.950
79.243
ppm (t1)
150
100
50
0
50000

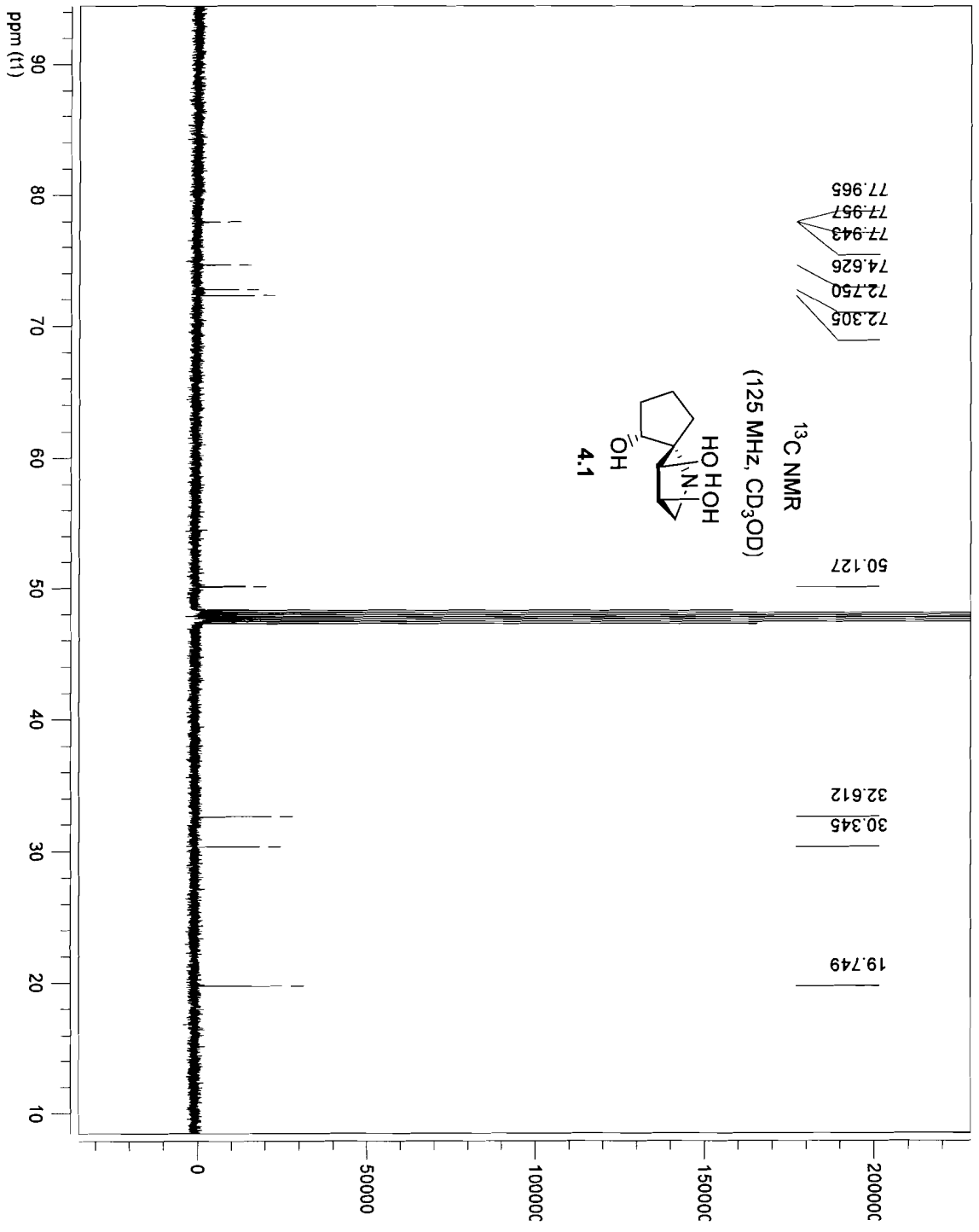

177.468
77.350
76.649
74.556
73.136
69.492
67.136
59.020
49.505
47.664
3.3
^{13}C NMR (125 MHz, D$_2$O)
ppm (t1)
150
100
50
0
5000
10000

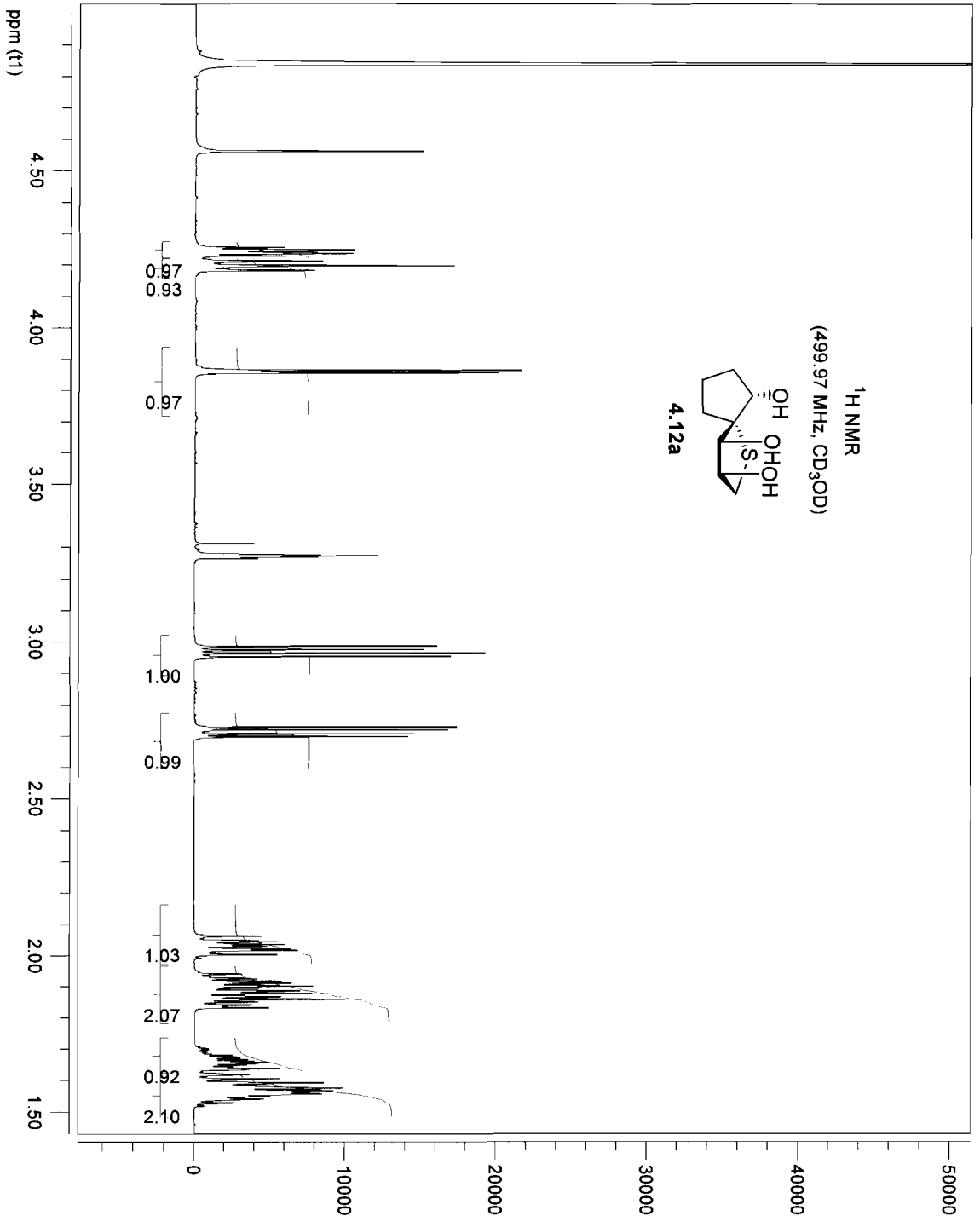

^{1}H NMR (499.97 MHz, D_2O)
3.3
ppm (t1)
4.50
4.00
0
50000

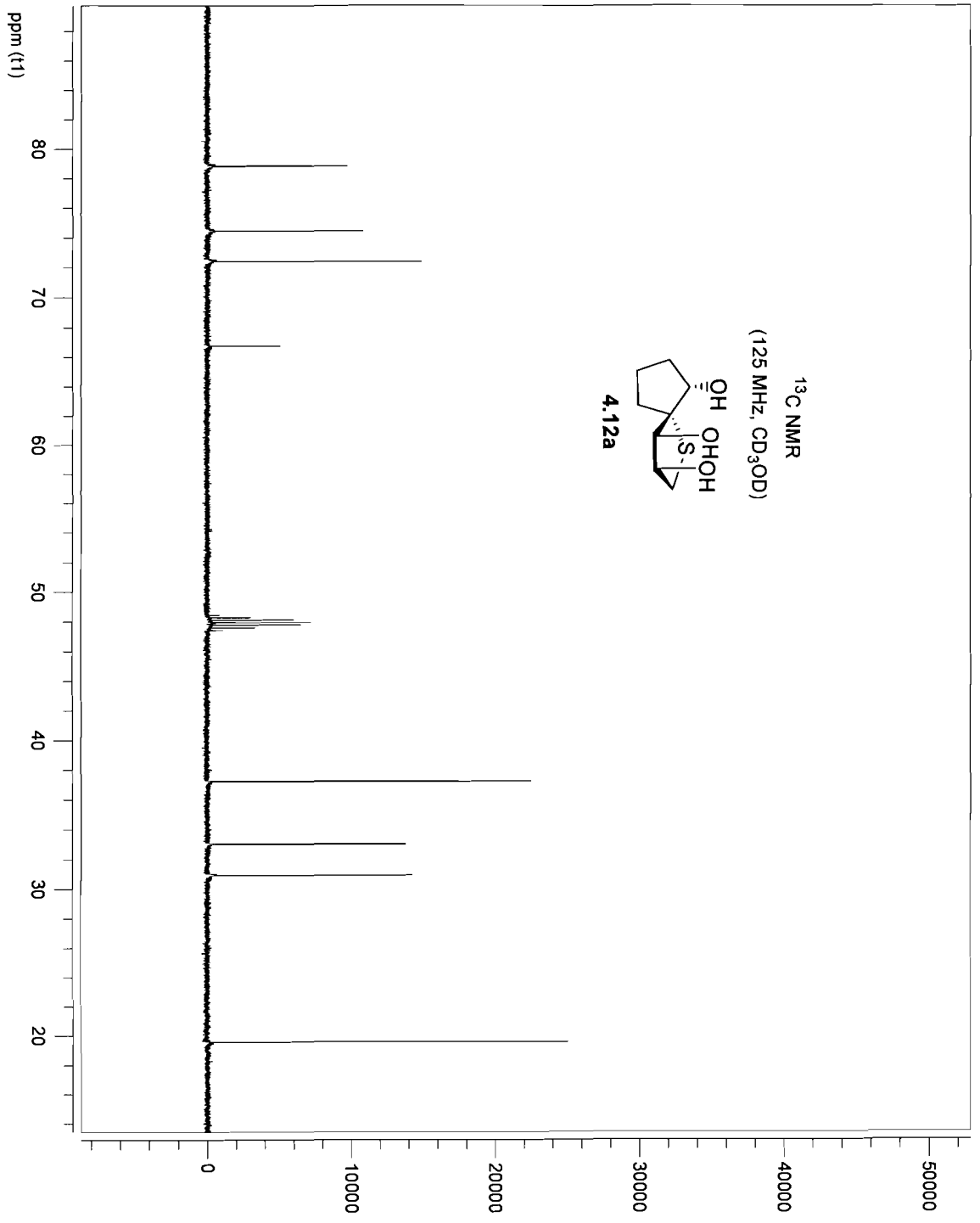

177.802
77.772
76.847
74.738
73.440
69.775
67.523
59.264
49.618
46.960
^{13}C NMR (125 MHz, D_2O)
3.2
ppm (t1)
200
150
100
50
0
0
100000
200000
300000
400000


^{1}H NMR (499.97 MHz, D_2O)
3.2
ppm (t1)
4.50
4.00
0
50000
10000


^{1}H NMR
(499.97 MHz, CD_3OD)
HO HOH
OH
N
4.1
ppm (t1)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
0.92
0.91
1.00
0.91
0.91
0.96
0.99
1.00
1.96
0.95
0
5000
10000


ppm (t1)
10
20
30
40
50
60
70
80
90
0
50000
100000
150000
200000
19.749
30.345
32.612
50.127
^{13}C NMR
(125 MHz, CD$_3$OD)
HO H OH
N
OH
4.1
72.305
72.750
74.626
77.943
77.957
77.965


^{1}H NMR
(499.97 MHz, CD_3OD)
4.12a
OH
OH
OH
S
ppm (t1)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
0.97
0.93
0.97
1.00
0.99
1.03
2.07
0.92
2.10
0
10000
20000
30000
40000
50000


^{13}C NMR
(125 MHz, CD_3OD)
OH OHOH
S
4.12a
ppm (t1)
80
70
60
50
40
30
20
0
10000
20000
30000
40000
50000

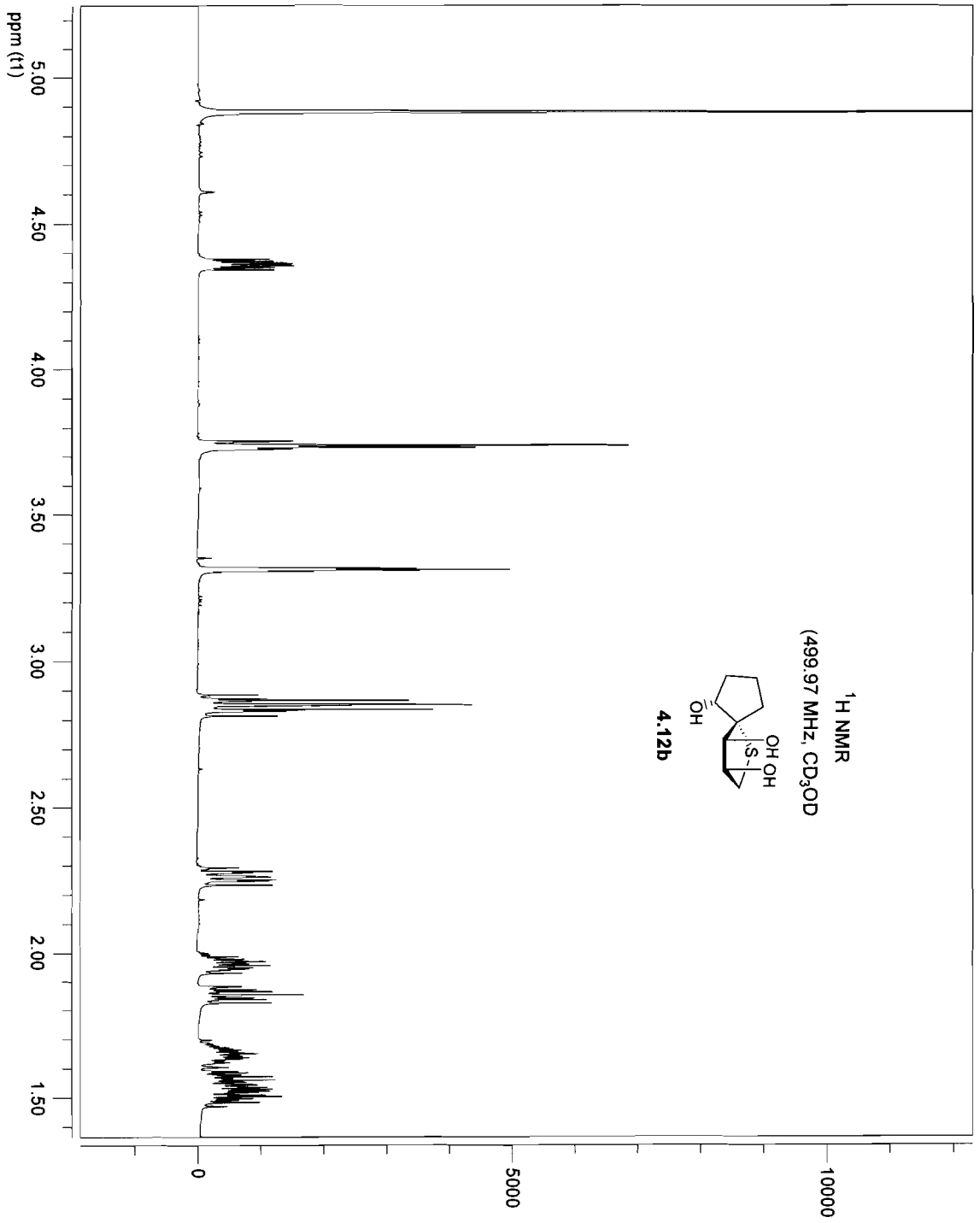
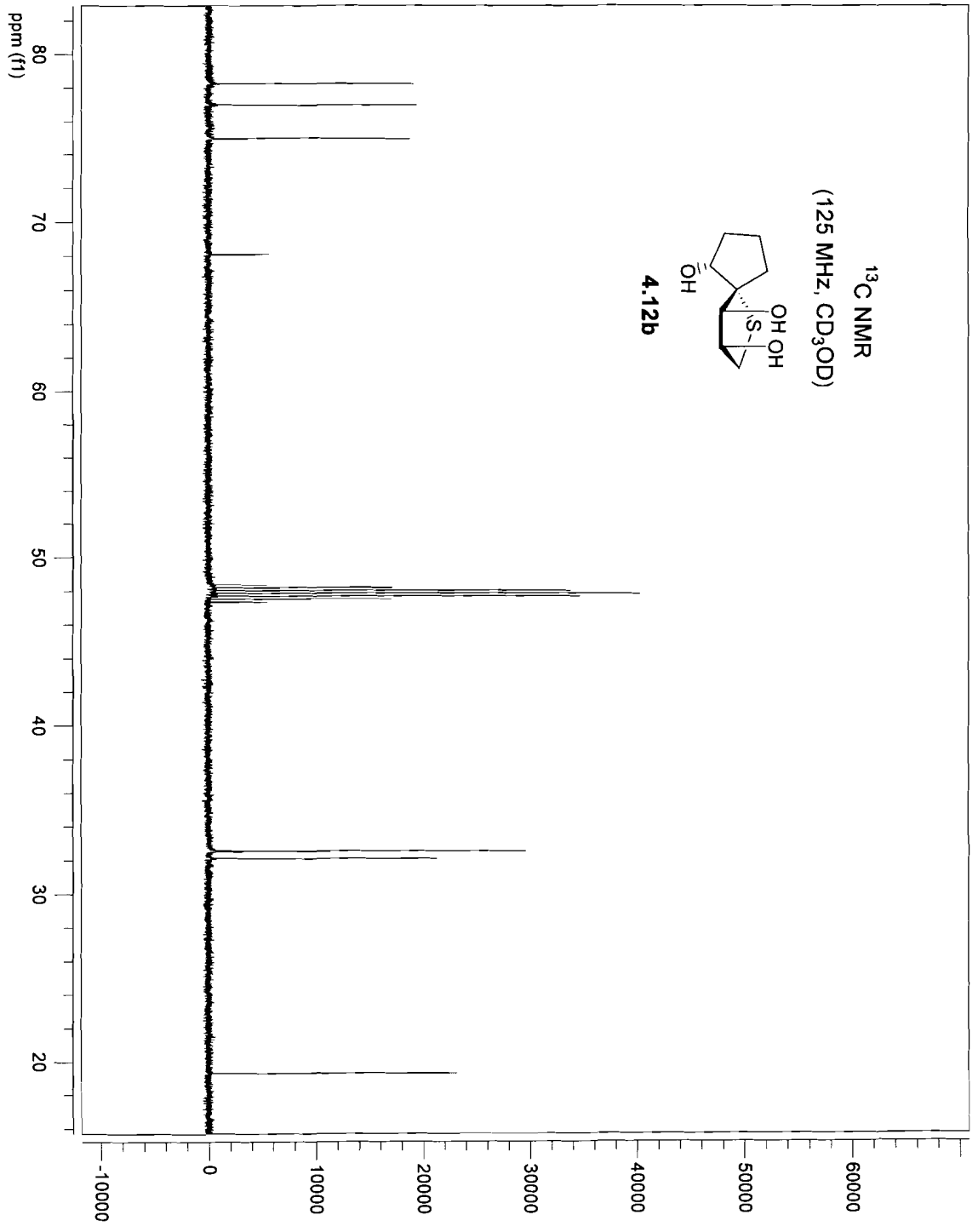

ppm (t1)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
0
5000
10000
^{1}H NMR
(499.97 MHz, CD_3OD
OH
OH OH
S
4.12b

Figure 51


^{13}C NMR
(125 MHz, CD_3OD)
OH OH
OH
4.12b
ppm (f1)
80
70
60
50
40
30
20
60000
50000
40000
30000
20000
10000
0
-10000