A NOVEL CLASS OF GLYCOSIDASE INHIBITORS: AMMONIUM, SELENONIUM, AND SULFONIUM SULFATES

by

Ahmad Ghavami

B.Sc., Chemistry, Shiraz University,

Shiraz, Iran, 1990

M.Sc., Chemistry, Sharif University of Technology,

Tehran, Iran, 1994

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Name: Ahmad Ghavami

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Examining Committee:

Chair: Dr. F.W.B. Einstein, Professor Emeritus

Dr. B.M. Pinto, Professor, Senior Supervisor

Dr. A.J. Bennet, Professor, Committee Member

Dr. P.D. Wilson, Assistant Professor, Committee Member

Dr. K.N. Slessor, Professor, Internal Examiner

Dr. T.G. Back, Professor, External Examiner
Department of Chemistry, University of Calgary

Date Approved: August 6, 2003

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ABSTRACT

This thesis focuses on the design and synthesis of a novel class of compounds, namely ammonium, selenonium, and sulfonium sulfates, as glycosidase inhibitors, and also the investigation of their enzyme inhibitory activities.

A sulfonium ion, salacinol, is one of the active principles in the aqueous extracts of the plant *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of type II diabetes. In order to establish the absolute configuration of salacinol and to further investigate the potential of this novel class of inhibitors, a general synthetic route has been designed that afforded salacinol, its different stereoisomers, and the hitherto unknown nitrogen and selenium congeners.

The inhibitory activities of the candidate glycosidase inhibitors have been examined with different amylases and glucoamylase G2. Enzyme inhibition assays showed that the type of heteroatom and stereochemistry at the different stereogenic centres of the candidate inhibitors play significant roles in discriminating between different glycosidase enzymes. It follows that alterations of these centres, based on an understanding of the atomic interactions between the compounds and their target enzymes, could be a powerful approach to the design of the next generation, high affinity inhibitors. These inhibitors have potential for inhibition of pancreatic α-amylase and intestinal glucosidases, and hence the treatment of type II diabetes.

Inhibition of glycosidase enzymes involved in carbohydrate processing of glycoproteins has also been effective in the treatment of some other disorders such as metastatic cancer. Accordingly, the salacinol-related family of compounds was examined as

potential inhibitors of Golgi α-mannosidase II (GMII), a key enzyme in the *N*-glycoprotein processing pathway.

Of all the candidate inhibitors tested, only four were found to inhibit the enzyme in the mM range. X-ray crystallographic analysis of the complexes of these four inhibitors in the active site of GMII indicates that electrostatic interactions of the positively charged heteroatom centre with Asp 204, the catalytic nucleophile, are important. In addition, a hypothesis is presented that a high-affinity inhibitor for GMII should satisfy a T_6 coordination with the Zn atom in the active site, as in the presumed transition state for the glycosidase catalyzed hydrolysis reaction.

DEDICATION

To my family, with gratitude for all their support and encouragement.

ACKNOWLEDGMENTS

I would like to thank my senior supervisor, Dr. B. Mario Pinto, for giving me the opportunity to work in his laboratory and for all his support and encouragement.

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I wish to thank Mrs. Marcy Tracey for acquiring numerous NMR spectra, Mr. M. K. Yang for performing the elemental analysis, and Dr. David McGillivray for acquiring the high resolution mass spectra.

My deepest thanks go to Dr. F. Matloubi-Moghaddam, my M.Sc. supervisor, who taught me how to work in a chemistry laboratory.

I would like to thank all my labmates, both past and present. It has been a great pleasure working with them. Special thanks to Joan Chen for being a wonderful friend and Rehana Hossany for her assistance in typing the table of contents.

I would also like to thank the people on my volleyball team, who let me release all my work-related stress on the ball!

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LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	acetic acid
All	Allyl
AMY1	barley α-amylase 1
aq	aqueous
Ar	aromatic
Arg	arginine
Asp	aspartic acid
ax	axial
B	boat
Bn	benzyl
bp	boiling point
br	broad
BSA	bovine serum albumin
c	concentration
Calcd	calculated
CelA	cellobiohydrolase A
CCD	charged coupled device
COSYDFTP	correlated spectroscopy with double quantum filtering time-proportional-phase-incrementation
d	doublet
dd	doublet of doublets

ddd	doublet of doublets of doublets
dt	doublet of triplets
dec	decomposition
DMF	*N*, *N*-dimethylformamide
DMNJ	deoxymannojirimycin
DNA	deoxyribonucleic acid
dp	degree of polymerization
E	envelope
eq	equatorial
equiv	equivalent
ER	endoplasmic recticulum
EtOAc	ethyl acetate
Fab	fragment with antigen binding
FAB	fast atom bombardment
Fuc	fucose
Fo-Fc	observed factor-calculated factor
Gal	galactose
Gal*N*Ac	*N*-acetylgalactosamine
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
His	histidine
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IC_{50}	concentration required to reduce binding by 50%
INVBTP	inverse C-H correlation using BIRD pulse sequence with time-proportional-phase-incrementation
J	coupling constant in Hz
K_M	Michaelis constant
K_i	inhibition constant
KDa	kilo Dalton
LSIMS	liquid secondary ionization mass spectrometry
Lit.	literature
m	multiplet
MALDI	matrix-assisted laser desorption ionization
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
Man	mannose
Me	methyl
MeOH	methanol
MES	2-(N-morpholino)ethanesulfonic acid
Mp	melting point
MS	mass spectrometry
NIDD	non-insulin-dependent diabetes
NMR	nuclear magnetic resonance

NOESY	nuclear Overhauser effect spectroscopy
PEG	poly ethylene glycol
Ph	phenyl
PMB	*para*-methoxybenzyl
PNP	*para*-nitrophenyl
Pr	propyl
psi	$lb/inch^2$
quant.	quantitative
Rf	retardation factor
RNA	ribonucleic acid
RP	resolving power
s	singlet
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
t	triplet
TFA	trifluoroacetic acid
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane
Trp	tryptophan
TS	transition state
Tyr	tyrosine

THESIS OVERVIEW

Chapter 1 presents an introduction to glycosidases, their mechanism of action, and some examples of naturally occurring and synthetic glycosidase inhibitors. It also describes the importance of factors such as shape or charge of the inhibitor on its inhibitory activity. Finally, the implications for treatment of type II diabetes with glycosidase inhibitors are featured.

Chapter 2 presents a manuscript (Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001**, *66*, 2312-2317) that describes the design and synthesis of the novel, naturally occurring glucosidase inhibitor, salacinol, and its stereoisomers. It also presents the first conclusive proof of the absolute configuration of salacinol. I performed all the experimental work and characterization of the compounds. B. D. Johnston assisted in the synthetic design.

Chapter 3 presents a manuscript (Ghavami, A.; Johnston, B. D.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2001**, *123*, 6268-6271) that describes the synthesis of the nitrogen analogues of salacinol and their evaluation as glycosidase inhibitors against glucoamylase G2, barley α-amylase and porcine pancreatic α-amylase. I performed all the synthetic work and characterizations of the compounds. B. D. Johnston assisted in the synthetic design. The enzyme inhibition studies were performed by M. T. Jensen and B. Svensson.

Chapter 4 presents a manuscript (Johnston, B. D.; Ghavami, A.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2002**, *124*, 8245-8250) that describes the synthesis of the selenium analogues of salacinol and their evaluation as glycosidase

inhibitors against glucoamylase G2, barley α-amylase and porcine pancreatic α-amylase. I performed the synthesis of the cyclic sulfates and the coupling reactions with the benzylated 4-seleno-D-arabinitol. B. D. Johnston performed the rest of the synthetic work. The enzyme inhibition studies were performed by M. T. Jensen and B. Svensson.

Chapter 5 presents a manuscript (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) that describes the synthesis of the D-xylitol heteroanalogues of salacinol and their evaluation as glycosidase inhibitors against glucoamylase G2, barley α-amylase and porcine pancreatic α-amylase. S. M. Chinapoo synthesized the cyclic amine and M. D. Maddess performed the coupling reaction of the cyclic amine and the D-cyclic sulfate. I performed the rest of the syntheses and the characterization of all compounds. The enzyme inhibition studies were performed by M. T. Jensen and B. Svensson.

Chapter 6 presents a manuscript (Ghavami, A.; Sadalapure, K. S.; Johnston, B. D.; Lobera, M.; Snider, B. B.; Pinto, B. M. *Synlett* **2003**, 1259-1262) that describes an improved method for the synthesis of salacinol and its stereoisomers. K. S. Sadalapure and I performed the synthesis and characterization of the compounds. B. D. Johnston assisted with synthetic design. M. Lobera and B. B. Snider used 1,1,1,3,3,3-hexafluoroisopropanol as a solvent for the coupling reaction in a model system, and informed us of the outcome of the reaction. Professional courtesy prompted us to include them as co-authors.

Chapter 7 presents an introduction to the function and structure of Golgi α-mannosidase II and describes the inhibitory activities of our novel class of inhibitors against this enzyme. It also presents the X-ray crystallographic analysis of complexes of the inhibitors with the enzyme, and the analysis of the enzyme-inhibitor interactions in the

active site. The enzyme inhibition and X-ray crystallographic studies were performed by D. A. Kuntz, and D. R. Rose. I performed the analysis of the interactions of the inhibitors in the active site. W. Xin assisted in the manipulation of coordinate files.

Chapter 8 presents the general conclusions resulting from the work in this thesis.

CHAPTER 1: INTRODUCTION

1.1 General introduction to carbohydrates

Carbohydrates are molecules made of carbon, oxygen, and hydrogen atoms and can be considered to be one of the most important classes of biomolecules. They are the main source of the energy, for example, starch in plants and glycogen in animals. Some carbohydrate structures present on cell surfaces are involved as recognition units for antibodies, toxins, and hormones. Carbohydrates also form the backbones of DNA and RNA, as deoxyribose and ribose units, respectively.

The best known and common member of this family, D-glucose, was known to the ancient Persians and Arabians as grape-sugar. Because of the complexity of their structures, carbohydrate structure elucidation has not been facile; however, there has been a great deal of work in this area over a period of forty years. The versatile properties of carbohydrates are due to the diversity of the structures that can be made from a limited number of monosaccharides as building blocks by linking them in a variety of ways. Compared to the amino acids that form one type of linkage, two identical monosaccharides for example, glucose, form more than 10 different disaccharides. Carbohydrates are often found covalently linked to other biomolecules such as proteins, and lipids which are called glycoconjugates.

1.2 Glycosidases

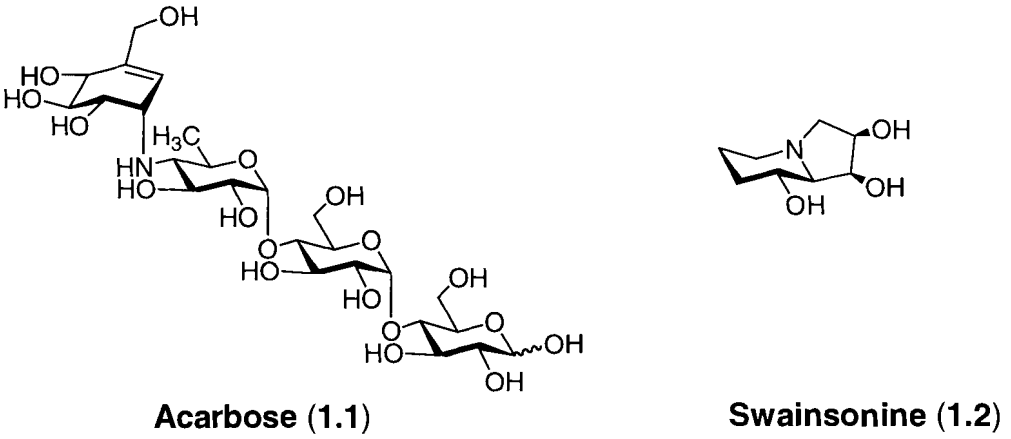
Glycosidases are enzymes that catalyze the hydrolysis of glycosidic bonds and convert polysaccharides to oligosaccharides or monosaccharides. Glycosidase enzymes with diverse functional specificity play important roles in the biochemical processing of biopolymers containing carbohydrates.[1] One important class of these enzymes is

responsible for the liberation of glucose from its higher oligomers or polymers. Disruption in the function and regulation of these enzymes can lead to disease states such as diabetes.

During a few hours after having a meal the blood glucose concentration is high. During this time, the pancreas secretes insulin that causes rapid transfer of glucose to the cells and lowers the blood glucose level. In type II (non-insulin-dependent) diabetes mellitus (NIDD), insulin secretion can be normal but the cells are not very sensitive to the insulin. In the treatment of NIDD, management of blood glucose levels is critical. One strategy for treating NIDD is to delay digestion of ingested carbohydrates, thereby lowering postprandial blood glucose concentration. This can be achieved by administering drugs which inhibit the activity of enzymes, such as pancreatic α-amylase which breaks down starch to oligosaccharides containing glucose and the glucosidases, which mediate the hydrolysis of oligosaccharides to glucose in the small intestine. For example, the carbohydrate analogue acarbose (**1.1**), which is currently used for the oral treatment of diabetes,[2,3] reversibly inhibits the function of pancreatic α-amylase and certain membrane-bound intestinal α-glucoside hydrolase enzymes. In patients suffering from type II diabetes, such enzyme inhibition results in delayed glucose absorption into the blood and a smoothing or lowering of postprandial hyperglycemia, resulting in improved glycemic control.

Inhibition of glycosidase enzymes involved in carbohydrate processing of glycoproteins has also been effective in the treatment of some nondiabetic disorders such as cancer.[4] While normal cells display characteristic oligosaccharide structures, tumor cells display very complex structures that are usually restricted to embryonic tissues.[4] It is believed that these complex structures provide signal stimuli for rapid proliferation and

metastasis of tumor cells. A possible strategy for the therapeutic use of glycosidase inhibitors is to take advantage of the different rates of normal vs cancer cell growth to inhibit assembly of complex oligosaccharide structures. For example, the indolizidine alkaloid swainsonine (**1.2**), an inhibitor of Golgi α-mannosidase II (GMII), a key component of the *N*-glycosylation pathway in protein synthesis, reportedly reduces tumor cell metastasis, enhances cellular immune responses, and slows tumor cell growth in mice (see also Chapter 7).[5] Swainsonine treatment has led to significant reduction of tumor mass in human patients with advanced malignancies, and is a promising drug therapy for patients suffering from breast, liver, lung, and other malignancies.[6,7] Therefore, natural or synthetic inhibitors of glycosidase enzymes have potential as new therapeutic agents.



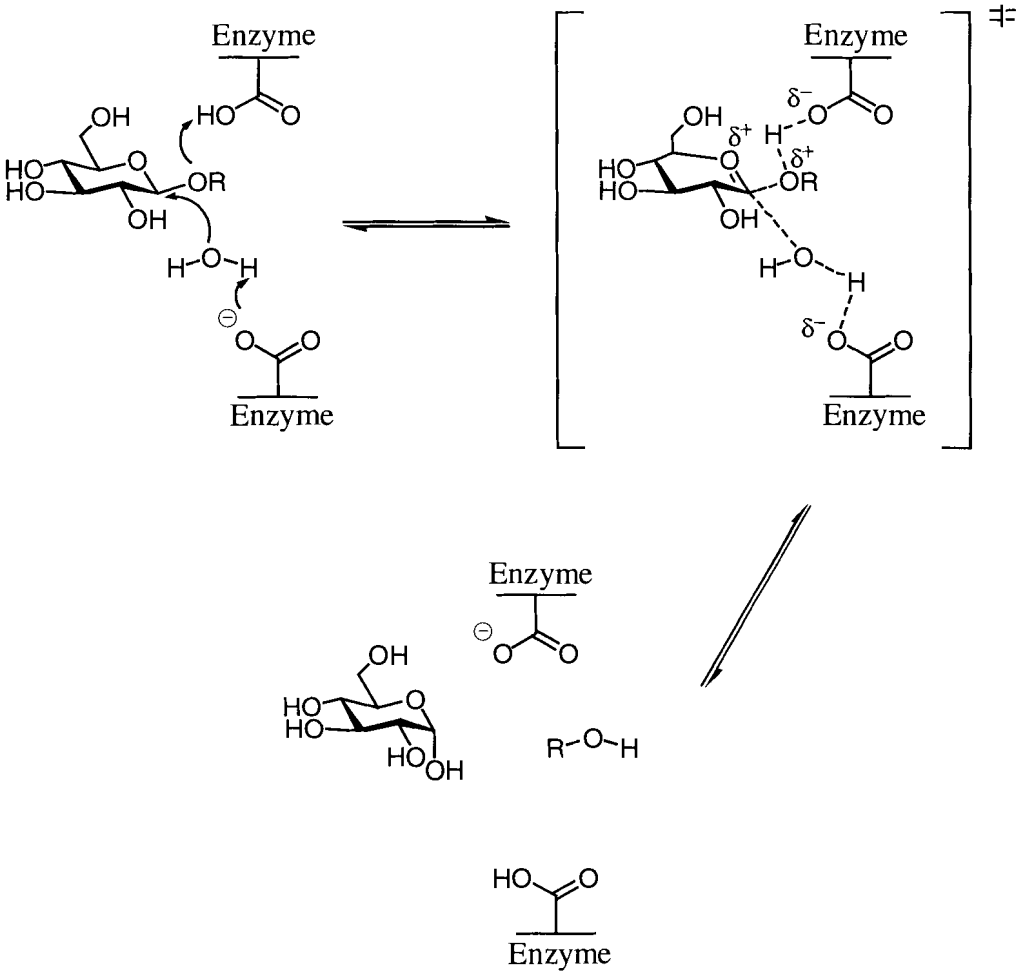
Acarbose (1.1) **Swainsonine (1.2)**

Chart 1.1. Structures of acarbose (**1.1**) and swainsonine (**1.2**).

In order to design highly specific and effective inhibitors for glycosidases, one should know the mechanism of action of these enzymes and also the structures of the active sites. The reaction is a nucleophilic substitution at the anomeric carbon which can occur by retention or inversion of the anomeric configuration. In 1953, Koshland proposed

the mechanisms for these two basic classes of glycosidases which are still widely accepted.[8]

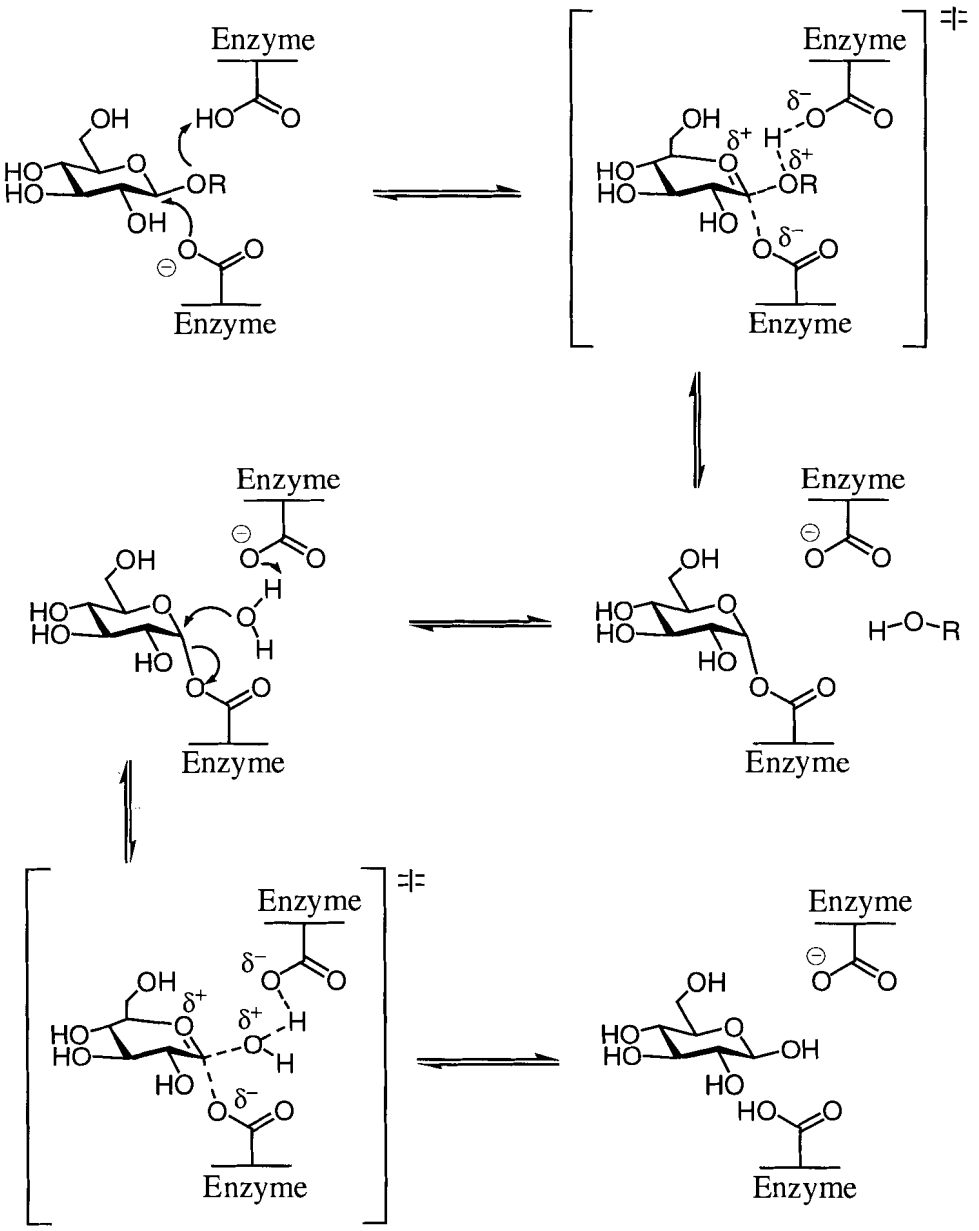
In the inverting glycosidases the reaction occurs via a single displacement mechanism. There are two carboxylic acid residues in the active site involved in the mechanism. One carboxylic acid acts as a general acid and protonates the aglycon to make a better leaving group while the second carboxylate group acts as a general base to remove a proton from the water and increase its nucleophilicity (Scheme 1.1).



Scheme 1.1. Inverting mechanism of glycosidases.

The retaining glycosidases act via a double displacement mechanism. In the first

step, one of the carboxylic acid residues acts as a general acid and protonates the aglycon and the second carboxylate acts as a nucleophile, forming the glycosyl-enzyme intermediate. In the second step, the carboxylate acts as a general base and deprotonates the nucleophilic water molecule which attacks the anomeric carbon and replaces the carboxylate leaving group (Scheme 1.2).



Scheme 1.2. Retaining mechanism of glycosidases.

The two carboxyl groups in inverting glycosidases are 10.5 Å apart on average, compared to the two carboxyl residues in the retaining glycosidases which are 5.5 Å apart on average.[9]

Both mechanisms involve a transition state (TS) in which there is a partial positive charge on the endocyclic oxygen atom and the bond between this oxygen and the anomeric carbon atom has a partial double bond character (Chart 1.2).

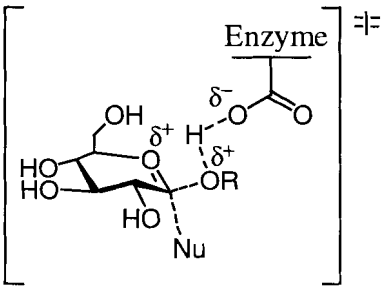


Chart 1.2. Proposed transition state for glycosidases.

In enzymatic catalysis, the TS is preferably stabilized by electrostatic or hydrophobic interactions with the enzyme active site. These interactions might be non-covalent and present at the ground state too, but they are optimized at the transition state and so the TS is stabilized much more; consequently, the activation energy is much less in an enzyme catalyzed reaction.[10]

Therefore, a highly effective inhibitor for these enzymes can be a stable molecule which can mimic the oxacarbenium ion transition state electronically and structurally. This compound theoretically would be able to bind to the active site of the enzyme with high affinity.

The stereoelectronic requirement for planarity of an oxacarbenium ion is unambiguous. In the case of a furanosyl cation, the conformation of the ring is probably

an envelope, whereas in the case of a pyranosyl cation it is probably a half-chair or classical boat (Chart 1.3).[11] In all these cases, portions of the molecule are planar.

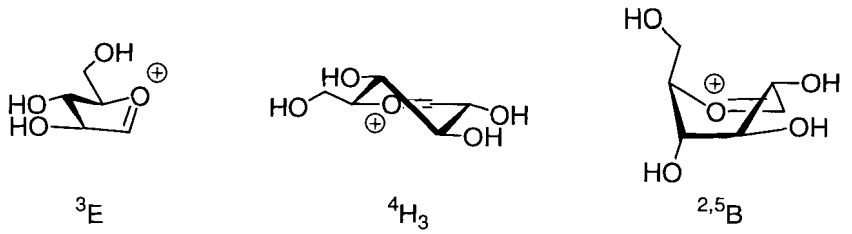


Chart 1.3. Proposed structures of oxacarbenium ions.

There are some criteria for an inhibitor to be considered a TS analogue.[12] For example:

a) Strong inhibition. As was mentioned before, a TS analogue will bind much more strongly to the enzyme compared to a substrate analogue. Theoretically, this difference in the inhibitory activities can be a factor equal to the ratios of the rate constants of the enzyme-catalyzed and the un-catalyzed reactions which can be as high as 10^{17} in some cases.[13] However, one should be very careful with this classification, since it does not mean that all the strong inhibitors are TS analogues.

b) Slow inhibition. Enzymes have a conformation which is complementary to the conformation of the substrate in the ground state. After binding to the substrate, the conformation of the enzyme is changed to another conformation which is more favored for the binding to the TS. A TS analogue can only bind to the conformationally changed enzyme, and only a small fraction of the enzyme is normally in the TS conformation. This fact will slow down the binding of the TS analogue inhibitor to the enzyme. There are some examples of slow inhibition which are not due to the enzyme conformational change.[14]

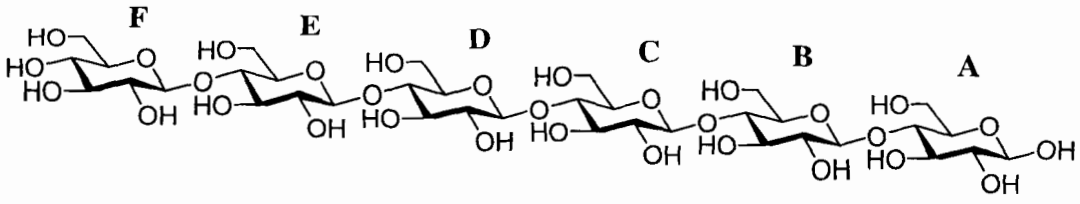
c) Specificity. The specificity of an enzyme depends on both the catalytic action of the enzyme and the binding to the substrate. This includes the interactions of the enzyme with the sugar hydroxyl groups and, to a lesser extent, the interactions with the aglycon. To be able to evaluate the specificities of inhibitors, one should know the specificities for the enzymes, because overlapping specificities can be misleading.

1.3 Glycosidase inhibitors

Small molecules that can inhibit the activity of an enzyme are very important in controlling many biological reactions. Glycosidase inhibitors have potential as drugs for disorders such as diabetes, viral infections, and cancer.[2-4] They can also provide information about the structure and mechanism of action of enzymes.

To design glycosidase inhibitors most of the focus has been on mimicking the conformation of the TS, and the proposed positive charge. The widely held, but incorrect, belief that all glycosidases perform catalysis by going through a $^4H_3$ shaped TS caused most research groups to try to mimic the half chair conformation of the TS in their designs, but there are also some reports of mimicking the boat conformation.[15]

To the best of my knowledge, there are three reports that support the possibility of formation of a boat TS for glycosidase hydrolysis. Guerin et al.[16] reported the crystal structure of cellobiohydrolase CelA, in complex with cellohexaose (**1.3**). The glucosyl residue **D** is bound to the active site in a distorted $^{2,5}B$ conformation, which facilitates the formation of an oxacarbenium ion intermediate and breakage of the **D** – **C** glycosidic bond.

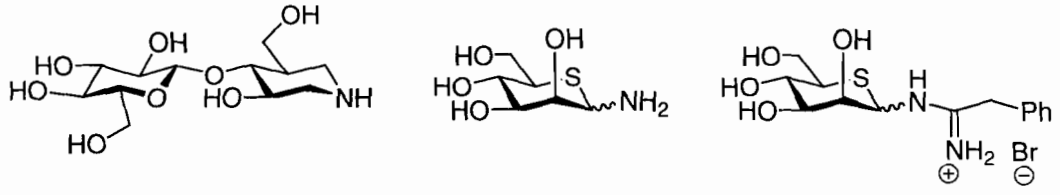


1.3

Chart 1.4. Structure of cellohexaose (**1.3**).

Varrot et al.[17] also reported the formation of a $^{2,5}B$ conformation for the isofagomine ring of compound **1.4**, in the active site of the inverting β-glucosidase, Cel6A.

In a study in our group, 5-thio-D-mannopyranosylamine (**1.5**) and 5-thio-D-mannopyranosylamidinium bromide (**1.6**) were synthesized as potential glycosidase inhibitors. Both these compounds were found to bind in a boat ($^{1,4}B$) conformation in the Golgi α-mannosidase II active site (Figure 1.1).[18]



1.4 **1.5** **1.6**

Chart 1.5. Structures of compounds **1.4 – 1.6**.

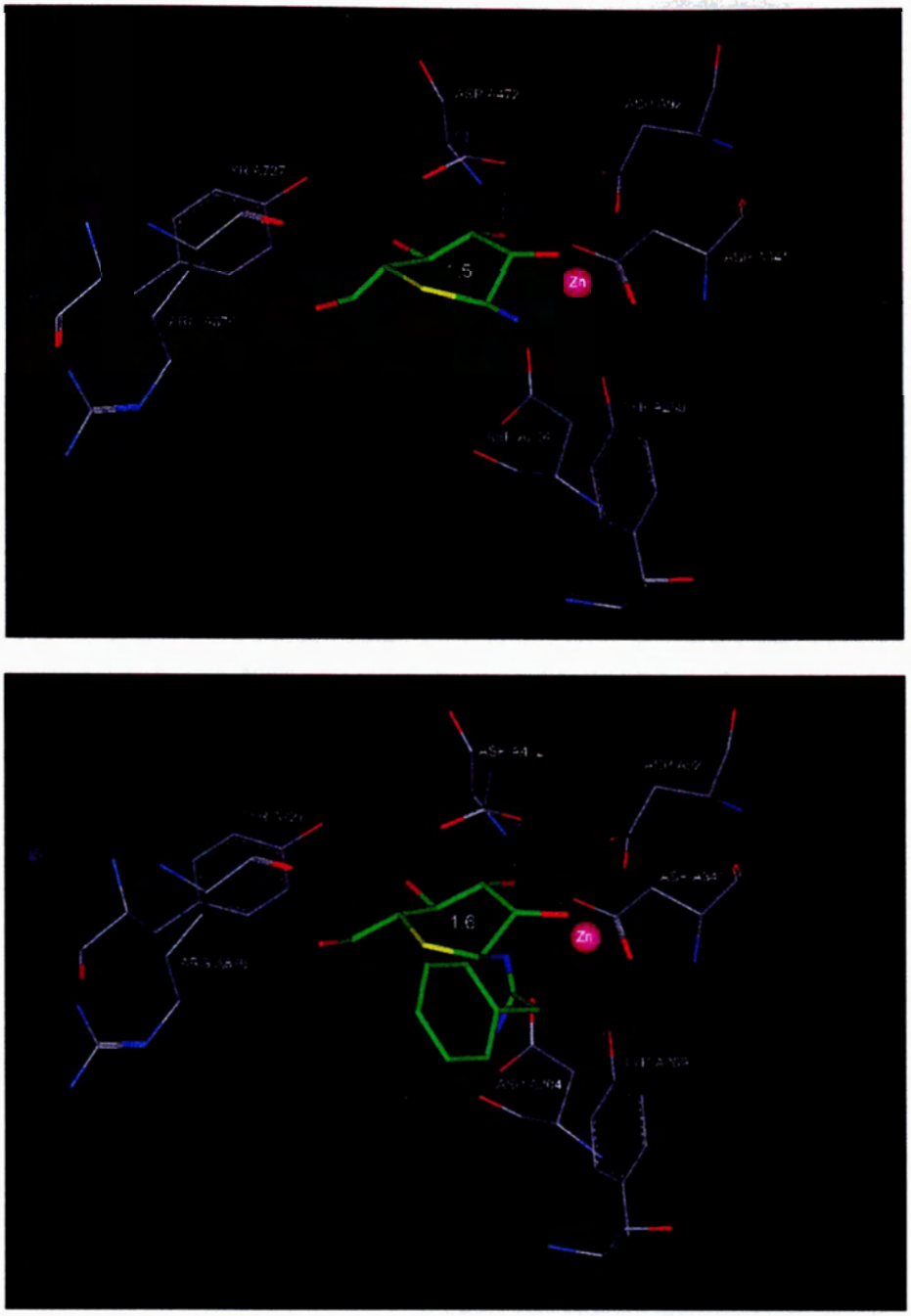


Figure 1.1. X-ray crystal structures of **1.5** and **1.6** in the active site of GMII. (Reproduced from reference 18. Copyright © 2003 Simon Fraser University. Reprinted with permission from Lizie Mehta Kavlekar.)

Designs focusing on charge have mimicked the positive charge in a number of positions in the molecule.[19] The first step in the catalytic hydrolysis of glycosides is the protonation of the exocyclic oxygen. In an early TS, there is a substantial build up of positive charge on this atom. Compounds like acarbose (**1.1**) that have an amine instead of the exocyclic oxygen atom can be considered as this class of TS analogues. There are also inhibitors that mimic the charge build up on the endocyclic oxygen atom. Nojirimycin (**1.7**) and swainsonine (**1.2**) are examples of this class. Another class mimics the positive charge build up on the anomeric carbon of the oxacarbenium ion (Chart 1.6). Isofagomine (**1.8**) in which the anomeric carbon atom of a sugar has been replaced by a nitrogen atom is an example of this class.[20]

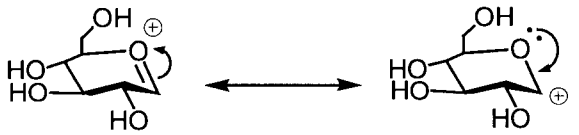
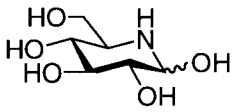
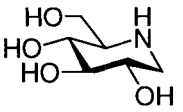


Chart 1.6. Resonance structures of an oxacarbenium ion.

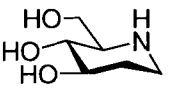


Nojirimycin (1.7)



Deoxynojirimycin (1.9)

Isofagomine (1.8)



Fagomine (1.10)

Chart 1.7. Structures of compounds **1.7** – **1.10**.

1.3.1 Iminosugars as glycosidase inhibitors

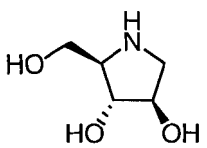
Naturally occurring analogues of the sugars which contain a nitrogen atom instead of the ring oxygen atom have been isolated from different sources and have shown very strong inhibitory activities against glycosidases. These sugar mimics are hydroxylated derivatives of the monocyclic and bicyclic systems found in piperidine, pyrrolidine, indolizidine, pyrrolizidine and nortropane alkaloids and are described in greater detail below.[21]

1.3.1.1 Piperidines

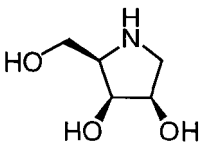
Nojirimycin[22] (**1.7**) is the oldest member of this family and the first iminosugar isolated from bacteria in 1966; it is a potent inhibitor of α- and β-glucosidases from various sources. 1-Deoxynojirimycin[23] (**1.9**), and fagomine[24] (**1.10**) are other examples of the same family with strong inhibitory activities against glycosidases.

1.3.1.2 Pyrrolidines

1,4-Dideoxy-1,4-imino-D-arabinitol (**1.11**), was isolated from the fruits of the plant *Angylocalyx boutiqueanus* and is a potent α-glucosidase inhibitor.[25] The D-lyxitol derivative (**1.12**) is an α-galactosidase inhibitor.[25]



1.11



1.12

Chart 1.8. Structures of compounds **1.11** and **1.12**.

1.3.1.3 Indolizidines

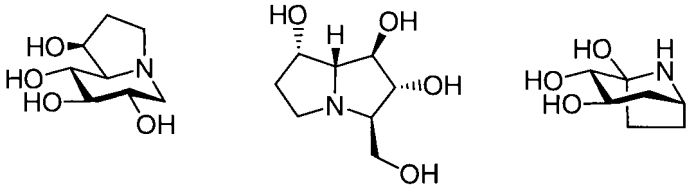
Indolizidines are polyhydroxy heterocycles with a five and a six membered ring fused together. Swainsonine (**1.2**) and castanospermine (**1.13**) were isolated from the plants *Swainsona canescens* and *Castanospermum australe*, respectively, which are toxic to livestock. Swainsonine (**1.2**) is also found in locoweed, which cause the disorder locoism, in the western United States. Swainsonine (**1.2**) is a nM inhibitor of Golgi α-mannosidase II (see Chapter 7), and castanospermine is a μM inhibitor of β-glucosidase.

1.3.1.4 Pyrrolizidines

These polyhydroxy heterocycles have two five membered rings fused together. Australine (**1.14**) has been also found in the seeds of *Castanospermum australe*, and is a μM inhibitor of glucoamylase.[26]

1.3.1.5 Nortropanes

Nortropanes also contain a five and a six membered fused ring system and were added to the previous four groups after the discovery of calystegines. Calystegines (**1.15**) are usually found in the roots and underground organs of plants, and they have shown mM inhibitory activities against α- and β-galactosidases and β-glucosidase.[27]

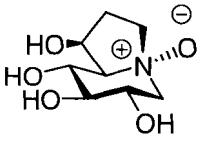


1.13 **1.14** **1.15**

Chart 1.9. Structures of compounds **1.13 – 1.15**.

Azasugars and glycosylamines are moderately basic and in the enzyme active site they are protonated; hence, they mimic the positive charge of the oxacarbenium ion. This protonation can take place via two different mechanisms. a) The amine equilibrates with the aqueous solvent (depending on the pK_a) first and the ammonium ion formed interacts with the carboxylate group of the enzyme. b) The amine binds to the enzyme first and becomes protonated by the carboxylic acid residue in the active site.

In order to introduce a permanent positive charge on the nitrogen atoms of azasugars, Kajimoto et al.[24] synthesized *N*-oxides for example castanospermine *N*-oxide (**1.16**). It was thought that, because of the zwitterionic character, these compounds might have a stronger electrostatic interaction with the residues in the active site. However, **1.16** is a weaker inhibitor of β-glucosidase compared to castanospermine (**1.13**). This is probably due to the proximity of the negatively charged oxygen to the ammonium ion and hence the carboxylate groups in the active site, which produces an electrostatic repulsion.



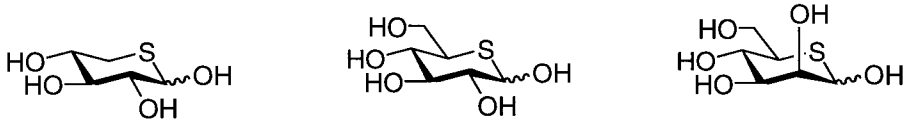
1.16

Chart 1.10. Structure of castanospermine *N*-oxide (**1.16**).

1.3.2 Thiosugars as glycosidase inhibitors

Early attempts to replace the oxygen atom in the sugars with another heteroatom were made with sulfur. In 1961 5-thio-D-xylopyranose (**1.17**) was synthesized independently by three different groups.[28] In 1962 Feather et al.[29] synthesized 5-thio-D-glucopyranose (**1.18**), which was found to be an inhibitor of the release of insulin.[30] 5-

Thio-D-mannopyranose (**1.19**) was the first naturally occurring thiosugar, isolated from the marine sponge *Clathria pyramida*. This compound shows antibacterial activity against both Gram positive and Gram negative bacteria.[31]



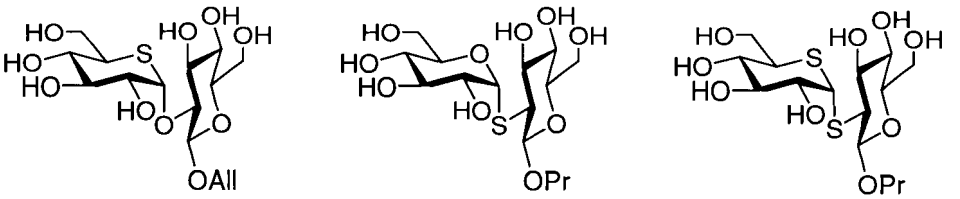
1.17 **1.18** **1.19**

Chart 1.11. Structures of compounds **1.17 – 1.19**.

Because of their potential value as glycosidase inhibitors and their use for probing recognition processes, thiosugars have become very important targets and there are many reviews on the synthesis and biological activities of these compounds.[32]

Replacement of the ring and interglycosidic oxygen atoms in disaccharides with sulfur has been well studied in our group and has been shown to give compounds with some glycosidase inhibitory activities. These modified biological substrates have been used as probes to study enzyme inhibition.

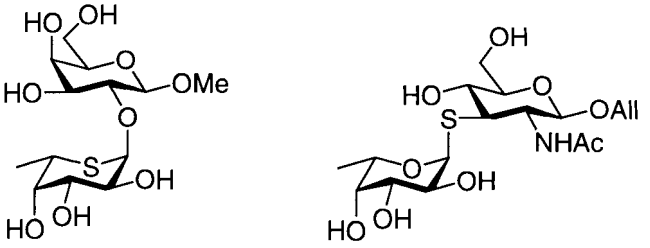
The syntheses of kojibioside analogues **1.20**[33] and **1.21**[34] were achieved in our laboratory. Compound **1.20** was found to be a poor inhibitor of glucosidase II but a competitive inhibitor of glucosidase I (K_i = 2.0 mM).[35] Compound **1.21** was determined to be a poor inhibitor of glucosidase I but a competitive inhibitor of glucosidase II (K_i = 1.0 mM).[35] The synthesis of the dithio analogue **1.22** of kojibiose as a potential inhibitor of glucosidase I was also achieved by our group.[36]



1.20 **1.21** **1.22**

Chart 1.12. Kojibioside analogues **1.20** – **1.22**.

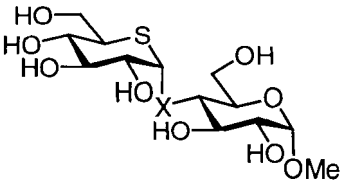
Hashimoto et al.,[37,38] synthesized a series of α-L-fucopyranosyl disaccharides containing sulfur in the ring or interglycosidic linkage in order to characterize α-fucosidases. Compound **1.23** was reported to have excellent inhibitory activity against α-L-fucosidase (K_i = 30 μM). Compound **1.24** was also found to be a potent competitive inhibitor against bovine kidney α-L-fucosidase (K_i = 0.65 mM).



1.23 **1.24**

Chart 1.13. Structures of compounds **1.23** and **1.24**.

Another study in our laboratory involved the synthesis of heteroanalogues of methyl maltoside containing sulfur in the nonreducing ring with oxygen **1.25**, sulfur **1.26**, selenium[39] **1.27** or nitrogen[40] **1.28** in the interglycosidic linkage. Compounds **1.25**, **1.26**, **1.27**, and **1.28** (Chart 1.14) are substrate analogues for glucosidases and were found to be competitive inhibitors of glucoamylase G2, with K_i values of 1.34, 2.04, 0.80, and 0.004 mM, respectively.



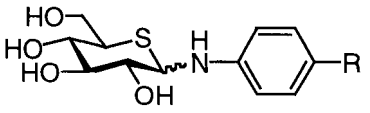
1.25 X = O **1.27** X = Se

1.26 X = S **1.28** X = NH

Chart 1.14. Synthetic heteroanalogues of methyl maltoside.

The methyl maltoside analogue with nitrogen in the interglycosidic linkage, compared with oxygen, sulfur, or selenium analogues, was found to be a strong inhibitor of glucoamylase G2 with a *Ki* value of 0.004 mM. This result reinforces the importance of a basic group adjacent to the anomeric carbon, which is capable of forming a positively charged centre and providing more effective binding to the active site.

Randell et al.[41] synthesized and evaluated the inhibitory activities of a series of 5-thio-D-glucopyranosylarylamines, with sulfur in the ring and nitrogen at the anomeric centre against glucoamylase G2.



1.29 R = H **1.31** R = NO_2

1.30 R = OMe **1.32** R = CF_3

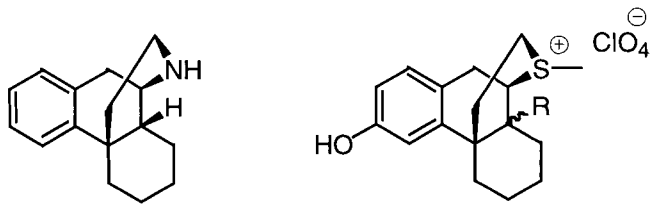
Chart 1.15. Synthetic 5-thio-D-glucopyranosylarylamines.

Compounds **1.29**, **1.30**, **1.31**, and **1.32** were found to be weak, competitive inhibitors of glucoamylase G2, with K_i values of 0.78, 0.47, 0.27, and 0.87 mM, respectively.

Thiosugars containing a sulfur atom in the ring have been classified as substrate analogues rather than TS analogues, due to the inability of the ring sulfur to be protonated in the manner analogues to the nitrogen inhibitors, and hence they do not mimic the positive charge of the putative TS of glycosidases.

1.3.3 Sulfonium salts as glycosidase inhibitors

To investigate the ability of a permanent positive sulfonium centre to provide the electrostatic stabilization of the inhibitor in an enzyme active site, Belleau et al.[42] synthesized sulfonium analogues of morphinan (**1.33**), levorphanol and isolevorphanol, **1.34**, and **1.35** and showed that they were agonists or antagonists of morphine for the opiate receptor.



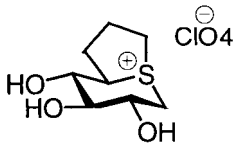
Morphinan (1.33)

1.34 R = β-H
1.35 R = α-H

Chart 1.16. Structures of morphinan (**1.33**), and sulfonium analogues **1.34** and **1.35**.

Inspired by the work of Belleau et al.,[42] our group designed and synthesized the castanospermine analogue **1.36**, in which the bridgehead nitrogen atom is replaced by a sulfonium ion.[43] We reasoned that **1.36**, which bears a permanent positive charge, would provide the necessary electrostatic stabilization to bind competitively to glycosidases. Compound **1.36** was shown to be a mM inhibitor (K_i = 1.32 mM) of glucoamylase G2.[44]

Siriwardena, et al.[45] designed and synthesized a pyrrolizidine alkaloid analogue **1.37** which showed selectivity for inhibition of human liver α-mannosidases.



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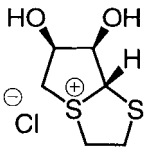
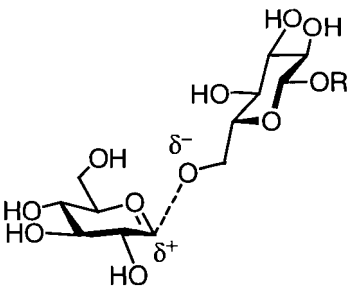


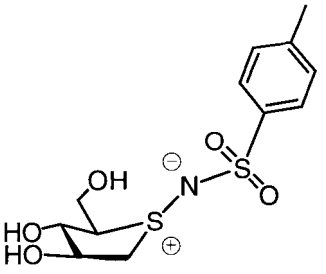
1.37

Chart 1.17. Structures of the sulfonium salts **1.36** and **1.37**.

Yuasa et al.[46] designed the iminothiasugar **1.38** as a mimic of the transition-state of glycosidases; however, compound **1.38** did not show any significant inhibitory activity against glycosidases.



TS for Glycosidases

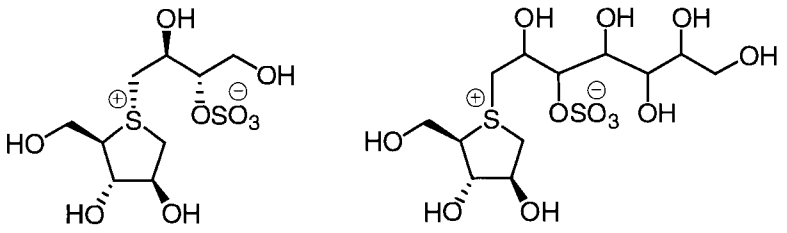


1.38

Chart 1.18. Compound **1.38** as a TS analogue for glycosidases.

The feasibility of the use of sulfonium ions as glycosidase inhibitors has been recently validated by the report of the isolation of the naturally occurring glucosidase inhibitors salacinol[47] (**1.39**) and kotalanol[48] (**1.40**), from *Salacia reticulata,* a Sri Lankan

plant known for its medicinal properties. The molecular structures of salacinol (**1.39**) and kotalanol (**1.40**) are unique in that they contain a sulfonium ion (1,4-anhydro-4-thio-D-pentitol cation) stabilized by an internal sulfate counterion. The inhibitory activities of salacinol (**1.39**) and kotalanol (**1.40**) against maltase and sucrase, respectively, are nearly equal to those of acarbose (**1.1**), an α-glucosidase inhibitor used clinically for the treatment of diabetes, while their inhibitory potencies against isomaltase are greater than that of acarbose (**1.1**).[48,49] The herb *Salacia reticulata*, is used in traditional medicine against diabetes. Inhibitory effects of salacinol (**1.39**) on serum glucose levels in maltose and sucrose loaded rats are stronger than those for acarbose (**1.1**).[49]



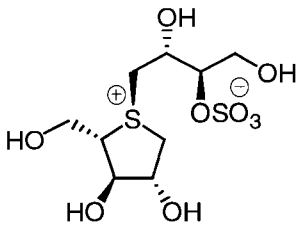
Salacinol (1.39) **Kotalanol (1.40)**

Chart 1.19. Structures of salacinol (**1.39**) and kotalanol (**1.40**)

The anti-obesity effects of the hot water-soluble extracts of *S. reticulata* using obese rat models has shown body weight depression of the rats after oral administration of the extracts.[50] The weight loss is expected due to the strong intestinal α-glucosidase inhibitory activity of the extract. The safety profile of the extract from *S. reticulata* showed no oral toxicity at a dose of 5,000 mg/kg body weight.[51] In other safety studies, salacinol extracts from *S. oblonga* which is also a woody climbing plant found in Sri Lanka and India, in a medial food in amounts much greater than proposed for human intake did not result in any toxic effects in rats.[52]

The initial structure proposed for salacinol (**1.39**) was based on X-ray crystallography, and revealed an intriguing inner-salt structure, composed of a 1,4-anhydro-4-thio-arabinitol moiety alkylated at sulfur by a 1-deoxy-erythritol-3-sulfate moiety. The configuration of the arabinitol moiety was assigned as L and that of the erythritol moiety as D (see **1.41**). The same group subsequently reported the isolation of a second compound, kotalanol (**1.40**), the degradation of which led to the release of 1,4-anhydro-4-thio-D-arabinitol. This apparently led to a revision of the structure of salacinol (**1.39**) to reflect the probable close biosynthetic relationship of the two inhibitors, and salacinol (**1.39**) was then assigned to be the enantiomer of the original structure **1.41**. Evidently, the original X-ray structure determination did not assign the hand of the molecule explicitly.

This thesis began with the task of unequivocally establishing the absolute configuration of salacinol (**1.39**). A synthetic scheme was designed to provide salacinol (**1.39**), and its enantiomer **1.41**. In order to probe structure-activity relationships further, the diastereomer **1.42**, nitrogen analogues, **1.43** and **1.44**, selenium analogues, **1.45**, **1.46** and xylitol heteroanalogues **1.47**, **1.48** were synthesized.



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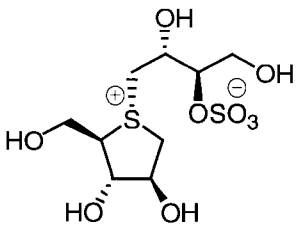

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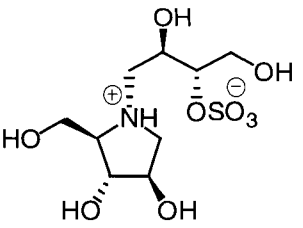
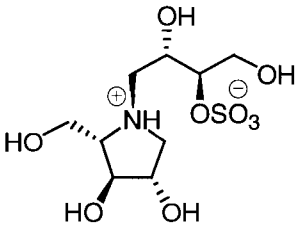


Ghavamiol (1.43)



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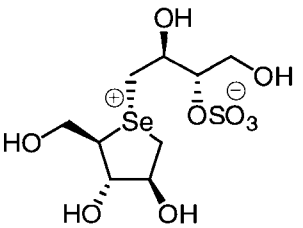
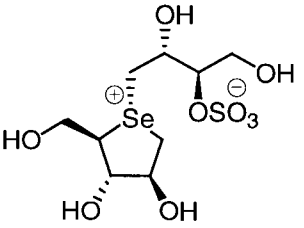


Blintol (1.45)



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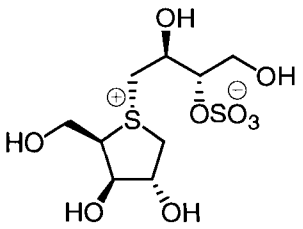

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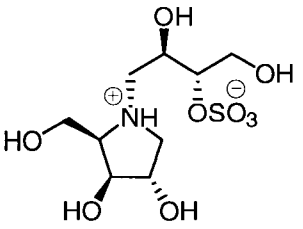

1.48

Chart 1.20. Salacinol analogues **1.41 – 1.48** studied in this thesis.

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CHAPTER 2: A NEW CLASS OF GLYCOSIDASE INHIBITOR: SYNTHESIS OF SALACINOL AND ITS STEREOISOMERS†

Ahmad Ghavami, Blair D. Johnston, and B. Mario Pinto*

Department of Chemistry

Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

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Tel: (604) 291-4327

Fax: (604) 291-3765

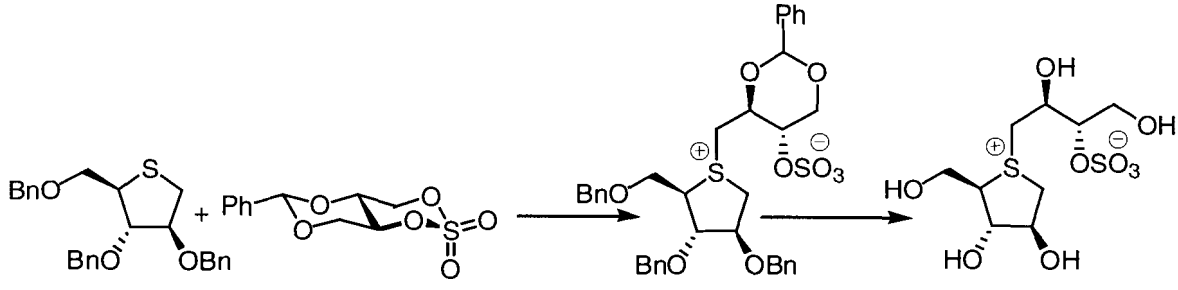
e-mail:bpinto@sfu.ca

2.1 Graphical Abstract

A New Class of Glycosidase Inhibitor: Synthesis of Salacinol and Its Stereoisomers

Ahmad Ghavami, Blair D. Johnston, and B. Mario Pinto*

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6



Keywords: glycosidase inhibitors, salacinol, salacinol analogues, sulfonium salt, cyclic sulfate.

2.2 Abstract

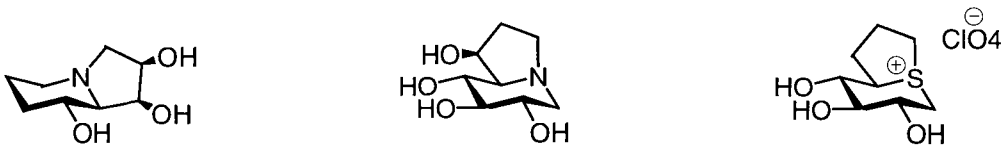
Salacinol (**2.4**) is one of the active principles in the aqueous extracts of *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of diabetes. The syntheses of salacinol (**2.4**), the enantiomer of salacinol (**2.5**), and a diastereomer (**2.7**) are described. The synthetic strategy relies on the selective nucleophilic attack of 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-thio-D- or L-arabinitol at C-1 of 2,4-*O*-benzylidene D- or L-erythritol-1,3-cyclic sulfate. The work serves to resolve the ambiguity about the exact structure of salacinol and establishes conclusively the structure of the natural product.

2.3 Introduction

Glycosidase enzymes with diverse functional specificity play important roles in the biochemical processing of biopolymers containing carbohydrates.[1] One important class of these enzymes is responsible for the liberation of glucose from its higher oligomers or polymers. Disruption in the function and regulation of these enzymes can lead to disease states such as diabetes. In the treatment of type II noninsulin dependent diabetes (NIDD) management of blood glucose levels is critical. One strategy for treating NIDD is to delay digestion of ingested carbohydrates, thereby lowering postprandial blood glucose concentration. This can be achieved by administering drugs which inhibit the activity of enzymes, such as the glucosidases, which mediate the hydrolysis of complex starches to oligosaccharides in the small intestine. For example, the carbohydrate analogue acarbose, which is currently used for the oral treatment of diabetes,[2,3] reversibly inhibits the function of pancreatic α-amylase and membrane-bound intestinal α-glucoside hydrolase enzymes. In patients suffering from type II diabetes, such enzyme inhibition results in delayed glucose absorption into the blood and a smoothing or lowering of postprandial hyperglycemia, resulting in improved glycemic control.

Inhibition of glycosidase enzymes involved in carbohydrate processing of glycoproteins has also been effective in the treatment of some nondiabetic disorders such as cancer.[4] While normal cells display characteristic oligosaccharide structures, tumor cells display very complex structures that are usually restricted to embryonic tissues.[4] It is believed that these complex structures provide signal stimuli for rapid proliferation and metastasis of tumor cells. A possible strategy for the therapeutic use of glycosidase inhibitors is to take advantage of the different rates of normal vs cancer cell growth to

inhibit assembly of complex oligosaccharide structures. For example, the indolizidine alkaloid swainsonine (**2.1**), an inhibitor of Golgi α-mannosidase II, reportedly reduces tumor cell metastasis, enhances cellular immune responces, and slows tumor cell growth in mice.[5] Swainsonine treatment has led to significant reduction of tumor mass in human patients with advanced malignancies, and is a promising drug therapy for patients suffering from breast, liver, lung and other malignancies.[6,7] Therefore, natural or synthetic inhibitors of glycosidase enzymes have potential as new therapeutic agents.

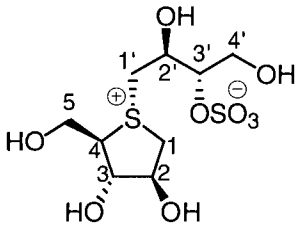


2.1 **2.2** **2.3**

Chart 2.1. Compounds **2.1** – **2.3**.

Known glycosidase inhibitors such as the indolizidine alkaloids swainsonine (**2.1**) and castanospermine (**2.2**) are known to carry a positive charge at physiological pH.[8] It is believed that the mechanism of action of such inhibitors may be at least partially explained by the establishment of stabilizing electrostatic interactions between the inhibitor and a carboxylate residue in the enzyme active site.[8] Recently, we reported the synthesis of the sulfonium salt (**2.3**) which might function as a mimic of castanospermine (**2.2**).[9] We reasoned that **2.3**, which bears a permanent positive charge, would provide the necessary electrostatic stabilization to bind competitively to glycosidases. The feasibility of such an approach has been recently validated by the report of the isolation of the glycosidase inhibitor salacinol (**2.4**) from the roots and stems of *Salacia reticulata* Wight, (known as

'Kothalahimbutu' in Singhalese).[10] This sulfonium salt was found to be one of the active principles in the aqueous extracts of *S. reticulata* (a woody climbing plant) that are traditionally used in Sri Lanka for the treatment of diabetes. Traditionally, Ayurvedic medicine advised that a person suffering from diabetes should drink water left overnight in a mug carved from Kothalahimbutu wood.

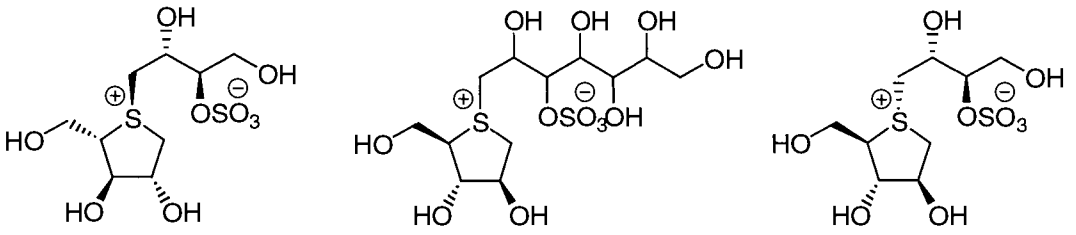


2.4

Chart 2.2. Salacinol (**2.4**).

The initial structural assignment of salacinol (**2.4**) revealed an intriguing inner-salt structure, composed of a 1,4-anhydro-4-thio-arabinitol alkylated at sulfur by a 1'-deoxy-erythritol-3'-sulfate moiety. The relative configuration of the chiral centres was elucidated by X-ray crystallography, and the structure was formulated as an anhydro-4-thio-L-arabinitol unit linked to a D-erythritol unit (**2.5**). Subsequently, the same group isolated another glucosidase inhibitor from *S. reticulata* with a related sulfonium-salt structure, namely kotalanol (**2.6**), which showed more potent inhibitory activity against sucrase than salacinol (**2.4**).[11] The configurations of the stereogenic centres in the longer heptitol side chain or at the sulfur atom were not determined, but degradation led to the release of 1,4-anhydro-4-thio-D-arabinitol. This apparently led to a revision of the structure of salacinol (**2.4**) to reflect the probable close biosynthetic relationship of the two inhibitors, and salacinol (**2.4**) was then assigned to be the enantiomer of the original structure **2.5**. The

recent report by Yuasa et al.[12] on the synthesis of salacinol (**2.4**) prompts us to report our own findings. [13]



2.5 **2.6** **2.7**

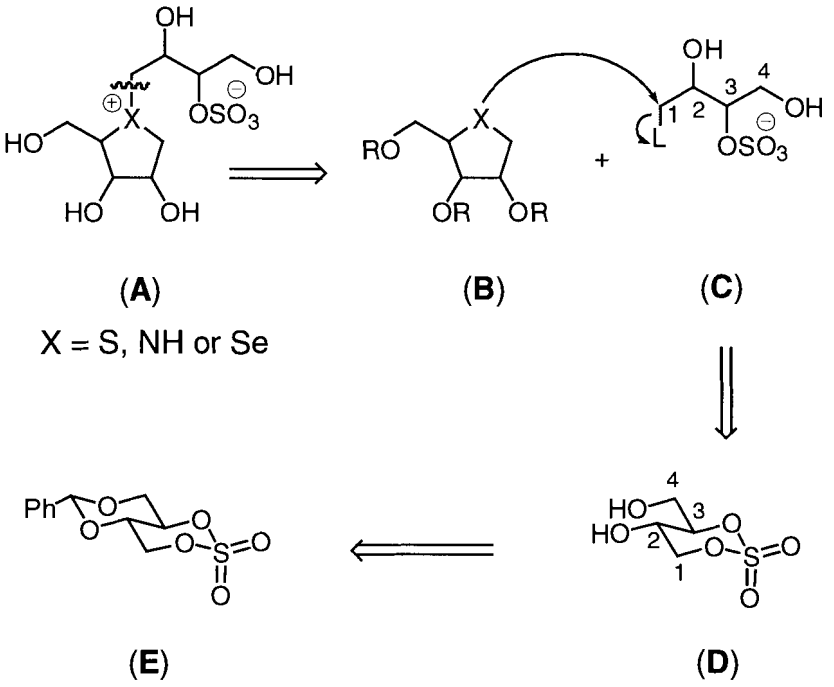
Chart 2.3. Compounds **2.5** – **2.7**.

To unequivocally establish the absolute configuration of salacinol (**2.4**), and to further investigate the inhibition of glycosidase enzymes by this new class of inhibitor, we now report the synthesis of salacinol (**2.4**) and its enantiomeric structure **2.5**. In addition, the diastereomeric sulfonium-salt (**2.7**) was synthesized in order to assess structure-activity relationships.

2.4 Results and Discussion

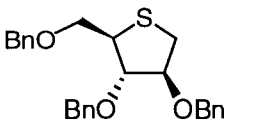
Retrosynthetic analysis indicated that salacinol (**2.4**) or its analogues (**A**) could be obtained by alkylation of anhydro-alditol derivatives at the ring heteroatom (Scheme 2.1). This strategy was chosen in order to provide flexibility for the synthesis of analogues having other heteroatoms such as nitrogen or selenium in the ring, and different configurations of the sugar rings. The alkylating agent could either be an open-chain electrophile (**C**) or a cyclic sulfate derivative such as **D** or **E**, whereby selective attack of the thioether at the least hindered primary centre should afford the desired sulfonium ions.

We have investigated the latter approach and have found that the opening of appropriately protected cyclic sulfate derivatives by thioether nucleophiles proceeds smoothly to give the desired compounds.

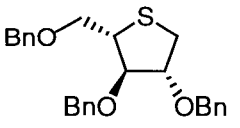


Scheme 2.1. Retrosynthetic analysis.

The thio-arabinitols **2.8**[14] and **2.9**[15] were synthesized from D-glucose and D-xylose, respectively.



2.8



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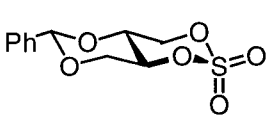

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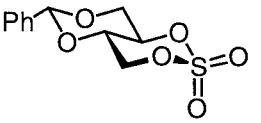
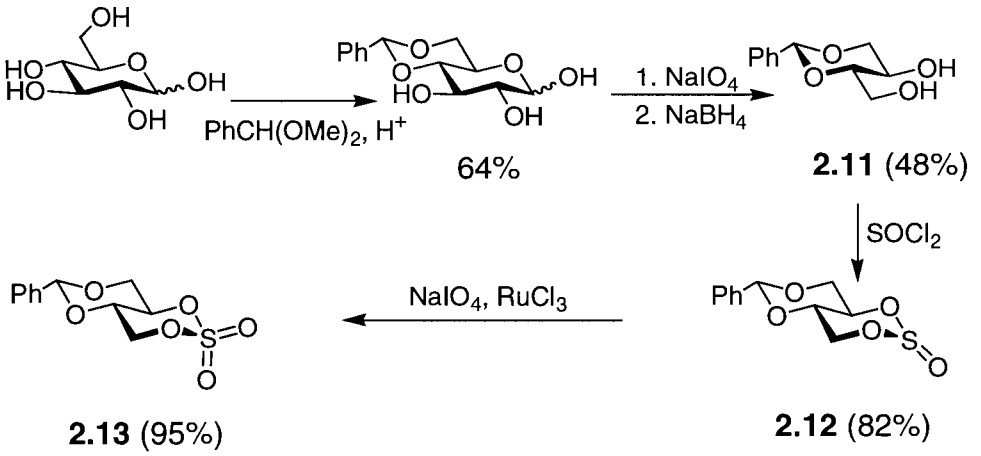


2.13

Chart 2.4. Compounds **2.8** – **2.10**, and **2.13**.

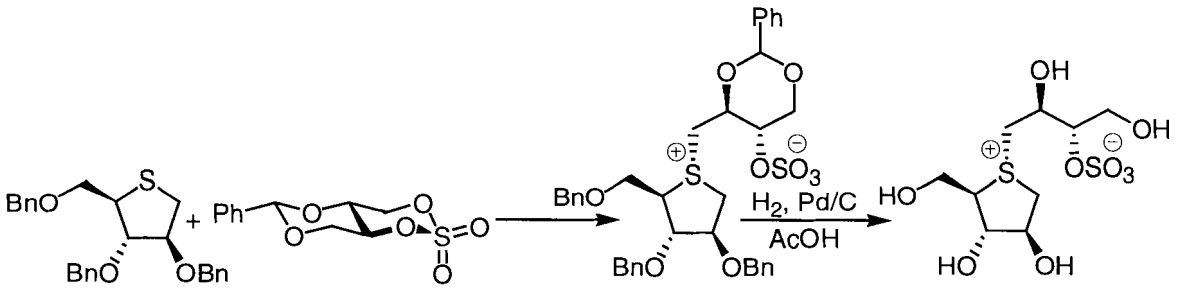
The 2,4-*O*-benzylidene-L- (**2.10**) and D- (**2.13**) erythritol-1,3-cyclic sulfates were synthesized from L- and D-glucose, respectively, in a manner similar to that described for the corresponding 2,4-*O*-ethylidene derivative (Scheme 2.2).[16]



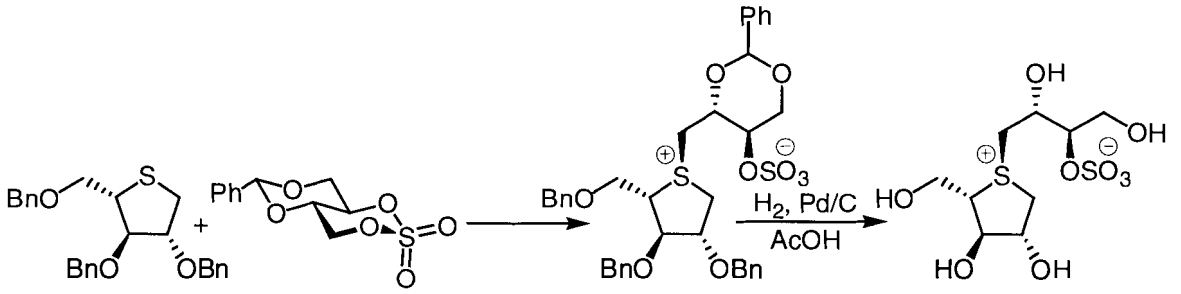
Scheme 2.2. Synthesis of the cyclic sulfate (**2.13**).

The target compounds were prepared by opening of the cyclic sulfates by nucleophilic attack of the sulfur atom in the 5-membered rings. Initially, reactions were

carried out at room temperature in methanol, but the reaction rates were too slow. Increasing the temperature resulted in competing nucleophilic attack of methanol and formation of methyl ethers. Dry acetone was found to be a more suitable solvent. The addition of K_2CO_3 was necessary to prevent decomposition due to the hydrolysis reactions of the cyclic sulfates. Yuasa et al.[12] have also noted the decomposition of the related cyclic sulfates in DMF when the temperature was increased to 60-70 °C.[12] Thus, compound **2.14** was synthesized by alkylation of the protected thio-arabinitol (**2.8**) with the cyclic sulfate (**2.10**) (1.2equiv) in dry acetone containing K_2CO_3, to give the protected compound **2.14** in 33% yield. Compound **2.14** exhibited the expected downfield shifts due to sulfonium ion formation for H-1 and H-4 and for C-1 and C-4 in the NMR spectra when compared to those of the precursor sulfide **2.8**. Sulfonium salt formation also resulted in broadening of the ^1H resonances of the arabinitol ring such that $J_{1a,2}$, $J_{2,3}$ and $J_{3,4}$ were no longer resolved. Compound **2.15** was similarly prepared in 40% yield from the enantiomeric thioether (**2.9**) and the cyclic sulfate (**2.13**). Comparison of the ^1H and ^{13}C NMR spectra for compounds **2.14** and **2.4** with those of compounds **2.15** and **2.5**, respectively, showed small chemical shift differences between the enantiomeric pairs (± 0.1 ppm for ^1H NMR spectra and ± 1 ppm for ^{13}C NMR spectra), but the coupling constants were identical. We attribute the chemical shift discrepancies to concentration or temperature differences between samples. Deprotection of the coupled products **2.14** and **2.15** by hydrogenolysis over a Pd/C catalyst gave compounds **2.4** (67%) and **2.5** (80%), respectively, each exhibiting ^1H and ^{13}C NMR spectra that were in complete accord with those reported for salacinol (**2.4**) (Scheme 2.3).[10]



2.8 **2.10** **2.14** (33%) **Salacinol (2.4)** (67%)

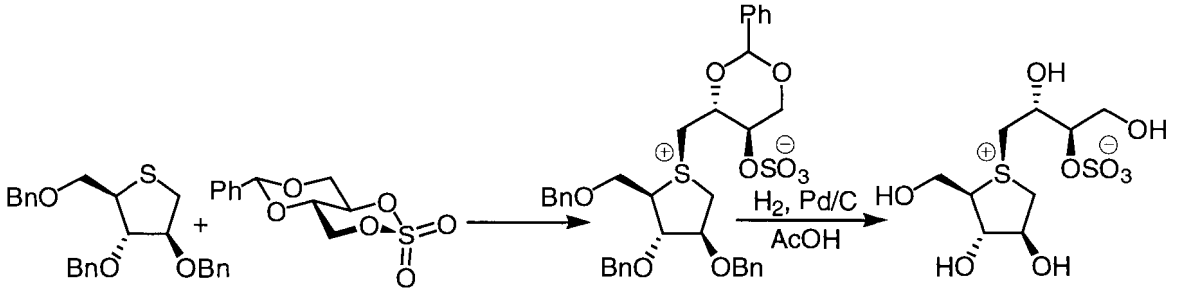


2.9 **2.13** **2.15** (40%) **2.5** (80%)

Scheme 2.3. Syntheses of salacinol (**2.4**), and its enantiomer **2.5**.

The stereochemistry at the stereogenic sulfonium centre in **2.4** and **2.5** was established by means of a NOESY experiment. A correlation between H-1' and H-4 for each isomer confirmed the trans relationship between the erythritol side chain and the C-4 substituent on the anhydroarabinitol moiety. Optical rotations for the two enantiomers indicated that the value for the dextrorotatory isomer **2.4** ($[\alpha]_D$ +2.1°) most closely matched the literature value reported for the naturally occurring compound salacinol ($[\alpha]_D$ +4.9°).[10] Since the X-ray crystal structure of the naturally occurring salacinol, with $[\alpha]_D$ +4.9°, had indicated a trans relative configuration between the anhydro-4-thio-D-arabinitol unit and the erythritol unit,[10] it is clear that the authentic structure of salacinol is

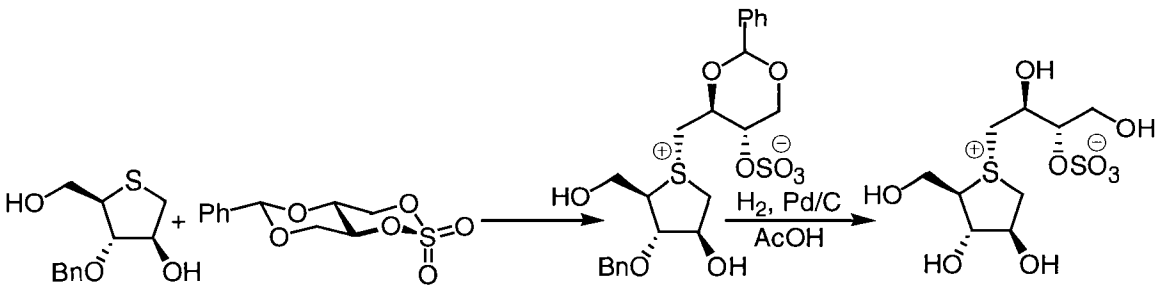
represented by structure (**2.4**), namely an anhydro-4-thio-D-arabinitol unit linked to an L-erythritol unit, and not the enantiomeric structure **2.5**. The diasteromeric compound **2.7** was similarly obtained by the reaction of compound **2.8** with the cyclic sulfate (**2.13**) to produce **2.16** in 79% yield. Deprotection, as before, gave compound **2.7** in 59% yield (Scheme 2.4), which exhibited distinctly different NMR spectra and optical rotation from salacinol (**2.4**). The stereochemistry at the stereogenic sulfur centre in **2.7** was confirmed by means of a NOESY experiment, as described above. These results constitute additional evidence that salacinol (**2.4**) is the sulfonium salt composed of 1-deoxy-L-erythritol-3-sulfate and 1,4-anhydro-4-thio-D-arabinitol.



2.8 **2.13** **2.16** (79%) **2.7** (59%)

Scheme 2.4. Synthesis of compound **2.7**.

To reduce the number of synthetic steps, the coupling reactions were attempted with partially protected or unprotected thio-arabinitols. Thus, the partially protected compound **2.17** was reacted with the cyclic sulfate (**2.10**) in acetone containing K_2CO_3, to give compound **2.18** in 32% yield. Deprotection yielded salacinol (**2.4**) in 36% yield (Scheme 2.5). 1,4-Anhydro-4-thio-D-arabinitol itself was not soluble in acetone, and the reaction in methanol produced several products.



2.17 **2.10** **2.18** (32%) **2.4** (36%)

Scheme 2.5. Synthesis of compound **2.4**.

The glucosidase inhibitory properties of compounds **2.4**, **2.5**, and **2.7** are under investigation, as are the syntheses of other analogues of this new class of glycosidase inhibitors.

2.5 Experimental Section

2.5.1 General Methods

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz. All assignments were confirmed with the aid of two-dimensional $^{1}H,^{1}H$ (COSYDFTP) or $^{1}H,^{13}C$ (INVBTP) experiments using standard Bruker pulse programs. MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix using a PerSeptive Biosystems Voyager-DE instrument. Column chromatography was performed with Merck Silica gel 60 (230-400 mesh). High-resolution mass spectra were LSIMS (Fab), run on a Kratos Concept H double focussing mass spectrometer at 10000 RP.

2,4-*O*-Benzylidene-D-erythritol (2.11). Compound (**2.11**) was prepared from 4,6-*O*-benzylidene-D-glucose according to standard procedures.[17,18] Compound (**2.11**) has been reported by MacDonald et al.,[18] without NMR characterization which is therefore dealt with here. Mp 138-139 °C, lit.[18] mp 135-136 °C; $[\alpha]_D$ –44° (*c* 1.0, MeOH) (lit.[18] –43° (*c* 2.0, MeOH)); ^{1}H NMR (CD_3OD): δ 7.53-7.28 (5H, m, Ar), 5.53 (1H, s, C*H*Ph), 4.20 (1H, m, H-4_{eq}), 3.92 (1H, dd, $J_{1a,1b}$ = 12.1, $J_{1a,2}$ = 1.7 Hz, H-1a), 3.74 (1H, dd, $J_{1b,2}$ = 5.7 Hz, H-1b), 3.67-3.55 (3H, m, H-3, H-2, H-4_{ax}); ^{13}C NMR (CD_3OD): δ 139.52 (C_{ipso}), 129.77 (C_{para}), 128.99 (2C) and 127.49 (2C) (C_{ortho} and C_{meta}), 102.36 (*C*HPh), 84.22 (C-3), 72.21 (C-4), 62.76 (C-1), 62.59 (C-2); MALDI-TOF MS: *m/e* 211 (M^+ + H), 233 (M^+ + Na). Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.96; H, 6.55.

2,4-*O*-Benzylidene-D-erythritol-1,3-cyclic sulfite (2.12). A solution of the diol (**2.11**) (4.5 g, 21 mmol) and Et_3N (11 mL, 4 equiv) in dry CH_2Cl_2 (90 mL) was added dropwise to a solution of $SOCl_2$ (2.4 mL, 1.5 equiv) in dry CH_2Cl_2 (60 mL), with stirring in an ice-bath under an N_2 atmosphere. Stirring was continued at 0 °C, until TLC (hexanes/EtOAc, 4:1) showed complete disappearance of the starting material. The mixture was diluted with CH_2Cl_2 (150 mL) and washed with H_2O (150 mL) and brine (150 mL). The organic solution was dried (Na_2SO_4) and concentrated on a rotary evaporator. The product was purified by flash chromatography [hexanes/EtOAc, 4:1 + 0.1% Et_3N] to give **2.12** as a 1:1 mixture of two diastereomers (4.5 g, 82%). The less polar isomer was selectively recrystallized from EtOAc/hexanes. Mp 137-139 °C; $[\alpha]_D$ +32° (*c* 1.0, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 7.48-7.36 (5H, m, Ar), 5.68 (1H, s, C*H*Ph), 5.04 (1H, ddd, $J_{3,4ax}$ = 10.4, $J_{2,3}$ = 9.5, $J_{3,4eq}$ = 5.0 Hz, H-3), 4.80 (1H, dd, $J_{1ax,2}$ = $J_{1ax,1eq}$ = 10.4 Hz, H-1ax), 4.24 (1H, dd, $J_{4eq,4ax}$ = 10.5 Hz, H-4eq), 4.18 (1H, ddd, $J_{1eq,2}$ = 4.8 Hz, H-2), 4.06 (1H, dd, H-1eq), 3.89 (1H, dd, H-4ax); ^{13}C NMR (CD_2Cl_2): δ 137.14 (C_{ipso}), 129.74 (C_{para}), 128.65 (2C) and 126.50 (2C) (C_{ortho} and C_{meta}), 102.72 (*C*HPh), 73.56 (C-2), 68.16 (C-4), 63.90 (C-3), 60.18 (C-1). Anal. Calcd for $C_{11}H_{12}O_5S$: C, 51.55; H, 4.72. Found: C, 51.80; H, 4.66.

2,4-*O*-Benzylidene-D-erythritol-1,3-cyclic sulfate (2.13). The cyclic sulfite (**2.12**) (3.5 g, 14 mmol) was dissolved in a mixture of MeCN (50 mL) and CCl_4 (50 mL), and $NaIO_4$ (4.1 g, 1.5 equiv) and $RuCl_3.H_2O$ (50 mg) were added followed by H_2O (50 mL). The mixture was stirred vigorously at room temperature until TLC (hexanes/EtOAc, 4:1) showed complete disappearance of the starting material. The mixture was diluted with Et_2O (200 mL) and washed with H_2O (200 mL) and brine (200 mL). The organic solution was dried

(Na_2SO_4) and concentrated on a rotary evaporator. The product was purified by flash chromatography [hexanes/EtOAc, 4:1 + 0.1% Et_3N] to yield a white solid (3.5 g, 95%). A portion of the product was recrystallized from EtOAc/hexanes. Mp 115-125 °C (dec); $[\alpha]_D$ +4° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.48-7.37 (5H, m, Ar), 5.65 (1H, s, C*H*Ph), 4.86 (1H, ddd, $J_{2,3} = J_{3,4ax} = 10.0$, $J_{3,4eq} = 5.0$ Hz, H-3), 4.76 (1H, dd, $J_{1ax,2} = 10.7$, $J_{1ax,1eq} = 10.5$ Hz, H-1ax), 4.65 (1H, dd, $J_{1eq,2} = 5.0$ Hz, H-1eq), 4.44 (1H, dd, $J_{4eq,4ax} = 10.5$ Hz, H-4eq), 4.25 (1H, ddd, H-2), 3.97 (1H, dd, H-4ax); ^{13}C NMR (CD_2Cl_2): δ 136.32 (C_{ipso}), 130.03 (C_{para}), 128.74 (2C) and 126.52 (2C) (C_{ortho} and C_{meta}), 102.98 (*C*HPh), 75.74 (C-3), 73.19 (C-1), 71.68 (C-2), 67.64 (C-4); MALDI-TOF MS: *m/e* 273 (M^+ + H). Anal. Calcd for $C_{11}H_{12}O_6S$: C, 48.53; H, 4.44. Found: C, 48.43; H, 4.39.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-arabinitol (2.8). A mixture of 1,4-anhydro-3-*O*-benzyl-4-thio-D-arabinitol (**2.17**)[14] (1.0 g, 4.2 mmol) and 60% NaH (0.85 g, 5 equiv) in DMF (20 mL) was stirred in an ice-bath for 1 h. A solution of benzyl bromide (1.9 mL, 3.8 equiv) in DMF (5 mL) was added and the solution was stirred at room temperature for 3 h. The mixture was added to ice-water (150 mL) and extracted with Et_2O (150 mL). The organic solution was dried (Na_2SO_4) and concentrated. The product was purified by flash chromatography [hexanes/EtOAc, 4:1] to give a syrup (1.6 g, 90%). $[\alpha]_D$ +5° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38-7.23 (15H, m, Ar), 4.61 (2H, s, CH_2Ph), 4.53 and 4.48 (2H, 2d, $J_{A,B} = 12.1$ Hz, CH_2Ph), 4.51 and 4.47 (2H, 2d, $J_{A,B} = 11.9$ Hz, CH_2Ph), 4.19 (1H, ddd, $J_{1b,2} = 4.6$ Hz, H-2), 4.11 (1H, dd, $J_{2,3} = 3.8$, $J_{3,4} = 3.6$ Hz, H-3), 3.69 (1H, dd, $J_{5a,5b} = 8.8$, $J_{4,5a} = 7.6$ Hz, H-5a), 3.57 (1H, ddd, $J_{4,5b} = 6.3$ Hz, H-4), 3.50 (1H, dd, H-5b), 3.08 (1H, dd, $J_{1a,1b} = 11.4$, $J_{1a,2} = 5.1$ Hz, H-1a), 2.90 (1H, dd, H-1b); ^{13}C NMR ($CDCl_3$): δ

138.16, 138.06, 137.88 ($3C_{ipso}$), 128.40-127.59 ($15C_{Ar}$), 85.08 (C-3), 85.04 (C-2), 73.01 (CH_2Ph), 72.34 (C-5), 71.85, 71.50 ($2CH_2Ph$), 48.99 (C-4), 33.10 (C-1). Anal. Calcd for $C_{26}H_{28}O_3S$: C, 74.25; H, 6.71. Found: C, 74.18; H, 6.53.

2.5.2 General Procedure for the Synthesis of the Protected Sulfonium Sulfates (2.14), (2.15), (2.16)

The thiosugar (3 mmol) and the cyclic sulfate (1.2 equiv) were dissolved in dry acetone (0.5 mL) and anhydrous K_2CO_3 (7 mg) was added. The mixture was stirred in a sealed tube in an oil-bath (75 °C) overnight. The solvent was removed under reduced pressure, and the product was purified by column chromatography.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(S)-[(2S,3S)-2,4-benzylidenedioxy-3-(sulfooxy) butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (2.14). Column chromatography [$CHCl_3$/MeOH, 10:1 + 0.1% Et_3N] of the crude product gave an amorphous solid (33%). $[\alpha]_D$ −11.9° (*c* 1.7, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 7.49-7.12 (20H, m, Ar), 5.54 (1H, s, C*H*Ph), 4.59 (1H, ddd, $J_{2',3'}$ = 9.6, $J_{3',4'ax}$ = 10.7, $J_{3',4'eq}$ = 5.4 Hz, H-3'), 4.54 (2H, s, CH_2Ph), 4.51 (1H, br d, H-2), 4.50 (1H, dd, $J_{4'eq,4'ax}$ = 10.7 Hz, H-4'eq), 4.48 and 4.37 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.38 (1H, dd, $J_{1'a,1'b}$ = 13.6, $J_{1'a,2'}$ = 2.7 Hz, H-1'a), 4.35 (1H, br s, H-3), 4.29 (1H, ddd, $J_{1a',2'}$ = 3.4 Hz, H-2'), 4.25 and 4.15 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.06 (1H, br d, $J_{1a,1b}$ = 13.3, H-1a), 4.00 (1H, dd, H-1'b), 3.98 (1H, br dd, H-4), 3.77 (1H, dd, H-4'ax), 3.74 (1H, dd, $J_{1b,2}$ = 3.8 Hz, H-1b), 3.62 (1H, dd, $J_{5a,5b}$ = 9.9, $J_{4,5a}$ = 8.7 Hz, H-5a), 3.53 (1H, dd, $J_{4,5b}$ = 7.2 Hz, H-5b); ^{13}C NMR (CD_2Cl_2): δ 137.34, 137.24, 136.56, 136.39 ($4C_{ipso}$), 129.73-126.62 ($20C_{Ar}$), 101.95 (*C*HPh), 83.75 (C-3), 82.82 (C-2), 76.80 (C-2'),

73.73, 72.84, 72.52 (3*C*H_2Ph), 69.54 (C-4'), 67.01 (C-5), 66.48 (C-3'), 65.27 (C-4), 49.67 (C-1'), 48.28 (C-1); MALDI-TOF MS: *m/e* 693 (M^+ + H). Anal. Calcd for $C_{37}H_{40}O_9S_2$: C, 64.14; H, 5.82. Found: C, 63.88; H, 5.83.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(R)-[(2R,3R)-2,4-benzylidenedioxy-3-(sulfooxy) butyl]-sulfoniumylidene]-L-arabinitol Inner Salt (2.15). Column chromatography [$CHCl_3$/MeOH, 10:1 + 0.1% Et_3N] of the crude product gave an amorphous solid (40%). $[\alpha]_D$ +14.3° (*c* 1.4, CH_2Cl_2). Anal. Calcd for $C_{37}H_{40}O_9S_2$: C, 64.14; H, 5.82. Found: C, 64.13; H, 5.74.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(R)-[(2R,3R)-2,4-benzylidenedioxy-3-(sulfooxy) butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (2.16). Column chromatography [$CHCl_3$/MeOH, 10:1 + 0.1% Et_3N] of the crude product gave an amorphous solid (79%). $[\alpha]_D$ –46.9° (*c* 0.65, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.43-7.10 (20H, m, Ar), 5.49 (1H, s, C*H*Ph), 4.59 and 4.51 (2H, 2d, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.54 and 4.42 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.56 (1H, ddd, $J_{2',3'} = J_{3',4'ax}$ = 9.7, $J_{3',4'eq}$ = 4.2 Hz H-3'), 4.50 (1H, dd, H-4'eq), 4.45 (1H, m, H-2), 4.44 (1H, dd, H-1'a), 4.41 (1H, m, H-3), 4.40 and 4.36 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.27 (1H, ddd, $J_{1'a,2'} = J_{1'b,2'}$ = 3.5 Hz, H-2'), 4.24 (1H, br dd, H-4), 3.96 (1H, dd, $J_{5a,5b}$ = 9.7, $J_{4,5a}$ = 6.2 Hz, H-5a), 3.90 (1H, dd, $J_{1'b,\,1'a}$ = 13.3 Hz, H-1'b), 3.82 (1H, dd, $J_{4,5b}$ = 9.7 Hz, H-5b), 3.76 (1H, dd, $J_{4'ax,4'eq}$ = 10.2 Hz, H-4'ax), 3.73 (1H, br d, H-1a), 3.51 (1H, dd, $J_{1b,1a}$ = 13.2, $J_{1b,2}$ = 3.9 Hz, H-1b); ^{13}C NMR (CD_2Cl_2): δ 137.62, 137.27, 136.48, 136.29 (4C_{ipso}), 129.80-126.56 (20C_{Ar}), 102.16 (*C*HPh), 84.25 (C-3), 82.56 (C-2), 77.07 (C-2'), 74.02, 72.74 (3*C*H_2Ph), 69.75 (C-4'), 67.19 (C-5), 66.82 (C-3'), 65.76 (C-4),

50.41 (C-1'), 49.60 (C-1); MALDI-TOF MS: *m/e* 693 (M^+ + H). Anal. Calcd for $C_{37}H_{40}O_9S_2$: C, 64.14; H, 5.82. Found: C, 64.16; H, 5.73.

3-*O*-Benzyl-1,4-dideoxy-1,4-[(S)-[(2S,3S)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (2.18). Column chromatography [$CHCl_3$/MeOH, 10:1 + 0.1% Et_3N] of the crude product gave an amorphous solid (32%). ^{1}H NMR (CD_2Cl_2): δ 7.49-7.26 (10H, m, Ar), 6.22 (1H, d, $J_{2,OH}$ = 4.4 Hz, 2-OH), 5.54 (1H, s, C*H*Ph), 4.96 (1H, br s, H-2), 4.65 and 4.56 (2H, 2d, $J_{A,B}$ = 11.6 Hz, CH_2Ph), 4.64 (1H, br m, 5-OH), 4.52 (1H, ddd, $J_{2',3'}$ = 9.6 Hz, H-3'), 4.46 (1H, dd, $J_{4'eq,4'ax}$ = 10.6, $J_{3',4'eq}$ = 5.4 Hz, H-4'eq), 4.32 (1H, br s, H-3), 4.30 (1H, br d, H-1a), 4.28 (1H, ddd, H-2'), 4.12 (1H, dd, $J_{1'a,2'}$ = 2.6 Hz, H-1'a), 4.10 (1H, dd, H-4), 4.01 (1H, dd, $J_{1'b,1'a}$ = 13.5, $J_{1'b,2'}$ = 3.5 Hz, H-1'b), 3.92-3.78 (2H, m, H-5a, H-5b), 3.78 (1H, dd, $J_{3',4'ax}$ = 10.1 Hz, H-4'ax), 3.67 (1H, dd, $J_{1b,1a}$ = 13.4, $J_{1b,2}$ = 3.9 Hz, H-1b); ^{13}C NMR (CD_2Cl_2): δ 136.92, 136.73 ($2C_{ipso}$), 129.97-126.61 ($10C_{Ar}$), 102.32 (*C*HPh), 88.45 (C-3), 76.61 (C-2'), 76.22 (C-2), 72.96 (*C*H_2Ph), 71.24 (C-4), 69.27 (C-4'), 66.96 (C-3'), 60.51 (C-5), 52.43 (C-1), 48.30 (C-1'); MALDI-TOF MS: *m/e* 513 (M^+ + H). Anal. Calcd for $C_{23}H_{28}O_9S_2$: C, 53.89; H, 5.51. Found: C, 53.64; H, 5.34.

2.5.3 General Procedure for the Deprotection of the Protected Sulfonium Sulfates

The protected compound (120 mg, 0.17 mmol) was dissolved in AcOH/H_2O, 4:1 (3 mL) and stirred with Pd-C (80 mg) under H_2 (52 psi). After 60 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The

combined filtrates were concentrated and the residue was purified by column chromatography.

1,4-Dideoxy-1,4-[(S)-[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (2.4). Column chromatography [$CHCl_3$/MeOH/H_2O, 7:3:1] of the crude product gave an amorphous solid (67%). $[\alpha]_D$ +2.1° (*c* 0.48, MeOH) (lit.[10] $[\alpha]_D^{28}$ +4.9° (*c* 0.35, MeOH)); ^1H NMR (pyridine-d5): δ5.25 (1H, ddd, $J_{2',3'}$ = 7.4, $J_{3',4'b}$ = 3.8, $J_{3',4'a}$ = 3.6 Hz, H-3'), 5.14-5.09 (2H, m, H-3, H-2), 5.00 (1H, m, H-2'), 4.78 (1H, dd, $J_{1'a,1'b}$ = 13.0, $J_{1'a,2'}$ = 4.9 Hz, H-1'a), 4.70 (1H, m, H-4), 4.63 (1H, dd, $J_{1'b,2'}$ = 4.0 Hz, H-1'b), 4.61 (1H, dd, $J_{4'a,4'b}$ = 11.8 Hz, H-4'a), 4.54 (1H, dd, $J_{5a,5b}$ = 11.6, $J_{4,5a}$ = 6.5 Hz, H-5a), 4.51 (1H, dd, $J_{4,5b}$ = 7.5 Hz, H-5b), 4.37 (1H, dd, H-4'b), 4.32 (2H, br s, H-1a, H-1b); ^{13}C NMR (pyridine-d5): δ 79.14 (C-3'), 79.06 (C-3), 78.18 (C-2), 72.30 (C-4), 67.44 (C-2'), 62.05 (C-4'), 59.98 (C-5), 52.46 (C-1'), 50.35 (C-1); HRMS. Calcd for $C_9H_{18}O_9S_2$ (M + H): 335.0471. Found: 335.0481.

1,4-Dideoxy-1,4-[(R)-[(2R,3R)-2,4-dihydroxy-3-(sulfooxy)butyl]-sulfoniumylidene]-L-arabinitol Inner Salt (2.5). Column chromatography [$CHCl_3$/MeOH/H_2O, 7:3:1] of the crude product gave an amorphous solid (80%). $[\alpha]_D$ –1.6° (*c* 0.6, MeOH); HRMS. Calcd for $C_9H_{18}O_9S_2$ (M + H): 335.0471. Found: 335.0466.

1,4-Dideoxy-1,4-[(R)-[(2R,3R)-2,4-dihydroxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (2.7). Column chromatography [$CHCl_3$/MeOH/H_2O, 7:3:1] of the crude product gave an amorphous solid (59%). $[\alpha]_D$ –35.6° (*c* 0.86, MeOH); ^1H NMR

(pyridine-d5): δ 5.19 (1H, ddd, $J_{2',3'}$ = 7.8, $J_{3',4'a}$ = $J_{3',4'b}$ = 3.7 Hz, H-3'), 5.17-5.12 (2H, m, H-2, H-3), 5.00 (1H, ddd, $J_{1'a,2'}$ = 5.1, $J_{1'b,2'}$ = 4.0 Hz, H-2'), 4.83 (1H, dd, $J_{1'a,1'b}$ = 13.0 Hz, H-1'a), 4.78 (1H, ddd, $J_{3,4}$ = 2.0 Hz, H-4), 4.65 (1H, dd, $J_{4'a,4'b}$ = 11.9 Hz, H-4'a), 4.65 (1H, dd, $J_{5a,5b}$ = 11.5, $J_{4,5a}$ = 5.0 Hz, H-5a), 4.60 (1H, dd, $J_{4,5b}$ = 6.4 Hz, H-5b), 4.53 (1H, dd, H-1'b), 4.40 (1H, dd, H-4'b), 4.29 (1H, dd, $J_{1a,1b}$ = 12.7, $J_{1a,2}$ = 3.9 Hz, H-1a), 4.17 (1H, dd, $J_{1b,2}$ = 2.6 Hz, H-1b); ^{13}C NMR (pyridine-d5): δ 79.46 (C-3'), 79.38 (C-3), 78.94 (C-2), 71.94 (C-4), 67.52 (C-2'), 62.02 (C-4'), 60.26 (C-5), 52.64 (C-1'), 51.01 (C-1); HRMS. Calcd for $C_9H_{18}O_9S_2$ (M + H): 335.0471. Found: 335.0486.

2.6 Acknowledgment

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CHAPTER 3: SYNTHESIS OF NITROGEN ANALOGUES OF SALACINOL AND THEIR EVALUATION AS GLYCOSIDASE INHIBITORS

Ahmad Ghavami,[a] Blair D. Johnston,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*

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Tel: (604) 291-4327

Fax: (604) 291-3765

e-mail:bpinto@sfu.ca

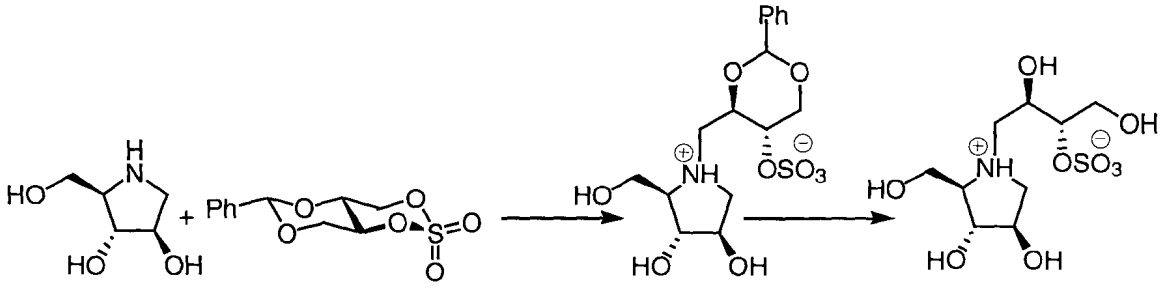
3.1 Graphical Abstract

Synthesis of Nitrogen Analogues of Salacinol and their Evaluation as Glycosidase Inhibitors

Ahmad Ghavami,[a] Blair D. Johnston,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*



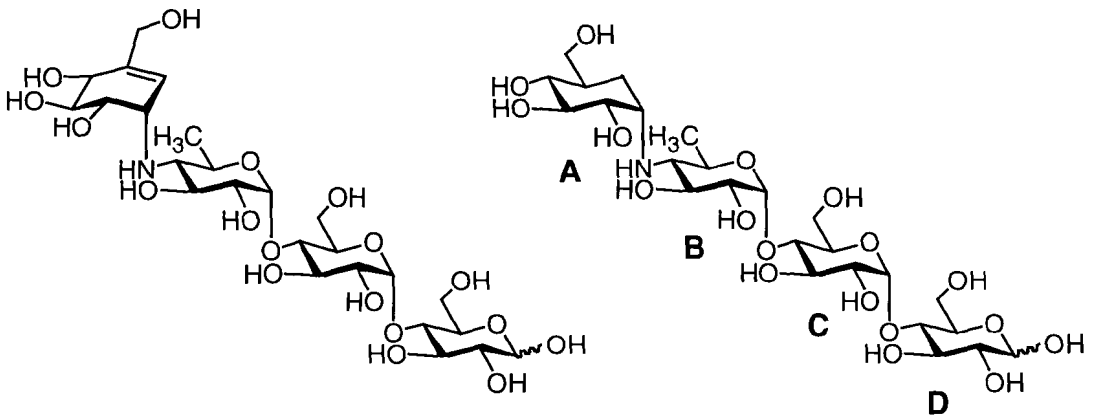
Keywords: glycosidase inhibitors, salacinol, nitrogen analogues, ammonium salts, cyclic sulfate.

3.2 Abstract

The syntheses of two nitrogen analogues (**3.11** and **3.12**) of the naturally occurring sulfonium ion, salacinol (**3.7**) are described. The latter compound 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 synthetic strategy relies on the nucleophilic attack of a 1,4-dideoxy-1,4-imino-D- or L-arabinitol at the least hindered carbon of 2,4-*O*-benzylidene D- or L-erythritol-1,3-cyclic sulfate. The nitrogen analogues bear a permanent positive charge and serve as mimics of the sulfonium ion. We reasoned that these ammonium derivatives should function in a similar manner to known glycosidase inhibitors of the alkaloid class such as castanospermine (**3.4**) and deoxynojirimycin (**3.5**). Enzyme inhibition assays indicate that salacinol (**3.7**) is a weak (K_i = 1.7 mM) inhibitor of glucoamylase, whereas compounds **3.11** and **3.12** inhibit glucoamylase with K_i values in the range ~10-fold higher. The nitrogen analogues **3.11** and **3.12** showed no significant inhibitory effect of either barley α-amylase (AMY1) or porcine pancreatic α-amylase (PPA) at concentrations of 5 mM. In contrast, salacinol (**3.7**) inhibited AMY1 and PPA in the micromolar range, with K_i values of 15 ± 1 μM and 10 ± 2 μM, respectively.

3.3 Introduction

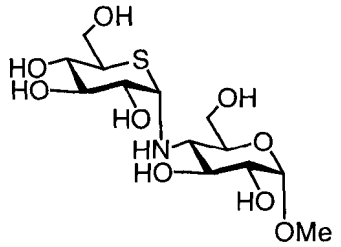
The controlled inhibition of glycosidase enzymes plays important roles in the biochemical processing of biopolymers containing carbohydrates.[1,2] The intrinsic low affinities of carbohydrate-protein interactions seem to have led Nature to select non-carbohydrate mimics as natural inhibitors of this important class of enzymes. Thus, for example, Nature uses protein inhibitors in order to inhibit amylase enzyme activity,[1] and carbohydrate mimics of the alkaloid class, such as polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines, and nortropanes, are widespread in plants and microorganisms, and have been shown to possess glycosidase inhibitory activity.[2] The naturally occurring glycosidase inhibitor acarbose (**3.1**),[3] which contains a nitrogen atom in one of the linkages between sugar and pseudosugar units gives the highest known carbohydrate affinity for a binding protein, and is currently used for the oral treatment of diabetes.[4,5]



3.1 **3.2**

Chart 3.1. Structures of acarbose (**3.1**) and dihydroglucoacarbose (**3.2**).

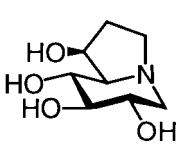
It was generally believed that the carbohydrate mimics containing nitrogen are protonated in the enzyme active site and act as glycosidase inhibitors because of their ability to mimic the shape or charge of the presumed transition state for enzymatic glycoside hydrolysis.[6] Considerable synthetic effort has therefore led to a variety of nitrogen-containing analogues, some of which have shown inhibitory activity.[6] However, detailed kinetic analysis indicated that although **3.1** provides some resemblance to a transition-state analogue, it is likely that electrostatic stabilization contributes significantly to its high affinity for glucoamylase.[7] That electrostatic stabilization is important is supported by the observations that the substrate analogues, dihydroglucoacarbose (GAC) (**3.2**)[8] and the maltoside heteroanalogue **3.3**[9] are also competitive inhibitors of glucoamylase, with low K_i values. The analogue **3.3** is envisaged to mimic rings A and B of GAC. In contrast, it was shown that castanospermine (**3.4**) and deoxynojirimycin (**3.5**) are good competitive inhibitors of the β-glucosidase from *Agrobacterium sp.* but do not function as transition state analogues, despite their high affinity binding.[7] We contend that it is the electrostatic stabilization that leads to the high affinity binding.



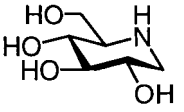
3.3

Chart 3.2. Maltoside heteroanalogue **3.3**.

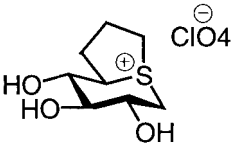
We have recently reported the synthesis of a sulfonium-ion mimic **3.6** of castanospermine (**3.4**).[10] We reasoned that the interaction of a permanent positive charge with active-site carboxylate residues would make a dominant contribution to the interaction energy.



3.4



3.5

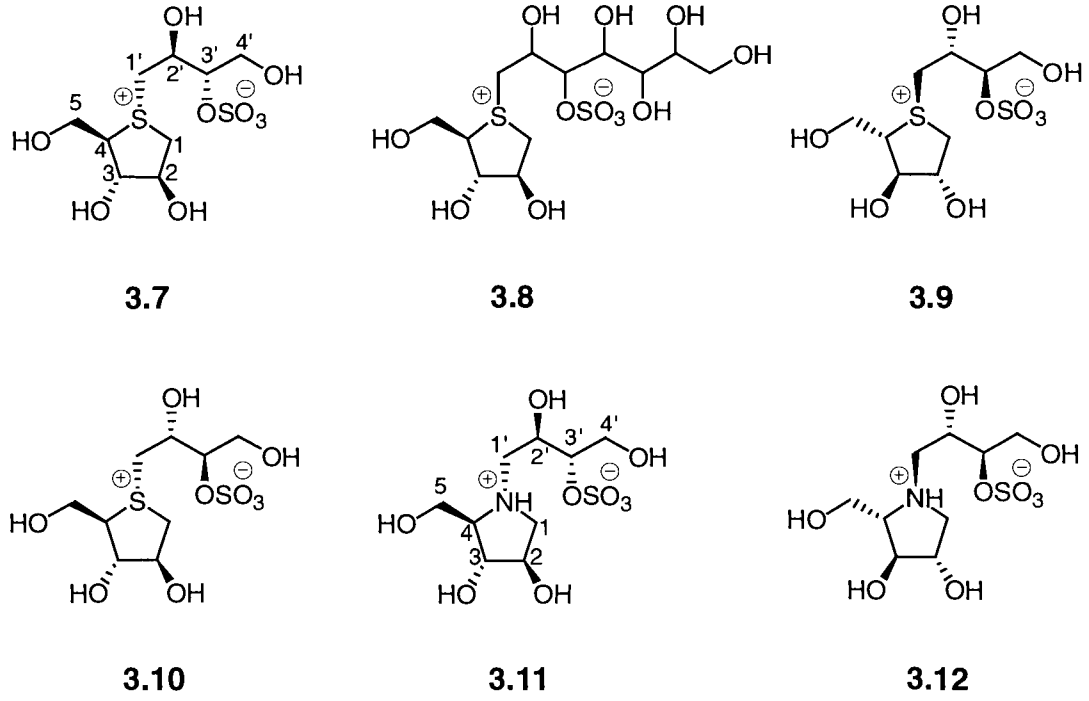


3.6

Chart 3.3. Compounds **3.4** – **3.6**.

The concept was validated by the recent isolation[11,12] of naturally occurring glucosidase inhibitors from *Salacia reticulata*, a plant from Sri Lanka ('Kothalahimbutu' in Singhalese) known for its antidiabetic properties. The compounds, salacinol (**3.7**) and kotalanol (**3.8**), contain a thiosugar sulfonium ion with an internal sulfate providing the counterion. In type II diabetes, insulin secretion may be normal but the entry into cells of glucose (normally mediated by insulin) is compromised, and levels of glucose in the blood are high.[13] Inhibition of pancreatic α-amylase, which hydrolyzes starch into smaller oligosaccharides, and α-glucosidases, which break down these oligosaccharides further to glucose in the intestinal membrane are therefore the targets of other glucosidase inhibitors, e.g. acarbose (**3.1**).[4,5] Such enzyme inhibition results in delayed glucose absorption into the blood and a smoothing or lowering of postprandial hyperglycemia, resulting in improved glycemic control. Salacinol (**3.7**) and kotalanol (**3.8**) may potentially have fewer long-term side effects than other existing oral antidiabetic agents. Recent animal studies

have shown that the oral ingestion of an extract from a *S. reticulata* trunk at a dose of 5,000 mg/kg had no serious acute toxicity or mutagenicity in rats.[14]



3.7 **3.8** **3.9**

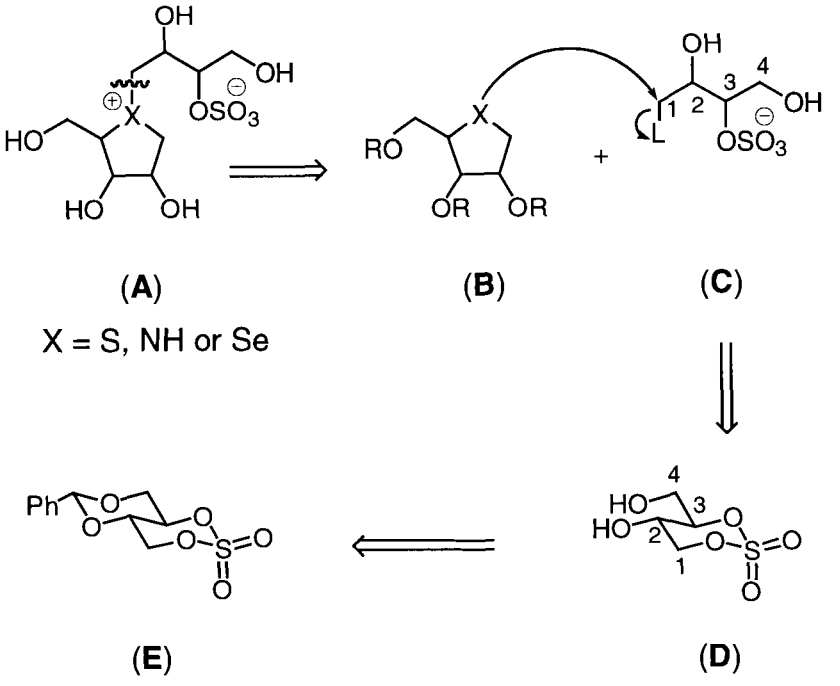
3.10 **3.11** **3.12**

Chart 3.4. Compounds **3.7** – **3.12**.

The synthesis of salacinol (**3.7**) and its stereoisomers **3.9**, **3.10**, and conclusive proof of structure of the natural product have recently been reported.[15-17] We now report the synthesis of the hitherto unknown nitrogen congeners **3.11**, **3.12** as potential glycosidase inhibitors.[16] We reasoned that the latter ammonium derivatives, bearing a permanent positive charge, should function in a manner similar to that of castanospermine (**3.4**) and deoxynojirimycin (**3.5**).

3.4 Results and Discussion

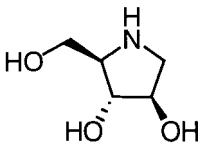
Retrosynthetic analysis indicated that salacinol (**3.7**) or its analogues (**A**) could be obtained by alkylation of anhydroalditol derivatives at the ring heteroatom (Scheme 3.1).[16,17] The alkylating agent could either be an open-chain electrophile (**C**) or a cyclic sulfate derivative such as **D** or **E**, whereby selective attack of the heteroatom at the least hindered primary centre should afford the desired sulfonium, ammonium, or selenonium ions. We have found that the opening of benzylidene-protected cyclic sulfates by the amines proceeded smoothly to give compound **3.11** and its enantiomer **3.12**.[16]



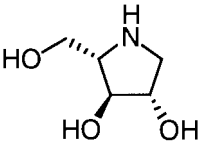
Scheme 3.1. Retrosynthetic analysis.

The iminoarabinitols **3.13** and **3.14** were synthesized from D-glucose[18] and D-xylose,[19] respectively. The 2,4-*O*-benzylidene-L- (**3.15**) and D- (**3.16**) erythritol-1,3- cyclic

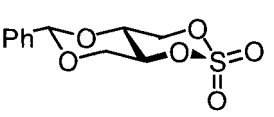
sulfates were synthesized from L- and D-glucose, respectively,[17] in a similar manner to that described for the corresponding 2,4-*O*-ethylidene derivative.[20]



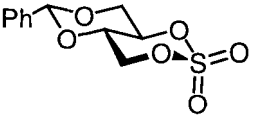
3.13



3.14



3.15

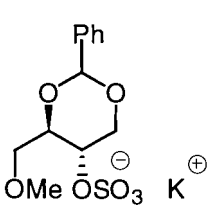


3.16

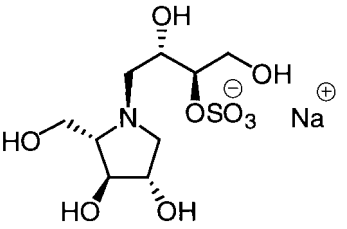
Chart 3.5. Compounds **3.13** – **3.16**.

Compound **3.17** was synthesized by alkylation of 1,4-dideoxy-1,4-imino-D-arabinitol (**3.13**) with the benzylidene protected cyclic sulfate (**3.15**) (1.2 equiv), in dry methanol containing K_2CO_3 at 60-75 °C to give the protected compound **3.17** in 72% yield (Scheme 3.2). A side product **3.18** was also formed in 16% yield, which was assigned to be the product of methanolysis of the cyclic sulfate **3.15**. Deprotection of the coupled product **3.17** by hydrogenolysis over a Pd/C catalyst gave compound **3.11** in 64% yield. Evidence of the tertiary ammonium structure was obtained by high-resolution FAB mass spectrometery, run in the positive ion mode. If the compound had been a tertiary amine with H^+ or Na^+ associated with the sulfate anion, only the H^+ or Na^+ ions would be observed; the negative sulfate ion would be grounded out on the source wall and would not leave the source. Since the M + H peaks are observed, the tertiary ammonium cation must

be present with the internal sulfate counterion.[21] The presence of a tertiary ammonium structure was also confirmed by ^{1}H-NMR spectroscopy. In compounds **3.11** and **3.12** the protons that are on carbons, which are α to the ring nitrogen are all deshielded and broadened at neutral pH. For compound **3.12**, altering the pH to ~12 with sodium hydroxide resulted in upfield shifts and sharper signals for these proton resonances. We attribute these shifts to the formation of the tertiary amine sulfate **3.19** at high pH. The stereochemistry at the stereogenic nitrogen centre in **3.11** was established by means of a NOESY experiment.



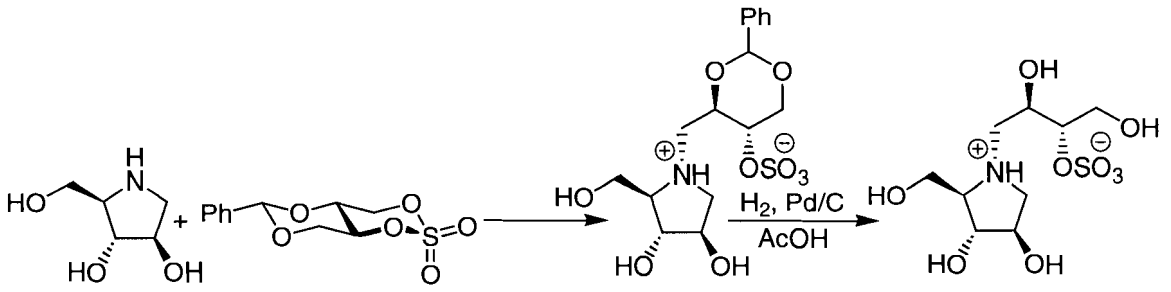
3.18



3.19

Chart 3.6. Compounds **3.18** and **3.19**.

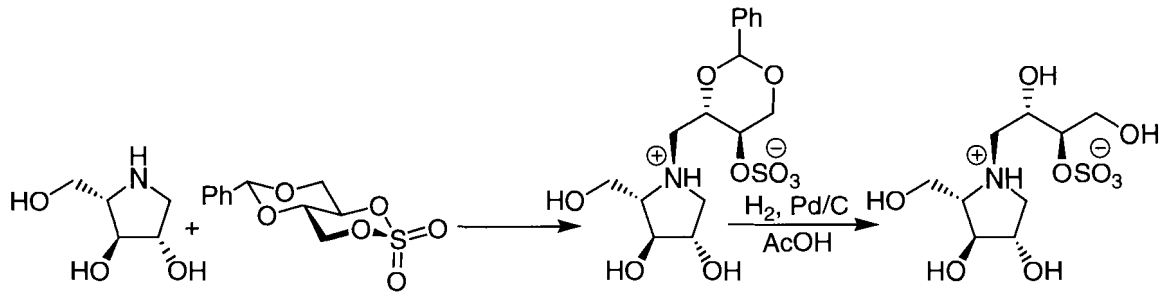
A correlation between H-1' and H-4, confirmed the trans relationship between the erythritol side chain and the C-2 and C-4 substituents on the anhydroarabinitol moiety, which is identical to the stereochemistry at the stereogenic sulfur atom in salacinol (**3.7**); no correlation between H-1' and H-3 was observed. We have chosen to name compound **3.11** ghavamiol.



3.13 **3.15** **3.17** (72%) **3.11** (64%)

Scheme 3.2. Synthesis of ghavamiol (**3.11**).

The enantiomer of ghavamiol (**3.12**) was similarly obtained by the reaction of compound **3.14** with the cyclic sulfate (**3.16**) to produce the ammonium salt **3.20** in 72% yield. Deprotection as before, produced compound **3.12** in 77% yield. The ^{1}H and ^{13}C NMR spectra for the enantiomer **3.12** were essentially identical to those of ghavamiol (**3.11**) except for small changes in chemical shifts due to concentration effects. Proof of structure and stereochemistry were obtained as described above.



3.14 **3.16** **3.20** (72%) **3.12** (77%)

Scheme 3.3. Synthesis of the enantiomer of ghavamiol (**3.12**).

3.5 Enzyme Inhibition Assays

Compounds **3.11** and **3.12** were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2,[22, 23] porcine pancreatic α-amylase, and barley α-amylase.[24] The effects were compared to those of salacinol (**3.7**). Glucoamylase G2 was weakly inhibited by salacinol (**3.7**) (K_i = 1.7 mM). In comparison, ghavamiol (**3.11**) and its enantiomer **3.12** showed very weak inhibition, with K_i values in the range ~10-fold higher than for **3.7**. We therefore estimate that compounds **3.11** and **3.12** cannot have K_i values that are less than 10 mM. The nitrogen analogues **3.11** and **3.12** showed no significant inhibitory effect of either barley α-amylase (AMY1) or porcine pancreatic α-amylase (PPA) at concentrations of 5 mM. In contrast, salacinol (**3.7**) inhibited AMY1 and PPA in the micromolar range, with K_i values of 15 ± 1 and 10 ± 2 μM, respectively. It would appear then that the nitrogen analogues **3.11** and **3.12** and salacinol (**3.7**) show discrimination or selectivity for certain glycosidase enzymes and further testing against a wider panel of enzymes that includes human small intestinal maltase-glucoamylase[25] and human pancreatic α-amylase[26] is planned to map the enzyme selectivity profiles of these compounds.

3.6 Experimental Section

3.6.1 General Methods

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz. All assignments were confirmed with the aid of two-dimensional $^{1}H,^{1}H$ (COSYDFTP) or $^{1}H,^{13}C$ (INVBTP) experiments using standard Bruker pulse programs. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix using a PerSeptive Biosystems Voyager-DE instrument. Column chromatography was performed with Merck Silica gel 60 (230-400 mesh). High resolution mass spectra were liquid secondary ionization fast atom bombardment (LSIMS (FAB)), run on a Kratos Concept H double focussing mass spectrometer at 10000 RP, using *meta*-NO_2-benzyl alcohol as matrix or, in the case of compounds **3.18** and **3.20**, negative LSIMS with glycerine as matrix and PEG-sulfate as the mass reference.

3.6.2 Enzyme Inhibition Assays

The glucoamylase G2 form from *Aspergillus niger* was purified from a commercial enzyme (Novo Nordisk, Bagsvaerd, Denmark) as described.[22,23] The initial rates of glucoamylase G2-catalyzed hydrolysis of maltose was tested with 1 mM maltose as substrate in 0.1 M sodium acetate pH 4.5 at 45 °C using an enzyme concentration of 7.0×10^{-8} M and five inhibitor concentrations in the range of 1 μm to 5 mM. The effects of the inhibition on rates of substrate hydrolysis were compared for the different compounds. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction

volume for the enzyme reaction mixtures of 150 or 300 μL.[27] The K_i values were calculated assuming competitive inhibition from $1/v = (1/V_{max}) + [(K_m)/(V_{max}[S]K_i)]$ x [I], where v is the rate measured in the presence or absence of inhibitor, [I] and [S] the concentrations of inhibitor and substrate, K_m 1.6 mM, and k_{cat} 11.3 s^{-1}, using ENZFITTER as described.[27,28]

Recombinant barley α-amylase isozyme 1 (AMY1) was produced and purified as described.[24] An aliquot of the porcine pancreatic α-amylase (PPA) crystalline suspension (in ammonium sulfate) was dialyzed extensively against the assay buffer without bovine serum albumin (BSA). The enzyme concentration was determined by aid of amino acid analysis as determined using an LKB model Alpha Plus amino acid analyzer. Porcine pancreatic α-amylase (PPA) and bovine serum albumin (BSA) were purchased from Sigma. Amylose EX-1 (DP17; average degree of polymerization 17) was purchased from Hayashibara Chemical Laboratories (Okayama, Japan). The inhibition of AMY1 (3×10^{-9} M) and PPA (9×10^{-9} M) activity towards DP17 amylose was measured at 37 °C in 20 mM sodium acetate, pH 5.5, 5 mM $CaCl_2$, 0.005 % BSA (for AMY1) and 20 mM sodium phosphate, pH 6.9, 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005 % BSA (for PPA). Six different final inhibitor concentrations were used in the range of 1 μM to 5 mM. The inhibitor was preincubated with enzyme for 5 min at 37 °C before addition of substrate. Initial rates were determined by measuring reducing sugar by the copper-bicinchoninate method as described.[24,29] The K_i values were calculated assuming competitive inhibition, as described above for the case of glucoamylase, and a K_m of 0.57 mg/mL and k_{cat} of 165 s^{-1} for AMY1 and 1 mg/mL and 1200s^{-1} for PPA, as determined in the substrate concentration range of

0.03 to 10 mg/mL using ENZFITTER.[28] For the K_i determinations, [S] = 0.7 mg/mL amylose DP 17 for AMY1 and [S] = 2.5 mg/mL amylose DP 17 for PPA.

1,4-Dideoxy-1,4-[(S)-[(2R,3S)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-iminium]-D-arabinitol Inner Salt (3.17) and potassium 2,4-*O*-benzylidene-1-*O*-methyl-L-erythritol-3-sulfate (3.18). A mixture of 1,4-dideoxy-1,4-imino-D-arabinitol (**3.13**) (100 mg, 0.7 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.15**) (235 mg, 1.2 equiv) were dissolved in dry MeOH (0.5 mL) and anhydrous K_2CO_3 (15 mg) was added. The mixture was stirred in a sealed tube in an oil-bath (75° C) overnight. The solvent was removed under reduced pressure and column chromatography [CH_2Cl_2/MeOH, 4.5:1] of the crude product gave **3.17** (219 mg, 72%) and **3.18** (40 mg, 16%) as amorphous solids. **3.17**: $[\alpha]_D$ +36.8° (*c* 0.4, MeOH); ^{1}H NMR (CD_3OD): δ 7.53-7.30 (5H, m, Ar), 5.61 (1H, s, C*H*Ph), 4.53 (1H, dd, $J_{4'ax,4'eq}$ = 11.0, $J_{3',4'eq}$ = 5.2 Hz, H-4′eq), 4.28 (1H, brt, $J_{1'b,2'} = J_{2'b,3'}$ = 9.8 Hz, H-2′), 4.20 (1H, ddd, $J_{3',4'ax}$ = 9.7 Hz, H-3′), 4.14 (1H, brs, H-2), 4.03 (1H, brd, $J_{1'a,1'b}$ = 10.7 Hz, H-1′), 3.94 (1H, brs, H-3), 3.92 (1H, dd, $J_{4,5a}$ = 5.1 Hz, H-5a), 3.86 (1H, dd, $J_{4,5b}$ = 7.2, $J_{5a,5b}$ = 12.3 Hz, H-5b), 3.81 (1H, dd, H-4′ax), 3.62 (1H, brd, $J_{1a,1b}$ = 11.0 Hz, H-1a), 3.47 (1H, brd, H-1b), 3.43 (1H, brs, H-4), 3.33 (1H, brd, H-1′b); ^{13}C NMR (CD_3OD): δ 138.66 (C_{ipso}), 130.15 (C_{para}), 129.23 (2C) and 127.40 (2C) (C_{ortho} and C_{meta}), 102.34 (*C*HPh), 77.81 (C-4), 77.52 (C-3), 77.40 (C-2′), 76.19 (C-2), 70.27 (C-4′), 68.92 (C-3′), 62.68 (C-1), 60.41 (C-5), 58.61 (C-1′); MALDI-TOF MS: *m/e* 428 (M^+ + Na) , 406 (M^+ + H); HRMS. Calcd for $C_{16}H_{23}O_9SN$ (M + H): 406.1179. Found: 406.1192. **3.18**: $[\alpha]_D$ +31.1° (*c* 0.8, MeOH); ^{1}H NMR (CD_3OD): δ 7.49-7.30 (5H, m, Ar), 5.55 (1H, s, C*H*Ph), 4.55 (1H, dd, $J_{4eq,4ax}$ = 10.6, $J_{3,4eq}$ = 5.4 Hz, H-4eq), 4.29 (1H, ddd, $J_{2,3}$ = 9.7, $J_{3,4ax}$

= 10.6 Hz, H-3), 3.90 (1H, ddd, $J_{1a,2}$ = 1.8, $J_{1b,2}$ = 6.5 Hz, H-2), 3.86 (1H, dd, $J_{1a,1b}$ = 11.1 Hz, H-1a), 3.76 (1H, dd, H-4ax), 3.61 (1H, dd, H-1b), 3.39 (3H, s, OCH_3); ^{13}C NMR (CD_3OD): δ 139.21 (C_{ipso}), 129.87 (C_{para}), 129.03 (2C) and 127.44 (2C) (C_{ortho} and C_{meta}), 102.42 (*C*HPh), 80.61 (C-3), 72.92 (C-1), 70.38 (C-2), 67.96 (C-4), 59.56 (O*C*H_3); MALDI-TOF negative ion MS: *m/e* 303 (M - K); HRMS. Calcd for $C_{12}H_{15}O_7S$ (M - K): 303.0538. Found: 303.0543.

1,4-Dideoxy-1,4-[(R)-[(2S,3RS)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-iminium]-L-arabinitol Inner Salt (3.20). A mixture of 1,4-dideoxy-1,4-imino-L-arabinitol (**3.14**) (80 mg, 0.6 mmol) and 2,4-*O*-benzylidene-D-erythritol-1,3-cyclic sulfate (**3.16**) (190 mg, 1.2 equiv) were dissolved in dry MeOH (0.5 mL) and anhydrous K_2CO_3 (10 mg) was added. The mixture was stirred in a sealed tube in an oil-bath (75° C) overnight. The solvent was removed under reduced pressure and column chromatography [CH_2Cl_2/MeOH, 5:1] of the crude product gave an amorphous solid (175 mg, 72%). $[\alpha]_D$ –32.5° (c 2.4, MeOH); MALDI-TOF MS: *m/e* 428 (M^+ + Na), 406 (M^+ + H); HRMS. Calcd for $C_{16}H_{23}O_9SN$ (M – H): 404.1015. Found: 404.1007.

3.6.3 General Procedure for the Deprotection of the Protected Ammonium Sulfates

The protected compound (200 mg, 0.5 mmol) was dissolved in AcOH/H_2O, 4:1 (5 mL) and stirred with Pd-C (30 mg) under H_2 (52 psi). After 60 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The combined filtrates were concentrated and the residue was purified by column chromatography.

1,4-Dideoxy-1,4-[(S)-[(2R,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]-iminium]-D-arabinitol Inner Salt (3.11). Column chromatography [$CHCl_3$/MeOH/H_2O, 7:3:1] of the crude product gave an amorphous solid (64%). $[\alpha]_D$ +7.2° (c 2.6, MeOH); ^1H NMR (CD_3OD): δ 4.26-4.20 (2H, m, H-2′, H-3′), 4.15 (1H, m, $J_{2,3}$ = 6.0 Hz, H-2), 3.98 (1H, brs, $J_{3,4}$ = 4.0 Hz, H-3), 3.94-3.87 (3H, m, $J_{3',4'a}$ = 4.0 Hz, H-5a, H-5b, H-4′a), 3.81 (1H, m, $J_{4'a,4'b}$ = 12.0, $J_{3',4'b}$ = 3.5 Hz, H-4′b), 3.74-3.62 (2H, m, $J_{1a,1b}$ = 13.0 Hz, $J_{1'a,1'b}$ = 14.0 Hz, H-1′a, H-1a), 3.49-3.42 (1H, m, $J_{1b,2}$ = 7.0 Hz, H-1b), 3.40-3.35 (1H, m, H-4), 3.15 (1H, m, $J_{1'b,2'}$ = 6.0 Hz, H-1′b); ^{13}C NMR (CD_3OD): δ 81.17 (C-3′), 78.27 (C-3), 77.86 (C-4), 76.19 (C-2), 68.07 (C-2′), 62.57 (C-1), 61.67 (C-4′), 60.72 (C-1′, C-5); HRMS. Calcd for $C_9H_{18}O_9SN$ (M + H): 318.0859. Found: 318.0863.

1,4-Dideoxy-1,4-[(R)-[(2S,3R)-2,4-dihydroxy-3-(sulfooxy)butyl]-iminium]-L-arabinitol Inner Salt (3.12). Column chromatography [$CHCl_3$/MeOH/H_2O, 7:3:1] of the crude product gave an amorphous solid (77%). $[\alpha]_D$ –7.7° (c 0.76, MeOH); HRMS. Calcd for $C_9H_{18}O_9SN$ (M + H): 318.0859. Found: 318.0856.

1,4-Dideoxy-1,4-[(R)-[(2S,3R)-2,4-dihydroxy-3-(sulfooxy)butyl]-imino]-L-arabinitol Inner Salt (3.19). A solution of **12** in CD_3OD was adjusted to pH 12 by the addition of aliquots of a concentrated solution of NaOH in CD_3OD. ^1H NMR (CD_3OD): δ 4.36 (1H, ddd, $J_{2',3'}$ = 5.8, $J_{3',4'a}$ = 4.2, $J_{3',4'b}$ = 4.8 Hz, H-3′), 4.00 (1H, ddd, $J_{1'a,2'}$ = 5.9, $J_{1'b,2'}$ = 6.9 Hz, H-2′), 3.94-3.90 (2H, m, H-3, H-2), 3.90 (1H, dd, $J_{4'a,4'b}$ = 12.0 Hz, H-4′a), 3.80 (1H, dd, H-4′b), 3.73 (1H, dd, $J_{5a,5b}$ = 11.4, $J_{4,5a}$ = 3.7 Hz, H-5a), 3.66 (1H, dd, $J_{4,5b}$ = 3.5 Hz, H-5b), 3.15 (1H, dd, $J_{1a,1b}$ = 10.5 Hz, H-1a), 3.10 (1H, dd, $J_{1'a,1'b}$ = 13.0 Hz, H-1′a), 2.77 (1H,

dd, $J_{1b,2}$ = 4.5 Hz, H-1b), 2.52 (1H, dd, H-1′b), 2.43 (1H, ddd, $J_{3,4}$ = 3.8 Hz, H-4); ^{13}C NMR (CD_3OD): δ 82.28 (C-3′), 80.68 (C-3), 77.56 (C-2), 75.24 (C-4), 71.15 (C-2′), 62.16 (C-4′), 61.85 (2C, C-1 and C-5), 58.69 (C-1′).

3.7 Acknowledgments

We are grateful to Sidsel Ehlers for technical assistance with the glucoamylase assays and the Natural Sciences and Engineering Research Council of Canada for financial support.

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CHAPTER 4: SYNTHESIS OF SELENIUM ANALOGUES OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR SALACINOL AND THEIR EVALUATION AS GLYCOSIDASE INHIBITORS†

Blair D. Johnston,[a] Ahmad Ghavami,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*

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Tel: (604) 291-4327

Fax: (604) 291-5424

e-mail:bpinto@sfu.ca

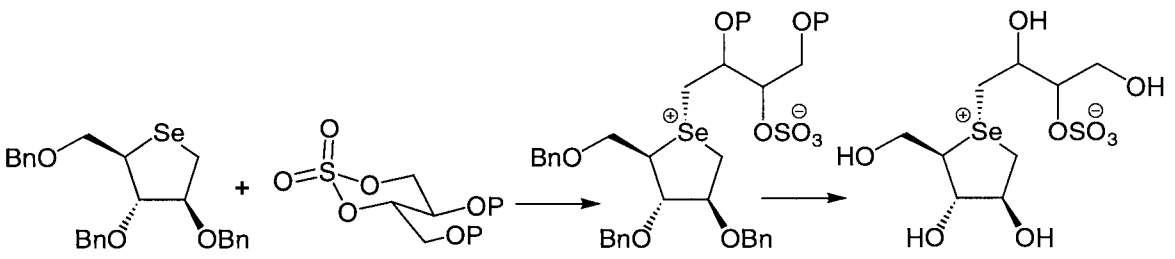
4.1 Graphical Abstract

Synthesis of Selenium Analogues of the Naturally Occurring Glycosidase Inhibitor Salacinol and their Evaluation as Glycosidase Inhibitors

Blair D. Johnston,[a] Ahmad Ghavami,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark*



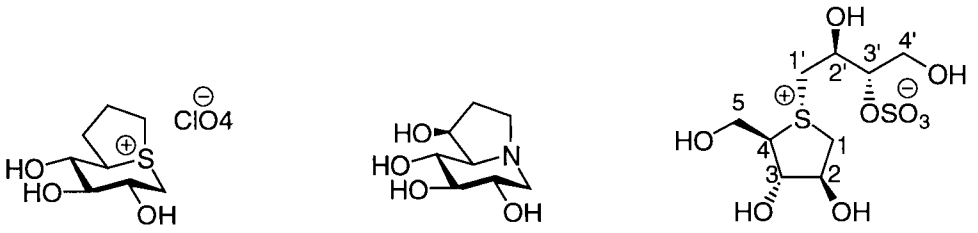
Keywords: salacinol, selenium analogues, cyclic sulfate, glycosidase inhibitors, enzyme inhibitory activities

4.2 Abstract

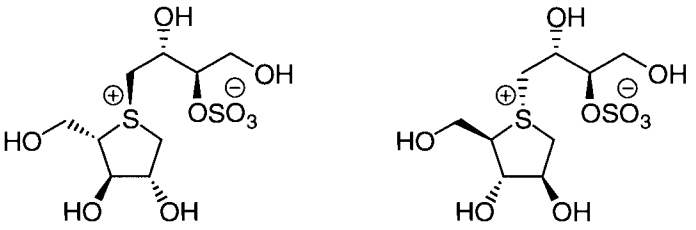
The syntheses of two selenium analogues **4.10** and **4.11** of the naturally occurring sulfonium ion, salacinol (**4.3**) are described. Salacinol (**4.3**) is one of the active principles in the aqueous extracts of *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of diabetes. The synthetic strategy relies on the nucleophilic attack of a 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol at the least hindered carbon of benzyl- or benzylidene-protected D- or L-erythritol-1,3-cyclic sulfate. The use of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as a solvent in the coupling reaction proves to be beneficial. Enzyme inhibition assays indicate that **4.10** is a better inhibitor (K_i = 0.72 mM) of glucoamylase than **4.3**, which has a K_i value of 1.7 mM. In contrast, **4.11** showed no significant inhibition of glucoamylase. Compounds **4.10** and **4.11** showed no significant inhibition of barley-α-amylase or porcine pancreatic-α-amylase.

4.3 Introduction

Glycomimetics in which specific oxygen atoms of carbohydrates have been replaced by different heteroatoms have been intensely investigated in the search for novel glycosidase inhibitors.[1] Our efforts in recent years have focused on a novel class of glycosidase inhibitors containing sulfonium ions as putative mimics of the oxacarbenium ion intermediates in glycosidase hydrolysis reactions.[2,3] Thus, we have described the synthesis and conformational analysis of a sulfonium-ion analogue **4.1** of the naturally occurring glycosidase inhibitor of the indolizidine alkaloid class, castanospermine (**4.2**).[2]



4.1 **4.2** **4.3**

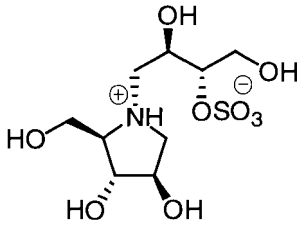


4.4 **4.5**

Chart 4.1. Structures of compounds **4.1** – **4.5**.

Yoshikawa et al.[4] also described the isolation of a naturally occurring glycosidase inhibitor containing a zwitterionic sulfonium-sulfate structure, namely salacinol (**4.3**), from the plant *Salacia reticulata*, which prompted us[3] and others[5] to synthesize **4.3** and its

stereoisomers, **4.4** and **4.5**, and provide conclusive proof of structure of the natural product. We have also reported the synthesis of the corresponding nitrogen congeners **4.6** and **4.7** as potential glycosidase inhibitors,[6] and this has been followed by a similar report from another group.[7] We have also reported the synthesis of 1,4-anhydro-D-xylitol heteroanalogues **4.8** and **4.9**, and their evaluation as glycosidase inhibitors.[8] Most recently, Yoshikawa et al.[9] reported the inhibitory activity of **4.3** against several α-glucosidases and also the inhibitory effects of extracts of *Salacia reticulata* on serum blood glucose levels in maltose- and sucrose-loaded rats. Those authors also assigned the "absolute stereostructure" of **4.3** to a zwitterionic compound comprised a 1,4-anhydro-4-thio-D-arabinitol moiety and a 1'-deoxy-D-erythrosyl-3'-sulfate unit.[9] However, we point out here that carbohydrate nomenclature dictates that the latter unit be designated a 1'-deoxy-L-erythrosyl-3'-sulfate unit, as also proven by our earlier work on the synthesis of the different stereoisomers of salacinol.[3]



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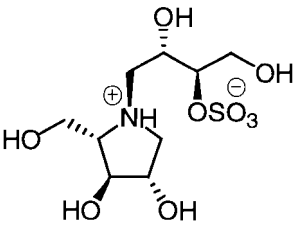

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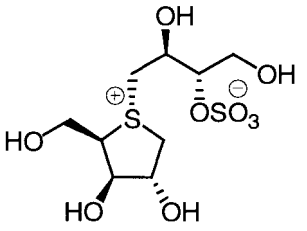

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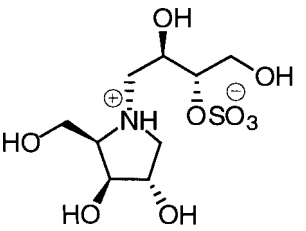
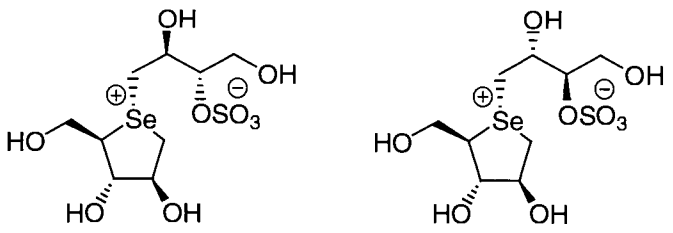


4.9

Chart 4.2. Compounds **4.6** – **4.9**.

As part of our continuing interest in evaluating the effect of the heteroatom substitution in the sugar ring on glycosidase inhibitory activity, we report in the present work the synthesis of novel analogues of **4.3** and its diastereomer **4.5**, in which the sulfur atom has been replaced by the heavier cognate atom, selenium, to give **4.10** and **4.11**, respectively. We report also their evaluation as glycosidase inhibitors of porcine pancreatic α-amylase (PPA), barley α-amylase (AMY1), and glucoamylase G2.

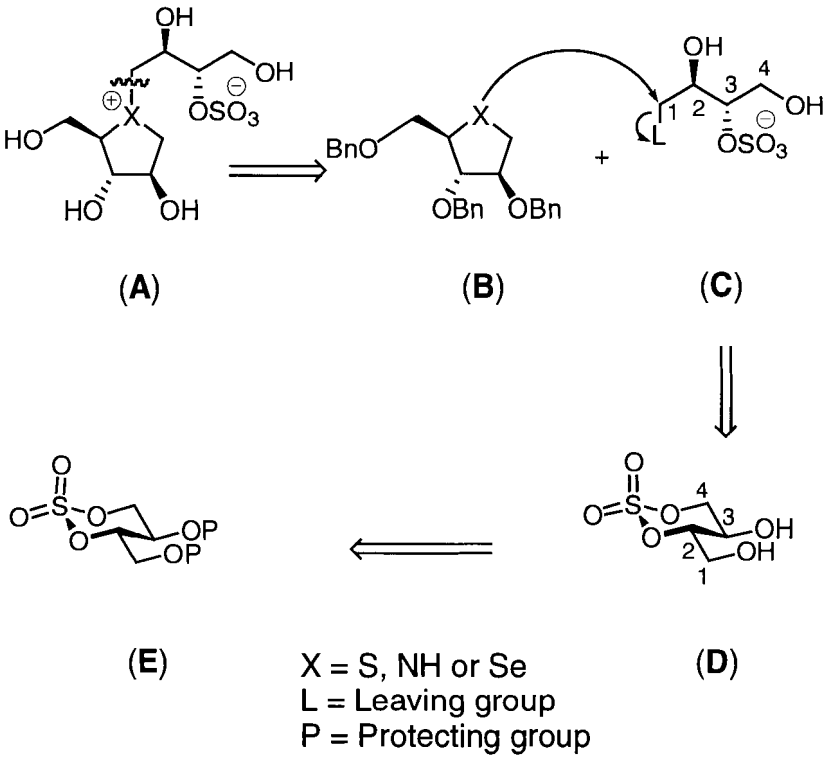


4.10 **4.11**

Chart 4.3. Blintol (**4.10**) and its diastereomer **4.11**.

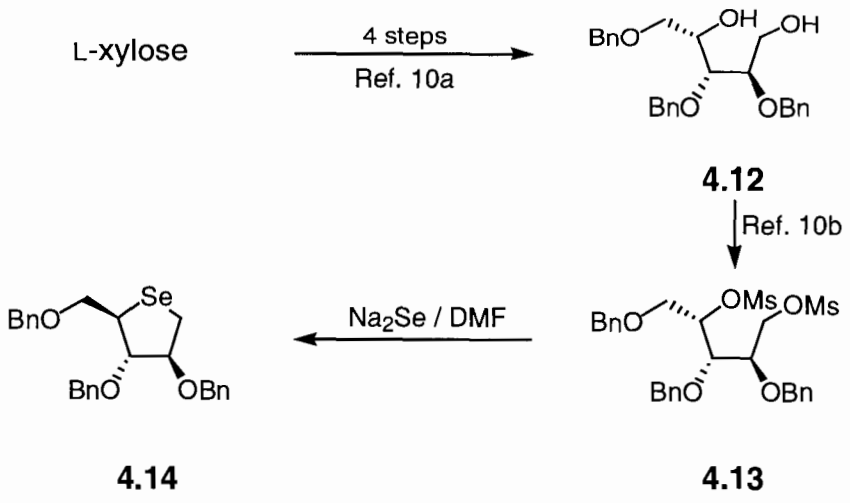
4.4 Results and Discussion

Retrosynthetic analysis indicated that salacinol (**4.3**) or its analogues **A** could be obtained by alkylation of anhydro-alditol derivatives at the ring heteroatom (Scheme 4.1).[3] This strategy was chosen in order to provide flexibility for the synthesis of analogues having different heteroatoms (X) in the ring. We envisaged that the opening of appropriately protected cyclic sulfates with selenoether nucleophiles (X = Se) would likely proceed as well, or better than, with the corresponding thioether derivatives.



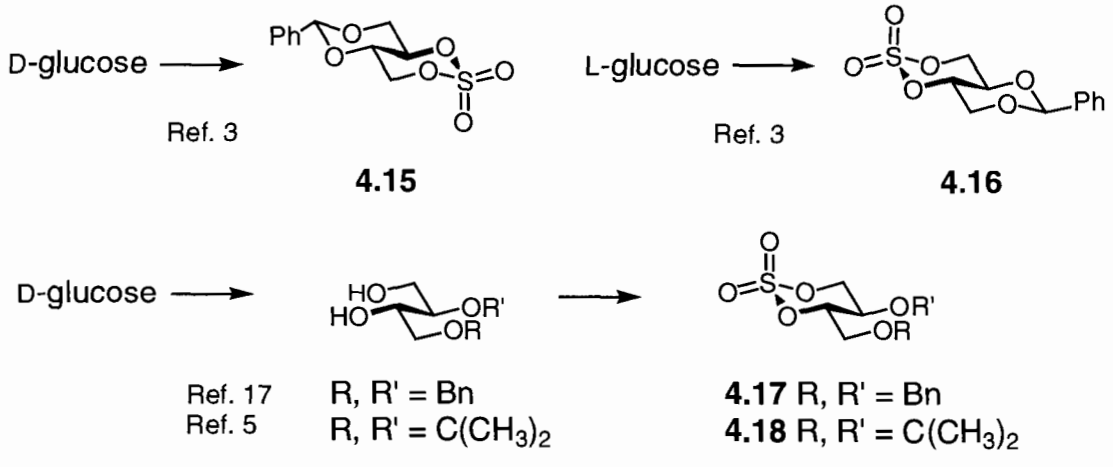
Scheme 4.1. Retrosynthetic analysis.

The required seleno-anhydroarabinitol, 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol (**4.14**), was prepared from L-xylose as shown in Scheme 4.2. Thus, 2,3,5-tri-*O*-benzyl-L-xylitol (**4.12**) was synthesized in four steps starting from L-xylose, as described by Satoh et al.[10a] for the synthesis of its enantiomer. Mesylation produced the dimesylate **4.13**[10b] that was reacted with freshly prepared Na_2Se to yield **4.14** in 80% yield (Scheme 4.2).



Scheme 4.2. Synthesis of the selenoarabinitol **4.14**.

The 2,4-*O*-benzylidene-L- and D- erythritol-1,3-cyclic sulfates **4.15** and **4.16** were synthesized from L- and D-glucose, respectively, using our methods reported previously (Scheme 4.3).[3]

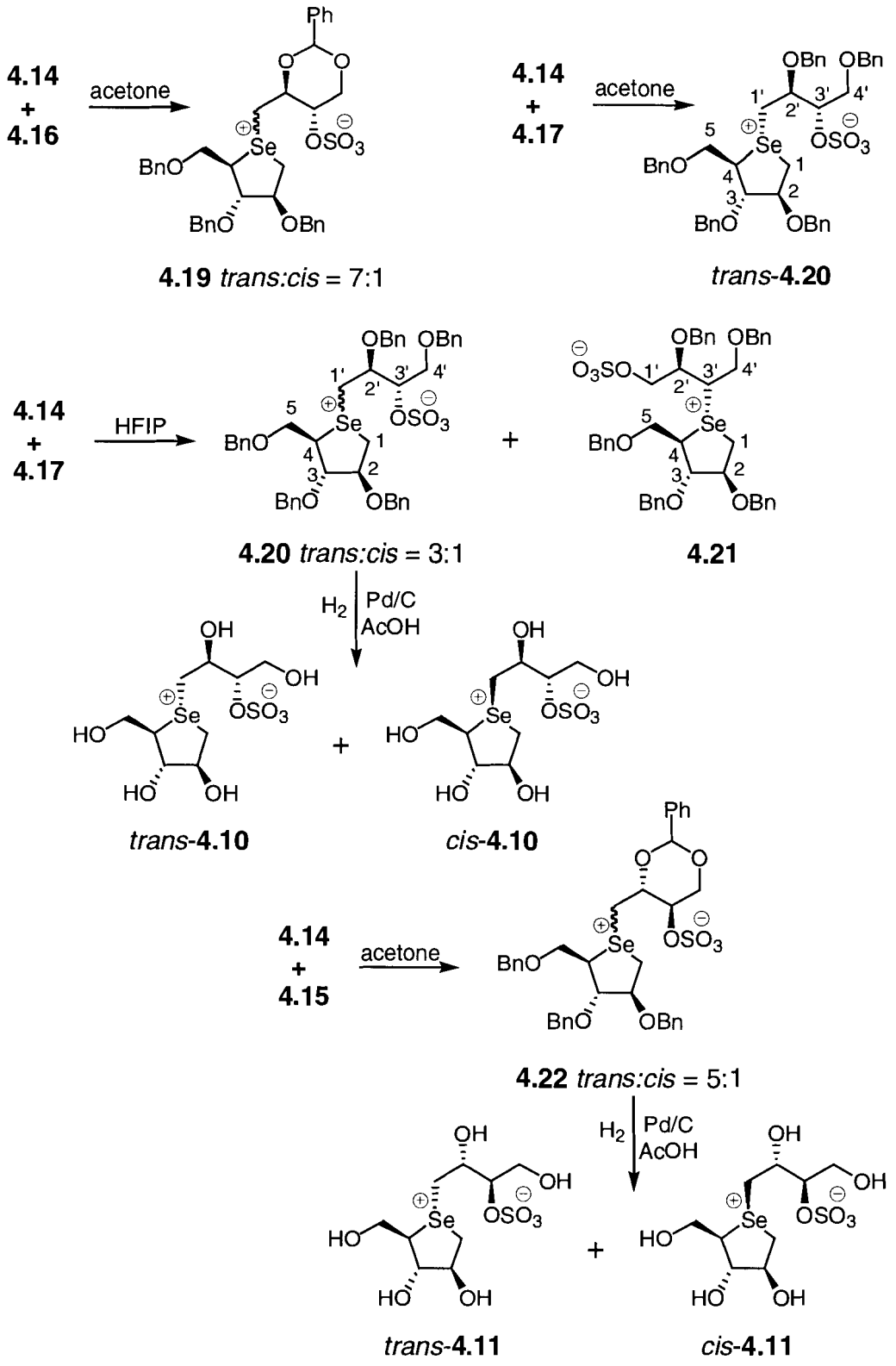


Scheme 4.3. Syntheses of the cyclic sulfates.

The selenonium salt **4.19** was synthesized by alkylation of the protected seleno-arabinitol **4.14** with the cyclic sulfate **4.16** (1.2 equiv) in dry acetone containing K_2CO_3, in

excellent yield (86%) (Scheme 4.4), but NMR spectroscopy showed the presence of two isomers in a ratio of 7:1.

We have also investigated an alternative route to compound **4.19** which avoided the use of expensive L-glucose as a starting material. Yuasa et al.[5] have reported the preparation of the cyclic sulfates **4.17** and **4.18** from D-glucose (Scheme 4.3) and investigated their utility in the synthesis of salacinol (**4.3**). They concluded that the more reactive isopropylidene derivative **4.18** was the reagent of choice under their conditions and that compound **4.17** decomposed at temperatures of 60-70° C in DMF. We also prepared **4.17** from D-glucose by a similar route (Scheme 4.3) and have confirmed that this derivative is a much less reactive alkylating agent than the corresponding benzylidene compound **4.15** that we had employed previously.[3] Thus, attempted reactions with **4.17** for the preparation of precursors to salacinol, under a variety of different reaction conditions, were unsuccessful. However, the more nucleophilic selenium derivative **4.14** did lead, under our standard conditions, to a low yield of the selenonium salt **4.20**, which was obtained as a single diastereomer (Scheme 4.4). The reaction proceeded very slowly at 85° C in acetone and was terminated before complete consumption of the selenoether because decomposition products were formed after extended periods. The NOESY spectrum of **4.20** showed a clear correlation between H-4 and H-1', thus indicating it to be the isomer with a trans relationship between C-5 and C-1'.



Scheme 4.4. Synthesis of blintol (**4.10**).

As part of a study of the influence of different solvents in such reactions, we investigated the effects of the unusual solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). This solvent has been shown to promote the nucleophilic ring-opening of epoxides by amines.[11] The beneficial effects were attributed to activation of epoxides through hydrogen bonding with the relatively acidic hexafluoro alcohol (pK_a = 9.3). Since cyclic sulfates are often considered to be synthetic equivalents of epoxides, we tested HFIP as a reaction medium for selenonium salt formation. The reaction of the selenoether **4.14** with the cyclic sulfate **4.17** in HFIP was much faster and proceeded essentially to completion in less than one day at 80 °C (Scheme 4.4). Two more polar products were formed and were separated by chromatography. The major product was obtained in 78% yield and proved to be compound **4.20**, isolated as a 3:1 mixture of diasteromers at the stereogenic selenium centre. The major diastereomer in the mixture was identical to the selenonium salt, *trans*-**4.20**, obtained from the reaction in acetone. The minor *cis*-**4.20** diastereomer showed the expected H-1'/H-5 correlation in the NOESY spectrum. The other minor product **4.21** (4%) was obtained isomerically pure and was assigned to be the product resulting from attack of the selenoether at the secondary carbon (C-3') of the cyclic sulfate. This mode of attack at the more hindered centre was not observed in the corresponding reaction of the thioether[3] and presumably arises because of the longer C-Se versus the C-S bond, thereby leading to fewer steric interactions during Se-C bond formation, and also because of the affinity of the softer Se atom for the softer secondary carbon centre. Alternatively, the acidic nature of HFIP could have resulted in an S_N1-type reaction. The ^1H NMR spectrum of **4.21**, compared to that of **4.20**, showed downfield shifts for the resonances of H-1' and an upfield shift of the H-3' resonance. Similar differences in the chemical shifts for C-1'

and C-3' in the ^{13}C NMR spectrum provided confirmation of the interchange of selenonium and sulfate centres. Thus, although HFIP resulted in a faster reaction and a higher overall yield, the selectivity for the desired *trans*-**4.20** isomer had decreased.

Hydrogenolysis of the benzyl protecting groups of **4.20** (trans:cis = 3:1) yielded **4.10** as a mixture of diastereomers. Precipitation from MeOH yielded amorphous selenosalacinol (*trans*-**4.10**) of >90% diastereomeric purity at the stereogenic selenium centre. We have chosen to name the selenium analogue of salacinol (*trans*-**4.10**) "blintol". The ^{1}H and ^{13}C NMR spectra of blintol (*trans*-**4.10**) were very similar to those of salacinol (**4.3**) except for slight upfield shifts for resonances corresponding to the nuclei in close proximity to the selenium centre and the magnitudes of the coupling constants. As previously noted for salacinol itself,[3] these spectra were sensitive to concentration effects due to solvation of molecular aggregates of charged zwitterionic species.

Since we had previously shown that a salacinol analogue having the side chain derived from D-erythritol instead of L-erythritol showed some glycosidase inhibitory activity,[6] the corresponding selenium analogue **4.22** was prepared starting from the seleno-arabinitol **4.14** and the cyclic sulfate **4.15**. Reaction of these partners in acetone under our standard conditions yielded the selenonium salt **4.22** (78%) as a mixture of two diasteromers (5:1) at the stereogenic selenium centre. The major component of the mixture was *trans*-**4.22**, having C-1' and C-5 in a *trans* relationship. Deprotection of **4.22** was problematic. Essentially no hydrogenolysis occurred under 1 atm of H_2, despite several additions of more Pd/C catalyst. Eventually the starting material was isolated and repurified to remove catalyst-poisoning impurites. Deprotection by hydrogenolysis was then achieved, albeit in very low yield (19%), and the selenonium salt **4.11** was obtained as

a crystalline solid (trans:cis, 8:1). We attribute the low yield to loss of the selenonium salt intermediates by adsorption on the large amounts of Pd/C used. Recrystallization of the diasteromeric mixture from MeOH gave pure *trans*-**4.11**.

4.5 Enzyme Inhibition Assays

Compounds *trans*-**4.10** and *trans*-**4.11** were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2,[12,13] porcine pancreatic α-amylase (PPA), and barley α-amylase (AMY1).[14] The effects were compared to those of salacinol (**4.3**). Glucoamylase G2 was weakly inhibited by **4.3** (K_i = 1.7 mM) whereas *trans*-**4.10** was a better inhibitor of this enzyme, with a K_i value of 0.72 mM. In contrast, *trans*-**4.11** showed no significant inhibition of glucoamylase. Salacinol (**4.3**) inhibited AMY1 and PPA, with K_i values of 15 ± 1 and 10 ± 2 μM, respectively. Surprisingly, compounds *trans*-**4.10** and *trans*-**4.11** showed no significant inhibition of either AMY1 or PPA. It would appear then that analogues *trans*-**4.10**, *trans*-**4.11**, and salacinol (**4.3**) show discrimination for certain glycosidase enzymes and could be promising candidates for selective inhibition of a wider panel of enzymes that includes human small intestinal maltase-glucoamylase[15] and human pancreatic α-amylase.[16] Such studies to map the enzyme selectivity profiles of these compounds are planned.

4.6 Experimental section

4.6.1 General Methods

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz, respectively, unless otherwise stated. All assignments were

confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard Bruker pulse programs. Column chromatography was performed with Merck Silica gel 60 (230-400 mesh). MALDI-TOF mass spectra were measured on a PerSeptive Biosystems Voyager-DE spectrometer, using 2,5-dihydroxybenzoic acid as a matrix.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-seleno-D-arabinitol (4.14). Selenium metal (1.1 g, 14 mmol) was added to liquid NH_3 (60 mL) in a –50 °C bath and small pieces of sodium (0.71 g) were added until a blue color appeared. A small portion of selenium (20 mg) was added to remove the blue color. NH_3 was removed by warming on a water bath and DMF was added and removed under high vacuum to remove the rest of the NH_3. A solution of the dimesylate **4.13** (7.4 g, 12.7 mmol) in DMF (100 mL) was added, and the mixture was stirred under N_2 in a 70 °C bath for 3 h. The mixture was cooled, and the solvent was removed under high vacuum. The product was partitioned between CH_2Cl_2 (150 mL) and water (50 mL), and the organic solution was washed with water (50 mL) and brine (50 mL), and dried ($MgSO_4$). The solvent was removed and the product was purified by flash chromatography [hexanes/EtOAc, 3:1] to give **4.14** as a pale yellow oil (4.74 g, 80%). $[\alpha]_D$ +22° (c 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.48-7.22 (15H, m, Ar), 4.67, 4.61 (2H, 2d, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.56, 4.48 (2H, 2d, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.53, 4.50 (2H, 2d, CH_2Ph), 4.22 (1H, ddd, H-2), 4.07 (1H, dd, $J_{2,3} = J_{3,4}$ = 4.6 Hz, H-3), 3.85 (1H, dd, $J_{5a,5b}$ = 9.2, $J_{4,5a}$ = 7.6 Hz, H-5a), 3.77 (1H, ddd, $J_{4,5b}$ = 6.9 Hz, H-4), 3.53 (1H, dd, H-5b), 3.11 (1H, dd, $J_{1a,1b}$ = 10.4, $J_{1a,2}$ = 5.1 Hz, H-1a), 2.96 (1H, dd, $J_{1b,2}$ = 5.3 Hz H-1b); ^{13}C NMR ($CDCl_3$): δ 138.24, 138.21, 138.06 ($3C_{ipso}$), 128.40-127.60 ($15C_{Ar}$), 85.93 (C-2), 85.63 (C-

3), 72.96 (2C C-5, CH_2Ph), 72.14, 71.50 ($2CH_2Ph$), 42.59 (C-4), 23.96 (C-1); MALDI-TOF MS: *m/e* 491.2 (M^+ + Na). Anal. Calcd for $C_{26}H_{28}O_3S$: C, 74.25; H, 6.71. Found: C, 74.18; H, 6.53.

2,4-Di-*O*-Benzyl-L-Erythritol-1,3-*O*-Cyclic Sulfate (4.17). A solution of 1,3-di-*O*-benzyl-D-erythritol[15] (10.0 g, 33.1 mmol) in CH_2Cl_2 and Et_3N (15 mL, 108 mmol) was cooled and stirred in an ice bath. Thionyl chloride (2.6 mL, 36 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 0.5 h. After an additional 5 min, the mixture was poured onto crushed ice (~100 g), and the aqueous phase was separated and extracted with additional CH_2Cl_2. The combined extracts were washed with cold water and dried over $MgSO_4$. The solvent was removed to give a mixture of the two diastereomeric cyclic sulfites as a pale brown oil. The mixture was passed through a short silica gel column with [hexanes/EtOAc, 3:1], without attempting to separate the isomers, and the mixture of cyclic sulfites (a pale yellow oil, 10.5 g) was immediately dissolved in 1:1 CH_3CN:CCl_4 (200 mL). Sodium periodate (10.6 g, 49.6 mmol) and $RuCl_3 \cdot xH_20$ (150 mg) were added and the mixture was stirred rapidly while H_2O (100 mL) was added. After 75 min, analysis by TLC showed formation of a single, slightly more polar product. The mixture was poured into a separatory funnel and the lower organic phase was separated. The dark red-brown aqueous phase was extracted with additional CCl_4 and the combined extracts were filtered and concentrated to a dark syrup that was dissolved in EtOAc (200 mL) and filtered to remove black material. The colorless filtrate was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl and dried over $MgSO_4$. Solvent removal gave an oil which was purified by flash chromatography [hexanes/EtOAc, 3:1] to yield **4.17** as a

colorless syrup (9.66 g, 80%). Storage at -20° C resulted in slow crystallization. A sample was recrystallized from Et_2O/hexanes. Mp: 63-65 °C; $[\alpha]_D$ –9.4° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40 – 7.20 (10H, m, Ar), 4.76 (1H, ddd, $J_{2,3}$ = 9.4, $J_{3,4a}$ = 3.3, $J_{3,4b}$ = 2.1 Hz, H-3), 4.64 and 4.53 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.59 and 4.50 (2H, 2d, $J_{A,B}$ = 11.6 Hz, CH_2Ph), 4.44 (1H, dd, $J_{1ax,1eq}$ = 11.0, $J_{1ax,2}$ = 10.1 Hz, H-1ax), 4.33 (1H, dd, $J_{1eq,2}$ = 5.2 Hz, H-1eq), 4.17 (1H, ddd, H-2), 3.88 (1H, dd, $J_{4a,4b}$ = 12.0 Hz, H-4a), 3.76 (1H, dd, H-4b); ^{13}C NMR ($CDCl_3$): δ 137.19 and 136.53, ($2C_{ipso}$), 128.75, 128.65, 128.56, 128.14, 128.07 and 127.90 (each $2C_{Ar}$, C_{ortho}, C_{meta}, C_{para}), 84.99 (C-3), 73.69 and 73.64 ($2CH_2$Ph), 71.59 (C-2), 66.92 and 66.50 (C-1, C-4). MALDI-TOF MS: *m/e* 387.2 (M^+ + Na), 403.1 (M^+ + K). Anal. Calcd for $C_{18}H_{20}O_6S$: C, 59.33; H, 5.53. Found: C, 59.38; H, 5.52.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-selenoniumylidene]-D-arabinitol Inner Salt (4.19). The selenoether **4.14** (0.20 g, 0.43 mmol) was reacted with the cyclic sulfate **4.16** (0.14 g, 1.2 equiv) in acetone (0.7 mL) by the procedure used previously for the corresponding sulfide.[3] Column chromatography [$CHCl_3$/MeOH, 15:1] of the crude product gave an amorphous solid (0.27 g, 86%). NMR showed the presence of two isomers (7:1) at the stereogenic selenium centre, which were separated on preparative HPLC [acetonitrile/H_2O]. Data for the major diastereomer *trans*-**4.19** follow. ^1H NMR (CD_2Cl_2) δ 7.50-7.10 (20H, m, Ar), 5.55 (1H, s, C*H*Ph), 4.58 (1H, m, H-2), 4.56-4.45 (5H, m, H-3', 3CH_2Ph, H-4'eq), 4.38 (1H, dd, $J_{1'a,2'}$ = 2.2 Hz, H-1'a), 4.38-4.34 (2H, m, H-3, CH_2Ph), 4.34 and 4.26 (2H, 2d, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.25 (1H, m, H-2'), 4.14-4.08 (2H, m, H-1a, H-4), 3.97 (1H, dd, $J_{1'a,1'b}$ = 12.1, $J_{1'b,2'}$ = 3.3 Hz, H-1'b), 3.80 (1H, dd, $J_{4'ax,4'eq}$ = $J_{3',4'ax}$ = 10.1 Hz, H-4'ax), 3.65-3.52 (3H, m, H-1b, H-5a, H-5b); ^{13}C

NMR (CD_2Cl_2): δ 137.28-126.68 ($24C_{Ar}$), 102.10 (*C*HPh), 84.55 (C-3), 83.36 (C-2), 77.18 (C-2'), 73.70, 72.81, 72.36 ($3CH_2Ph$), 69.57 (C-4'), 67.76 (C-3'), 67.02 (C-5), 66.30 (C-4), 48.77 (C-1'), 46.43 (C-1). Anal. Calcd for $C_{37}H_{40}O_9SSe$: C, 59.99; H, 5.45. Found: C, 59.91; H, 5.44.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*)-2,4-di(benzyloxy)-3-(sulfooxy)butyl]-selenoniumylidene]-D-arabinitol Inner Salt (4.20).

(a) By reaction in $(CH_3)_2CO$

The selenoether **4.14** (117 mg, 0.250 mmol), cyclic sulfate **4.17** (84 mg, 0.23 mmol) and K_2CO_3 (80 mg, 0.58 mmol) were added to anhydrous acetone (3.0 mL) and the mixture was stirred in a sealed tube with heating at 70 °C for 20 h and then at 85 °C for 48 h. Periodic analysis by TLC ($CHCl_3$/MeOH, 10:1) showed that the reaction was proceeding very slowly and that substantial fractions of the cyclic sulfate **4.17** and the selenoether **4.14** remained unreacted. Slow decomposition of the cyclic sulfate to produce polar impurities was noted at the higher temperature. At the end of 68 h, the extent of reaction to yield the desired product was estimated to be <30% but decomposition products were becoming significant, and thus, the reaction was terminated at this point. The mixture was cooled and filtered through Celite with the aid of CH_2Cl_2. The solvents were removed, and the residue was purified by column chromatography [gradient of $CHCl_3$ to $CHCl_3$/MeOH, 10:1]. The selenonium salt **4.20** (39 mg, 20%) was obtained as a colorless syrup. Compound **4.20** was isomerically pure and was assigned to be the isomer with a trans relationship between C-5 and C-1' by analysis of the NOESY spectrum. Data for *trans*-**4.20** follow. $[\alpha]_D$ –15° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38-7.05 (25H, m, Ar), 4.65

and 4.46 (2H, 2d, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.64 (1H, dt, $J_{2',3'}$ = 6.9 Hz, H-3'), 4.63 and 4.47 (2H, 2d, $J_{A,B}$ = 11.6 Hz, CH_2Ph), 4.48 and 4.33 (2H, 2d, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.46 (1H, dd, H-2), 4.43 and 4.38 (2H, 2d, $J_{A,B}$ = 11.2 Hz, CH_2Ph), 4.38 and 4.28 (2H, 2d, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.28 (1H, ddd, H-2'), 4.24 (1H, br d, $J_{2,3}$ = 1.6 Hz, H-3), 4.22 (1H, br d, H-1a), 4.17 (1H, dd, $J_{1'a,1'b}$ = 12.1, $J_{1'a,2'}$ = 1.1 Hz, H-1'a), 4.04 (1H, dd, $J_{4'a,4'b}$ = 11.1, $J_{3',4'a}$ = 2.9 Hz, H-4'a), 3.87 (1H, dd, $J_{3',4'b}$ = 2.5 Hz, H-4'b), 4.50 (1H, br t, H-4), 3.67 (1H, dd, $J_{1'b,2'}$ = 4.3 Hz, H-1'b), 3.60 (1H, dd, $J_{1a,1b}$ = 12.8, $J_{1b,2}$ = 3.0 Hz, H-1b), 3.57 (1H, dd, $J_{5a,5b}$ = 10.1, $J_{4,5a}$ = 7.1 Hz, H-5a), 3.54 (1H, dd, $J_{4,5b}$ = 9.0 Hz, H-5b); ^{13}C NMR ($CDCl_3$): δ 137.07, 137.00, 136.87, 136.15 and 135.88 ($5C_{ipso}$), 128.81-127.60 ($25C_{Ar}$), 83.83 (C-3), 81.16 (C-2), 74.99 (C-3'), 73.79 and 73.40 ($2CH_2Ph$), 75.18 (C-2'), 72.85, 72.01, and 71.59 ($3CH_2Ph$), 69.14 (C-4'), 67.13 (C-5), 64.83 (C-4), 50.08 (C-1'), 46.34 (C-1); MALDI-TOF MS: *m/e* 833.8 (M^+ + H), 753.7 (M^+ + H - SO_3). Anal. Calcd for $C_{44}H_{48}O_9SSe$: C, 63.53; H, 5.82. Found: C, 63.39; H, 5.86.

(b) By reaction in $(CF_3)_2CHOH$

The selenoether **4.14** (633 mg, 1.35 mmol), cyclic sulfate **4.17** (531 mg, 1.46 mmol), and K_2CO_3 (73 mg, 0.53 mmol) were added to 1,1,1,3,3,3-hexafluoro-2-propanol (2.0 mL) and the mixture was stirred in a Carius tube while being warmed slowly. The K_2CO_3 dissolved with evolution of gas between 60 °C and 80 °C. The tube was cooled to room temperature, opened to relieve pressure and then resealed and kept at 80 °C for 22 h. Analysis by TLC [$CHCl_3$/MeOH, 10:1] showed virtually complete consumption of the cyclic sulfate **4.17**, although some of the selenoether **4.14** remained unreacted. Two products of increased polarity had been formed (major, Rf 0.40; minor, Rf 0.35). The mixture was cooled and

filtered through Celite with the aid of CH_2Cl_2. The solvents were removed and the residue was purified by column chromatography (two successive silica gel columns: first with a gradient of $CHCl_3$ to $CHCl_3$/MeOH, 10:1 to separate the starting materials, and then with EtOAc/MeOH, 25:1 to separate the two products). The selenonium salts **4.20** (827 mg, 78%) and **4.21** (45 mg, 4%) were obtained as syrupy oils. Compound **4.20** proved to be a 3:1 mixture of isomers at the stereogenic selenium centre. The major component (*trans*-**4.20**) was identical to the compound obtained from the reaction in acetone (that is the trans C-5, C-1' isomer), while the minor component was assigned to be the corresponding *cis*-**4.20** isomer. Partial ^{13}C NMR data for the cis isomer were obtained from a spectrum of the mixture, and assignments were made by analogy to those of the trans isomer. Compound **4.21** was isomerically pure and was assigned to be the isomer with a trans relationship between C-5 and C-3' by analysis of the NOESY spectrum.

Data for the *cis*-**4.20** isomer follow. ^{13}C NMR ($CDCl_3$): 84.31 (C-3), 82.78 (C-2), 75.42 (C-3'), 73.84 and 73.52, (2*C*H_2Ph), 73.18 (C-2'), 72.86 (*C*H_2Ph), 71.72 (2C, 2*C*H_2Ph), 68.78(C-4'), 65.46 (C-5), 58.33 (C-4), 42.71 (C-1'), 40.16 (C-1).

Data for **4.21** follow. $[\alpha]_D$ –68° (*c* 2.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.38-7.00 (25H, m, Ar), 4.76 (1H, dt, $J_{2',3'}$ = 7.0 Hz, H-3'), 4.71 and 4.42 (2H, 2d, $J_{A,B}$ = 10.9 Hz, C*H*$_2$Ph), 4.68 and 4.51 (2H, 2d, $J_{A,B}$ = 12.4 Hz, C*H*$_2$Ph), 4.50 and 4.36 (2H, 2d, $J_{A,B}$ = 11.6 Hz, C*H*$_2$Ph), 4.50 (1H, ddd, H-4), 4.38 (1H, dd, $J_{1'a,1'b}$ = 12.6, $J_{1'a,2'}$ = 5.1 Hz, H-1'a), 4.36-4.32 (2H, m, H-2, H-3), 4.32 and 4.14 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C*H*$_2$Ph), 4.29 (1H, dd, $J_{1'b,2'}$ = 3.1 Hz, H-1'b), 4.28 and 4.21 (2H, 2d, $J_{A,B}$ = 11.8 Hz, C*H*$_2$Ph), 4.13 (1H, ddd, H-2'), 4.10 (1H, dd, $J_{4'a,4'b}$ = 11.8, $J_{3',4'a}$ = 3.8 Hz, H-4'a), 3.84 (1H, dd, $J_{3',4'b}$ = 3.8 Hz, H-4'b), 3.48 (1H, dd, $J_{5a,5b}$ = 10.0, $J_{4,5a}$ = 9.1 Hz, H-5a), 3.44 (1H, dd, $J_{1a,1b}$ = 12.0 Hz, H-1a), 3.42 (1H, dd,

$J_{4,5b}$ = 9.9 Hz, H-5b), 3.14 (1H, dd, $J_{1b,2}$ = 2.2 Hz, H-1b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.15, 136.63, 136.57, 136.34 and 136.11 ($5C_{ipso}$), 128.89-127.50 (25C, Ar), 83.43 (C-3), 81.12 (C-2), 75.35 (C-2'), 73.55 (C-1'), 73.06 72.40, 71.85, and 71.65 ($4CH_2Ph$), 66.31 (C-5), 65.90 (C-4'), 63.79 (CH_2Ph), 62.65 (C-4), 61.73 (C-3'), 39.77 (C-1); MALDI-TOF MS: *m/e* 833.8 (M^+ + H), 753.8 (M^+ + H - SO_3). Anal. Calcd for $C_{44}H_{48}O_9SSe$: C, 63.53; H, 5.82. Found: C, 63.79; H, 5.83.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*R*,3*R*)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-selenoniumylidene]-D-arabinitol Inner Salt (4.22). The selenoether **4.14** (760 mg, 1.63 mmol), cyclic sulfate **4.15**[3] (467 mg, 1.72 mmol) and K_2CO_3 (102 mg, 0.74 mmol) were added to anhydrous acetone (3.0 mL) and the mixture was stirred in a sealed tube with heating at 80 °C for 13 h. Analysis by TLC ($CHCl_3$/MeOH, 10:1) showed that the cyclic sulfate **4.15** had been consumed but that there was a substantial amount of the selenoether **4.14** remaining. Another portion of the cyclic sulfate (180 mg, 0.66 mmol) was therefore added and the reaction was continued at 80 °C for a further 12 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 and processed and purified as described for compound **4.19**. Compound **4.22** was obtained as a colorless foam (0.939 g, 78%). Analysis by NMR showed that the product was a mixture of two isomers (~5:1) at the stereogenic selenium centre. The major component of the mixture was assigned to be the diastereomer with a trans relationship between C-5 and C-1' on the basis of analysis of the NOESY spectrum.

Data for the major diastereomer (*trans*-**4.22**) follow. ^{1}H NMR (CD_2Cl_2) δ 7.50-7.10 (25H, m, Ar), 5.54 (1H, s, C*H*Ph), 4.58 and 4.50 (2H, 2d, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.55 (1H, dd,

$J_{4'ax,4'eq}$ = 10.6, $J_{3',4'eq}$ = 5.1 Hz, H-4'eq), 4.50 (1H, dd, H-2), 4.45 (1H, br d, $J_{2,3}$ = 2.6 Hz, H-3), 4.44 (1H, ddd, H-3'), 4.40 and 4.35 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.35 and 4.23 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.34 (1H, ddd, H-2'), 4.33 (1H, br t, H-4), 4.16 (2H, br d, $J_{1',2'}$ = 4.9 Hz, H-1'a, H-1'b), 3.91 (1H, dd, $J_{1a,2}$ = 1.5, $J_{1a,1b}$ = 12.1 Hz, H-1a), 3.78 (1H, dd, $J_{3',4'ax}$ = 9.8 Hz, H-4'ax), 3.67-3.59 (2H, m, H-5a, H-5b), 3.56 (1H, dd, $J_{1b,2}$ = 3.4 Hz, H-1b); ^{13}C NMR (CD_2Cl_2): δ 137.66, 137.31, 136.72, and 136.49 ($4C_{ipso}$), 129.73-126.66 ($25C_{Ar}$), 102.04 (C*H*Ph), 84.27 (C-2), 83.04 (C-3), 77.04 (C-2'), 73.60, 72.51 and 72.14 ($3CH_2$Ph), 69.77 (C-4'), 68.82 (C-3'), 67.05 (C-5), 64.81 (C-4), 48.19 (C-1'), 46.35 (C-1); MALDI-TOF MS: *m/e* 741.6 (M^+ + H), 661.5 (M^+ + H - SO_3). Anal. Calcd for $C_{37}H_{40}O_9SSe$: C, 60.08; H, 5.45. Found: C, 59.91; H, 5.45.

1,4-Dideoxy-1,4-[[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]-selenoniumylidene]-D-arabinitol Inner Salt (4.10). To a solution of selenonium salt **4.20** (744 mg, 0.894 mmol, 3:1 mixture of isomers) in HOAc (10 mL) was added 10% Pd/C catalyst (200 mg) and the mixture was stirred under an atmosphere of H_2 for 16h. More Pd/C (200 mg) was added and the hydrogenolysis was continued for an additional 24 h. The mixture was filtered through Celite with MeOH (80 mL) and concentrated to give a syrup. Purification by column chromatography (EtOAc/MeOH/H_2O, 6:3:1) gave compound **4.10** as a colorless gum (225 mg, 66%). Analysis by ^{1}H NMR indicated a mixture of isomers (5:1). The product was dissolved in a minimum amount of warm MeOH and cooled slowly to deposit an amorphous solid (112 mg). This proved to be >90% pure **4.10** which was assigned, by analysis of the NOESY spectrum, to be the major *trans*-**4.10** isomer corresponding to the configuration of salacinol. Data for the diastereomer *trans*-**4.10** follow. $[\alpha]_D$ +20° (*c* 0.5,

D_2O); ^1H NMR (600 MHz, D_2O): δ 4.84 (1H, ddd, H-2), 4.53 (1H, dd, $J_{2,3}$ = 3.5 Hz, H-3), 4.43 (1H, ddd, $J_{2',3'}$ = 7.0 Hz, H-2'), 4.37 (1H, ddd, H-3'), 4.22 (1H, ddd, $J_{3,4}$ = 3.2 Hz, H-4), 4.12 (1H, dd, $J_{4,5a}$ = 5.1, $J_{5a,5b}$ = 12.5 Hz, H-5a), 4.04 (1H, dd, $J_{1'a,2'}$ = 3.7, $J_{1'a,1'b}$ = 12.4 Hz, H-1'a), 3.98 (1H, dd, $J_{4,5b}$ = 8.9 Hz, H-5b), 3.97 (1H, dd, $J_{3',4'a}$ = 3.6, $J_{4'a,4'b}$ = 12.8 Hz, H-4'a), 3.90 (1H, dd, $J_{1'b,2'}$ = 7.5 Hz, H-1'b), 3.88 (1H, dd, $J_{3',4'b}$ = 3.4 Hz, H-4'b), 3.86 (1H, dd, $J_{1a,2}$ = 3.2 Hz, H-1a), 3.83 (1H, dd, $J_{1b,2}$ = 4.0, $J_{1a,1b}$ = 12.2 Hz, H-1b); ^{13}C NMR (100MHz, D_2O): δ 83.10 (C-2'), 80.98 (C-3), 80.27 (C-2), 72.58 (C-4), 68.32 (C-2'), 62.34 (C-4'), 61.93 (C-5), 50.29 (C-1'), 47.75 (C-1); MALDI-TOF MS: *m/e* 383.2 (M^+ + H), 303.2 (M^+ + H - SO_3). Anal. Calcd for $C_6H_{18}O_9SSe$: C, 28.35; H, 4.76. Found: C, 28.12; H, 4.83.

1,4-Dideoxy-1,4-[[(2*R*,3*R*)-2,4-dihydroxy-3-(sulfooxy)butyl]-selenoniumylidene]-D-arabinitol Inner Salt (4.11). Hydrogenolysis of the selenonium salt **4.22** (0.906 g, 1.22 mmol, trans:cis = 5:1) by the same procedure reported above for compound **4.19** was extremely sluggish. After a total of 1.2 g of Pd/C had been added in 3 portions over 3 days of stirring under an atmosphere of H_2, little reaction had occurred. The reaction was stopped and the catalyst removed by filtration through Celite with methanol. The solvents were removed and the residue, consisting mostly of unreacted starting material **4.22**, was repurified by column chromatography. The fractions containing **4.22** were combined and concentrated to a syrup. Hydrogenolysis of this material in acetic acid (6 mL) with Pd/C (0.4 g) was now relatively rapid and TLC indicated complete reaction after 24 h. Processing and purification as described for **4.10** gave **4.11** as a colorless foam (88 mg, 19%). This low yield was attributed to losses of the selenonium salt by adsorption on the

large amounts of Pd/C that had been used. Analysis by ^{1}H NMR indicated that **4.11** was a mixture of isomers (8:1) at the selenium centre. The major component of the mixture was assigned to be the diastereomer with a trans relationship between C-5 and C-1' on the basic of observation of a strong H-1'/H-4 correlation in the NOESY spectrum. The pure *trans*-**4.11** was obtained by crystallization from MeOH.

Data for the major isomer *trans*-**4.11** follow. Mp: 137-140 °C; $[\alpha]_D$ -33 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.83 (1H, ddd, H-2), 4.54 (1H, dd, $J_{2,3}$ = 3.6 Hz, H-3), 4.43 (1H, td, $J_{1'a,2'} = J_{2',3'}$ = 6.9, $J_{1'b,2'}$ = 5.5 Hz, H-2'), 4.36 (1H, ddd, H-3'), 4.28 (1H, ddd, $J_{3,4}$ = 3.2 Hz, H-4), 4.10 (1H, dd, $J_{4,5a}$ = 5.3, $J_{5a,5b}$ = 12.7 Hz, H-5a), 4.00 (1H, dd, $J_{4,5b}$ = 8.0 Hz, H-5b), 3.98 (1H, dd, $J_{3',4'a}$ = 3.1 Hz, H-4'a), 3.96 (2H, m, H-1'a, H-1'b), 3.87 (1H, dd, $J_{3',4'b}$ = 3.4, $J_{4'a,4'b}$ = 12.8 Hz, H-4'b), 3.82 (2H, d, $J_{1a,2} = J_{1b,2}$ = 4.0 Hz, H-1a, H-1b); ^{13}C NMR (D_2O): δ 83.33 (C-3'), 81.15 (C-3), 80.34 (C-2), 75.54 (C-4), 68.67 (C-2'), 62.36 (C-4'), 61.86 (C-5), 49.96 (C-1'), 47.30 (C-1); MALDI-TOF MS: *m/e* 383.0 (M^+ + H), 303.0 (M^+ + H - SO_3). Anal. Calcd for $C_6H_{18}O_9SSe$: C, 28.35; H, 4.76. Found: C, 28.30; H, 5.01.

4.6.2 Enzyme Inhibition Assays

The glucoamylase G2 form from *Aspergillus niger* was purified from a commercial enzyme (Novo Nordisk, Bagsvaerd, Denmark) as described.[12,13] The initial rates of glucoamylase G2-catalyzed hydrolysis of maltose were tested with 1 mM maltose as substrate in 0.1 M sodium acetate pH 4.5 at 45 °C using an enzyme concentration of 7.0 x 10^{-8} M and five inhibitor concentrations in the range from 1 μm to 5 mM. The effects of the inhibition on rates of substrate hydrolysis were compared for the different compounds. The glucose released was analyzed in aliquots removed at appropriate time intervals using

a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL.[18] The K_i values were calculated assuming competitive inhibition from $1/v = (1/V_{max}) + [(K_m)/(V_{max}[S]K_i)]$ x [I], where v is the rate measured in the presence or absence of inhibitor, [I] and [S] the concentrations of inhibitor and substrate, K_m = 1.6 mM and k_{cat} = 11.3 s^{-1}, using ENZFITTER.[19]

Porcine pancreatic α-amylase (PPA) and bovine serum albumin (BSA) were purchased from Sigma. Amylose EX-1 (DP17; average degree of polymerization 17) was purchased from Hayashibara Chemical Laboratories (Okayama, Japan). Recombinant barley α–amylase isozyme 1 (AMY1) was produced and purified as described.[14] An aliquot of the porcine pancreatic α-amylase (PPA) crystalline suspension (in ammonium sulfate) was dialyzed extensively against the assay buffer without BSA. The enzyme concentration was determined by aid of amino acid analysis as determined using an LKB model Alpha Plus amino acid analyzer. The inhibition of AMY1 (3×10^{-9} M) and PPA (9×10^{-9} M) activity towards DP17 amylose was measured at 37 °C in 20 mM sodium acetate, pH 5.5, 5 mM $CaCl_2$, 0.005 % BSA (for AMY1) and 20 mM sodium phosphate, pH 6.9, 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005 % BSA (for PPA). Six different final inhibitor concentrations were used in the range from 1 μM to 5 mM. The inhibitor was preincubated with enzyme for 5 min at 37 °C before addition of substrate. Initial rates were determined by measuring reducing sugar by the copper-bicinchoninate method as described.[14,20] The K_i values were calculated assuming competitive inhibition, as described above for the case of glucoamylase, and a K_m of 0.57 mg/mL and k_{cat} of 165 s^{-1} for AMY1 and 1 mg/mL and 1200 s^{-1}, respectively, for PPA, as determined in the substrate

concentration range 0.03 - 10 mg/mL using ENZFITTER.[19] For the K_i determinations, [S] = 0.7 mg/mL amylose DP 17 for the AMY1 binding and [S] = 2.5 mg/mL amylose DP 17 for the PPA binding.

4.7 Acknowledgments

We thank Professor Barry B. Snider for a fruitful discussion and for the suggestion to investigate HFIP as a reaction solvent. We are grateful to Sidsel Ehlers for technical assistance with the glucoamylase assays. We also thank the Natural Sciences and Engineering Research Council of Canada for financial support and the Michael Smith Foundation for Health Research for a trainee fellowship (to A.G.).

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CHAPTER 5: SYNTHESIS OF 1,4-ANHYDRO-D-XYLITOL HETEROANALOGUES OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR SALACINOL AND THEIR EVALUATION AS GLYCOSIDASE INHIBITORS[†]

Ahmad Ghavami,[a] Blair D. Johnston,[a] Matthew D. Maddess,[a] Sarah M. Chinapoo,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*

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[†]This work is dedicated, with respect and affection to the memory of R. U. Lemieux.

* Author to whom correspondence should be addressed.

Tel: (604) 291-4327

Fax: (604) 291-3765

e-mail:bpinto@sfu.ca

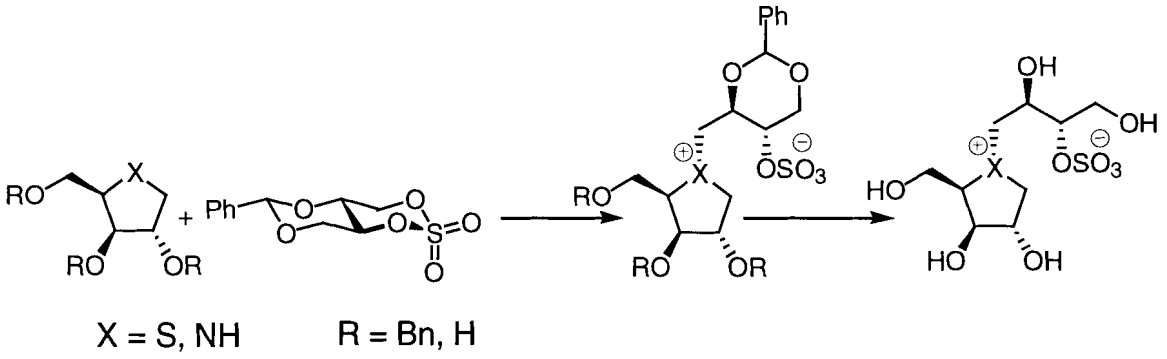
5.1 Graphical Abstract

Synthesis of 1,4-Anhydro-D-Xylitol Heteroanalogues of the Naturally Occurring Glycosidase Inhibitor Salacinol And Their Evaluation as Glycosidase Inhibitors

Ahmad Ghavami,[a] Blair D. Johnston,[a] Matthew D. Maddess,[a] Sarah M. Chinapoo,[a] Morten T. Jensen,[b] Birte Svensson,[b] and B. Mario Pinto[a]

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*



Keywords: glycosidase inhibitors, salacinol analogues, anhydro-D-xylitol heteroanalogues, enzyme inhibition

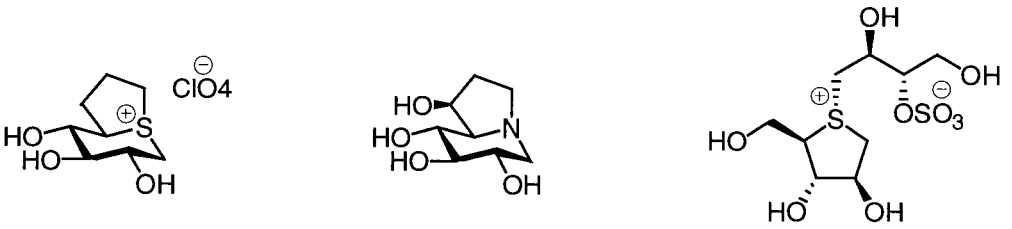
5.2 Abstract

The syntheses of two 1,4-anhydro-D-xylitol heteroanalogues (**5.8** and **5.9**) of the naturally occurring sulfonium ion, salacinol (**5.3**), containing a sulfur or nitrogen atom in the ring are described. Salacinol (**5.3**) 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 type II diabetes. The synthetic strategy relies on the nucleophilic attack of sulfur or nitrogen analogues of 1,4-anhydro-D-xylitol at the least-hindered carbon of 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate. The sulfonium ion **5.8** inhibited barley-α-amylase (AMY1) and porcine pancreatic-α-amylase (PPA), with K_i values of 109 ± 11 and 55 ± 5 μM, respectively. In contrast, the ammonium ion **5.9** showed no significant inhibition of either AMY1 or PPA. Compounds **5.8** and **5.9** also showed no significant inhibition of glucoamylase.

5.3 Introduction

A program of research to investigate the nature and origin of carbohydrate mimicry is in progress in our laboratory. Thus, we have recently reported the study of the peptide mimicry of carbohydrates recognized by antibodies directed against the Group A *Streptococcus* cell-wall polysaccharide[1] and have recently communicated our results with an antibody directed against the *Shigella flexneri* Y *O*-antigen.[2] In the latter study, the crystal structures of the Ab-peptide mimetic and Ab-pentasaccharide complexes were compared. The results indicated that although both ligands engage some common groups on the Ab receptor in H-bonding and hydrophobic interactions, each ligand also displays unique interactions with groups on the protein, lending support to our previous hypothesis that "functional" and not "structural" mimicry might be the mode of mimicry with peptide mimetics.[1]

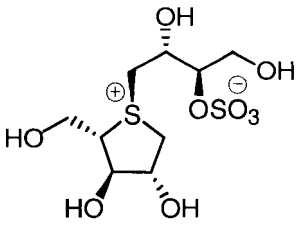
We have also studied the mimicry of carbohydrates by glycomimetics as potential glycosidase inhibitors. Thus, for example, we have described the synthesis and conformational analysis of a sulfonium-ion analogue (**5.1**) of the glycosidase inhibitor castanospermine (**5.2**).[3] Our reasoning was inspired by the pioneering work of the late B. Belleau who synthesized sulfonium-ion analogues of the morphinans, levorphanol and isolevorphanol, and showed that they were agonists or antagonists of morphine for the opiate receptor.[4a-d] Recently, a new class of glycosidase inhibitor with an intriguing inner-salt sulfonium-sulfate structure was isolated from the roots and stems of the plant *Salacia reticulata*. Extracts of this plant have been traditionally used in the Ayurvedic method of Indian medicine as a treatment for diabetes. One of the active ingredients of these extracts is the sulfonium salt salacinol (**5.3**).[5]



5.1 **5.2** **5.3**

Chart 5.1. Compounds **5.1** – **5.3**.

We[6] and others[7] have recently reported the synthesis of salacinol (**5.3**) and its stereoisomers **5.4**, **5.5**, and provided conclusive proof of structure of the natural product. We have also reported the syntheses of the hitherto unknown nitrogen congeners **5.6**, **5.7** as potential glycosidase inhibitors.[8] Enzyme inhibition assays indicated that salacinol (**5.3**) is a weak (K_i = 1.7 mM) inhibitor of glucoamylase, whereas compounds **5.6** and **5.7** inhibit glucoamylase with K_i values in the range about 10-fold higher. The nitrogen analogues **5.6** and **5.7** showed no significant inhibitory effect of either barley α-amylase (AMY1) or porcine pancreatic α-amylase (PPA) at concentrations of 5 mM. In contrast, salacinol (**5.3**) inhibited AMY1 and PPA in the micromolar range, with K_i values of 15 ± 1 μM and 10 ± 2 μM, respectively.[8]



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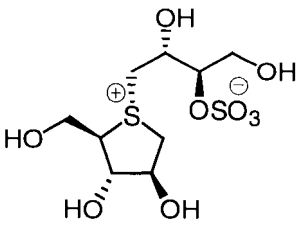

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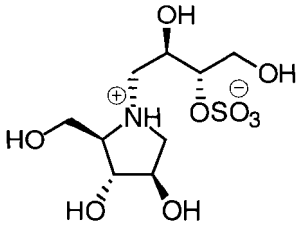

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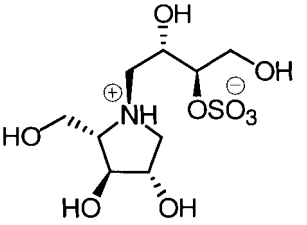


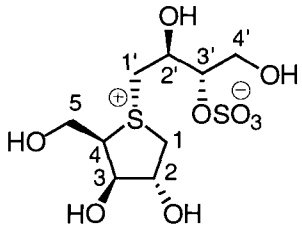
5.7

Chart 5.2. Compounds **5.4** – **5.7**.

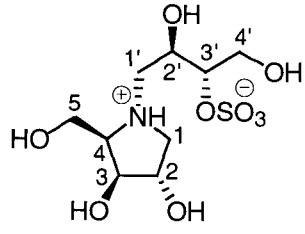
Yuasa et al.[9] have also investigated the glucosidase inhibitory activities of compounds **5.3** and **5.5** and showed that although salacinol (**5.3**) is a better inhibitor (IC_{50} = 1.1 μM) of rice α-glucosidase than its diastereomer **5.5** (IC_{50} = 0.38 mM), the inhibitory activities are comparable for almond α-mannosidase (IC_{50} = 2.1 mM for **5.3**; 3.6 mM for **5.5**). In the case of almond β-glucosidase, **5.5** is a better inhibitor than **5.3** (**5.3** showed no activity; IC_{50} = 3.4 mM for **5.5**).

In a recent study, Muraoka et al.[10] showed that salacinol (**5.3**) inhibits intestinal α-glucosidases: maltase, sucrase and isomaltase with IC_{50} values of 9.6 μM, 2.5 μM, and 1.8 μM, respectively, whereas compound **5.6** inhibits these enzymes with IC_{50} values of 306 μM, 44 μM, and 136 μM, respectively.

The results described above suggest that the stereochemistry at the different stereogenic centres and the nature of the ring heteroatom in the candidate inhibitors play a significant role in discriminating between different glycosidase enzymes. Therefore, in order to probe these structure-function studies further, we now report the syntheses of the thio and iminoxylitol analogues **5.8**, **5.9** of salacinol (**5.3**) and their evaluation as glycosidase inhibitors of AMY1, PPA, or glucoamylase.



5.8

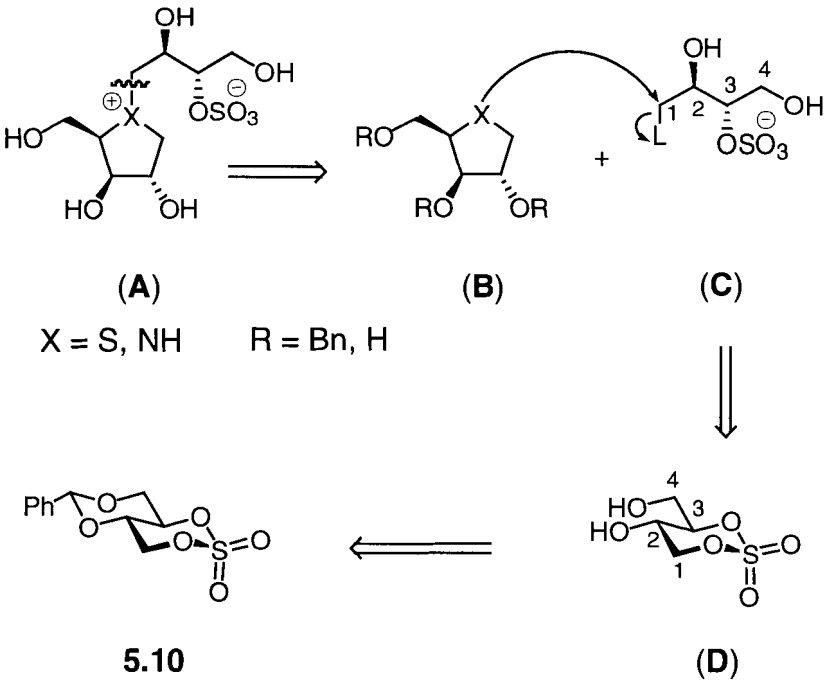


5.9

Chart 5.3. Xylitol heteroanalogues **5.8** and **5.9**.

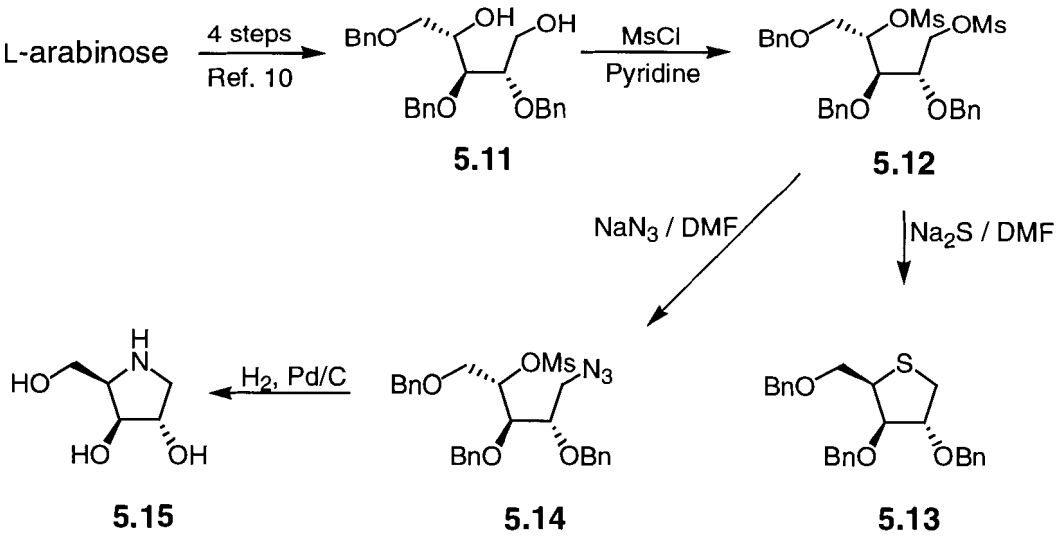
5.4 Results and Discussion

Retrosynthetic analysis indicated that salacinol (**5.3**) or its analogues (**A**) could be obtained by alkylation of anhydroalditol derivatives at the ring heteroatom (Scheme 5.1). As in our previous work,[6,8] the benzylidene acetal **5.10** of **D** was chosen as the alkylating agent. We envisaged that selective attack of the heteroatom at the least-hindered primary centre would afford the desired sulfonium or ammonium ions.



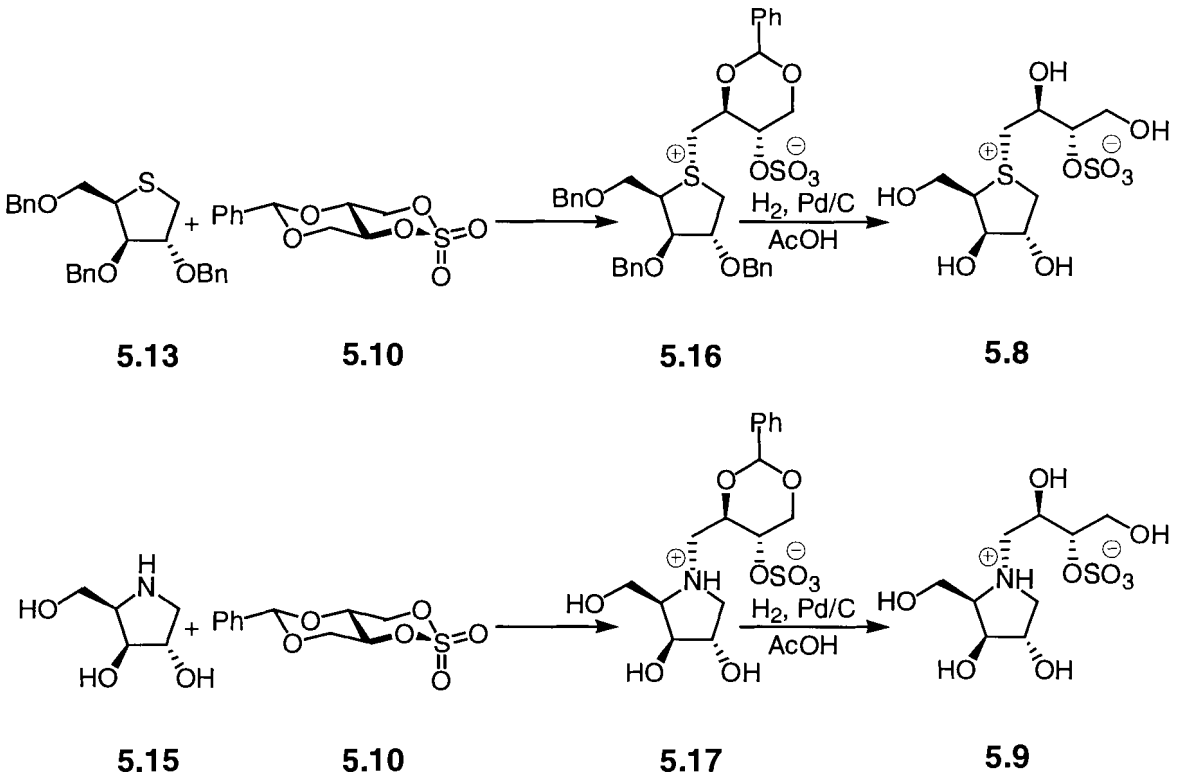
Scheme 5.1. Retrosynthetic analysis.

The cyclic sulfate **5.10** was synthesized in five steps starting from L-glucose.[6] The thio-and iminoxylitols **5.13**, **5.15** were synthesized from L-arabinose. Thus, the diol **5.11** was synthesized from L-arabinose in four steps according to the procedure used by van der Klein et al.[11] to synthesize its enantiomer (Scheme 5.2). Treatment of the diol **5.11** with methanesulfonyl chloride in pyridine then afforded the di-mesylated compound **5.12** (88% yield). Compound **5.12** was used as a key intermediate to synthesize both the thio- and iminoxylitols. Treatment of **5.12** with sodium sulfide in DMF produced compound **5.13** in 95% yield, whereas treatment with sodium azide in DMF followed by hydrogenolysis afforded the iminoxylitol **5.15** in 55% yield for the two steps.[12]



Scheme 5.2. Synthesis of the thio-and iminoxylitols **5.13**, **5.15**.

To synthesize the target sulfonium ion **5.8**, compound **5.16** was first synthesized by alkylation of 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-xylitol (**5.13**) with the cyclic sulfate **5.10** (1.2 equiv) in acetone containing K_2CO_3 at 60-70 °C in 72% yield. Compound **5.16** was obtained as the sole coupled product (Scheme 5.3). The stereochemistry at the stereogenic sulfonium centre in **5.16** was established by means of a NOESY experiment. Thus, a correlation between H-1' and H-4, confirmed the trans relationship between the erythritol side chain and C-4 substituent on the anhydroxylitol moiety, which is similar to the stereochemistry at the stereogenic sulfur atom in salacinol (**5.3**). Deprotection of **5.16** by hydrogenolysis over a palladium hydroxide catalyst on carbon was problematic because of poisoning of the catalyst but afforded compound **5.8** in 50% yield.



Scheme 5.3. Syntheses of compounds **5.8** and **5.9**.

The corresponding nitrogen congener **5.17** was synthesized in an analogous manner although, in this case, the increased nucleophilicity of the nitrogen atom did not necessitate benzylation of the hydroxyl groups. Thus alkylation of 1,4-dideoxy-1,4-imino-D-xylitol (**5.15**) with the cyclic sulfate **5.10** (1.2 equiv) in methanol containing K_2CO_3 at 60-70 °C afforded compound **5.17** in 63% yield. The stereochemistry at the stereogenic nitrogen centre in **5.17** was established by means of a NOESY experiment, as above. In this case, a correlation between H-1' and H-3, confirmed the trans relationship between the erythritol side chain and the C-3 substituent on the iminoxylitol moiety. Deprotection of **5.17** by hydrogenolysis over a Pd/C catalyst afforded compound **5.9** in 83% yield.

5.5 Enzyme Inhibition Assays

Compounds **5.8** and **5.9** were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2,[13,14] porcine pancreatic α-amylase, and barley α-amylase.[15] The effects were compared to those of salacinol (**5.3**). Glucoamylase G2 was weakly inhibited by salacinol (**5.3**) (K_i = 1.7 mM) whereas compounds **5.8** and **5.9** showed no significant inhibition of glucoamylase. The sulfonium ion **5.8** inhibited barley α-amylase (AMY1) and porcine pancreatic α-amylase (PPA), with K_i values of 109 ± 11 and 55 ± 5 μM, respectively, as compared to salacinol (**5.3**), with K_i values of 15 ± 1 and 10 ± 2 μM, respectively. In contrast, the ammonium ion **5.9** showed no significant inhibition of either AMY1 or PPA (Table 5.1). It would appear then that analogues **5.8** and **5.9** and salacinol (**5.3**) show discrimination or selectivity for certain glycosidase enzymes, and further testing against a wider panel of enzymes that includes human small intestinal maltase-glucoamylase,[16] and human pancreatic α-amylase[17] is planned to map the enzyme selectivity profiles of these compounds.

Table 5.1. K_i (mM) values of compounds **5.1 – 5.9** against barley α-amylase (AMY1), porcine pancreatic α-amylase (PPA), and glucoamylase G2.

Compound	AMY1	PPA	Glucoamylase
5.1	>5	>5	1.32
5.3	0.015	0.01	1.71
5.4	>5	>5	2.17
5.5	>5	>5	1.06
5.6	>5	>5	>2.5
5.7	>5	>5	>8
5.8	0.109	0.052	>5
5.9	>5	>5	>30

5.6 Experimental section

5.6.1 General Methods

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard Bruker pulse programs. Column chromatography was performed with Merck Silica gel 60 (230-400 mesh). High resolution mass spectra were measured with liquid secondary ionization fast atom bombardment (LSI-MS (FAB)), run on a Kratos Concept H double focussing mass spectrometer at 10000 RP, using *meta*-NO_2-benzyl alcohol as the matrix.

5.6.2 Enzyme Inhibition Assays

The glucoamylase G2 formed from *Aspergillus niger* was purified from a commercial enzyme (Novo Nordisk, Bagsvaerd, Denmark) as described.[13,14] The initial rates of glucoamylase G2-catalyzed hydrolysis of maltose was tested with 1 mM maltose as substrate in 0.1 M sodium acetate pH 4.5 at 45 °C using an enzyme concentration of 7.0 x 10^{-8} M and five inhibitor concentrations in the range from 1 μm to 5 mM. The effects of the inhibition on rates of substrate hydrolysis were compared for the different compounds. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL.[18] The K_i values were calculated assuming competitive inhibition from $1/v = (1/V_{max}) + [(K_m)/(V_{max}[S]K_i)]$ x [I], where v is the rate measured in the presence or absence of inhibitor, [I] and [S] the

concentrations of inhibitor and substrate, K_m 1.6 mM and k_{cat} 11.3 s^{-1}, using ENZFITTER.[19]

Porcine pancreatic α-amylase (PPA) and bovine serum albumin (BSA) were purchased from Sigma. Amylose EX-1 (DP17; average degree of polymerization 17) was purchased from Hayashibara Chemical Laboratories (Okayama, Japan). Recombinant barley α-amylase isozyme 1 (AMY1) was produced and purified as described.[15] An aliquot of the porcine pancreatic α-amylase (PPA) crystalline suspension (in ammonium sulfate) was dialyzed extensively against the assay buffer without BSA. The enzyme concentration was determined by aid of amino acid analysis as determined using an LKB model Alpha Plus amino acid analyzer. The inhibition of AMY1 (3×10^{-9} M) and PPA (9×10^{-9} M) activity towards DP17 amylose was measured at 37 °C in 20 mM sodium acetate, (pH 5.5, 5 mM $CaCl_2$, 0.005 % BSA (for AMY1)) and 20 mM sodium phosphate, (pH 6.9, 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005 % BSA (for PPA)). Six different final inhibitor concentrations were used in the range from 1 μM to 5 mM. The inhibitor was preincubated with enzyme for 5 min at 37 °C before addition of substrate. Initial rates were determined by measuring reducing sugar by the copper-bicinchoninate method as described.[15,20] The K_i values were calculated assuming competitive inhibition, as described above for the case of glucoamylase, and a K_m of 0.57 mg/mL and k_{cat} of 165 s^{-1} for AMY1 and 1 mg/mL and 1200s^{-1} for PPA, as determined in the substrate concentration range from 0.03 to 10 mg/mL using ENZFITTER.[19] For the K_i determinations, [S] = 0.7 mg/mL amylose DP 17 for the AMY1 binding and [S] = 2.5 mg/mL amylose DP 17 for the PPA binding.

2,3,5-Tri-*O*-benzyl-L-arabinitol (5.11). The diol **5.11** was synthesized from L-arabinose according to the procedure used by van der Klein P. A. M. et al.,[11] to synthesize its enantiomer. $[\alpha]_D$ –4.6° (*c* 1.0, CH_2Cl_2) (lit.[11] +6.8° (*c* 1, $CHCl_3$) for enantiomer); ^1H NMR (CD_2Cl_2): δ 7.38-7.25 (15H, m, Ar), 4.65 and 4.61 (2H, 2d, $J_{A,B}$ = 11.4 Hz, CH_2Ph), 4.60 and 4.57 (2H, 2d, $J_{A,B}$ = 11.2 Hz, CH_2Ph), 4.55 and 4.51 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.00 (1H, dddd, H-4), 3.81-3.74 (3H, m, H-2, H-1a, H-1b), 3.70 (1H, dd, $J_{3,4}$ = 7.0, $J_{2,3}$ = 3.6 Hz, H-3), 3.67 (1H, dd, $J_{5a,5b}$ = 12.2, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.63 (1H,dd, $J_{4,5b}$ = 5.2 Hz, H-5b), 2.90 (1H, d, $J_{OH,4}$ = 5.4 Hz, 4-OH), 2.20 (1H, dd, $J_{OH,1a}$ = 6.1, $J_{OH,1b}$ = 4.6 Hz, 1-OH); ^{13}C NMR (CD_2Cl_2): δ 138.68, 138.58 (3C_{ipso}), 128.76-128.07 (15C_{Ar}), 80.08 (C-2), 79.16 (C-3), 74.14, 73.72, 73.15 (3CH$_2$Ph), 71.63 (C-5), 70.84 (C-4), 61.82 (C-1).

2,3,5-Tri-*O*-benzyl-1,4-di-*O*-methanesulfonyl-L-arabinitol (5.12). To a solution of the diol **5.11** (4.0 g, 9.5 mmol) in pyridine (20 mL) at 0 °C was added a solution of methanesulfonyl chloride (1.8 mL, 2.5 equiv) in pyridine (3.0 mL). Stirring was continued at 0 °C, and under an N_2 atmosphere until TLC (hexanes/EtOAc, 3:2) showed complete disappearance of the starting material. The solvent was removed under high vacuum, and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1 M aqueous HCl (2 x 30 mL), H_2O (30 mL) and saturated aqueous $NaHCO_3$ (30 mL), and dried (Na_2SO_4). The solution was concentrated on a rotary evaporator and the product was purified by flash chromatography (hexanes/EtOAc, 3:2) to give **5.12** as a colourless oil (4.8 g, 88%). $[\alpha]_D$ +4.1° (*c* 1.5, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 7.42-7.20 (15H, m, Ar), 5.01 (1H, ddd, $J_{4,5b}$ = 6.8, $J_{4,5a}$ = $J_{3,4}$ = 3.2 Hz, H-4), 4.72 and 4.59 (2H, 2d, $J_{A,B}$ = 11.1 Hz, CH_2Ph), 4.65 and 4.61 (2H, 2d, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.53 (2H, s, CH_2Ph), 4.34-4.29 (2H, m, H-1a, H-1b),

3.96-3.91 (2H, m, H-2, H-3), 3.87 (1H, dd, $J_{5a,5b}$ = 11.3 Hz, H-5a), 3.81 (1H, dd, H-5b), 3.00 (3H, s, OSO_2CH_3), 2.93 (3H, s, OSO_2CH_3); ^{13}C NMR (CD_2Cl_2): δ 137.95, 137.87, 137.72 ($3C_{ipso}$), 128.84-128.29 ($15C_{Ar}$), 81.52 (C-4), 77.98 (C-2), 77.32 (C-3), 74.75, 73.96, 73.77 ($3CH_2Ph$), 69.16 (C-5), 68.64 (C-1), 39.04 (OSO_2CH_3), 37.67(OSO_2CH_3). Anal. Calcd for $C_{28}H_{34}O_9S_2$: C, 58.12; H, 5.92. Found: C, 58.21; H, 6.02.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-xylitol (5.13). Compound **5.12** (1.6 g, 2.8 mmol) was dissolved in DMF (10 mL) and $Na_2S.H_2O$ (1.1 g, 1.5 equiv) was added. The mixture was stirred at 100 °C until TLC (hexanes/EtOAc, 4:1) showed complete disappearance of the starting material. The solvent was removed under high vacuum, and the residue was dissolved in EtOAc (100 mL) and washed with H_2O (30 mL). The organic phase was dried (Na_2SO_4) and concentrated on a rotary evaporator. The product was purified by flash chromatography (hexanes/EtOAc, 4:1) to give a colorless syrup (1.1 g, 95%). $[\alpha]_D$ +67° (c 1.3, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 7.37-7.25 (15H, m, Ar), 4.57-4.47 (6H, m, $3CH_2Ph$), 4.21 (1H, ddd, H-2), 4.13 (1H, dd, $J_{2,3}$ = 3.8, $J_{3,4}$ = 3.5 Hz, H-3), 3.85 (1H, dd, $J_{4,5a}$ = 7.5 Hz, H-5a), 3.80 (1H, ddd, H-4), 3.60 (1H, dd, $J_{5a,5b}$ = 8.2, $J_{4,5b}$ = 5.5 Hz, H-5b), 3.10 (1H, dd, $J_{1a,1b}$ = 11.4, $J_{1a,2}$ = 4.4 Hz, H-1a), 2.85 (1H, dd, $J_{1b,2}$ = 2.1 Hz, H-1b); ^{13}C NMR (CD_2Cl_2): δ 138.87, 138.56 ($3C_{ipso}$), 128.70-127.86 ($15C_{Ar}$), 83.52 (C-3), 83.47 (C-2), 73.52, 72.95, 71.60 ($3CH_2Ph$), 69.85 (C-5), 48.55 (C-4), 33.46 (C-1). Anal. Calcd for $C_{26}H_{28}O_3S$: C, 74.25; H, 6.71. Found: C, 74.05; H, 6.63.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(S)-[(2S,3S)-2,4-benzylidenedioxy-3-(sulfooxy) butyl]-sulfoniumylidene]-D-xylitol inner salt (5.16). A mixture of the thioxylitol **5.13**

(100 mg, 0.24 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**5.10**) (80 mg, 1.2 equiv) was dissolved in dry acetone (0.5 mL) and anhydrous K_2CO_3 (15 mg) was added. The mixture was stirred in a sealed tube in an oil-bath (60-70 °C) overnight. The solvent was removed under reduced pressure and column chromatography ($CHCl_3$/MeOH, 10:1 + 0.1% Et_3N) of the crude product gave an amorphous solid (120 mg, 72%). $[\alpha]_D$ +28° (*c* 0.3, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.54-7.07 (20H, m, Ar), 5.52 (1H, s, C*H*Ph), 4.60 and 4.49 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.57-4.47 (2H, m, H-3', H-4'eq), 4.47 and 4.44 (2H, 2d, $J_{A,B}$ = 11.3 Hz, CH_2Ph), 4.41(1H, dd, H-1'a), 4.41-4.36 (2H, m, H-2, H-3), 4.27 (1H, ddd, , $J_{2',3'}$ = 9.1, $J_{1'a,2'}$ = $J_{1'b,2'}$ = 3.3 Hz, H-2'), 4.09 (1H, ddd, $J_{4,5a}$ = 9.4, $J_{4,5b}$ = 6.0, $J_{3,4}$ = 3.2 Hz, H-4), 4.02 and 3.96 (2H, 2d, $J_{A,B}$ = 11.4 Hz, CH_2Ph), 3.92 (1H, dd, $J_{1'b,1'a}$ = 13.6 Hz, H-1'b), 3.87 (1H, dd, $J_{1a,1b}$ = 14.4, $J_{1a,2}$ = 3.1 Hz, H-1a), 3.82 (1H, dd, $J_{5a,5b}$ = 9.4 Hz, H-5a), 3.76 (1H, dd, $J_{4'ax,4'eq}$ = $J_{3',4'ax}$ =12.2 Hz, H-4'ax), 3.71 (1H, br d, H-1b), 3.67 (1H, dd, H-5b); ^{13}C NMR (CD_2Cl_2): δ 137.42, 137.17, 136.46 ($4C_{ipso}$), 129.82-126.65 ($20C_{Ar}$), 101.76 (*C*HPh), 82.64 (C-3), 81.74 (C-2), 76.63 (C-2'), 73.92, 73.84, 72.64 ($3CH_2$Ph), 69.54 (C-4'), 66.56 (C-4), 66.40 (C-3'), 64.00 (C-5), 51.51 (C-1'), 47.42 (C-1). Anal. Calcd for $C_{37}H_{40}O_9S_2$: C, 64.14; H, 5.82. Found: C, 64.45; H, 5.85.

1,4-Dideoxy-1,4-[(S)-[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-xylitol inner salt (5.8). The protected compound **5.16** (150 mg, 0.22 mmol) was dissolved in AcOH/H_2O, 4:1 (3 mL) and stirred with palladium hydroxide catalyst on carbon (100 mg) under H_2 (52 psi). After 72 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The combined filtrates were concentrated and the residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O,

7:3:1) to give an amorphous solid (36 mg, 50%). $[\alpha]_D$ +20° (*c* 1.2, MeOH); ^{1}H NMR (CD_3OD): δ 4.64-4.59 (1H, m, H-2), 4.55 (1H, dd, $J_{2,3} = J_{3,4} = 2.9$ Hz, H-3), 4.37 (1H, ddd, H-4), 4.35 (1H, ddd, $J_{1'a,2'} = 3.5$ Hz, H-2'), 4.25 (1H, ddd, $J_{2',3'} = 7.2$, $J_{3',4'a} = J_{3',4'b} = 3.4$ Hz, H-3'), 4.16 (1H, dd, $J_{5a,5b} = 11.7$, $J_{4,5a} = 6.0$ Hz, H-5a), 4.07 (1H, dd, $J_{4,5b} = 9.0$ Hz, H-5b), 3.95 (1H, dd, $J_{1'a,1'b} = 13.4$ Hz, H-1'a) 3.93 (1H, dd, H-4'a), 3.91 (1H, dd, H-1a) 3.88 (1H, dd, $J_{1'b,2'} = 8.0$ Hz, H-1'b), 3.81 (1H, dd, $J_{4'b,4'a} = 12.1$ Hz, H-4'b), 3.58 (1H, br d, $J_{1b,1a} = 13.7$ Hz, H-1b); ^{13}C NMR (CD_3OD): δ 81.05 (C-3'), 79.55 (C-2), 78.56 (C-3), 71.69 (C-4), 67.71 (C-2'), 61.66 (C-4'), 58.92 (C-5), 52.60 (C-1'), 49.26 (C-1); HRMS. Calcd for $C_9H_{18}O_9S_2$ (M + H): 335.0471. Found: 335.0471.

1,4-Dideoxy-1,4-[(S)-[(2R,3S)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-iminium]-D-xylitol Inner Salt (5.17). A mixture of 1,4-Dideoxy-1,4-imino-D-arabinitol (**5.15**) (100 mg, 0.74 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**5.10**) (240 mg, 1.2 equiv) was dissolved in dry MeOH (0.5 mL) and anhydrous K_2CO_3 (15 mg) was added. The mixture was stirred in a sealed tube in an oil-bath (60-70 °C) overnight. The solvent was removed under reduced pressure, and column chromatography (CH_2Cl_2/MeOH, 5:1) of the crude product gave a white solid (191 mg, 63%) that was recrystallized from methanol. Mp: 202-204 °C; $[\alpha]_D$ +30° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 7.73-7.58 (5H, m, Ar), 5.93 (1H, s, C*H*Ph), 4.68 (1H, dd, $J_{4'eq,4'ax} = 11.0$, $J_{3',4'eq} = 5.5$ Hz, H-4′eq), 4.54 (1H, br t, $J_{2',3'} = J_{1'b,2'} = 9.9$ Hz, H-2′), 4.50-4.41 (3H, m, H-3, H-3′, H-2), 4.20 (1H, dd, $J_{5a,5b} = 12.6$, $J_{4,5a} = 5.5$ Hz, H-5a), 4.18-4.12 (2H, m, H-1′a, H-5b), 4.10 (1H, dd, $J_{3',4'ax} = 11.0$ Hz, H-4′ax), 4.11-4.01 (2H, m, H-1a, H-4), 3.66 (1H, br dd, $J_{1'b,1'a} = 11.6$ Hz, H-1′b), 3.56-3.50 (1H, br d, H-1b); ^{13}C NMR (D_2O): δ 138.50 (C_{ipso}), 132.45 (C_{para}), 131.26 (2C) and 128.62

(2C) (C_{ortho} and C_{meta}), 103.38 (*C*HPh), 78.08 (C-2′), 77.38 (C-3), 76.70 (C-2), 73.31 (C-4), 70.77 (C-4′), 70.41 (C-3′), 63.18 (C-1), 60.01 (C-1′), 59.55 (C-5). Anal. Calcd for $C_{16}H_{22}O_9SN$: C, 47.51; H, 5.49; N, 3.47. Found: C, 47.29; H, 5.80; N, 3.22.

1,4-Dideoxy-1,4-[(S)-[(2R,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]-iminium]-D-xylitol Inner Salt (5.9). The protected compound **5.17** (75 mg, 0.18 mmol) was dissolved in AcOH/H_2O, 4:1 (5 mL) and stirred with Pd-C (30 mg) under H_2. After 16 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The combined filtrates were concentrated and the residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O, 7:3:1) to give an amorphous solid (49 mg, 83%). $[\alpha]_D$ –5.3° (c 0.8, H_2O); ^{1}H NMR (D_2O): δ 4.59-4.40 (4H, m, H-3, H-2, H-2′, H-3′), 4.27-4.17 (2H, m, H-5a, H-5b), 4.18-4.06 (2H, m, H-1a, H-4), 4.11 (1H, dd, $J_{4'a,4'b}$ = 12.6, $J_{3',4'a}$ = 2.0 Hz, H-4′a), 4.02 (1H, dd, $J_{3',4'b}$ = 3.3 Hz, H-4′b), 4.11-3.95 (1H, m, H-1′a), 3.68-3.57 (1H, m, H-1b), 3.57-3.45 (1H, m, H-1′b); ^{13}C NMR (D_2O): δ 82.52 (C-3′), 77.46 (C-3), 76.56 (C-2′), 73.20 (C-4), 68.42 (C-2), 63.09 (C-1), 62.38 (C-1′), 61.93 (C-4′), 59.38 (C-5). HRMS Fab. Calcd for $C_9H_{18}O_9SN$ (M + H): 318.0859. Found: 318.0859.

5.7 Acknowledgments

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CHAPTER 6: IMPROVED SYNTHESES OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR SALACINOL

Ahmad Ghavami,[a] Kashinath S. Sadalapure,[a] Blair D. Johnston,[a]

Mercedes Lobera,[b] Barry B. Snider,[b] and B. Mario Pinto[a]*#

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Brandeis University, MS#015, 415 South Street, Box 9110*

Waltham, Massachusetts 02454-9110, USA

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* Author to whom correspondence should be addressed

Tel: (604) 291-4884

Fax: (604) 291-5424

e-mail:bpinto@sfu.ca

#This work is dedicated, with respect, to the memory of Raymond U. Lemieux.

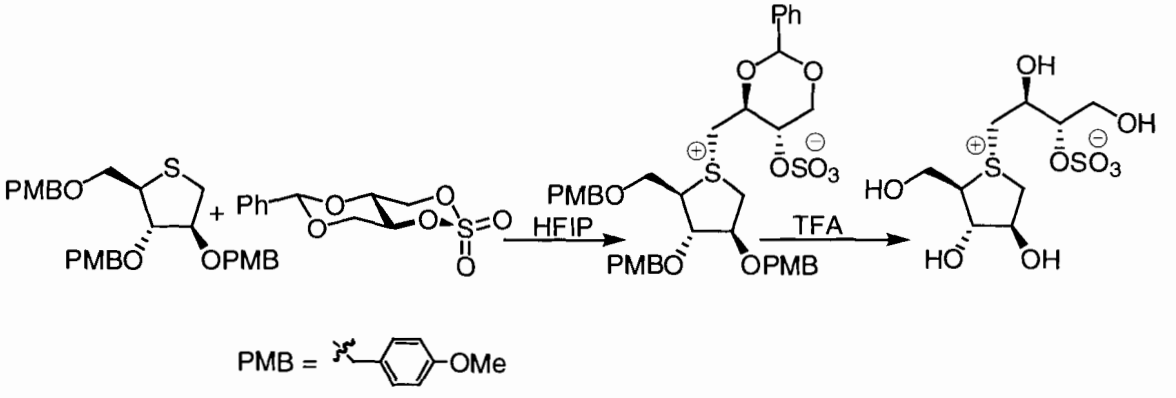
6.1 Graphical Abstract

Improved Syntheses of the Naturally Occurring Glycosidase Inhibitor Salacinol

Ahmad Ghavami,[a] Kashinath S. Sadalapure,[a] Blair D. Johnston,[a] Mercedes Lobera,[b] Barry B. Snider,[b] and B. Mario Pinto[a]*

[a]*Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6*

[b]*Department of Chemistry, Brandeis University, MS#015, 415 South Street, Box 9110 Waltham, Massachusetts 02454-9110, USA*



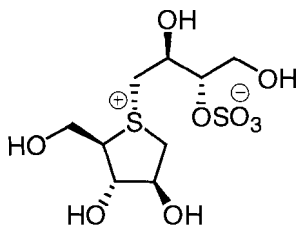
Keywords: glycosidase inhibitors, salacinol, efficient synthesis, sulfonium salt, cyclic sulfate.

6.2 Abstract

Improved syntheses of the naturally occurring sulfonium ion, salacinol are described. Salacinol 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 type II. The synthetic strategy relies on the nucleophilic attack of 2,3,5-tri-*O*-benzyl- or 2,3,5-tri-*O*-*p*-methoxybenzyl -1,4-anhydro-4-thio-D-arabinitol at the least hindered carbon of benzylidene-protected L-erythritol-1,3-cyclic sulfate in 1,1,1,3,3,3-hexafluoro-2-propanol as a solvent. The reactions are compared to those with the benzyl-protected L-erythritol-1,3-cyclic sulfate and also to those in acetone and 2-propanol. Excellent yields are obtained for the reactions with the benzylidene-protected cyclic sulfate. The synthetic route employing *p*-methoxybenzyl ether protecting groups is advantageous since all protecting groups in the adduct may be removed with trifluoroacetic acid to yield salacinol, thereby obviating the problematic deprotection of benzyl ethers by hydrogenolysis.

6.3 Introduction

Salacinol (**6.1**) is a potent glycosidase inhibitor isolated from the aqueous extracts of *Salacia reticulata* that are used in Sri Lanka and India for the treatment of diabetes.[1-3] The molecular structure of this inhibitor is unique in that it contains a sulfonium ion (1,4-anhydro-4-thio-D-pentitol cation) stabilized by an internal sulfate counterion (1'-deoxy-L-erythrosyl-3'-sulfate anion). Glycosidase inhibitors containing sulfonium ions are of interest as mimics of oxacarbenium intermediates in glycosidase hydrolysis reactions.[4-6] In this regard, we[5,7] and others[6] have previously reported the syntheses of salacinol (**6.1**) and its stereoisomers. In the search for novel glycosidase inhibitors, we have also reported the synthesis and glycosidase inhibitory properties of the heteroatom congeners of salacinol in which the ring sulfur atom has been substituted by the cognate atoms nitrogen[8] and selenium.[9] We report here an improved method for the synthesis of the natural product salacinol (**6.1**) that exploits an unusual solvent effect provided by 1,1,1,3,3,3-hexafluoroisopropanol (HFIP). The previous syntheses of salacinol and its stereoisomers employed either acetone[5,7] or DMF[6] as solvents.

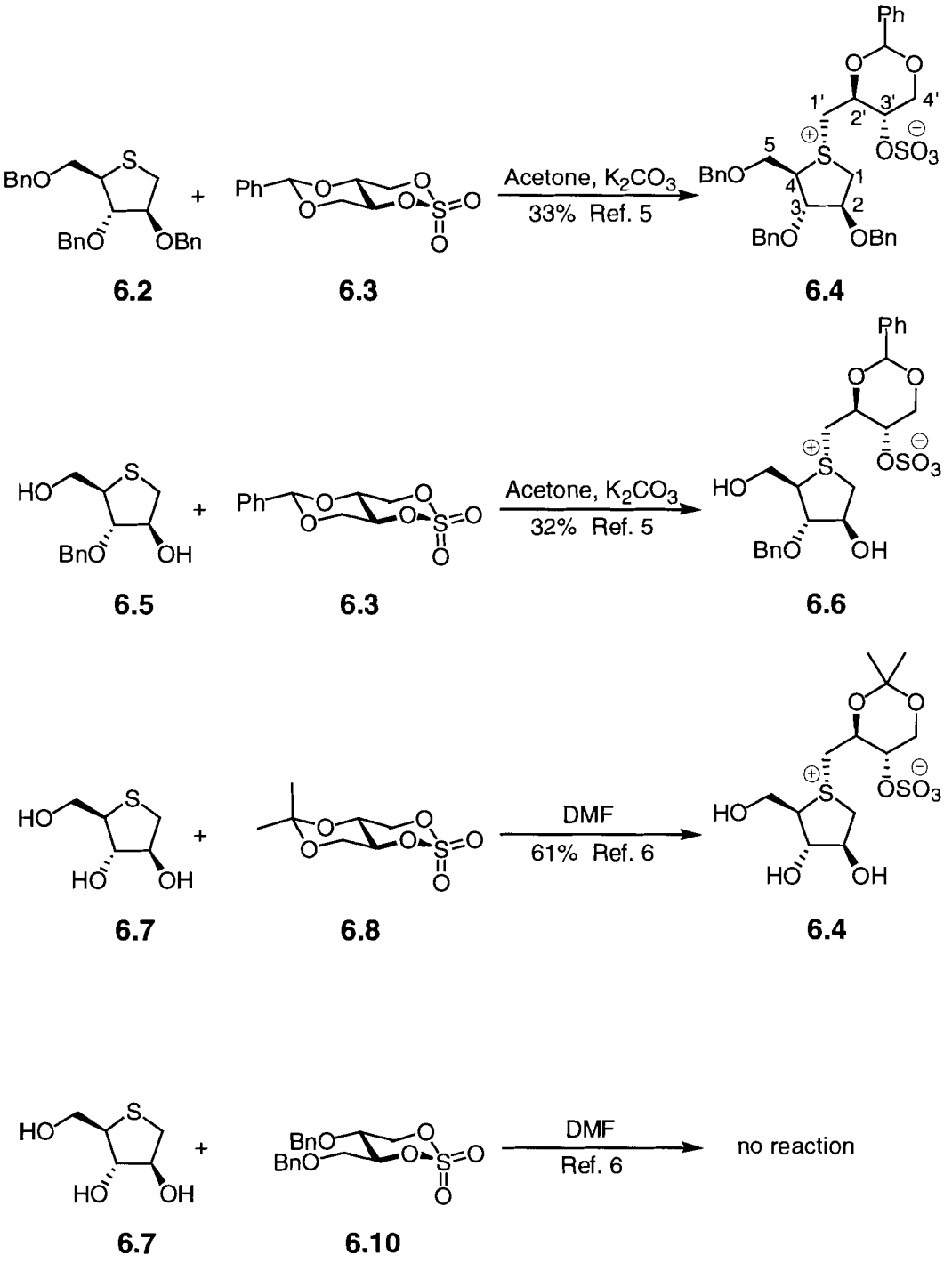


Salacinol (**6.1**)

Chart 6.1. Salacinol (**6.1**).

6.4 Results and Dscussion

The key step in the published syntheses of salacinol (**6.1**)[5,6] is the ring opening reaction of a cyclic sulfate by nucleophilic attack of the ring sulfur atom of 1,4-anhydro-4-thio-D-pentitol (**6.2**) (Scheme 6.1). The alkylation reaction involving these partners is critically dependent on the protecting groups on the cyclic sulfate. Thus, the unoptimized reaction of the per-benzylated thioether **6.2** with the benzylidene-protected cyclic sulfate **6.3** in acetone, containing potassium carbonate, proceeded in 33% yield (Scheme 6.1).[5] A similar yield was obtained in the reaction with the monobenzylated thioether **6.5**.[5] Reaction of the unprotected thioether **6.7** with the isopropylidenated-cyclic sulfate **6.8** in DMF proceeded in 61% yield although its reaction with the corresponding benzylated-cyclic sulfate **6.10** did not proceed.[6] The latter derivative **6.10** is clearly a much less reactive alkylating agent than **6.8**. Significant decomposition of the cyclic sulfates **6.8** and **6.10** at temperatures of 60-70 °C in DMF was also observed.[6]

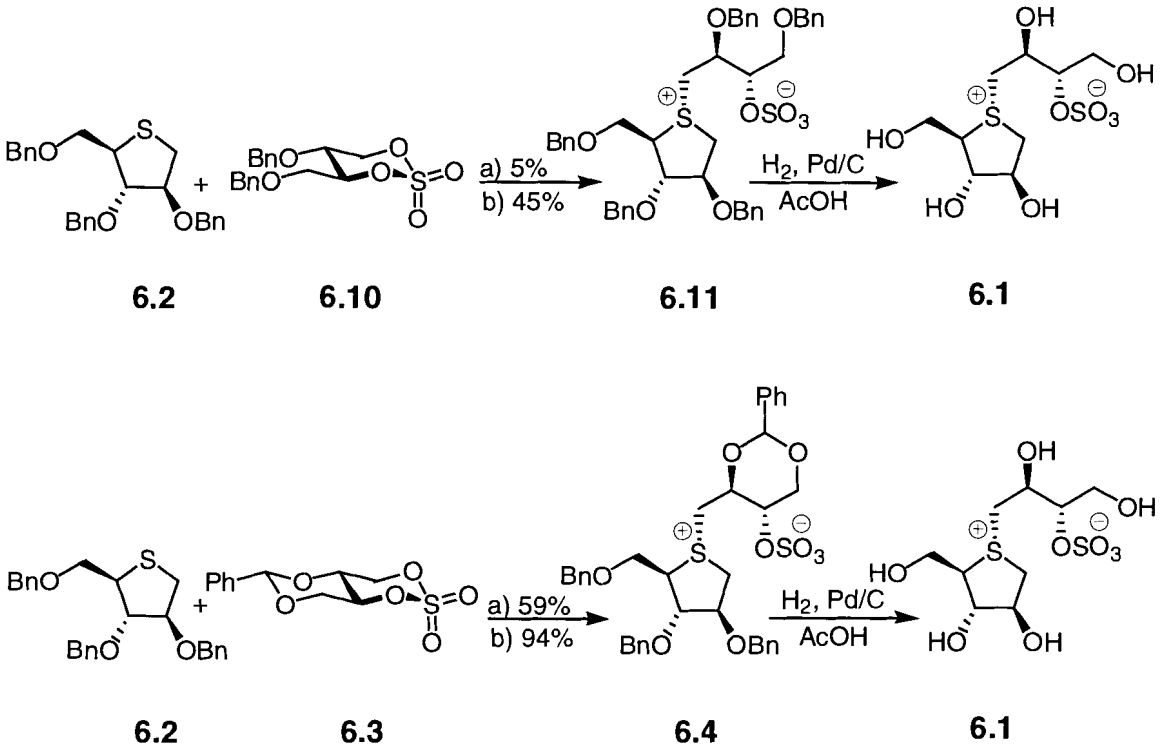


Scheme 6.1. Coupling reactions to give precursors of salacinol (**6.1**).

The biological importance of salacinol (**6.1**)[1-3] prompted us to investigate a more efficient method for its synthesis. The Hughes-Ingold rules indicate that the S_N2 reaction between a neutral nucleophile, such as **6.2** or **6.5**, and a neutral electrophile, such as **6.3**, **6.8** or **6.10**, should show a large increase in rate on increasing solvent polarity.[10] 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) has a higher normalized Dimroth-Reichardt solvent polarity parameter, E_T^N = 1.068, than water, E_T^N =1.00.[10] In contrast, the E_T^N values for acetone and DMF are only 0.355 and 0.404, respectively. Furthermore, HFIP, bp = 59 °C, is volatile, thus facilitating product purification. Preliminary studies indicated that tetrahydrothiophene reacted cleanly with **6.3** and **6.10** in HFIP at 45 °C for 2 days to give the desired alkylation products in >90% yield.[11]

Therefore, a systematic evaluation of the role of solvent in the alkylation reactions of **6.2** with benzyl- or benzylidene-protected cyclic sulfates **6.10** or **6.3**, respectively was undertaken. The reactions were carried out in acetone and hexafluoroisopropanol (HFIP) concurrently under identical conditions of concentration, temperature, and duration (Scheme 6.2). Reaction of the thioether **6.2** (1 equiv) and the cyclic sulfate **6.10** (1.2 equiv) in acetone containing K_2CO_3 at 75-80 °C in a sealed tube proceeded very slowly and yielded the desired alkylated product **6.11** in only 5% yield; the remainder of the starting materials was recovered. Prolonged heating and use of excess cyclic sulfate did not improve the yields. In addition, when excess cyclic sulfate **6.10** was used, its slow decomposition complicated the purification of the product **6.11** formed. However, the analogous reaction between **6.2** and the cyclic sulfate **6.10** in HFIP yielded the adduct **11** in 45% yield, with recovery of the unreacted starting materials (Scheme 6.2). It is noteworthy that the analogous reaction between **6.2** and the cyclic sulfate **6.10** in the polar,

protic solvent 2-propanol at 83°C for 26 h did not yield any desired product, the starting materials being recovered. It would appear, therefore, that it is the highly polar nature of HFIP that is critical in facilitating this reaction.



a) Acetone, K_2CO_3, 75-80 °C
b) HFIP, K_2CO_3, 75-80 °C

Scheme 6.2. Syntheses of salacinol (**6.1**).

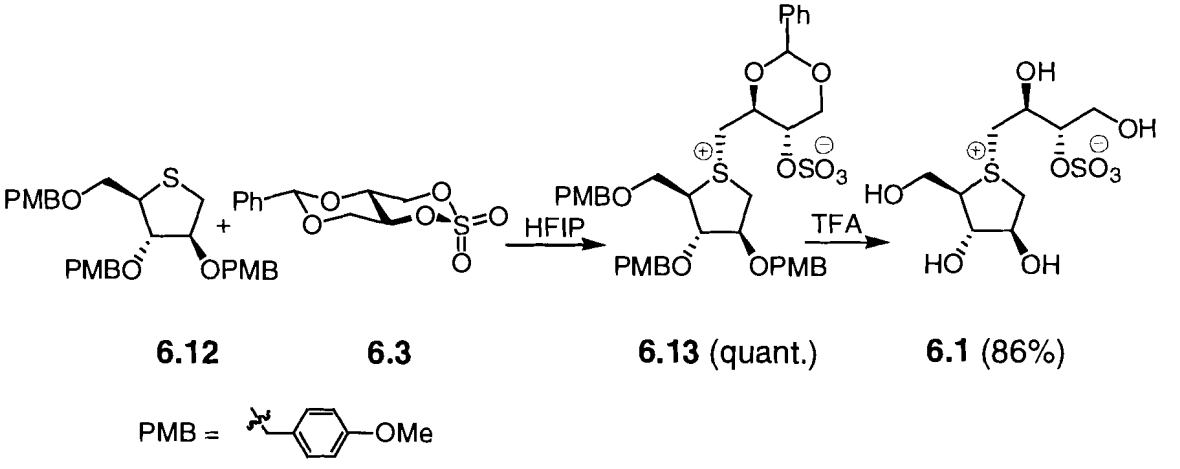
The previous results of Yuasa et al.[6] had indicated a far lesser reactivity of the benzylated cyclic sulfate relative to the cyclic sulfate containing an acetal protecting group (Scheme 6.1). Thus, the reactions of the benzylidene-protected cyclic sulfate **6.3** in acetone and HFIP, containing potassium carbonate, under identical conditions of concentration, temperature, and duration were examined next (Scheme 6.2). The

alkylation reaction of **6.2** with **6.3** in acetone proceeded with a dramatic increase in the yield (59%) of the alkylated product **6.4** relative to the reaction with **6.10**. The improvement from our earlier reported unoptimized yield of 33% is due to the use of a more concentrated reaction mixture.

More significantly, the desired product **6.4** was obtained in 94% yield when the reaction was performed in HFIP. Higher temperatures (> 80 °C) and prolonged reaction times led to the decomposition of the cyclic sulfate, although the stability of the cyclic sulfate was greater in the presence of K_2CO_3. The increased yields in HFIP may be accounted for by better solvation of the transition states for the reactions and of the adducts. The increased reactivity of the cyclic sulfate with the benzylidene protecting group **6.3** may be accounted for by the relief of ring strain accompanying the reaction, unlike in the corresponding reaction of the benzyl-protected cyclic sulfate **6.10**. Finally, the reaction of the unprotected thioether **6.7** with the benzylidene-protected cyclic sulfate **6.3** in HFIP was examined. At 60 °C, decomposition of the cyclic sulfate was observed, with no significant formation of the desired coupled product. Hydrogenolysis of the protected derivatives **6.4**[5] and **6.11** afforded salacinol (**6.1**), although this step was problematic because of poisoning of the catalyst, and only afforded the product in 65% yield.

In order to obviate the problematic hydrogenolysis step, we next chose to examine the reaction of the thioether containing *p*-methoxybenzyl ether protecting groups with the benzylidene-protected L-erythritol-1,3-cyclic sulfate; we reasoned that the removal of all protecting groups by acid hydrolysis would be facile. Thus, 2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-D-arabinitol (**6.12**), synthesized in 87% yield from **6.7**, was reacted

with the cyclic sulfate **6.3** in HFIP to afford the sulfonium salt **6.13** in quantitative yield (Scheme 6.3). Deprotection of **6.13** proceeded smoothly (86%) in aqueous trifluoroacetic acid to afford salacinol **6.1** in 75% overall yield. The latter sequence represents, therefore, an efficient synthesis of the biologically important natural product salacinol **6.1**.



Scheme 6.3. Improved synthesis of salacinol (**6.1**).

As a final point of interest, we comment on the stereochemistry at the stereogenic sulfonium centre in **6.4**, **6.11**, and **6.13**. These reactions proceeded stereoselectively irrespective of the solvent used in the reaction. The stereochemistry was confirmed by means of NOESY experiments that showed clear correlations between H-4 and H-1', thus indicating the presence of the isomer with a trans relationship between C-5 and C-1'. The barrier to inversion at the sulfonium ion centre must be substantial since no evidence for isomerization in these and related derivatives[7] has been noted.

6.5 Experimental Section

6.5.1 General Methods

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz. All assignments were confirmed with the aid of two-dimensional $^{1}H,^{1}H$ (COSYDFTP) or $^{1}H,^{13}C$ (INVBTP) experiments using standard Bruker pulse programs. MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix using a PerSeptive Biosystems Voyager-DE instrument. Column chromatography was performed with Merck Silica gel 60 (230-400 mesh). High-resolution mass spectra were LSIMS (Fab), run on a Kratos Concept H double focussing mass spectrometer at 10000 RP.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(S)-[(2S,3S)-2,4-di-(benzyloxy)-3-sulfooxy) butyl]- sulfoniumylidene]-D-arabinitol Inner Salt (6.11). A mixture of the thioether **6.2**[5] (270 mg, 0.64 mmol) and 2,4-di-*O*-benzyl-1,3-cyclic sulfate (**6.10**)[6,9] (280 mg, 0.77 mmol) in either acetone or HFIP (0.5 mL), containing anhydrous K_2CO_3 (16 mg, 0.10 mmol) was stirred in a sealed tube in an oil-bath (75-80 °C) for 14h. The solvent was removed under reduced pressure and the residue was purified by column chromatography using (CH_2Cl_2:MeOH, 10:1) as eluant to give the *title compound* **6.11**, as an amorphous solid (29 mg, 5%) in acetone and (229 mg, 45%) in HFIP. R_f 0.40 (CH_2Cl_2:MeOH, 10:1); $[\alpha]_D$ - 26° (c 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38-7.05 (25H, m, Ar), 4.67 and 4.45 (2H, 2d, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.60 and 4.45 (2H, 2d, $J_{A,B}$ = 9.5 Hz, CH_2Ph), 4.59 and 4.44 (2H, 2d, $J_{A,B}$ = 11.2 Hz, CH_2Ph), 4.58 (1H, dt, $J_{2',3'}$ = 5.0 Hz, H-3'), 4.42 and 4.28 (2H, 2d, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.36 (1H, m, H-2), 4.32 (1H, ddd,

J = 1.7, 4.1, 6.3 Hz, H-2'), 4.30 and 4.20 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.23 (1H, m, H-3), 4.13 (1H, dd, $J_{1'a,1'b}$ = 13.4, $J_{1'a,2'}$ = 2.0 Hz, H-1'a), 4.05 (1H, d, $J_{2,3}$ = 13.3 Hz, H-1a), 4.00 (1H, dd, $J_{4'a,4'b}$ = 11.1, $J_{3',4'a}$ = 2.7 Hz, H-4'a), 3.86 (1H, dd, $J_{3',4'b}$ = 2.4, $J_{4'a,4'b}$ = 11.3 Hz, H-4'b), 3.71 (1H, brt, J = 9.2 Hz, H-4), 3.69 (1H, dd, $J_{1'b,2'}$ = 3.8, $J_{1'b,1'a}$ = 9.2 Hz, H-1'b), 3.60 (1H, dd, $J_{1a,1b}$ = 13.5, $J_{1b,2}$ = 3.8 Hz, H-1b), 3.51 (1H, dd, $J_{5a,5b}$ = 13.6, $J_{4,5a}$ = 9.7 Hz, H-5a), 3.49 (1H, dd, $J_{4,5b}$ = 9.7 Hz, H-5b); ^{13}C NMR ($CDCl_3$): δ 137.97, 136.77, 136.71, 136.05 and 135.77 ($5C_{ipso}$), 128.81-127.66 ($25C_{Ar}$), 83.14 (C-3), 81.65 (C-2), 74.59 (C-3'), 73.81, 73.53, 73.39, 72.12, 71.84 ($5CH_2$Ph), 73.10 (C-2'), 68.79 (C-4'), 66.62 (C-5), 65.53 (C-4), 50.89 (C-1'), 48.07 (C-1). MALDI-TOF MS: m/e 785.41 (M^++H), 808.32 (M^++Na). Anal. Calcd for $C_{44}H_{48}O_9S_2$: C, 67.32; H, 6.16. Found: C, 67.36; H, 6.10.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[(S)-[(2S,3S)-2,4-benzylidenedioxy-3-(sulfooxy) butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (6.4). A mixture of the thioether 6.2[5] (260 mg, 0.62 mmol) and 2,4-di-*O*-benzylidene-1,3-cyclic sulfate (6.3)[5] (200 mg, 0.74 mmol) in either acetone or HFIP (0.5 ml) containing K_2CO_3 (13 mg, 0.09 mmol) was treated as described above to yield the *title compound* 6.4[5] as an amorphous solid (252 mg, 59% in acetone) and (406 mg, 94% in HFIP).

1,4-Anhydro-2,3,5-tri-*O*-(*p*-methoxybenzyl)-4-thio-D-arabinitol (6.12). To an ice cold mixture of 1,4-anhydro-4-thio-D-arabinitol **6.7**[5] (0.98 g, 6.52 mmol) and 60% NaH (1.56 g, 39.15 mmol, 6 equiv.) in THF (15 mL), a solution of *p*-methoxybenzyl chloride (4.59 g, 29.34 mmol, 4.5 equiv.) in THF (10 mL) was added over 30 min. The reaction

mixture was allowed to attain room temperature and further stirred for 1h before heating to 55 °C for 12h. The reaction mixture was cooled and poured in to ice-water (150 mL) and extracted with Et_2O (150 mL). The organic solution was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography [hexanes:EtOAc, 7:3] to give a colorless syrup (2.96 g, 87%). $[\alpha]_D$ + 6° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.20-6.80 (12H, m, Ar), 4.55 (2H, s, C*H*$_2$Ph), 4.48 and 4.45 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C*H*$_2$Ph), 4.42 and 4.39 (2H, 2d, $J_{A,B}$ = 12.0 Hz, C*H*$_2$Ph), 4.13 (1H, dd, $J_{1a,2}$ = 4.6 , $J_{2,3}$ = 9.1 Hz, H-2), 4.05 (1H, dd, $J_{2,3}$ = $J_{3,4}$ = 3.7 Hz, H-3), 3.81 (3H, s, OC*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 3.76 (3H, s, OC*H*$_3$), 3.64 (1H, dd, $J_{5a,5b}$ = 8.9, $J_{4,5a}$ = 7.5 Hz, H-5a), 3.50 (1H, ddd, $J_{4,5b}$ = 6.3 Hz, H-4), 3.45 (1H, dd, H-5b), 3.04 (1H, dd, $J_{1a,1b}$ = 11.4, $J_{1a,2}$ = 5.2 Hz, H-1a), 2.85 (1H, dd, H-1b). ^{13}C NMR ($CDCl_3$): δ 159.24, 159.16 (3C_{para}), 130.31, 130.19, 130.01 (3C_{ipso}), 129.48, 129.28, 129.22 (6C_{ortho}), 113.80, 113.74 (6C_{meta}), 84.77 (C-3), 84.70 (C-2), 72.66, 71.49, 71.20 (3*C*H$_2$Ph), 72.15 (C-5), 55.24 (3O*C*H$_3$), 48.96 (C-4), 33.07 (C-1). Anal. Calcd for $C_{29}H_{34}O_6S$: C, 68.21; H, 6.71. Found: C, 67.99; H, 6.69.

2,3,5-Tri-*O*-*p*-methoxybenzyl-1,4-dideoxy-1,4-[(S)-[(2*S*,3*S*)-2,4-benzylidenedioxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (6.13). A mixture of the thioether **6.12** (1.5 g, 2.94 mmol), and the cyclic sulfate **6.3** (0.96 g, 1.2 equiv) in HFIP (2.5 mL) containing anhydrous K_2CO_3 (30 mg) was stirred in a sealed tube in an oil-bath (55°C) overnight. TLC analysis (CH_2Cl_2:MeOH, 10:1) showed that the thioether **6.12** was completely consumed. The solvent was removed under reduced pressure and the product was purified by column chromatography (gradient of CH_2Cl_2 to CH_2Cl_2:MeOH,

10:1) to give compound **6.13** (2.30 g, 100%) as a colorless foam. $[\alpha]_D$ –10.5° (*c* 1.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.51-6.81 (17H, m, Ph), 5.53 (1H, s, C_6H_5CH), 4.57 (1H, ddd, $J_{2',3'} = J_{3',4'ax} = 10.0$, $J_{3',4'eq} = 5.5$ Hz, H-3'), 4.49 (1H, dd, $J_{4'ax,4'eq} = 10.8$ Hz, H-4'eq), 4.44 (2H, s, CH_2Ph), 4.42-4.39 (1H, m, H-2), 4.39 and 4.29 (2H, 2d, $J_{A,B} = 11.4$ Hz, CH_2Ph), 4.33 (1H, dd, $J_{1'a,1'b} = 13.4$, $J_{1'a,2'} = 2.6$ Hz, H-1'a), 4.29-4.26 (1H, m, H-3), 4.26 (1H, ddd, H-2'), 4.19 and 4.09 (2H, 2d, $J_{A,B} = 11.5$ Hz, CH_2Ph), 4.03 (1H, br d, $J_{1a,2} < 1$ Hz, H-1a), 3.96-3.89 (2H, m, H-4, H-1'b), 3.80 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.77 (1H, dd, H-4'ax), 3.63 (1H, dd, $J_{1a,1b} = 13.3$, $J_{1b,2} = 3.8$ Hz, H-1b), 3.58 (1H, dd, $J_{5a,5b} = 9.9$, $J_{4,5a} = 8.5$ Hz, H-5a), 3.49 (1H, dd, $J_{4,5b} = 7.3$ Hz, H-5b); ^{13}C NMR (CD_2Cl_2): δ 160.30, 160.23, 159.97, 137.20 and 130.27-126.61 ($21C_{Ar}$), 114.45, 114.36 and 114.18 ($3C_{ipso}$, OMBn), 101.96 (*C*HPh), 83.29 (C-3), 82.37 (C-2), 76.76 (C-2'), 73.36, 72.43, and 72.14 ($3CH_2Ph$), 69.50 (C-4'), 66.71 (C-5), 66.55 (C-4), 66.45 (C-3'), 55.61 (3C, $3OCH_3$), 49.55 (C-1'), 48.48 (C-1). Anal. Calcd for $C_{40}H_{46}O_{12}S_2$: C, 61.36; H, 5.92. Found: C, 61.13; H, 6.00.

1,4-Dideoxy-1,4-[(S)-[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]-sulfoniumylidene]-D-arabinitol Inner Salt (6.1). Compound **6.13** (2.30 g, 2.94 mmol) was dissolved in trifluoroacetic acid (24 mL) and while stirring, water (2.4 mL) was added. The mixture was stirred at room temperature for 0.5 h. The solvent was removed under reduced pressure and the gummy residue was washed with CH_2Cl_2 (3 × 20 mL). Water (15 mL) was added to dissolve the crude product, and then evaporated under reduced pressure to remove the traces of acid left. Salacinol **6.1** (0.67 g, 68%) was crystallized from MeOH.

The mother liquor was concentrated and purified by column chromatography (EtOAc:MeOH:H_2O, 7:3:1) to give more salacinol **6.1** as a white solid (0.18 g, 18%).

6.6 Acknowledgments

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6.7 References

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CHAPTER 7: X-RAY CRYSTALOGRAPHIC ANALYSIS OF GOLGI-α-MANNOSIDASE II IN COMPLEX WITH SALACINOL ANALOGUES

7.1 Introduction

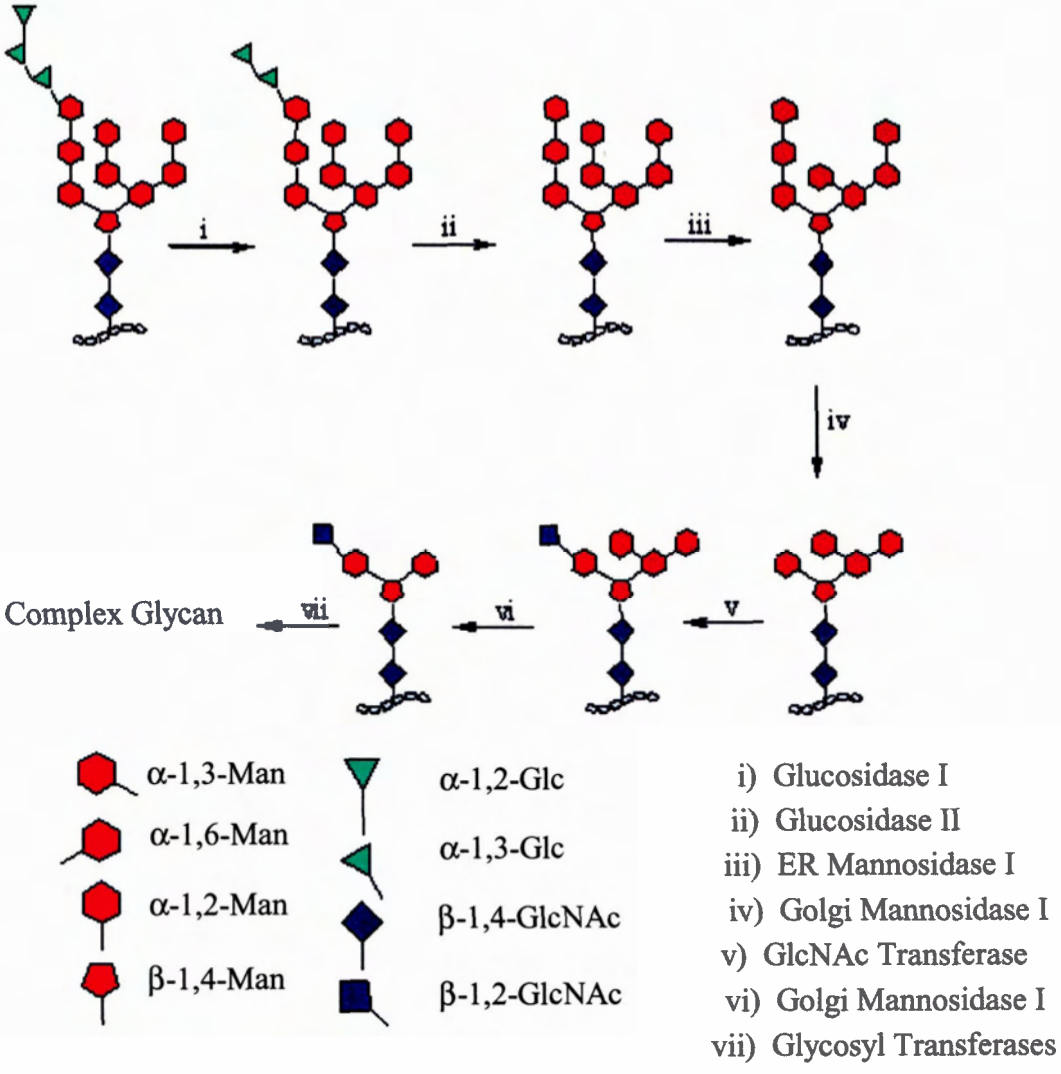
7.1.1 *N*-Glycans

Oligosaccharide units of glycoproteins are linked to either asparagine side chains by *N*-glycosidic bonds or serine and threonine residues by *O*-glycosidic bonds. The *N*-linked glycosylation pathway is the process that involves formation and maturation of the oligosaccharides connected to the asparagine side chain of proteins and is necessary for the proper processing of proteins formed in the endoplasmic reticulum (ER) and Golgi compartment.[1]

7.1.2 Biosynthesis of *N*-glycans

Different *N*-glycans are formed from the same oligosaccharide precursor, $Glc_3Man_9GlcNAc_2$, that is attached through a pyrophosphate linkage to a lipid called dolichol, in most species. The dolichol-linked oligosaccharide precursor is formed in the membranes of the ER by stepwise addition of monosaccharides to dolichol phosphate. It is then transferred from dolichol to specific asparagine residues in the growing polypeptide chain.

When attached to protein, the oligosaccharide precursor undergoes a maturation process or processing. Processing of *N*-glycans is controlled by enzymatic reactions catalyzed by glycosidases and glycosyltransferases and takes place in the ER and Golgi compartment (Scheme 7.1).



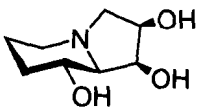
Scheme 7.1. *N*-linked glycan biosynthesis.

***N*-Glycan processing starts with removal of glucose residues.** Glucosidase I cleaves the terminal α-(1→2) and glucosidase II removes the two α-(1→3)-glucose residues; and one α-(1→2)-linked mannose is then removed in the ER, and $Man_8GlcNAc_2$ is formed. Three more α-(1→2)-linked mannose residues are then removed in the cis Golgi and then, following the action of GlcNAc transferase I, Golgi α-mannosidase II removes the terminal α-(1→3) and α-(1→6)-linked mannose residues to yield

$GlcNAcMan_3GlcNAc_2$. This oligosaccharide can be extended by other GlcNAc transferases to initiate the branches that are subsequently modified by Golgi glycosyltransferases specific for the addition of Gal, GalNAc, GlcNAc, Fuc, and sialic acid residues.

7.1.3 Golgi α-mannosidase II

In breast, skin, and colon cancer, the unusual quantitative distributions of complex structures on the cell surface are associated with metastasis.[2] This altered distribution is associated with abnormalities in the *N*-glycosylation pathway, and inhibition of key enzymes in this pathway therefore has clinical potential in cancer treatment. Golgi α-mannosidase II (GMII) is a key component of the *N*-glycosylation pathway in protein synthesis. Inhibition of GMII by oral administration of swainsonine (**7.1**) has shown promise in clinical trials as an anti-metastatic agent by interfering with the expression of complex carbohydrates.[3]



7.1

Chart 7.1. Structure of swainsonine (**7.1**).

GMII is an exo-glycosidase, which cleaves α-(1→3) and α-(1→6)-linked mannose residues from the substrate $GlcNAcMan_5GlcNAc_2$ in the oligosaccharide processing pathway. It is a protein, which has a molecular weight of 125 kDa according to SDS-

PAGE. This enzyme is very specific and does not cleave the mannose residues unless the GlcNAc transferase has already attached the β-(1→2) GlcNAc to the substrate. The hydrolysis of both glycosidic bonds occurs with retention of anomeric configuration, which is due to a double displacement mechanism.[4]

In order to design highly specific and effective inhibitors for this enzyme, which have the potential as anti-cancer drugs, one should know the mechanism of action of the enzyme and also the structure of the active site. Van den Elsen et al.[4] have reported previously the atomic structure by X-ray crystallography of the *Drosophila* homologue of GMII (dGMII) and its complex with some inhibitors including swainsonine (**7.1**). Since mammalian GMII is very hard to purify in high quantities, GMII from *Drosophila melanogaster* (dGMII), which is very similar to human GMII has been used in the structural studies of GMII (Figure 7.1). Specifically, as can be seen in Figure 1B, the active site in dGMII is very similar to human GMII and consists of conserved and identical amino acid residues, which are mostly negatively charged.

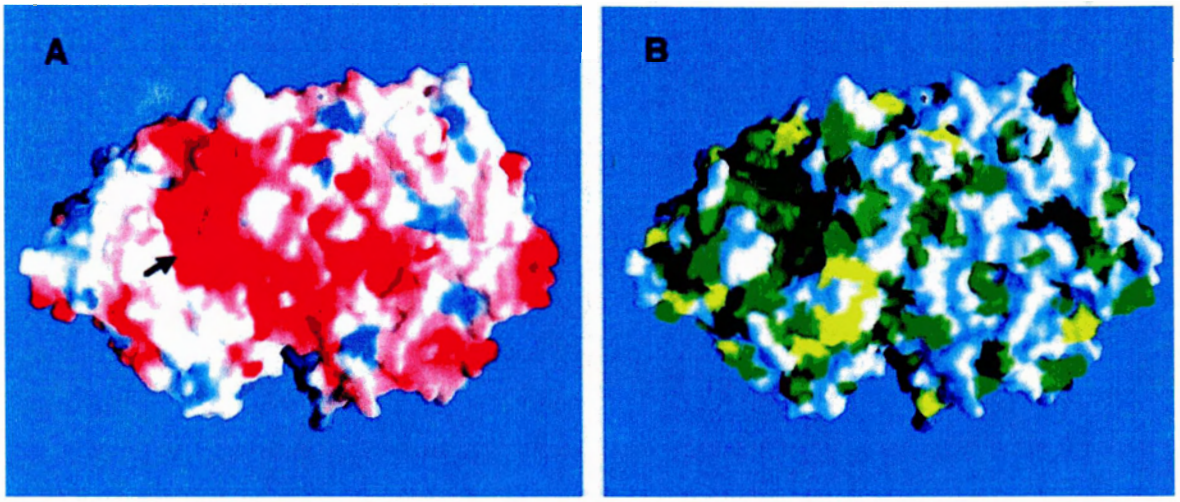


Figure 7.1. Molecular surface representation of dGMII. Arrow indicates the active site. (**A**) Colored for electrostatic potential (red for negative and blue for positive). (**B**) Colored for electrostatic homology with the sequence of human GMII (dark green for identical, light green for conserved, yellow for similar, and white for different). (Reproduced from reference 4. Copyright © 2001 European Molecular Biology Organization. Reprinted with permission from European Molecular Biology Organization)

The binding of inhibitors to this enzyme involves both hydrophobic and hydrogen bonding interactions. Amino acids with aromatic rings, for example Trp 95, Phe 206, or Tyr 727 are involved in hydrophobic interactions. The hydrocarbon rings of the inhibitor swainsonine (**1**) are stacked against these aromatic rings (Figure 7.2).[4]

Another very important feature is the presence of a Zn ion in the active site. This ion is coordinated to the OD1 oxygen of Asp 204 and Asp 92 and also the NE2 nitrogen of His 90 and His 471 (Figure 7.2).[4]

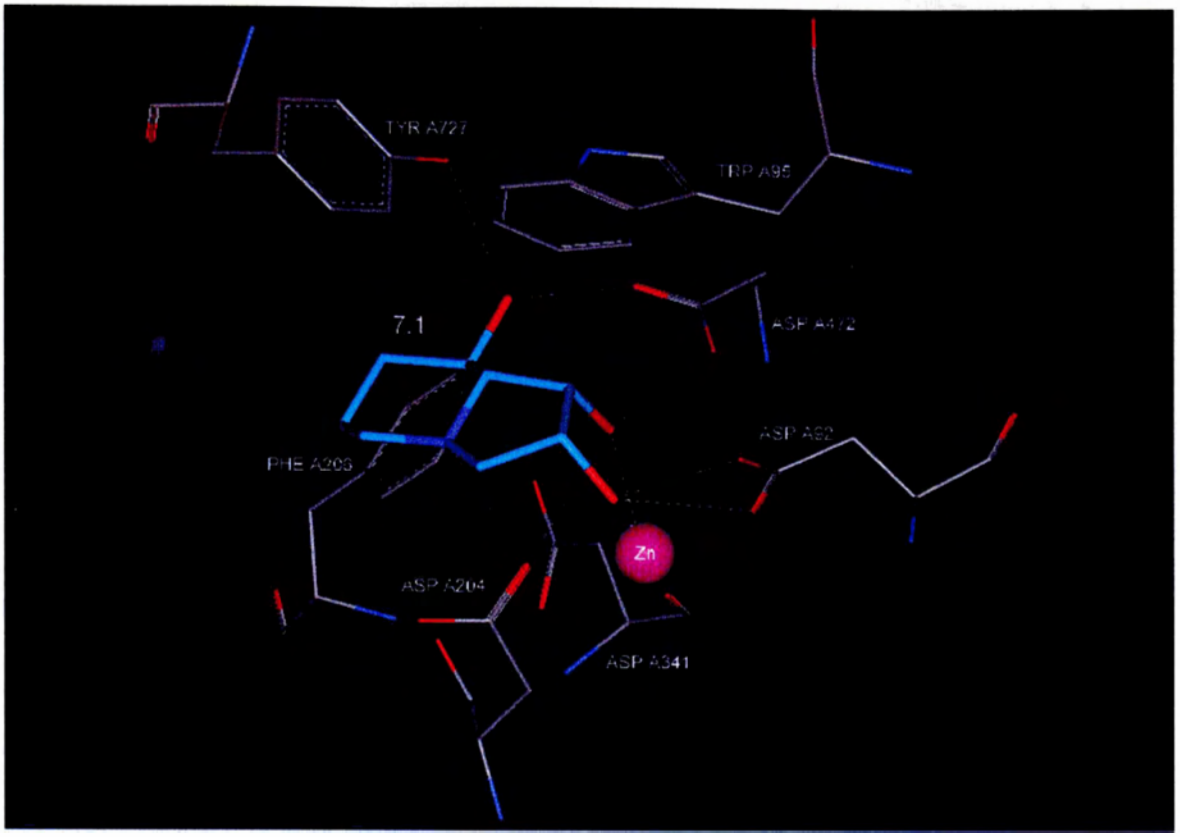
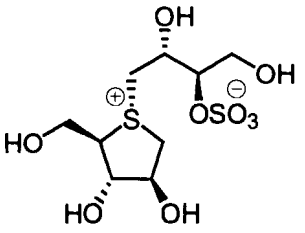


Figure 7.2. X-ray crystal structure of swainsonine (**7.1**) in the active site of dGMII.

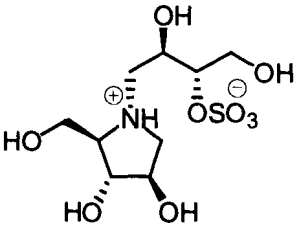
GMII operates by the classical double displacement mechanism of glycosidases, in which the product retains the anomeric configuration of the substrate at the active C1 position.[5] In this mechanism, the reaction passes through an oxacarbenium-like transition state into a glycosylated covalent intermediate with the nucleophilic side-chain of Asp 204, followed by a second similar transition state, leading to release of the product. Asp 341 or Asp 472, more likely the former because of the proper geometry, acts as the general acid/base (Figure 7.2).

7.2 Structural analysis of complexes of GMII with 7.2 – 7.5

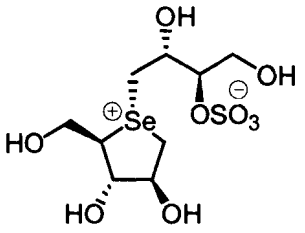
We now discuss our observations from the structural analysis of dGMII complexes with a series of our novel inhibitors in the active site. Examination of the interactions of the enzyme with a diastereomer of salacinol (**7.2**), ghavamiol (**7.3**), and selenium analogues **7.4**, and **7.5** explains the binding properties of these inhibitors to dGMII and provides information on further characteristics of the binding site that will be useful in the design of new inhibitors.



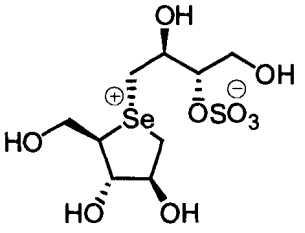
7.2



Ghavamiol **7.3**



7.4



Blintol **7.5**

Chart 7.2. Structures of compounds **7.2** – **7.5**.

7.3 Experimental

7.3.1 Synthesis

The syntheses of compounds **7.2**,[6,7] **7.3**,[8,9] **7.4**,[10] and **7.5**[10] used in this study have been reported previously.

7.3.2 Enzyme Inhibition Assays

These studies were performed by D. A. Kuntz. The following description was also provided by him. Inhibition of mannosidase activity was carried out in micro-titre plates in a final volume of 50 µL. Inhibitors were dissolved in water to a final concentration of 200 mM. The reaction mixture consisted of 25 µL of 10 mM *p*-nitrophenyl-α-D-mannopyranoside (PNP-mannose), 0.10 µL of 200 mM buffer and 10 µL of water or inhibitor. The buffer used was 2-N-morpholinoethanesulfonic acid (MES) pH 5.75 in the case of GMII, determined previously,[11] to be optimal for this enzyme. The reaction mixture was pre-warmed to 37 °C and 5 µL of mannosidase diluted in 10 mM Tris pH 8, 100 mM NaCl, was added to initiate the reaction. The amount of enzyme added was that which was necessary to keep the reaction in the linear range. In the case of the GMII this represented approximately 350 ng of protein for a 15 minute reaction. At the endpoint, the reaction was stopped using 50 µL of 0.5 M sodium carbonate. The absorbance of the reaction mixture was measured at 405 nm with 520 nm background correction on a micro-titre plate reader. 100% activity was the activity of the enzyme in the absence of any inhibitor. Activity remaining was calculated as a percentage of this uninhibited activity and the value of 50% inhibition (IC_{50}) was taken from plots of remaining activity vs. inhibitor concentration.

7.3.3 Crystallization

These studies were performed by D. A. Kuntz. The following description was also provided by him. Crystallization of dGMII was carried out using hanging drop vapor diffusion as described previously.[4] In all cases, crystals were less than 24 h old at the time

of crystal evaluation and freezing. In the cases of compounds **7.2** and **7.3**, co-crystallization was successful in producing large well-diffracting crystals. Co-crystallization trials of the seleno-containing analogues **7.4** and **7.5** only produced showers of small crystals. For the seleno-containing compounds and for salacinol, the crystals were grown in the absence of inhibitor and then soaked with inhibitor for approximately 30 min. Prior to freezing, the crystals were passed through drops containing 10, 15, 20 and 25% 2-methyl-2,4-pentanediol. These cryo-solutions all contained 10 mM inhibitor. Inclusion of inhibitor in the cryo-solution was essential for visualizing clear electron density of these weakly binding compounds. Subsequent to cryo-solution exposure, the crystals were mounted frozen in nylon CryoLoops (Hampton Research) directly in a liquid nitrogen cryostream.

7.3.4 Data Collection

Data were collected at 100 K either at the Ontario Cancer Institute on a MAR Research 2300 image plate detector mounted on a rotating anode generator with Cu target, operated at 50 kV and 100 mA with beam focusing using Osmic optics, or at the Cornell High Energy Synchrotron Source, beamline F1 using an ADSC Quantum 4 CCD detector in the rapid readout mode. Typically 300-400 frames of 0.5° oscillation were collected for each data set. Data reduction and scaling were carried out using Denzo and Scalepack, respectively.[12]

7.3.5 Refinement

The structures of the complexes were solved by molecular replacement. Briefly, rigid body refinement was carried out against the published structure of native dGMII with Tris and waters in the region of the active site removed. This was followed by simulated annealing to 3500 K, group B-factor refinement and individual B-factor refinement, prior to generation of electron density maps. At this initial stage R-factors were typically in the range of 22%, and the Fo-Fc density clearly showed the presence of bound compound and unassigned waters.

7.4 Results

We[6] and others[7] have recently reported the synthesis of compound **7.2**, and we have also reported the syntheses of the hitherto unknown nitrogen congener **7.3**[8] as well as the selenium analogues **7.4**,[10] and **7.5**[10] as potential glycosidase inhibitors.

Previous studies of this novel type of glycosidase inhibitors have focused on pancreatic-α-amylase[6,8-10] and intestinal glucosidases[13,14] as possible mammalian physiological targets for inhibition. In this work, we begin to address the salacinol-derived family of compounds as a starting point for a novel set of inhibitors of the Golgi α-mannosidase II. Besides affinity itself, the specificity of inhibitors for GMII in preference to related enzymes, in particular the lysosomal mannosidase, is an important issue in limiting the side effects of any potential anti-metastatic or anti-inflammatory therapeutic.

The results of the comparison of the inhibitory activities of this series of compounds with α-amylases and glucoamylase G2 have emphasized the importance of the heteroatom, S, Se or N, as well as the stereochemistry at the centres on the sulfate-

containing aliphatic arm in defining specificity. Therefore, these portions of the structures are presumed to make significant direct interactions with atoms in the enzyme active sites, or in defining critical structural or chemical characteristics of the ligands required for inhibition.

Examination of the structure of the complex of GMII with the sulfonium ion **7.2** indicates that the hydroxyl groups on the five membered ring in the sulfonium salt **7.2** interact with Asp 472, Arg 876, Asp 92 and Zn in the enzyme active site. The hydroxyl groups on the side chain interact with Tyr 269, Asp 341 and Asp 340. The sulfonium centre has a weak interaction (3.24 Å) with Asp 204 (Figure 7.3).

The Zn atom coordinates with Asp 204, Asp 92, His 90, His 471, and OH-2, probably in a T_5 square pyramidal geometry (Figure 7.4).

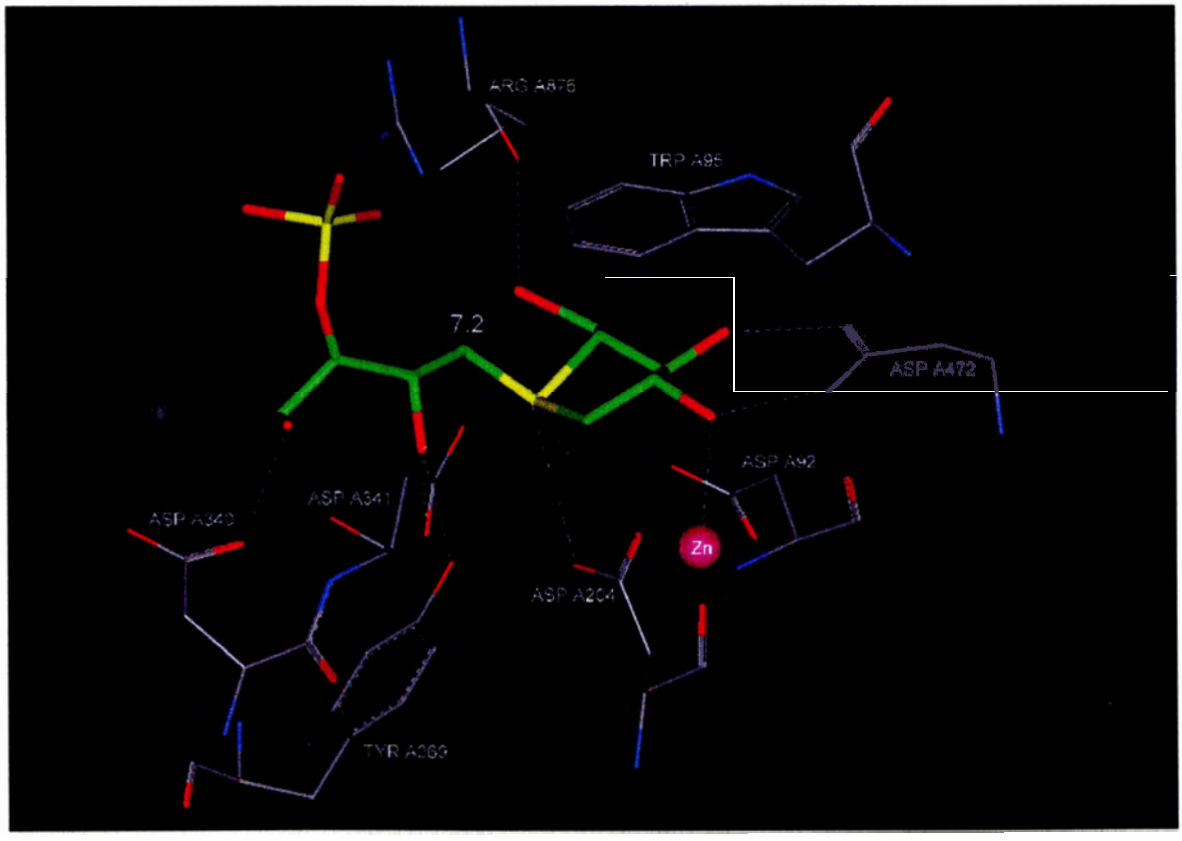


Figure 7.3. X-ray crystal structure of **7.2** in the active site of GMII.

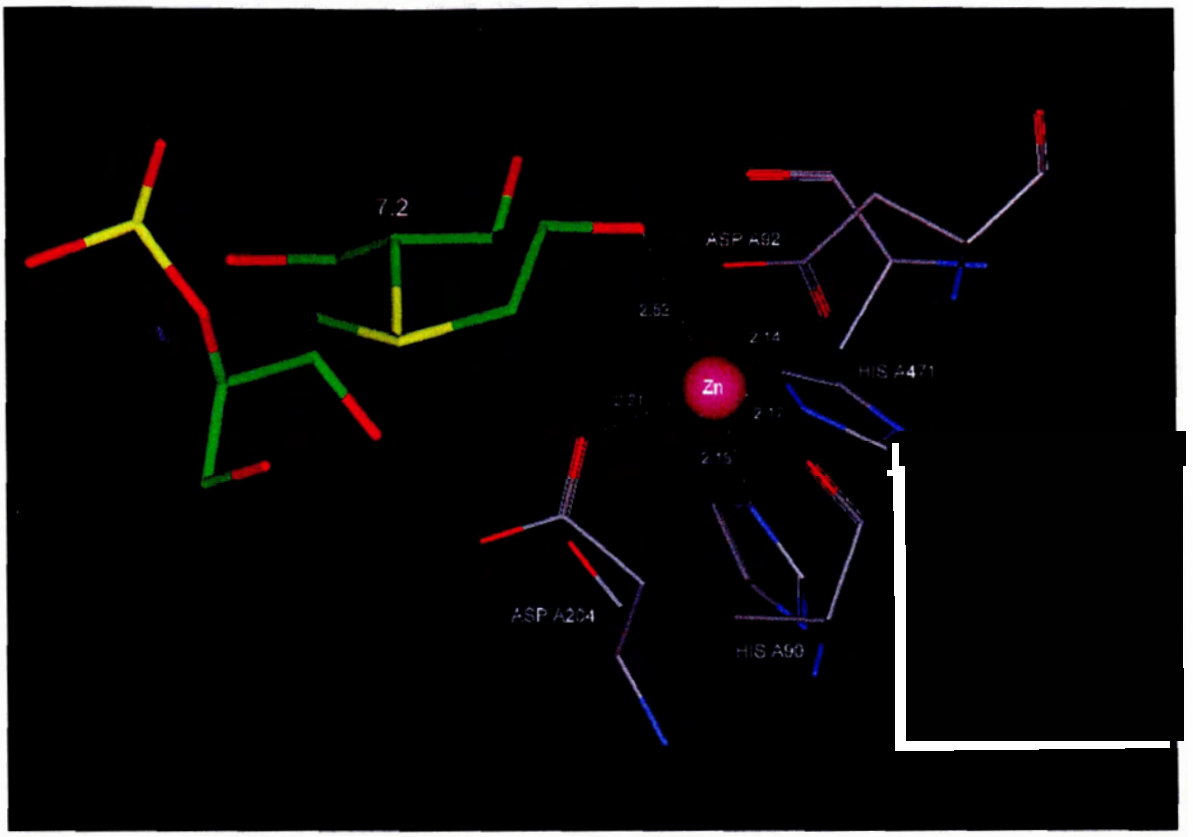


Figure 7.4. Coordination of Zn to **7.2**.

The corresponding structure of GMII with the nitrogen analogue **7.3** shows that the ring hydroxyl groups in **7.3** interact with Asp 472, Arg 876, Tyr 727, Asp 204, Asp 92 and Zn. The side chain, however, only interacts with Tyr 269 through OH-2' and is free to move easily. The ammonium centre has a strong interaction (3.00 Å) with Asp 204 (Figure 7.5).

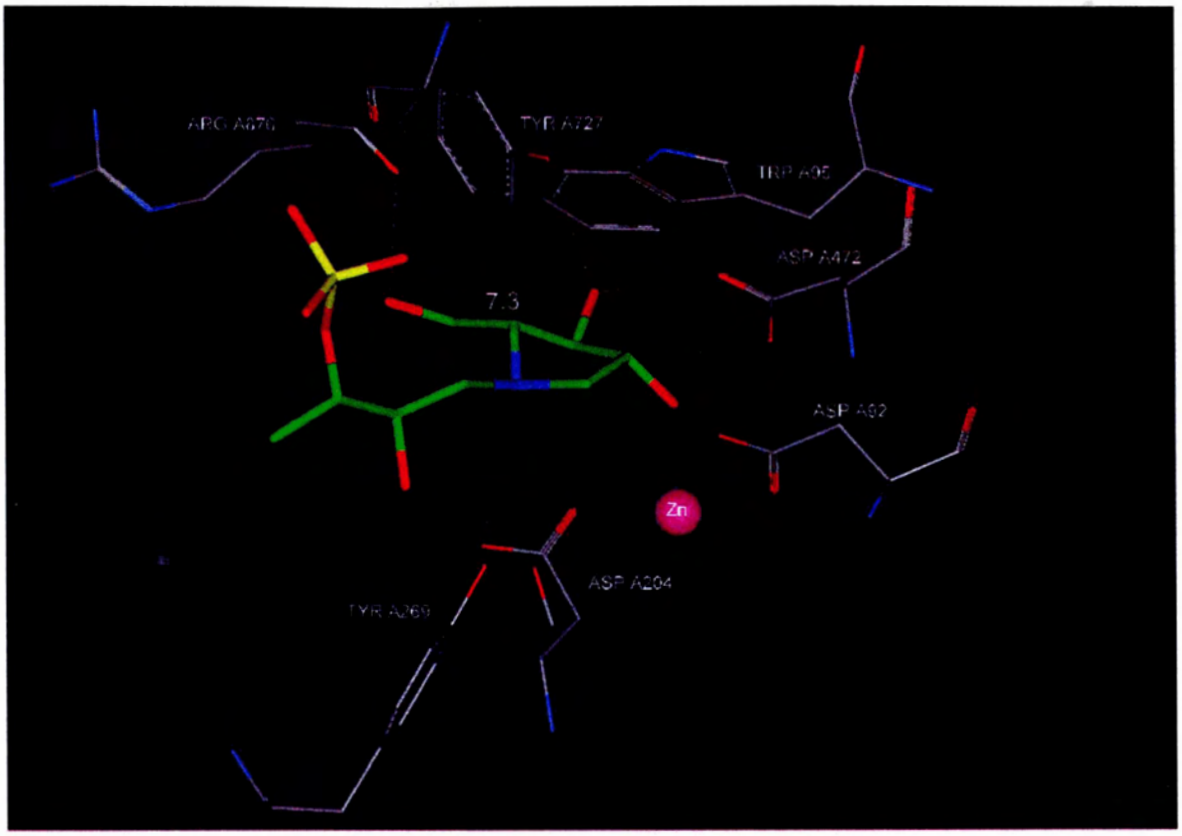


Figure 7.5. X-ray crystal structure of **7.3** in the active site of GMII.

The Zn atom coordinates to Asp 204, Asp 92, His 90, His 471, and has a strong interaction with OH-2 (2.20 Å). This T_5 geometry matches with a trigonal bi-pyramidal or square-based pyramidal geometry. However, this complex was superimposable on the Tris (**7.6**)-Zn complex, which has a square-based pyramidal geometry according to van den Elsen et al.[4] We believe, therefore, that our complex has the same square-based pyramidal geometry (Figure 7.6).

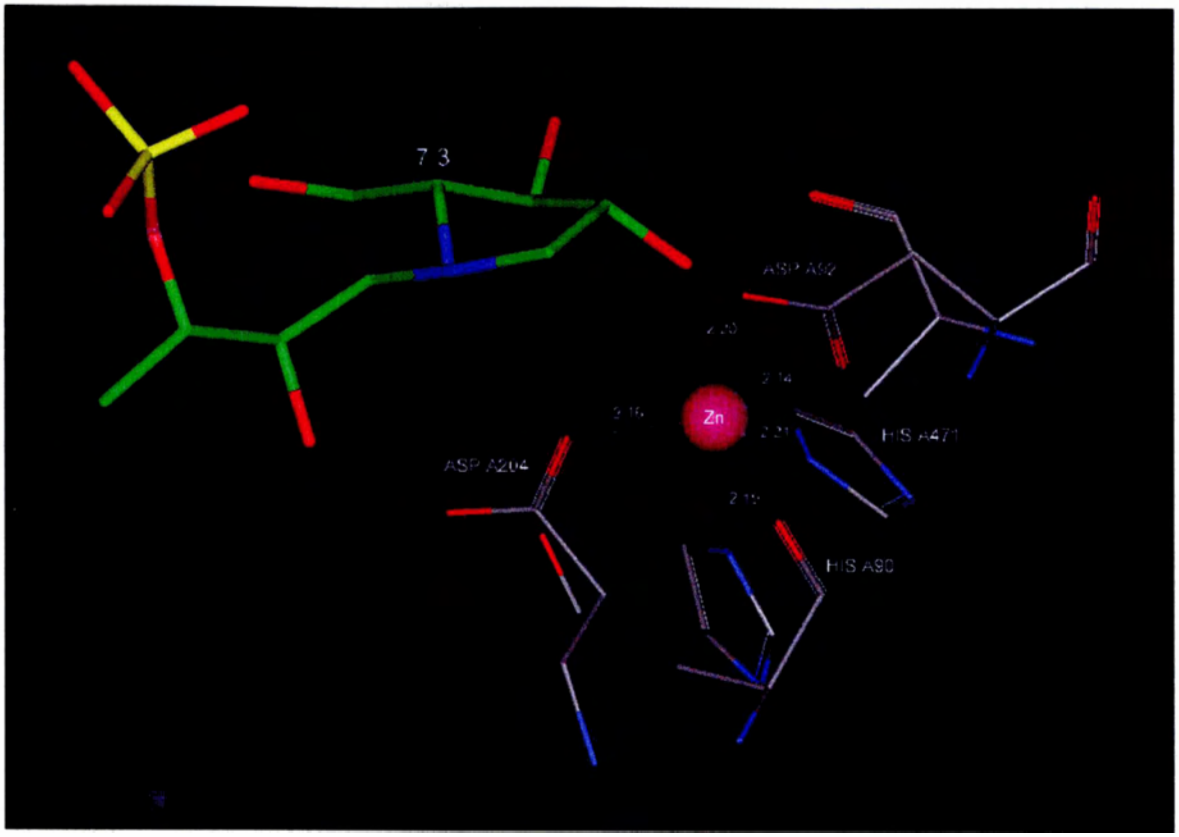


Figure 7.6. Coordination of Zn to **7.3**.

The structure of the complex of GMII with the selenium analogue **7.4**, indicates that the ring hydroxyl groups interact with Asp 472, Arg 876, Asp 92, and Zn. The side chain has only one significant interaction with Tyr 269 through OH-2' which is very similar to **7.3** (Figure 7.7). The Zn atom coordination with the OH-2 hydroxyl group on the other hand is weak (2.53 Å), which is very similar to the sulfur analogue, **7.2** and the geometry is square-based pyramidal, as shown in Figure 7.8.

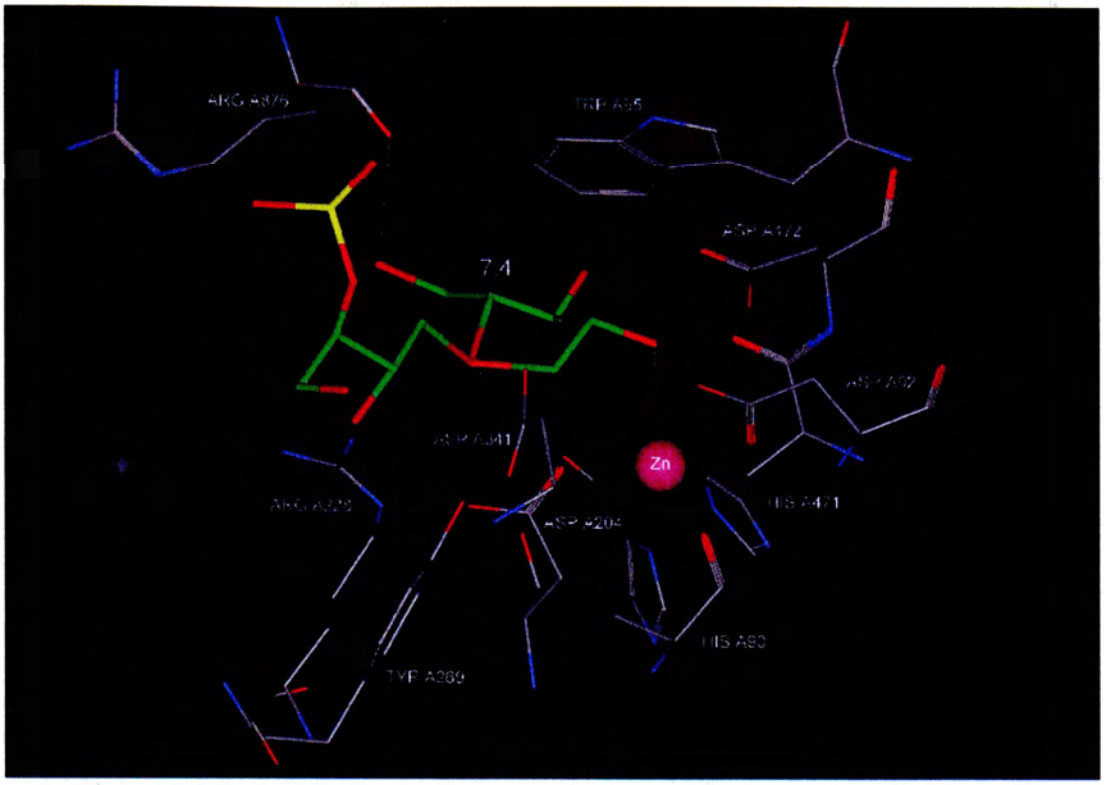


Figure 7.7. X-ray crystal structure of **7.4** in the active site of GMII.

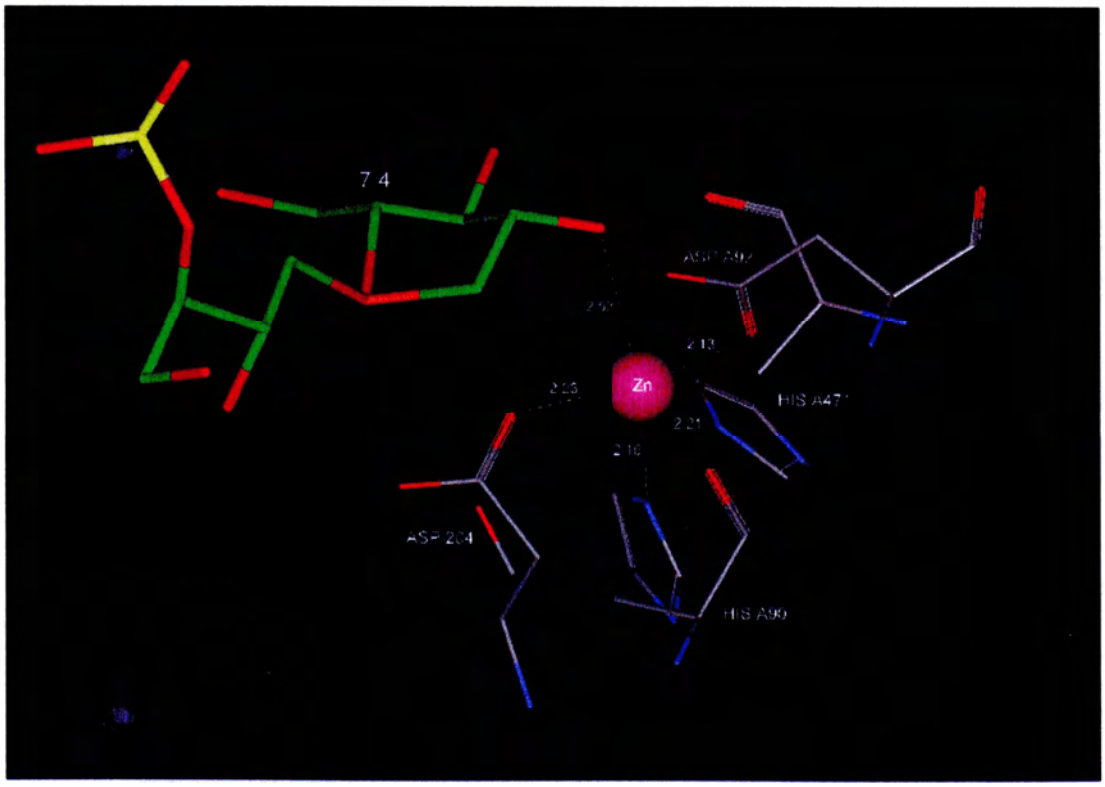


Figure 7.8. Coordination of Zn to **7.4**.

Finally, the structure of the complex of GMII with the diastereomeric selenium analogue **7.5** indicates that many residues interact with the ring hydroxyl groups such as Asp 472, Arg 876, Asp 204, Asp 92, Tyr 727, and Zn. The hydroxyl group on the side chain only interacts weakly with Tyr 269, through OH-2' (2.95 Å), which is similar to the other inhibitors (Figure 7.9).

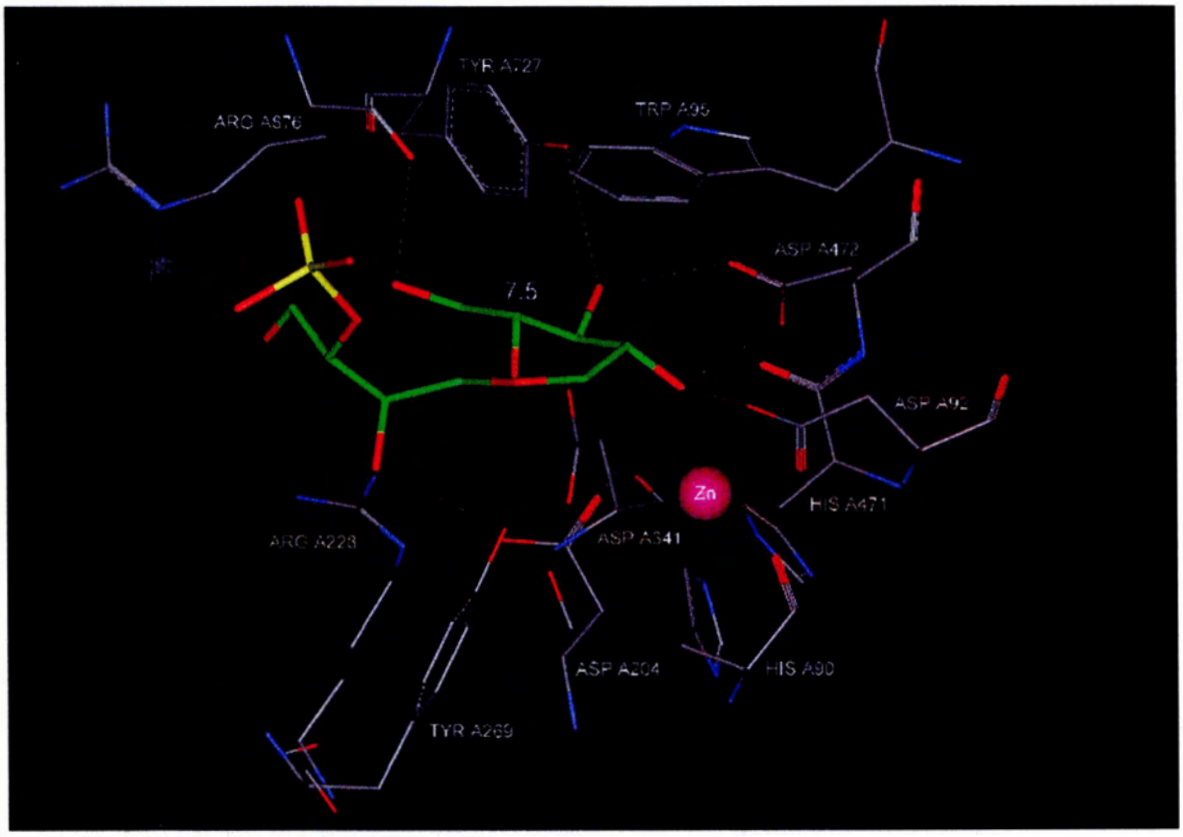


Figure 7.9. X-ray crystal structure of **7.5** in the active site of GMII.

The coordination with the Zn atom in this case is very similar to the nitrogen analogue where the interaction of Zn to the OH-2 group is strong (2.18 Å). Here too, the geometry is probably square-based pyramidal (Figure 7.10).

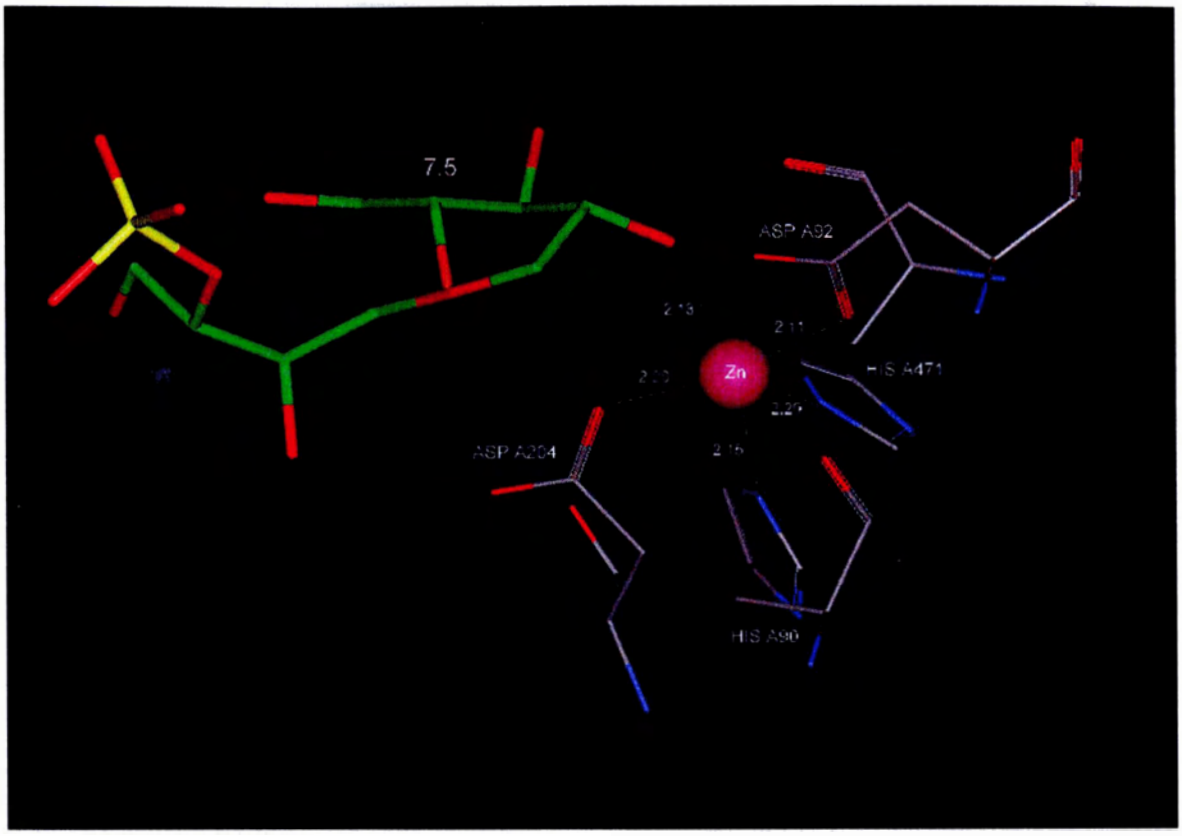
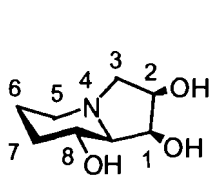


Figure 7.10. Coordination of Zn to **7.5**.

The compounds **7.2** – **7.5** are weak inhibitors of GMII, with IC_{50} values of about 7.5 mM. The comparison of the interactions of these inhibitors, with each other and also with swainsonine (**7.1**), a nM inhibitor of GMII is summarized in Table 7.1.

Table 7.1. Interactions of swainsonine (**7.1**) and compounds **7.2** – **7.5** in the active site of GMII.

Residue	Compound **7.2**		Compound **7.3**		Compound **7.4**		Compound **7.5**		swainsonine (**7.1**)	
Asp 204 (OD1)	S^+	3.24 Å	N^+	3.00 Å	Se^+	3.17 Å	Se^+	3.12 Å	N	2.88 Å
			OH-2	2.90 Å			OH-2	2.75 Å	OH-1	2.83 Å
Asp 204 (OD2)	S^+	3.64 Å	N^+	3.93 Å	Se^+	3.58 Å	Se^+	3.74 Å	N	3.55 Å
Tyr 269 (OH)	S^+	3.84 Å	N^+	4.14 Å	Se^+	3.46 Å	Se^+	3.53 Å	N	4.23 Å
	OH-2'	2.27 Å	OH-2'	2.58 Å	OH-2'	2.83 Å	OH-2'	2.95 Å		
Asp 472 (OD1)	OH-3	2.52 Å	OH-3	2.62 Å	OH-3	2.60 Å	OH-3	2.84 Å	OH-8	2.51 Å
Asp 472 (OD2)	OH-2	2.38 Å	OH-2	2.40 Å	OH-2	2.37 Å	OH-2	2.61 Å	OH-1	2.61 Å
Asp 92 (OD1)	----------		OH-2	2.95 Å	----------		----------		OH-2	2.91 Å
Asp 92 (OD2)	OH-2	2.84 Å	----------		OH-2	2.83 Å	OH-2	2.85Å	OH-2	2.43 Å
Arg 876	OH-5	2.72 Å	OH-5	2.65 Å	OH-5	2.82 Å	OH-5	2.69 Å	----------	
Tyr 727 (OH)	----------		OH-3	2.84 Å	----------		OH-3	2.94 Å	OH-8	2.69 Å
Zn	OH-2	2.52 Å	OH-2	2.20 Å	OH-2	2.53 Å	OH-2	2.18 Å	OH-1	2.31 Å
									OH-2	2.30 Å
IC_{50}	7.5 mM		7.5 mM		7.5 mM		7.5 mM		20 nM	



7.1

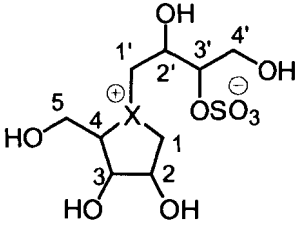


Chart 7.3. Numbering used for swainsonine (**7.1**) and salacinol analogues **7.2** – **7.5** in the above Table.

7.5 Discussion

The data in Table 7.1 indicate that the interactions of the charged heteroatoms with the residues (shown in red), especially Asp 204, is very important and the stronger the inhibitor, the stronger this interaction. In addition, the interaction of the inhibitor and the Zn atom is very important, and in the case of ghavamiol (**7.3**), blintol (**7.5**), and swainsonine (**7.1**), strong interactions with Zn are observed (Table 7.1).

The compounds are bound in an envelope conformation in the active site. The selenonium centre in blintol (**7.5**) is located in a similar position to the nitrogen atom in swainonine (**7.1**). The hydroxyl groups OH-2, and OH-3 overlap very favorably with the OH-1, and OH-8 hydroxyl groups of swainsonine (**7.1**) (Figure 7.11). The dominant electrostatic interactions in these inhibitors are with Asp 204 (Table 7.1). It is curious, therefore, that the selenium analogue **7.5** is only an inhibitor in the mM range.

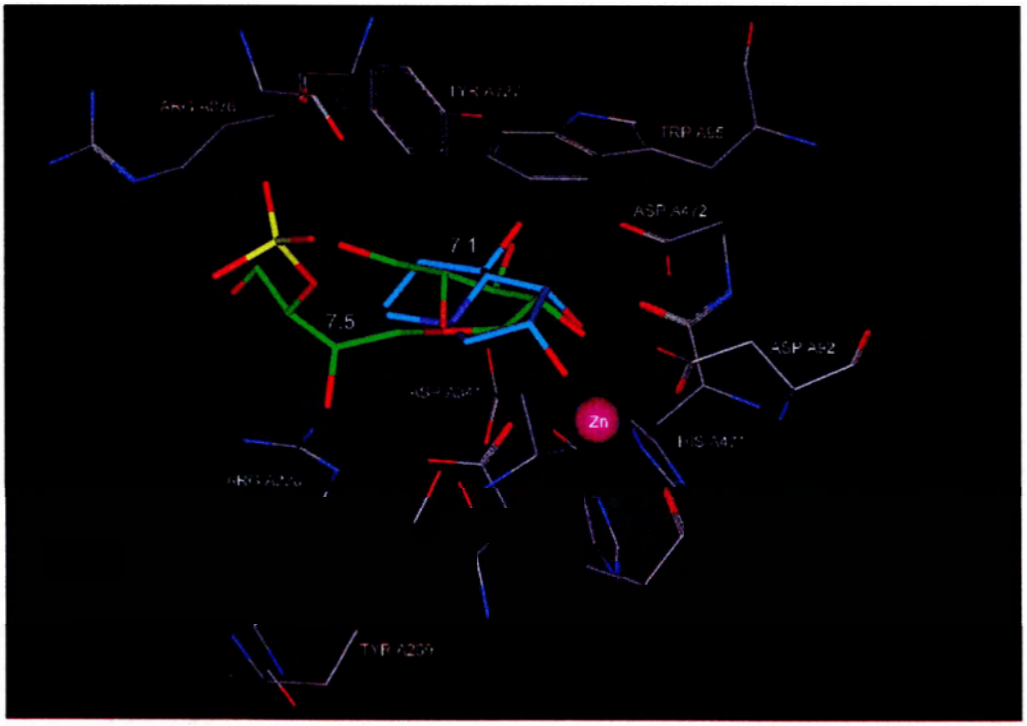
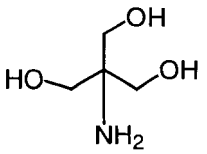


Figure 7.11. Superimposition of blintol (**7.5**) and swainsonine (**7.1**) in the active site of GMII.

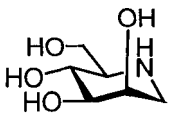
The observations reported in this study show that for GMII, interaction is mediated through the hydroxyl moieties on the five-membered ring, and variations in the sulfate arm have little effect on binding. This result is supported by inhibition data indicating that, for GMII, the stereochemistry of these hydroxyl groups is a crucial mediator of the interaction. Thus, stereoisomers of **7.2** and **7.3**, differing in stereochemistry at the centres on the 5-membered ring were not effective inhibitors.

In the case of swainsonine (**7.1**), the distance (2.88 Å) with Asp 204 is much less than that (3.12 Å) in **7.5**. It might also be of great significance that with all our inhibitors, the coordination with Zn is T_5, but in swainsonine (**7.1**) it is T_6 with both OH-1 and OH-2 binding very closely to the Zn atom.

Comparison of the structures of the complexes of GMII with compounds **7.1 – 7.5,** Tris (**7.6**),[2] and deoxymannojirimycin (DMNJ) (**7.7**),[2] indicate that in the case of Tris (**7.6**), and compounds **7.2 – 7.5** the Zn atom has a T_5 coordination and all these compounds show weak inhibitory activities (IC_{50} values in the mM range) against GMII; however, in DMNJ (**7.7**) which is a μM inhibitor, and swainsonine (**7.1**) (IC_{50} = 20 nM), the coordination to the Zn atom is T_6. Presumably, the T_6 coordination of the Zn atom is present in the transition state (TS) of the glycosidase mediated hydrolysis reaction. An effective TS mimic might therefore require T_6 coordination with Zn. It is my hypothesis that a high-affinity inhibitor should satisfy a T_6 coordination with the Zn atom in the enzyme active site, and provide good electrostatic contact with Asp 204.



7.6



7.7

Chart 7.4. Structures of Tris (**7.6**), and DMNJ (**7.7**).

In addition to the interactions with the Zn atom, the interactions with the other residues in the active site are also important accounting for the 1000 fold increase of inhibitory activity of swainsonine (**7.1**) compared to DMNJ (**7.7**).

It is interesting to note that the conformations of the five-membered rings in the complexes of **7.2 – 7.5** with GMII share characteristics with the presumed transition states in the catalytic mechanism for the glycosidase reaction. Specifically, the rings resemble the half-chair conformation of a putative oxacarbenium-ion transition state, and the transient positive charge of that state is mimicked by the permanent positive charge provided by the ammonium, selenonium, or sulfonium ions in these compounds. The interactions between these positive centres with the side-chain of Asp 204, as also observed in the swainsonine (**7.1**) complex, are indicative of the transition state stabilization capability of the enzyme. Thus, the mode of interactions that we report here for the salacinol family of compounds are consistent with an active site suited to stabilize a transition state predicted by the catalytic mechanism.

Our results give some useful insights into the properties of the GMII active site and possible approaches to new inhibitors. The close interaction of the hydroxyl groups with the active site Zn atom and the geometry of the coordinated Zn atom (see above) reinforce the importance of the Zn in binding substrate and transition state analogues. The zinc

coordination geometries T_4, T_5, and T_6 have been observed in proteins with frequencies of 48, 44, and 6%, respectively, for catalytic sites (Zn ion involved in catalysis), and 79, 6, and 12%, respectively, for structural sites (Zn plays a structural role).[15] In GMII the Zn atom is in the catalytic site and probably has a T_5 geometry in the absence of an inhibitor. A T_6 coordination geometry might be essential in the design of the next generation, high affinity inhibitor (see above).

Secondly, the lack of well-defined electron density for the side-chains of the salacinol-based compounds is likely an indication of their flexibility and weak interaction with the GMII binding site. This flexibility would also introduce an unfavorable conformational entropy of binding. An approach to modifying this family of compounds to generate higher affinity inhibitors would be to induce direct contacts between binding site residues and this side-chain by incorporating the hydroxyl and sulfate groups into more rigid, cyclic structures.

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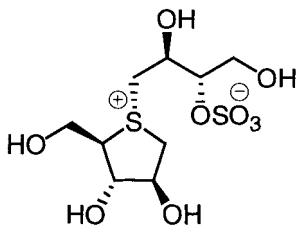
CHAPTER 8: GENERAL CONCLUSIONS

This thesis has described my studies of a novel class of glycosidase inhibitors.

8.1. Synthesis

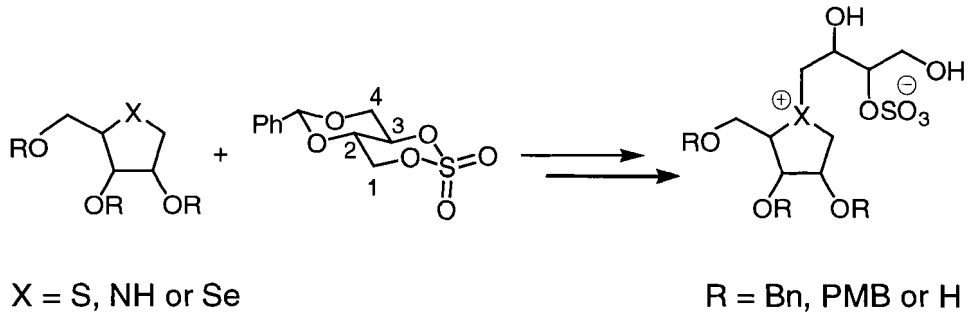
We synthesized the naturally occurring glycosidase inhibitor, salacinol (**8.1**), as a potential drug for the treatment of diabetes type II. We were also able to establish the absolute configuration of the natural product, and resolve the ambiguity about the exact structure of this compound. To further investigate the inhibition of glycosidase enzymes by this new class of inhibitor, and to assess structure-activity relationships, we also synthesized different stereoisomers of salacinol (**8.1**).



8.1

Chart 8.1. Structure of salacinol (**8.1**).

A general synthetic strategy was designed that also provided flexibility for the synthesis of analogues having other heteroatoms such as nitrogen or selenium in the ring, and different configurations at the stereogenic centres on the ring and the acyclic side chain (Scheme 8.1). The enantiomeric cyclic sulfates were designed to provide variation in the relative stereochemistry at the centres in the side chain (Chart 8.2).



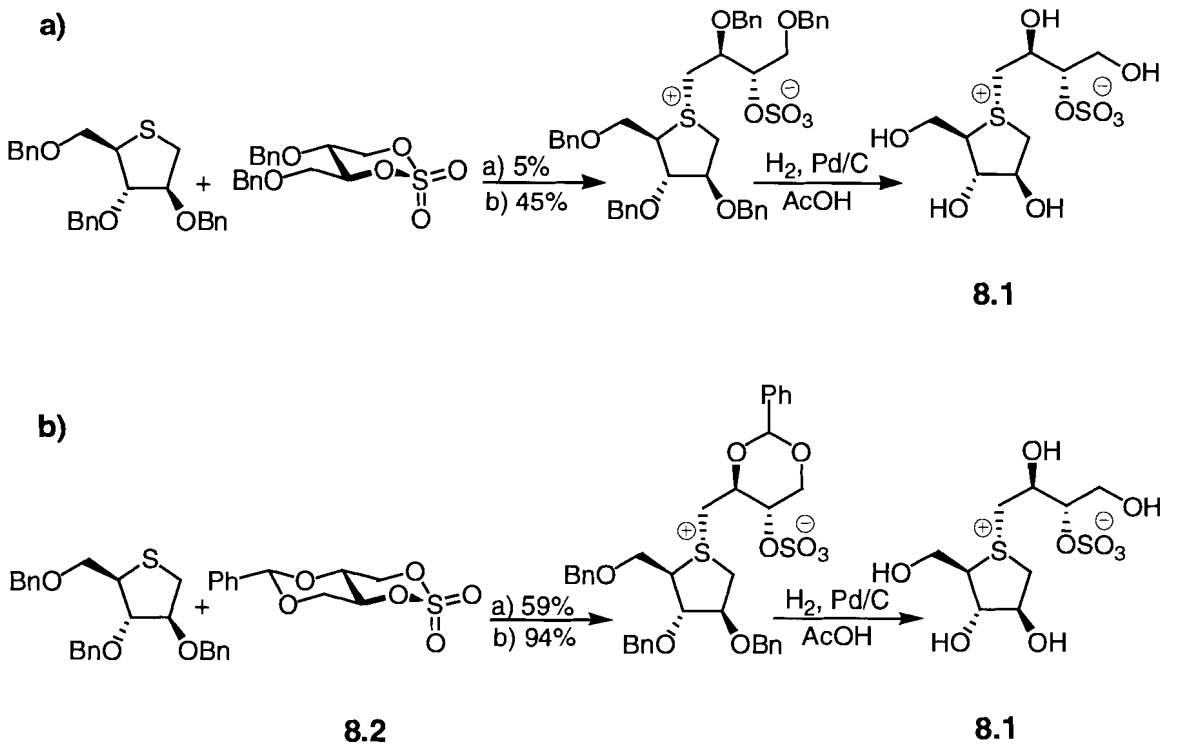
Scheme 8.1. General synthetic scheme.

8.2 **8.3**

Chart 8.2. Structures of the cyclic sulfates (**8.2**) and (**8.3**).

We have also investigated an alternative route for the synthesis of these compounds with a benzyl-protected cyclic sulfate, that avoided the use of expensive L-glucose as a starting material, but unfortunately, this cyclic sulfate was not very reactive (Scheme 8.2a).

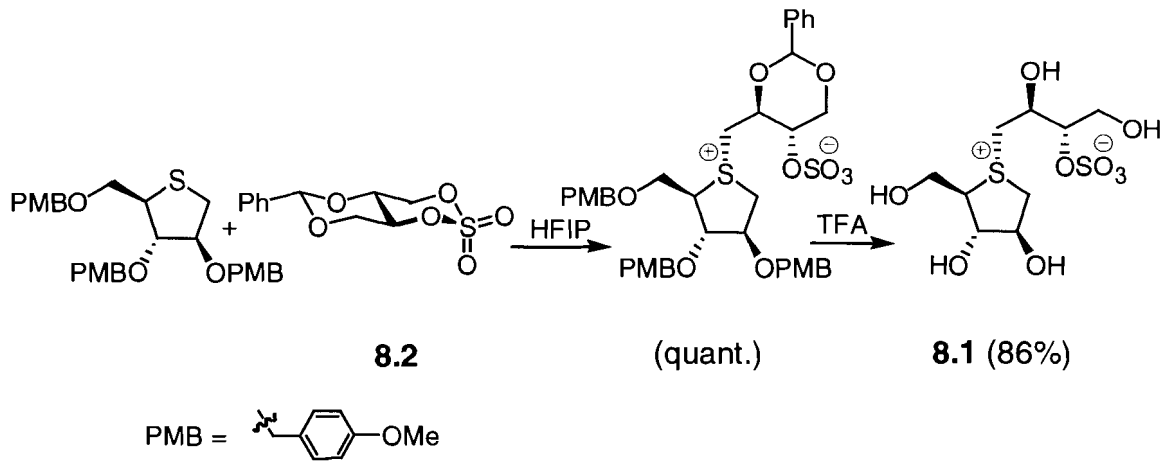
However the effects of the unusual solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were found to be spectacular in increasing the yields of the coupling reaction (Scheme 8.2a). The reaction of the benzylidene-protected cyclic sulfate in this solvent also proceeded in excellent yield (Scheme 8.2b).



a) Acetone, K_2CO_3, 75-80 °C
b) HFIP, K_2CO_3, 75-80 °C

Scheme 8.2. Syntheses of salacinol (**8.1**).

An optimized synthesis of salacinol (**8.1**) was finally achieved by the use of *p*-methoxybenzyl ether protecting groups instead of the benzyl ethers since facile deprotection was now achieved with trifluoroacetic acid instead of the problematic hydrogenolysis reaction (Scheme 8.3).



Scheme 8.3. Improved synthesis of salacinol (**8.1**).

8.2. Enzyme Inhibition

The inhibitory activities of the candidate glycosidase inhibitors were examined initially with different amylases and glucoamylase G2. Enzyme inhibition assays indicated that the type of heteroatom and stereochemistry at the different stereogenic centres of the candidate inhibitors play a significant role in discriminating between different glycosidase enzymes. It follows that alterations of these centres, based on an understanding of the atomic interactions between the compounds and their target enzymes, could be a powerful approach to the design of the next generation, high affinity inhibitors.

Inhibition of glycosidase enzymes involved in carbohydrate processing of glycoproteins has also been effective in the treatment of some other disorders such as metastatic cancer. Accordingly, the salacinol-derived family of compounds was examined as potential inhibitors of Golgi α-mannosidase II, a key enzyme in the *N*-glycoprotein processing pathway.

Of all the candidate inhibitors tested, only four were found to inhibit the enzyme in the mM range. The stereochemistry of the hydroxyl groups on the 5-membered ring was the

same in these derivatives. X-ray crystallographic analysis of the complexes of these four inhibitors in the active site of GMII showed that the interactions of the hydroxyl groups on the 5-membered ring, with groups on the protein were very important. The side chain on the other hand did not have strong interactions with the enzyme.

The most important interactions are probably those between the charged ammonium, selenonium, or sulfonium centre in the heterocyclic ring with the nucleophilic (basic) residue (Asp 204); this residue is the likely nucleophile in the glycosidase-catalyzed hydrolysis reaction. The interactions of the inhibitors with the Zn atom also appear to be of great importance.

I propose that the Zn atom has a T_6 coordination in the transition state (TS) of the glycosidase mediated hydrolysis reaction, and to mimic the TS, an effective inhibitor must be involved in T_6 coordination to the Zn atom. It is my hypothesis, therefore, that a high-affinity inhibitor should satisfy a T_6 coordination with the Zn atom in the enzyme active site, and provide good electrostatic contact with Asp 204.

In addition, since there is no important interaction between the enzyme and the side-chain in our inhibitors, an approach to modifying this family of compounds to generate higher affinity inhibitors would be to induce direct contacts between binding site residues and this side-chain by incorporating the hydroxyl and sulfate groups into more rigid, cyclic structures.