SYNTHESIS OF 2-SUBSTITUTED DERIVATIVES OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR, SALACINOL AND ITS NITROGEN ANALOGUES

by

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ABSTRACT

This thesis describes the synthesis of 2-amino- and amido- derivatives of the nitrogen analogue of the naturally occurring glycosidase inhibitor, salacinol, the attempted synthesis of 2-amino- and acetamido derivatives of salacinol itself, the synthesis of 2-fluorinated and 1,2-ene derivatives of salacinol, and the synthesis of a suitable precursor for elaboration of 4'-thionucleosides and 4'-thiooligonucleotides.

Glycosidases are involved in many biological processes such as cell-cell or cell-virus recognition, immune responses, cell growth, and viral and parasitic infections. The controlled inhibition of glycosidases has potential for the treatment of many diseases such as diabetes, viral infections, and cancer. The synthesis of potential inhibitors of the enzyme human maltase glucoamylase (MGA) that catalyzes the breakdown of glucose-containing oligosaccharides, and hexosaminidase enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glucopyranosides are described.

The compounds were intended to mimic the oxacarbenium ion transition state in the enzyme-catalyzed reactions. Seven 2-amino- or 2-amido- derivatives of the nitrogen analogues of salacinol were synthesized. Interestingly, alkylation reactions of the nitrogen atom were observed during hydrogenation of the 2-azido derivatives. Circumvention of this side reaction yielded the 2-substituted analogues which showed marginal activity (<33% at 250μM) against human *O*-GlcNAcase and *Vibrio cholerae* NagZ enzymes.

The attempted synthesis of 2-amido and 2-amino derivatives of salacinol itself is also described. These compounds undergo rearrangement reactions during or before their formation due to nucleophilic participation of the neighboring amino or acetamido functions, and result in opening of the heterocycle.

2-Deoxy-2-fluorosalacinol and 1,2-ene derivatives of salacinol were synthesized for structure activity studies with human maltase glucoamylase (MGA). The 2-deoxy-1,2-ene derivative of salacinol and 2-deoxy-2-fluorosalacinol inhibited recombinant human maltase glucoamylase with an IC_{50} values of 150 μM and a K_i value of 6 ± 1 μM, respectively.

Finally, the synthesis of 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose, a suitable precursor for the synthesis of 4'-thionucleosides is described. This compound was elaborated into 4'-thionucleosides and oligonucleotides by my collaborator, J. K. Watts.

Keywords:

Salacinol, inhibitors, cyclic sulfate, glycosidases, human maltase glucoamylase (MGA), hexosaminidase enzymes, sulfonium ions, *O*-GlcNAcase, NagZ enzymes, 4'-thionucleosides, 2-substituted derivatives of salacinol, nitrogen analogues of salacinol, zwitterionic structures.

DEDICATION

To my mother and my husband, with love.

ACKNOWLEDGEMENTS

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

I would like to thank my collaborators for assistance: Dr. D. J. Vocadlo, Dr. Keith A. Stubbs and Scott Yuzwa for enzyme assays, Dr. Ramakrishna G. Bhat for his assistance in the project described in chapter 2, Lyann Sim and Dr. David R. Rose for enzyme assays, and Jonathan K. Watts for collaboration on the project discussed in chapter 5.

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My thanks to my labmates, with whom it has been a pleasure to work, especially Rehana and Laleh for their help.

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Ac	acetyl
AcOH	acetic acid
aq	aqueous
Ar	aromatic
ADP	adenosine diphosphate
AMY1	barley α-amylase 1
ATP	adenosine triphosphate
Bn	benzyl
Bz	Benzoyl
bp	boiling point
br	broad
c	concentration
Calcd	calculated
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
dt	doublet of triplets
DMF	*N*,*N*-dimethylformamide
DNA	deoxyribonucleic acid
EtOAc	ethyl acetate
equiv	equivalent

FAB	fast atom bombardment
GAA	acid α-glucosidase
Glc	glucose
GlcNAc	*N*-acetylglucosamine
h	hour
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HPA	human pancreatic α-amylase
HRMS	high-resolution mass spectrometry
IC_{50}	concentration required to reduce binding by 50%
J	coupling constant
K_m	Michaelis constant
K_i	inhibition constant
m	multiplet
MALDI	matrix-assisted laser desorption ionization
TOF	time of flight
Man	mannose
Me	methyl
MeOH	methanol
MGA	maltase glucoamylase
mp	melting point
Nag Z	1,4-*N*-acetylglucosaminidase
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy

NIDD	non-insulin-dependent diabetes
O-GlcNAcase	*N*-acetyl-β-D-glucosaminidase
Ph	phenyl
PMB	*para*-methoxybenzyl
PMN	polymorphonuclear leukocytes
PPA	porcine pancreatic α-amylase
Pr	propyl
psi	pound/inch^2
RNA	ribonucleic acid
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
tert-	tertiary
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TS	transition state
UDP-Gal*f*	uridine 5′-diphosphate galactofuranose
UDP-Gal*p*	uridine 5′-diphosphate galactopyranose
VCNagZ	vibrio cholerae 1,4-*N*-acetylglucosaminidase

THESIS OVERVIEW

Chapter 1 presents an introduction to carbohydrates, glycosidase enzymes, and their mechanism of action. This is then followed by a discussion on the transition state of glycosidase-mediated hydrolysis reactions. Some examples of glycosidase inhibitors which belong to one of the categories of disaccharides, iminosugars, carbasugars, thiosugars, and sulfonium salts are then presented. The background on the naturally occurring sulfonium salt glycosidase inhibitor, salacinol, a core molecule in this research project, is then discussed.

Chapter 2 presents the work in the manuscript (Choubdar, N.; Bhat, R. G.; Stubbs, K. A.; Yuzwa, S.; Pinto, B. M. *Carbohydr. Res.* **2008**, *343*, 1766-1777) that describes the synthesis of seven 2-substituted nitrogen analogues of salacinol that were designed as potential inhibitors of *O*-GlcNAcase and NagZ enzymes. I performed all the experimental work and characterization of the compounds, with the exception of compound **2.16** that was synthesized by R. G. Bhat. K. A. Stubbs and S. Yuzwa performed the enzyme inhibition studies.

Chapter 3 presents the work in the manuscript (Choubdar, N.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 4671-4674) that describes the attempted synthesis of 2-azido and 2-amino derivatives of salacinol that were designed as potential glycosidase inhibitors. Some new rearrangement reactions were observed in this project that have potential for the mechanism of action of this class of compounds. I performed all the experimental work and the characterization of the compounds.

Chapter 4 presents the manuscript (Choubdar, N.; Sim, L.; Rose, D. R.; Pinto, B. M. *Carbohydr. Res.* **2008**, *343*, 951-956) that describes the synthesis of 2-fluorosalacinol and the 1,2-ene derivative of salacinol, as potential inhibitors of recombinant human maltase glucoamylase. I performed all the experimental work and characterization of the compounds. L. Sim and D. R. Rose performed the enzyme inhibition studies.

Chapter 5 presents portions of two manuscripts (Watts, J. K.; Sadalapure, K.; Choubdar, N.; Pinto, B. M. and Damha, M. J. *J. Org. Chem.* **2006**, *71*, 921-925; Watts, J. K.; Choubdar, N.; Sadalapure, K.; Robert, F.; Wahba, A. S.; Pelletier, J.; Pinto, B. M. and Damha, M. J. *Nucleic Acid Res.* **2007**, *35*, No.5, 1441-1451) that describe the synthesis of a suitable precursor of 4'-thionucleosides and 4'-thiooligonucleotides. I performed all the experimental work and characterization of the precursor molecule, 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose. Synthesis of the 4'-thionucleoside and its elaboration into 4'-thiooligonucleotides were performed by J. K. Watts and M. J. Damha. The conformational analysis was performed by B. M. Pinto and J. K. Watts.

Chapter 6 presents the general conclusion resulting from the work in this thesis.

CHAPTER 1. INTRODUCTION

1.1 Carbohydrates

During the nineteenth century Carbohydrates were called a family of compounds, the hydrates of carbon, with the general formula $C_n(H_2O)_n$. This definition has been modified to include new compounds that do not fit into this formula. Today, carbohydrates refer to polyhydroxy aldehydes, ketones, alcohols, acids, their simple derivatives, their heterocyclic analogues, and their polymers that have bonds of the acetal type.

Carbohydrates are the most abundant class of compounds of natural source. They are present and play different functions in plants and animals. Plants are the main source of carbohydrates. Carbohydrates are synthesized from plants using water and carbon dioxide in the photosynthesis process. Carbohydrates can be highly branched molecules. Their monomeric units may be connected to one another by many different linkage types. This complexity allows carbohydrates to provide almost unlimited variations in their structure.[1]

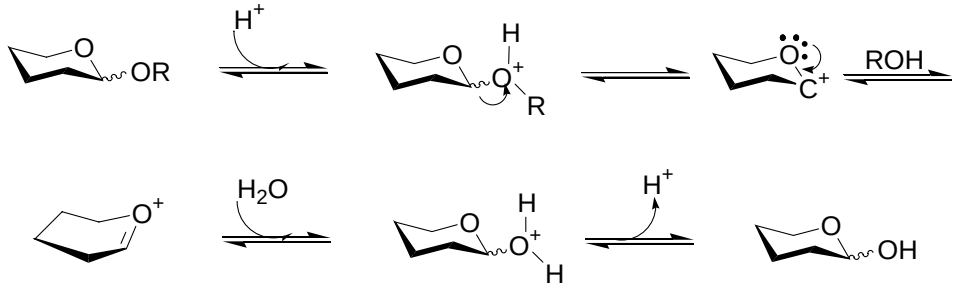
Carbohydrates are not only a very important group of molecules by themselves but also exist in many natural products. Streptomycin and puromycin are two natural antibiotics that contain aminosugars as their constituents. Nucleic acids that control the biosynthesis of proteins and transfer genetic information are another group of compounds that consist of carbohydrates. Adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP) which are two important molecules responsible for the energy balance of metabolic reactions also consist of carbohydrates. Glycoproteins that are

another important class of compounds are proteins that have appended carbohydrate residues.

Carbohydrates can be classified into two main groups, simple and complex. Simple carbohydrates are molecules that only contain carbohydrates in their structure. Complex carbohydrates are molecules in which carbohydrates are covalently bound to other molecules such as lipids, proteins, and aglycons. Many natural products fall into this category such as complex glycosides, carbohydrate antibiotics, nucleic acids, glycoproteins, and glycolipids.[2]

1.2 Glycosidases

Glycosidases are enzymes that catalyze the hydrolysis of glycosidic bonds and convert polysaccharides or oligosaccharides to monosaccharides (Scheme 1.1). Glycosidases are involved in the catabolism of glycoconjugates and glycoproteins and the biosynthesis of oligosaccharides.[3] Disruption in the function and regulation of glycosidases can lead to diseases such as diabetes.



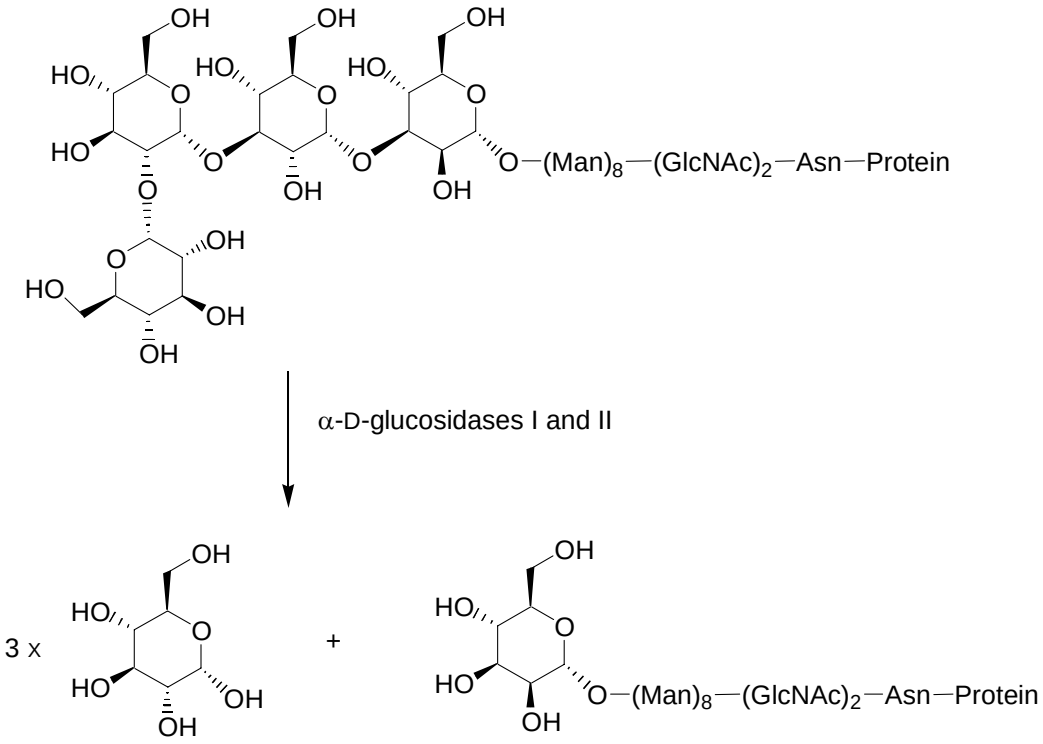
Scheme 1.1. Glycoside bond hydrolysis.

In type II diabetes (non-insulin-dependent, NIDD), insulin secretion can be normal but cells are not very sensitive to the insulin and glucose uptake is compromised. In the treatment of type II diabetes, control of blood glucose levels is critical. One strategy is to delay digestion of ingested carbohydrates, thereby lowering postprandial blood glucose concentration.[4] This can be achieved by inhibiting the activity of pancreatic α-amylase, which mediates the hydrolysis of complex starches to oligosaccharides, and/or membrane-bound intestinal α-glucosidases, which hydrolyze these oligosaccharides to glucose in the small intestine.[4,5]

Glycosidases are responsible for the cleavage of glycosidic bonds based on position, number, or configuration of the sugar molecule. There are two types of glycosidases: α-glycosidases and β-glycosidases. α-Glycosidases are able to catalyze cleavage of the terminal α-glucose, whereas, β-glycosidases can cleave terminal β-glucose. The transition-state structure for the substrates of these enzymes is proposed to have pseudoaxial orientation of the C-O bond and a skew conformation, suggesting that the main difference between α- and β- glucosidases are concerned with positioning of the catalytic nucleophile and the catalytic proton donor, represented by two carboxylic acids units.[6-8]

The glucosidase enzymes I and II are involved in the key steps of trimming of *N*-linked oligosaccharides by cleaving Glc(1-2)Glc and Glc(1-3)Glc linkages, respectively, and liberating the three glucose terminal residues of the $Glc_3Man_9GlcNAc_2$ oligosaccharide on the glycoprotein (Scheme 1.2).[9] Subsequently, this immature glycoprotein is processed by the concomitant action of glycosidases and transferases to give specific glycoconjugates, which play fundamental roles in biological processes such

as the immune response, intercellular recognition, cellular differentiation, the stability and solubility of proteins, and in inflammation and cancer.



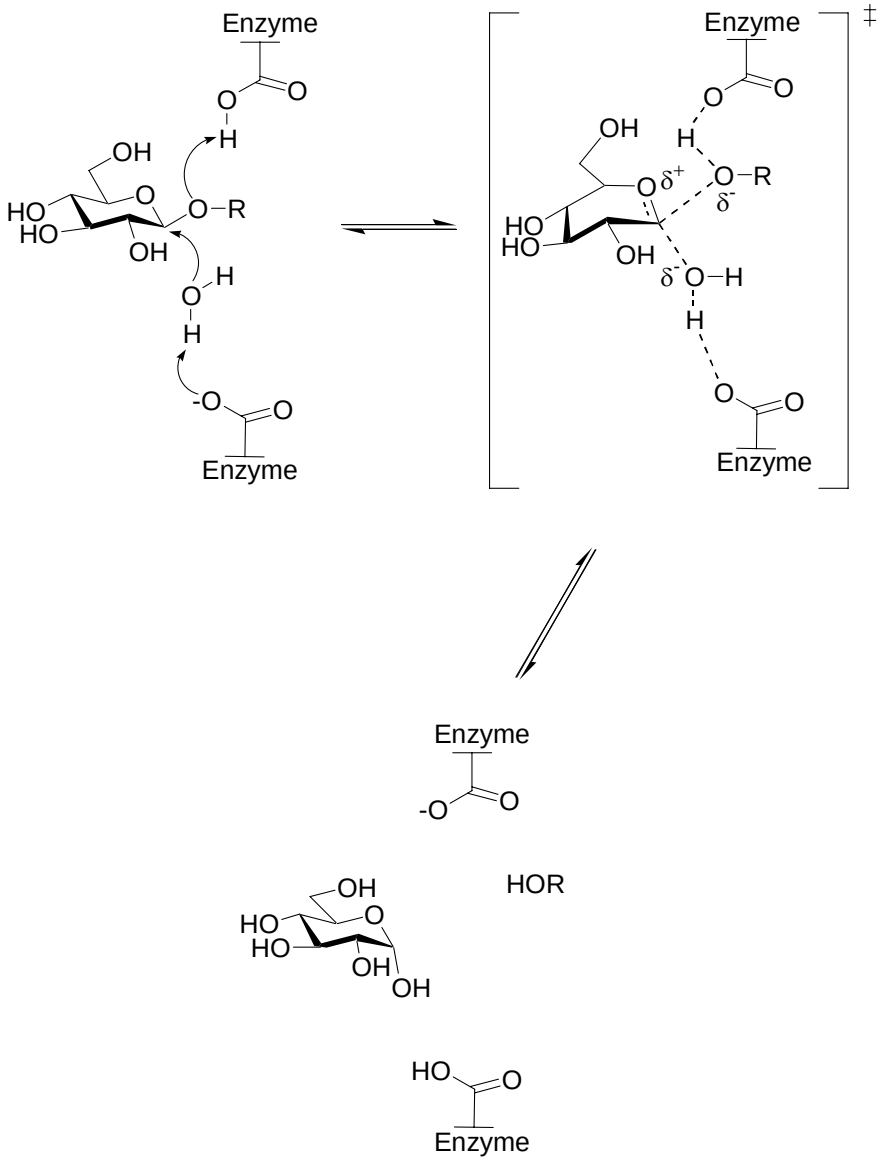
Scheme 1.2. Processing of the oligosaccharide ($Glc_3Man_9GlcNAc_2$) portion of the immature *N*-glycoprotein by the action of glucosidases I and II.

1.3 Mechanism of action of glycosidases

Hydrolysis of glycosidic bonds can occur via one of two possible pathways: inversion or retention of anomeric configuration. This will require at least two different mechanisms for glycosidases enzymes. Koshland[10] proposed two types of mechanism for glycosidase hydrolysis in 1953: inverting glycosidases and retaining glycosidases.[11]

1.3.1 Inverting glycosidases

In this mechanism, the two carboxyl groups serve as general acid and general base catalysts and are suitably placed (10.5 Å apart on average) to allow the substrate and a water molecule to bind between them (Scheme 1.3). 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. Reaction occurs via a single-displacement mechanism involving an oxacarbenium ion-like transition state.[10,11]

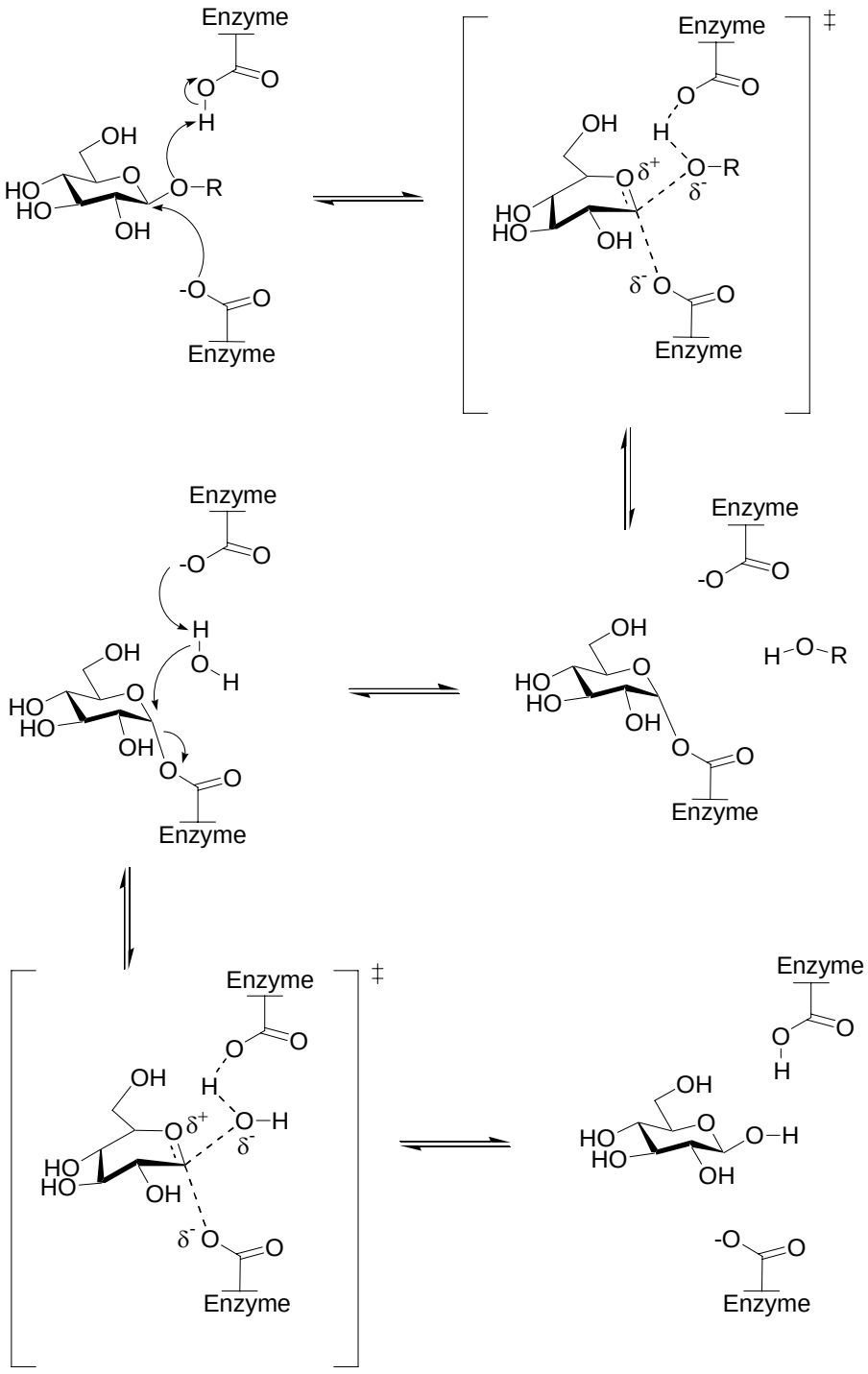


Scheme 1.3. Mechanism of inverting glycosidases.

1.3.2 Retaining glycosidases

Carbonyl groups in retaining glycosidases are only 5.5 Å apart, consistent with a double displacement mechanism involving a covalent glycosyl-enzyme intermediate. In this mechanism, one of the carboxyl groups functions as a general acid catalyst and protonates the glycosidic oxygen concomitant with bond cleavage. The other carboxyl

group acts as a nucleophile and forms a covalent glycosyl-enzyme intermediate. In the second step, the carboxylate side chain deprotonates the incoming water molecule, which attacks at the anomeric center and displaces the active site electrophile. Both steps occur via transition states that have substantial oxacarbenium ion-like character (Scheme 1.4).



Scheme 1.4. Mechanism of retaining glycosidases.

1.4 Transition state in glycosidase-mediated reactions

Both inverting and retaining glycosidase enzymes mechanisms proceed through a transition state (TS) with substantial oxacarbenium ion-like character (Chart. 1.1).[12,13] The positive charge on the anomeric carbon is shared with the endocyclic oxygen. This requires a double bond character to be present in the ring between C-1 and the endocyclic oxygen (Chart 1.1).

Formation of the oxacarbenium ion has been supported from the measurements of α-secondary deuterium kinetic isotope effects.[14-17] α-Secondary deuterium kinetic isotope effects provide insight into changes in hybridization at the sugar anomeric center between the ground and the transition states. Evidence for sp^2 hybridization, therefore, oxacarbenium ion character in the transition state of inverting and retaining glycosidases has been obtained.[18-21]

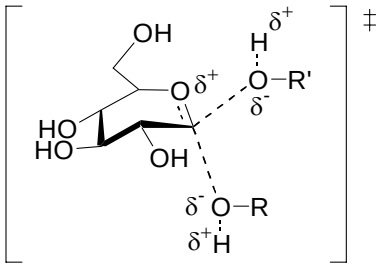
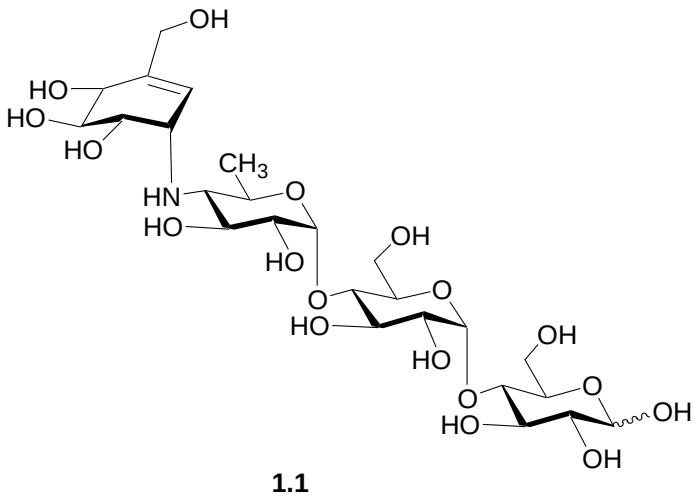


Chart 1.1. Transition state proposed for the glycosidase-mediated hydrolysis reaction.

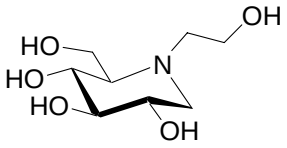
1.5 Glycosidase Inhibitors

Inhibition of glycosidases can have profound effects on quality control, maturation, transport, and secretion of glycoproteins and can alter cell-cell or cell-virus recognition processes. This principle is the basis for the potential use of glycosidase inhibitors for treatment of cancer,[22-25] HIV [26-29] and certain genetic disorders.[23]

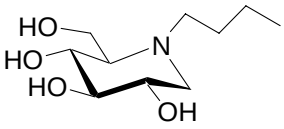
Currently, acarbose (**1.1**) and miglitol (**1.2**) are used therapeutically for the treatment of type II diabetes (non-insulin-dependent) (Chart 1.2). These drugs reduce postprandial hyperglycemia by interfering with the digestion of dietary carbohydrates. *N*-Butyl-1-deoxynojirimycin (**1.3**), which is also a glucosidase inhibitor, is used for the treatment of Gaucher's disease (a disease related to disturbed lysosomal storage) (Chart 1.2).[3]



1.1



1.2



1.3

Chart 1.2. Acarbose (**1.1**), miglitol (**1.2**), and *N*-Butyl-1-deoxynojirimycin (**1.3**).

In enzyme-catalyzed reactions, the transition state is stabilized by hydrophobic and electrostatic interactions. These interactions might be present at the ground state too, but they are optimized at the transition state so that the activation energy for the enzyme-catalyzed reactions is less than the activation energy of the non-catalyzed reaction.[30] For glycosidases, it is believed that this stabilization is so large that the transition state is bound with a dissociation constant of up to 10^{-20} M, which means that it is potentially possible to create transition-state analogue-inhibitors with K_i values of similar magnitude. However, no inhibitor has yet been discovered that comes even close to this value, due to the impossible task of precisely mimicking the transition state.[8] The most powerful inhibitor synthesized to date is a femtomolar transition-state analogue inhibitor of 5′-methylthioadenosine/S-adenosyl homocysteine nucleosidase designed by the Schramm group.[31] A strong inhibitor of these enzymes can be a stable molecule that is able to mimic both charge and shape of the oxacarbenium ion-like structure formed in the transition state of the glycosidase-mediated hydrolysis reactions (Chart 1.1).

In the design of transition-state analogues of glycosidase hydrolysis, much of the focus has been on mimicking the assumed geometry of the transition state or its assumed charge. Most designs focusing on geometry have tried to create inhibitors that are in a half chair conformation (**1.4**), which is commonly accepted to be the conformation of the transition state. The transition state structure of the sugar is distorted relative to the ground state, either in the form of a half chair (**1.4**), a boat (**1.5**) or a skew boat (**1.6**) in six-membered ring sugars (Chart 1.3).[18]

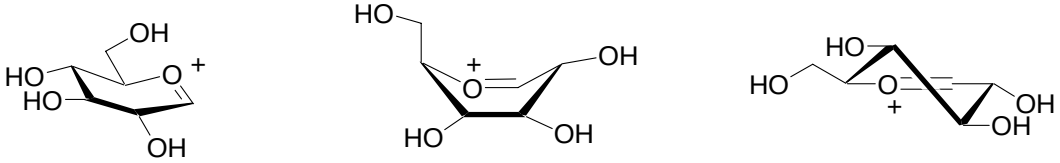


Chart 1.3. Half chair (**1.4**), boat (**1.5**), and skew boat (**1.6**) conformations of D-glucopyranose.

The transition state of the five-membered ring sugars has to be distorted in the form of an envelope either ^{3}E (**1.7**) or E_3 (**1.8**) (Chart 1.4).[18]

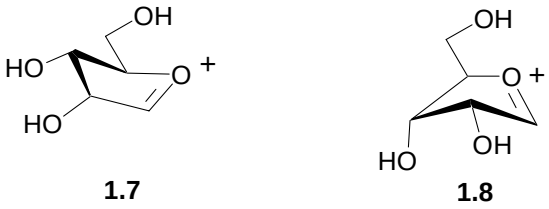
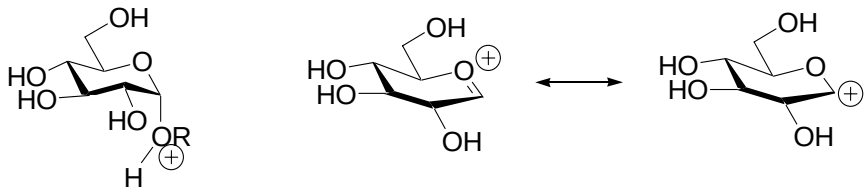


Chart 1.4. Envelope conformations of five-membered ring sugars (**1.7**) and (**1.8**).

Designs focusing on charge have mimicked charge build up in a number of different positions. Some inhibitors mimic charge by protonation at the exocyclic oxygen (**1.9**), some have charge build up at the endocyclic oxygen (**1.10**) along with its resonance form (**1.11**) (Chart 1.5).[8]



1.9 **1.10** **1.11**

Chart 1.5. Charge build up at different positions of the sugar ring in glycosidase-mediated hydrolysis reactions.

The classification of inhibitors as transition-state analogues has led to the definition of (a) strong inhibition, (b) slow inhibition, (c) dependence of $1/K_i$ on *p*H, and (d) specificity.

1.5.1 Strong inhibition

The strength of an inhibitor expressed by its binding constant $1/K_i$ in relation to substrate affinity is a prime criterion to judge transition state resemblance. A comparison of K_s/K_i with k_{cat}/k_{uncat} is not possible for many enzymes because k_{uncat} is too slow to be measured.[32] As K_i values < 10^{-9} M are quite rare and values for K_s range from 10^{-2} to 10^{-5} M, $K_s/K_i \approx 10^7$ for the most favorable cases which is, by orders of magnitude, only half-way to the rate acceleration factor k_{cat}/k_{uncat}. The models of transition state are only rough approximations and details such as bond lengths, bond angles, and orientation of the hydroxyl groups and the leaving aglycon are not precisely specified. As transition state stabilization requires many weak interactions with complementary groups of the active site, a perfect match will be difficult to achieve with a stable compound.[21]

1.5.2 Slow inhibition

Enzymes generally bind to the substrate in a ground state conformation and then undergo a conformational change in order to bind the transition state better. Transition-state analogue inhibitors might only bind to the conformationally changed enzymes. Slow inhibition is related to the time needed for the enzyme to change conformation. Transition state-like inhibitors bind slowly because only a minute fraction of the enzyme is present in the latter conformation, thus greatly reducing successful encounters. [21]

1.5.3 Dependence of $1/K_i$ on pH

This criterion has been used to disqualify inhibitors whose binding profile as a function of pH did not correlate with that of catalysis. This will occur when the inhibitor changes charge under the observed pH range, and though this may be regarded as an imperfection, the inhibitor could still be a transition-state analogue at one pH and not another.[33]

1.5.4 Specificity

The catalytic specificity of glycosidases expressed by k_{cat}/K_m depends, in addition to the stabilization of the transition state by the catalytic machinery, on specific interactions with the hydroxyl groups and, to a lesser extent, on the structural and electronic features of the aglycon. Transition state resemblance of a given type of inhibitor should thus be manifested in the extent to which variations of the sugar structure are reflected in its inhibitory strength with enzymes acting on substrates having the same

aglycon linked to different sugars. Thus, $1/K_i$ should depend in the same way on sugar structure as k_{cat}/K_m.[21]

$\ln K_i$ values of an inhibitor toward a series of enzymes or enzyme mutants are compared with the $\ln(k_{cat}/K_M)$ values of a substrate toward those enzymes. Alternatively, the same comparison is performed with one enzyme but with a series of similar structural analogues of an inhibitor and the substrates. In both cases, a linear relationship should be obtained if the inhibitor or inhibitor-type is a transition state analogue of the enzymatic reaction of that substrate provided that $k_{cat}/K_M \approx k_{cat}/K_S$, which is a good assumption. It should be noted that a linear relationship will only reveal similarity between the transition state and the inhibitor with regard to what has been varied.[34] Parts of the inhibitor that are not varied may not resemble the transition state. [35]

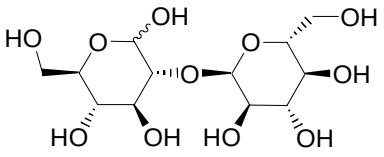
1.6 Disaccharides as glycosidase inhibitors

The isolation of kojibiose (**1.12**) and nigerose (**1.13**), two disaccharides with glycosidase inhibitory activity, opened a new perspective for the development of novel drugs for the treatment of HIV infections. Kojibiose (**1.12**), a disaccharide containing an α-(1→2) glycosidic bond, is an α-D-glucosidase I inhibitor[36] which was isolated in 1953 from sake extracts and also from its primary source, koji, a product related to rice fermentation by *Aspergillus oryzae* (Chart 1.6).[37]

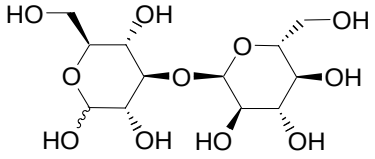
Nigerose (**1.13**), a disaccharide containing an α-(1→3) linkage, is an α-D-glucosidase II inhibitor which is produced by acid hydrolysis of amylopectin.[38,39] Nigerose and nigerosylmaltooligosaccharides have been shown to influence the immune

function and quality of life in healthy elderly people when administered as a supplemental syrup in food (Chart 1.6).[40]

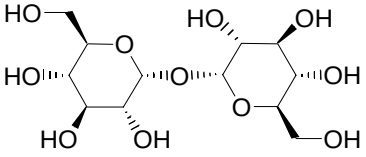
D-(+)-Trehalose (**1.14**), a disaccharide containing an α-(1→1) linkage of two glucose units, is another example that shows α-glucosidase inhibitory activity. Trehalose has been isolated from the extracts of *Mormodica charantia* seeds and of *Grifola frondosa* fruits. Trehalose is employed in the preparation of foods and manufacture of cosmetics and was recently suggested as a drug to be used in the treatment of osteoporosis (Chart 1.6).[41]



1.12



1.13



1.14

Chart 1.6. Examples of disaccharides as glycosidase inhibitors (**1.12**-**1.14**).

1.7 Iminosugars

Iminosugars are structural analogues of sugars in which the ring oxygen atom is replaced by a nitrogen atom. In recent years, there has been much interest in synthetic

and naturally occurring iminosugars. The biological properties of iminosugars arise because they interfere with the function of carbohydrate processing enzymes.[42]

Iminosugars isolated from plants or microorganisms are considered potential glycosidase inhibitors due to their ability to mimic the conformation and/or charge of the oxacarbenium ion-like transition state (Chart 1.1) of pyranosidic or furanosidic units of natural glycosidase substrates.[11,21]

Iminosugars are moderately basic and in the active site of the enzyme they are protonated; therefore, 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.

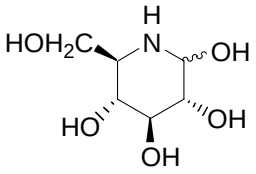
Naturally occurring sugar mimics with a nitrogen atom in the ring are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines, and nortropanes.[43]

1.7.1 Piperidines

Nojirimycin (**1.15**) was the first endocyclic nitrogen analogue of glucose that was discovered in 1966. Nojirimycin,[44,45] which was first isolated from several strains of *Bacillus*, *Streptomyces*, and mulberry tree leaves as an antibiotic, was shown to be a potent inhibitor of α- and β-glucosidases. Reduction of nojirimycin results in 1-deoxynojirimycin (**1.16**), a more stable compound, which is a glucosidase inhibitor (Chart 1.7).[46]

A series of different glycosylated deoxynojirimycins have been synthesized but among them, compound (**1.17**) showed potent inhibition against α-glucosidases. Compound (**1.17**) was approved for clinical testing for the treatment of postprandial hyperglycemia in patients with diabetes (Chart 1.7).[47]

Isofagomine (**1.18**), another example of iminosugars, is about 440 times more potent than 1-deoxynojirimycin toward β-glucosidases but is only a moderate inhibitor of α-glucosidases (Chart 1.7).[48]



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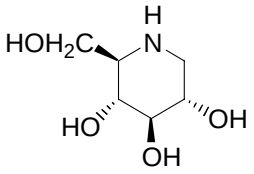

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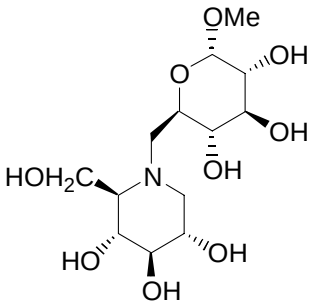

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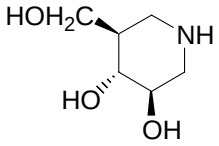


1.18

Chart 1.7. Nojirimycin (**1.15**), 1-deoxynojirimycin (**1.16**), glycosylated deoxynojirimycin (**1.17**), isofagomine (**1.18**).

1.7.2 Pyrrolidines

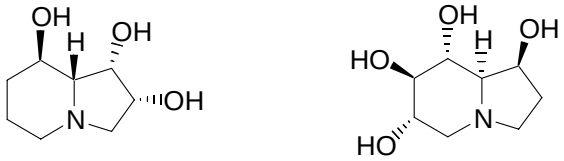
One example of a potent α-glucosidase inhibitor of the pyrrolidine class is 1,4-dideoxy-1,4-imino-D-arabinitol (**1.19**) which was first found in the fruits of *Angylocalyx boutiqueanus*.[49] The D-lyxitol analogue (**1.20**) is an α-galactosidase inhibitor (Chart 1.8).[50]

1.19 **1.20**

Chart 1.8. 1,4-Dideoxy-1,4-imino-D-arabinitol (**1.19**) and the D-lyxitol analogue (**1.20**).

1.7.3 Indolizidines

Fusion of the piperidine and pyrrolidine rings results in the indolizidine class of alkaloids. Swainsonine (**1.21**), one example of this group of compounds, was isolated from *Swansona canescens*, *Astragalus lentiginosus*, and *Ipomoea carnea*, and is the first alkaloid with strong inhibition (nM) toward α-mannosidase II.[51] Castanospermine (**1.22**), another indolizidine, extracted initially from seeds of *Castanospermum australe*, is a μM inhibitor of α-glucosidases (Chart 1.9).[52]

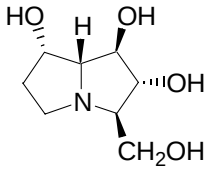


1.21 **1.22**

Chart 1.9. Swainsonine (**1.21**), castanospermine (**1.22**).

1.7.4 Pyrrolizidines

Pyrrolizidines are heterocycles formed from two fused five membered rings. Australine (**1.23**), from *Castanospermum australe*[53], is constituted of two fused pyrrolidine rings with a common nitrogen atom at the junction. This compound is a competitive inhibitor of α-glucosidase and amyloglucosidase (Chart 1.10).[54]

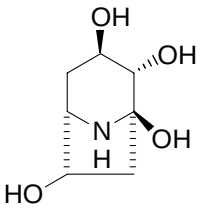


1.23

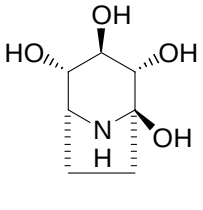
Chart 1.10. Australine (**1.23**).

1.7.5 Nortropanes

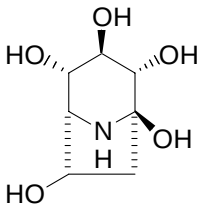
Nortropanes (**1.24-1.26**) are another class of natural products that are bicyclic and are potential β-glucosidase inhibitors. This class of compounds is called calystegins and is isolated from plants such as *Calystegia sepium*, *Ipomoea carnea*, and *Physalis alkekengi* var. *francheti*. Compounds **1.24** and **1.25** are α,β-glucosidase inhibitors and compound **1.26** has shown strong β-glucosidase inhibitory activity (Chart 1.11).[55]



1.24



1.25



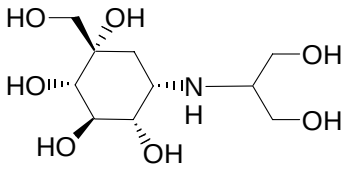
1.26

Chart 1.11. Examples of nortropanes (**1.24-1.26**).

1.8 Carbasugars and pseudoaminosugars

Carbasugars are compounds in which the oxygen atom of the pyranose ring is substituted by a carbon. However, the greater interest in this class of compounds is in pseudoaminosugars in which the oxygen atom of the pyranose ring is substituted by carbon, the oxygen atom of the glycosidic bond is replaced by nitrogen and the overall configuration is similar to D-glucose. One of the most important representatives of this class of compounds is acarbose (**1.1,** Chart 1.2)[56] which has been effective in the micro molar range against bacterial, fungal, and plant glycosidases.

Voglibose (**1.27**) is another example of this class of compounds that has been used for the treatment of diabetes in Japan since 1994. It was shown that this compound is 20 to 30 times more potent than acarbose (**1.1**) in α-glucosidase inhibitory activity,[57] with fewer side effects (Chart 1.12).[58]



1.27

Chart 1.12. Voglibose (**1.27**).

1.9 Thiosugars

Thiosugars are a class of carbohydrates in which the oxygen atom in the sugar ring is replaced by a sulfur atom. 5-Thio-D-xylopyranose (**1.28**) is the first thiosugar that was synthesized in 1961 by three groups independently (Chart 1.13).[59-61] 5-Thio-D-

glucopyranose (**1.29**) [62] was synthesized in 1962, and was shown to be an inhibitor of insulin release (Chart 1.13).[63]

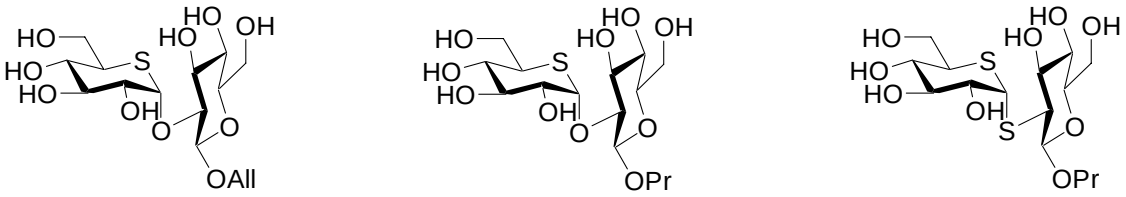
1.28 **1.29**

Chart 1.13. 5-Thio-D-xylopyranose (**1.28**) and 5-thio-D-glucopyranose (**1.29**).

Thiosugars have become very important targets because of their potential glycosidase inhibitory activities, and there are many reviews on the synthesis and biological activities of these compounds.[3]

Replacement of the ring oxygen with sulfur has been studied in our group. This substitution results in compounds with some inhibitory activity.

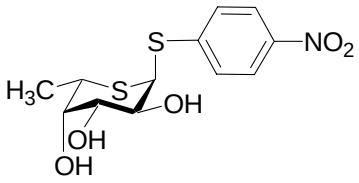
The synthesis of kojibioside analogues **1.30** and **1.31** was achieved in our laboratory.[64,65] Compound **1.30** is a poor inhibitor of glucosidase II but a competitive inhibitor of glucosidase I with K_i = 2.0 mM. Compound **1.31** is a poor inhibitor of glucosidase I but a competitive inhibitor of glucosidase II with K_i = 1.0 mM.[66] The dithio analogue **1.32** was also synthesized in our laboratory (Chart 1.14).[67]



1.30 **1.31** **1.32**

Chart 1.14. Kojibioside analogues (**1.30**-**1.32**).

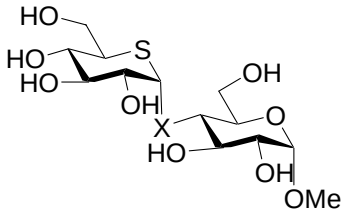
Hashimoto et al., synthesized a series of fucopyranosyl disaccharides.[68,69] The best inhibition was obtained with *p*-nitrophenyl-1,5-dithio-α-L-fucopyranoside (**1.33**) with K_i = 3.3 μM (Chart 1.15).



1.33

Chart 1.15. *p*-Nitrophenyl-1,5-dithio-α-L-fucopyranoside (**1.33**).

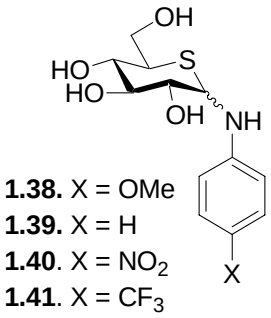
Another study in our laboratory involved the synthesis of heteroanalogues of methyl maltoside containing sulfur in the nonreducing ring with oxygen, sulfur, selenium or nitrogen atoms in the interglycosidic linkage (**1.34**-**1.37**). The K_i values of compounds **1.34**-**1.36** are in the range of 0.8 to 2.0 mM, and K_i of compound **1.37** is 4 ± 0.3 μM (Chart 1.16).[70,71]



1.34. X = O
1.35. X = S
1.36. X = Se
1.37. X = NH

Chart 1.16. Heteroanalogues of methyl maltoside (**1.34**-**1.37**).

A series of 5-thio-D-glucopyranosylamines (**1.38-1.41**) were synthesized in our group. These compounds are inhibitors of α-glucosidases with mM activity (Chart 1.17).[72]



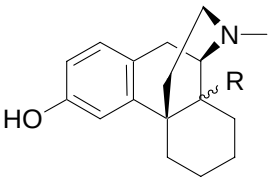
1.38. X = OMe
1.39. X = H
1.40. X = NO_2
1.41. X = CF_3

Chart 1.17. 5-Thio-D-glucopyranosylamines (**1.38-1.41**).

Thiosugars containing a sulfur atom in the ring have been classified as substrate analogues rather than transition state analogues, due to the inability of the sulfur atom to be protonated similar to nitrogen analogues; hence, thiosugars do not mimic the positive charge of the TS of glycosidases.

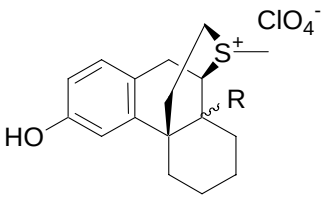
1.10 Sulfonium salts and their glycosidase inhibitory activities

Belleau et al. synthesized sulfonium-ion analogues of levorphanol (**1.42**) and isolevorphanol (**1.43**), *S*-methyl sulforphanol (**1.44**), and *S*-methyl isosulforphanol (**1.45**) respectively as the first example of the sulfur analogues of morphinans (Chart 1.18).[73]



1.42. R = β-H
1.43. R = α-H

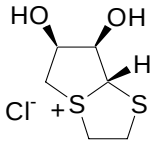
Morphinans



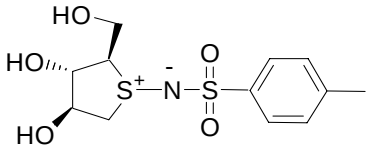
1.44. R = β-H
1.45. R = α-H

Chart 1.18. Levorphanol (**1.42**), isolevorphanol (**1.43**), *S*-methyl sulforphanol (**1.44**), *S*-methyl isosulforphanol (**1.45**).

In addition, the synthesis of various sulfonium salts such as the bicyclic compound **1.46**[74] and compound **1.47**[75] have been reported, and these compounds have been tested for their glycosidase inhibitory activities (Chart 1.19). Compound **1.46** is an inhibitor of α-mannosidases (76% at pH 6.5 and 48% at pH 4).[74]



1.46

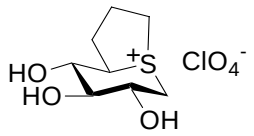


1.47

Chart 1.19. Pyrrolizidine analogue **1.46** and iminothiosugar **1.47**.

The synthesis of a bridgehead sulfonium salt analogue of the indolizidine alkaloid castanospermine (**1.48**) as well as its conformational study was achieved in our laboratory, as a model to test the theory that formation of a sulfonium salt carrying a

permanent positive charge might be advantageous in providing the necessary electrostatic interactions between the inhibitor and the active site carboxylate residues (Chart 1.20).[76,77]

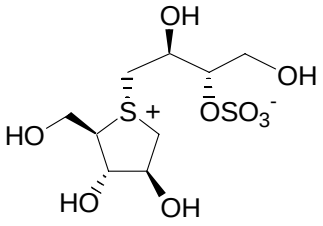


1.48

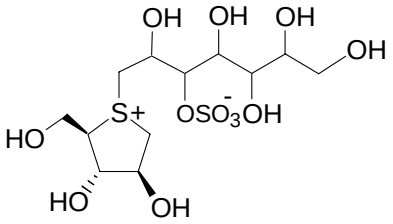
Chart 1.20. Sulfonium salt analogue of castanospermine (**1.48**).

1.10.1 Salacinol

The hypothesis of having a sulfonium ion that functions as a glycosidase inhibitor was validated by a report of the isolation of the two naturally occurring glycosidase inhibitors salacinol (**1.49**)[78] and kotalanol (**1.50**)[79] from extracts of the roots and stems of *Salacia reticulata*, known as 'Kotalahimbutu' in Singhalese, a large woody climbing plant widespread in Sri Lanka and South India (Chart 1.21). Aqueous extracts of this plant have been traditionally used in Indian medicine for treating type II diabetes. Ancient medicine advised that a person suffering from type II diabetes should drink water that is stored overnight in mugs carved from *Salacia reticulata*.[78-80] It was demonstrated that salacinol (**1.49**) and kotalanol (**1.50**) are two active components of the extracts of this plant.



1.49



1.50

Chart 1.21. Salacinol (**1.49**) and kotalanol (**1.50**).

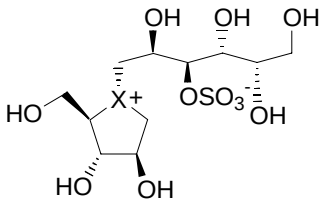
Salacinol and kotalanol consist of a common zwitterionic sulfonium-sulfate structure that comprises a 1,4-anhydro-4-thio-D-arabinitol core and a polyhydroxylated acyclic chain. Although the exact mechanism of the action of salacinol and kotalanol has not yet been discovered, it is postulated that the positively charged sulfur atom can act in the same way as the protonated ammonium center of the amine-based glycosidase inhibitors when binding in the active site of glycosidases.[81,82]

Salacinol shows strong blood glucose level control in vivo and inhibits intestinal α-glucosidases such as maltase, sucrase, and isomaltase. The inhibitory activity of salacinol against isomaltase was even higher than that of acarbose (**1.1**). The inhibitory activity of kotanalol (**1.50**), another active component of *Salacia reticulata* against sucrase is higher than that of salacinol (**1.49**) and acarbose (**1.1**) (Table 1.1).[78,79]

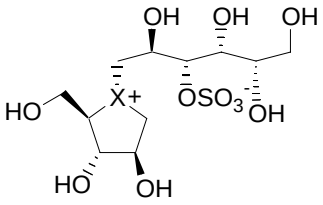
Table 1.1. K_i (μM) values of salacinol (**1.49**), kotalanol (**1.50**), and acarbose (**1.1**) for rat small intestinal disaccharidase.

	K_i (μg/ml)		
Substrate	Salacinol	Kotalanol	Acarbose
Maltase	0.31	0.23	0.12
Sucrase	0.32	0.18	0.37
Isomaltase	0.47	1.8	75

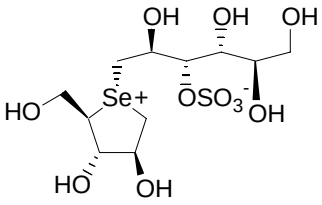
Some of the sulfonium and selenonium chain-extended analogues of salacinol synthesized in our laboratory (**1.51**-**1.56**, Chart 1.22), have shown comparable inhibitory activities against MGA relative to salacinol (Table 1.2).[83]



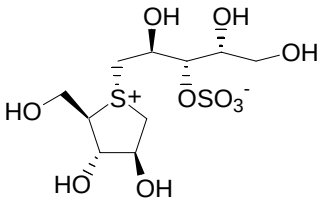
1.51 : X = S
1.52 : X = Se



1.53 : X = S
1.54 : X = Se



1.55



1.56

Chart 1.22. Compounds **1.51**-**1.56**.

Table 1.2. K_i (μM) values of salacinol (**1.49**) and its sulfonium and selenonium chain-extended analogues (**1.51**-**1.56**) against MGA.

Inhibitor	MGA K_i (μM)
Salacinol (**1.49**)	0.19
1.51	0.25
1.52	0.10
1.53	0.26
1.54	0.10
1.55	0.14
1.56	0.17

Salacinol (**1.49**) was first isolated from water-soluble fractions obtained from dried roots of *Salacia reticulata*.[78] Salacinol was then isolated from other plant species of *Salacia genus*, *Salacia oblonga* and *Salacia chinensis*.[84] Pure salacinol was collected from column chromatographic purification of the water soluble fraction[78] followed by repeated HPLC separations.

Studies have shown that the oral ingestion of an extract from *Salacia reticulata* trunk at a dose of 5000 mg/kg had no serious toxicity or mutagenicity in rats.[85] Furthermore, a double-blind study of the effects of the extract from *Salacia reticulata* on patients with type II diabetes has shown that the extract is an effective treatment of type II diabetes, with side effects comparable to the placebo control group.[86]

Since sulfonium salts have been exploited by Nature as glycosidase inhibitors, the synthesis of new sulfonium-ion analogues could be a promising approach for the discovery of new inhibitors.

This thesis will describe the design and synthesis of novel C2-substituted analogues of salacinol and C2-substituted nitrogen analogues of salacinol and their biological inhibitory activities. It will also describe the synthesis of 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose, a novel precursor for the synthesis of 4'-thionucleosides and 4'-thiooligonucleotides.

CHAPTER 2. SYNTHESIS OF 2-AMIDO, 2-AMINO, AND 2-AZIDO DERIVATIVES OF THE NITROGEN ANALOGUE OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR SALACINOL AND THEIR INHIBITORY ACTIVITIES AGAINST *O*-GLCNACASE AND NAGZ ENZYMES.

Reproduced in part from "Synthesis of 2-Amido, 2-Amino, and 2-Azido Derivatives of the Nitrogen Analogue of the Naturally Occurring Glycosidase Inhibitor Salacinol and Their Inhibitory Activities Against *O*-GlcNAcase and NagZ Enzymes" *Carbohydrate Research* **2008**, *343*, 1766-1777.

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2.1 Nitrogen analogues of salacinol

Substitution of the sulfur heteroatom of salacinol (**1.49**, Chapter 1) by a nitrogen atom (**2.1**, Chart 2.1) produces a new family of salacinol analogues (Chart 2.1). The nitrogen analogue **2.1** and its stereoisomers (for instance compound **2.2**) are potential glycosidase inhibitors.[87] Since the nitrogen atom is protonated, these compounds are able to mimic the oxacarbenium ion-like structure (Chart 1.1, Chapter 1) proposed as the transition state of the glycosidase mediated hydrolysis reactions or form electrostatic contacts with the active site carboxylate residues.

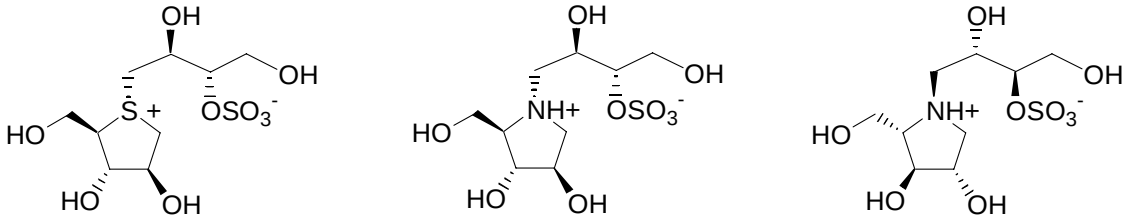


Chart 2.1. Salacinol (**1.49**) and nitrogen analogues of salacinol (**2.1**, **2.2**).

Nitrogen analogues of salacinol (**2.1**, **2.2**) were synthesized in our laboratory[87] and were tested against three glucosidase enzymes, namely glucoamylase G2, porcine pancreatic α-amylase (PPA), and barley α-amylase (AMY1), and the effects were compared to those of salacinol (**1.49**). It was observed that the ammonium compounds **2.1** and **2.2** were not active against AMY1 and PPA at concentrations of 5 mM compared to salacinol (**1.49**) that showed stronger inhibition against AMY1 and PPA (K_i = 15 ± 1 and 10 ± 2 μM, respectively). Salacinol showed weak inhibition of glucoamylase G2 (K_i

= 1.7 mM) whereas the ammonium compounds **2.1** and **2.2** inhibited glucoamylase with K_i values in the range ~10-fold higher than salacinol (Table. 2.1).

Screening of salacinol and the nitrogen analogues against human pancreatic α-amylase (HPA) showed that compound **2.2** was an inhibitor of this enzyme with a K_i value of 0.4 mM, compared to salacinol that showed stronger inhibition with a K_i value of 75 μM. In contrast, **2.1** was not active against this enzyme (Table. 2.1).[88]

Compound **2.1** was also synthesized by Muraoka et al. and was tested for its α-glucosidase inhibitory activities against rat small-intestinal disaccharidases; maltase, sucrase, and isomaltase. When compared to salacinol (**1.49**), the inhibitory activities of **2.1** against these enzymes decreased considerably (Table. 2.1).[89]

Interestingly, at a concentration of 1 mmol/L compound **2.1** inhibited ~96% of the recombinant lysosomal acid α-glucosidase (GAA) activity, whereas this enzyme was relatively insensitive to the α-glucosidase inhibitor, acarbose.[90] In addition, compound **2.1** showed selective inhibition of the activity of GAA in the detergent extract of human polymorphonuclear leukocytes (PMNs), which also contains a second acid α-glucosidase known as maltase glucoamylase (RAAG). Inhibition studies in which recombinant GAA was added to PMN extract showd that all of the recombinant enzyme was inhibited by 1 mmol/L of **2.1** in the presence of all the other components from the PMN lysate.[90] This selective inhibition of the enzyme GAA has been used to advantage in the design of a diagnostic test for Pompe's disease.

Table 2.1. Glycosidase inhibitory activities of salacinol (**1.49**) and its nitrogen analogues (**2.1**) and (**2.2**).[83]

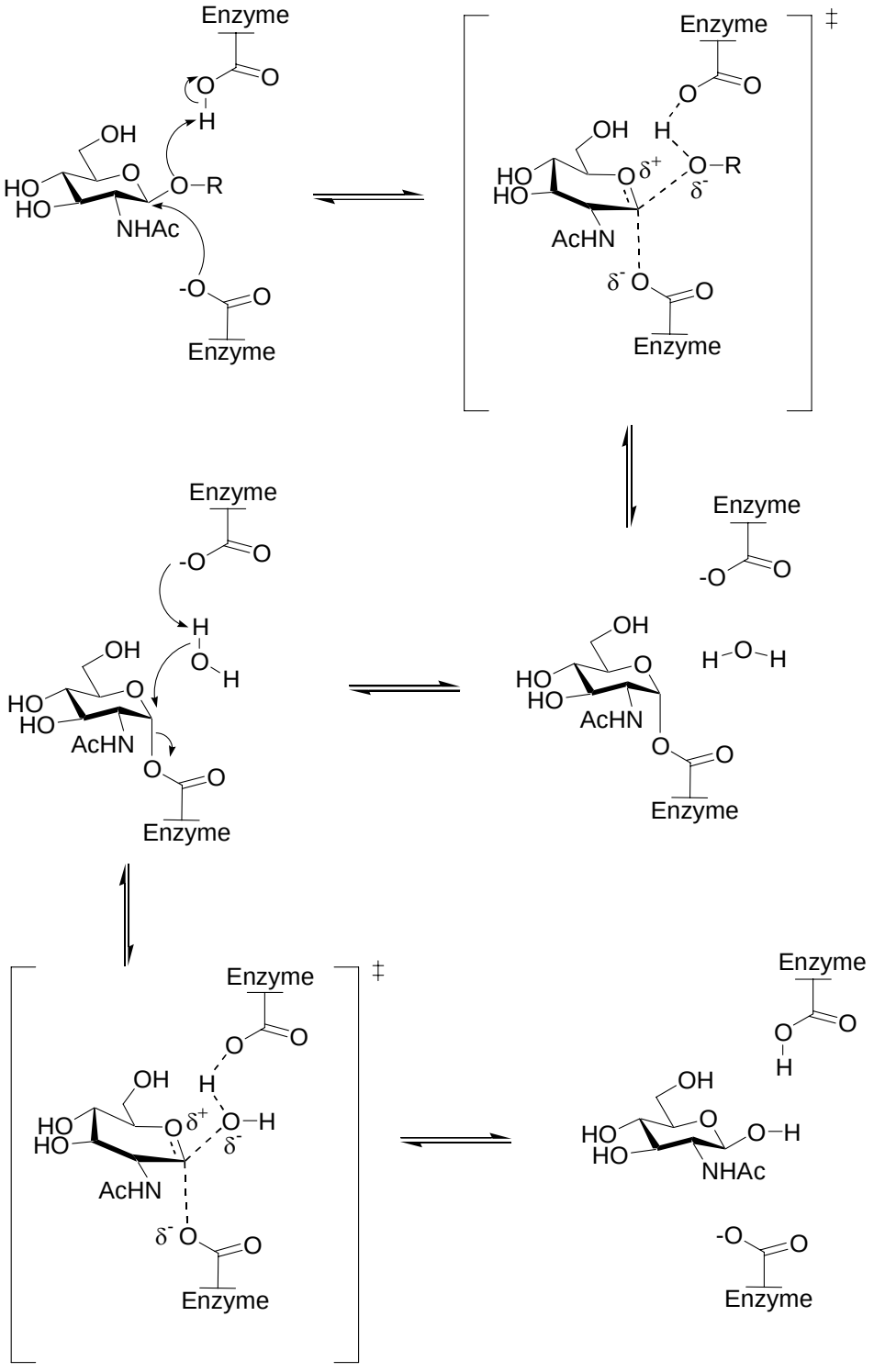
Inhibitor	Rat intestinal disaccharidase K_i (μM)	Gluco-amylase G2 K_i (mM)	PPA K_i (mM)	AMY1 K_i (mM)	MGA K_i (μM)	HPA K_i (mM)
Salacinol (**1.49**)	0.31 Maltase 0.32 Sucrase 0.47 Isomaltase	1.71	0.01	0.015	0.19	0.075
Nitrogen analogue (**2.1**)	306 Maltase 44 Sucrase 136 Isomaltase	> 2.5	> 5	> 5	NI	NI
Nitrogen analogue (**2.2**)	_	> 8	> 5	> 5	NI	0.4

2.2 *N*-Acetyl-*β*-D-glucosaminidase and NagZ enzymes

β-*N*-Acetylgulcosaminidases are glycosyl hydrolases that catalyze the hydrolysis of *N*-acetylglucosamine from the nonreducing end of oligosaccharide and glycoconjugates. In the past few years, there has been much interest in these enzymes due to their importance in mediating certain lysosomal storage diseases, their uses in

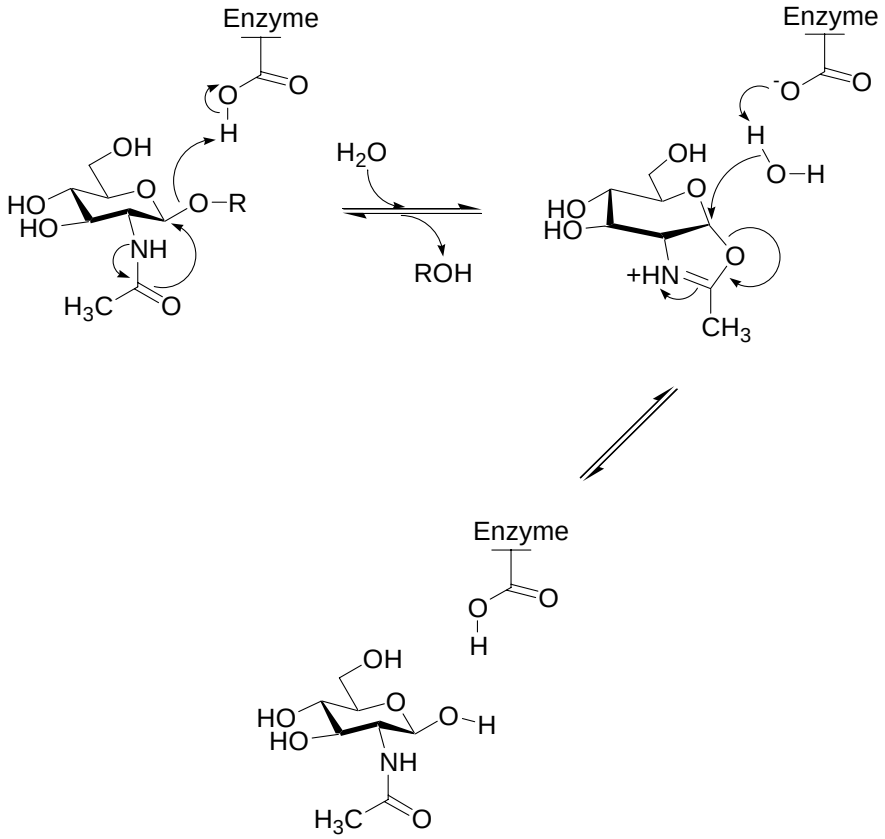
biotechnology, and as targets for antifungal agents.[91-93] *N*-Acetyl-*β*-glucosaminidases have been found in family 20, 84, and 3 of the glycosyl hydrolases, in the classification devised by Henrissat.[94,95]

The mechanism of family 3 *β*-glucosidases, of which *Vibrio cholerae* NagZ (VCNagZ) is a member, has been shown to operate via a retaining mechanism. Thus, *β*-*N*-acetylglucosaminidases catalyze the hydrolysis of their substrates using a double displacement mechanism in which two active site carboyl groups assist in the formation and breakdown of a glycosyl-enzyme intermediate. The first step involves the attack by one of the carboxylates at the anomeric center, along with general acid catalysis from the other carboxyl group that results in inversion of the configuration at the anomeric center. In a second step, the intermediate is hydrolyzed by general base-catalyzed attack of water at the anomeric center, resulting in cleavage of the glycosidic bond with overall retention of the anomeric configuration (Scheme 2.1).[96]



Scheme 2.1. Proposed mechanism for family 3 *β*-*N*-acetylglucosaminidases.

However, X-ray structural data and kinetic studies with inhibitors for the family 20 of *β*-*N*-acetylglucosaminidases provided strong evidence for catalysis involving neighboring group participation from the 2-acetamido group of the substrate. In this mechanism, the 2-acetamido group of the substrate acts to generate an enzyme-bound oxazolinium ion intermediate (Scheme 2.2).[97,98] This intermediate is then hydrolyzed by general base-catalyzed attack of water at the anomeric center in a manner analogous to a normal double displacement mechanism (Scheme 2.2).



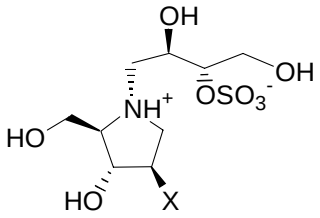
Scheme 2.2. Proposed mechanism for family 20 *β*-*N*-acetylglucosaminidases.

It was of interest to design compounds that might function as inhibitors of the hexosaminidase enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glycopyranosides. Our initial foray into this area involved the attempted synthesis of 2-acetamido- and 2-amino derivatives of salacinol (**1.49**, Chapter 1). Ring opening reactions were observed by nucleophilic participation of the amide or amine moieties that resulted in acyclic amido and ammonium sulfate compound respectively (Chapter 3).[99] We were intrigued by this result since it seemed to suggest that irreversible inhibition of enzymes might occur through nucleophilic participation of active-site residues. We further questioned whether this type of reactivity would be general with the 2-amino and 2-amido compounds, and whether the corresponding *nitrogen* analogues of salacinol containing such functionalities at C-2 would be stable. We report herein synthesis of seven target compounds **2.3-2.9** containing acetamido, amino, azido, and longer-chain amido substituents at C-2 (Chart 2.2).

A very recent report describing the activity of the parent iminoalditol **2.10**, its enantiomer **2.11**, and the corresponding *N*-benzyl derivatives **2.12**, **2.13** (Chart 2.3) against a D-hexosaminidase enzyme indicated that whereas the D-isomers are weak competitive inhibitors, the L-isomers are potent, non-competitive inhibitors.[100] Accordingly, we report also our own syntheses of the parent compounds, the iminoarabinitol **2.10**, *N*-Boc-1,2,4-trideoxy-2-amino-1,4-imino-D-arabinitol **2.14**, and *N*-Boc-1,2,4-trideoxy-2-acetamido-1,4-imino-D-arabinitol **2.15** (Chart 2.3).

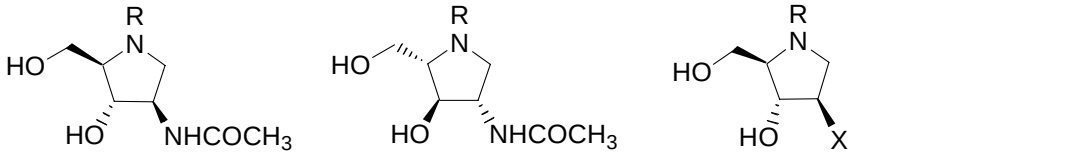
Finally, we report the enzyme inhibitory activities of compounds **2.3**-**2.9**, their parent 2-acetamido (**2.10**) and 2-azido (**2.17**) iminoalditols, *N*-Boc protected 2-acetamido

(**2.15**) and 2-amino (**2.14**) compounds against two enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glycopyranosides.



2.3 : X = N_3
2.4 : X = NH_2
2.5 : X = $NHCOCH_3$
2.6 : X = $NHCOCH_2CH_3$
2.7 : X = $NHCOCH_2CH_2CH_2CH_3$
2.8 : X = $N(CH_3)_2$
2.9 : X = $NHCH_2CH_3$

Chart 2.2. Target compounds **2.3**-**2.9**.



2.10 : R = H
2.12 : R = $CH_2C_6H_5$

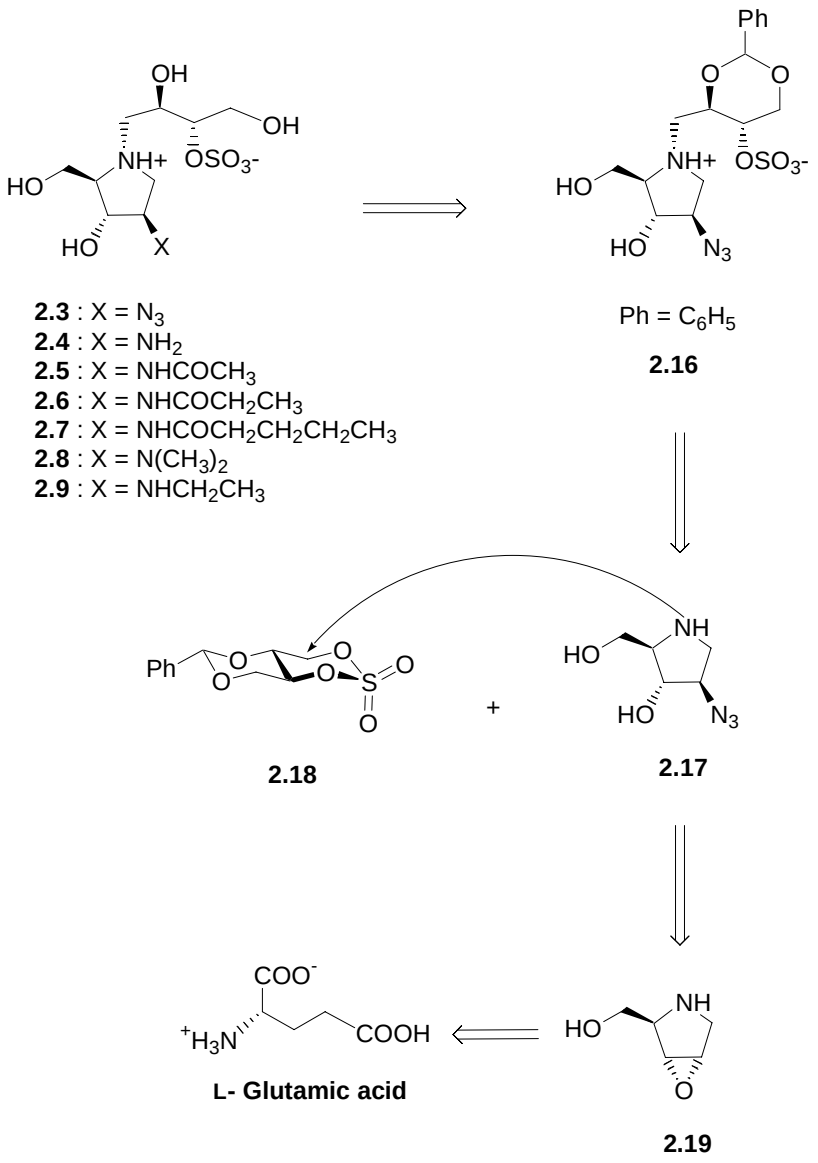
2.11 : R = H
2.13 : R = $CH_2C_6H_5$

2.14 : R = Boc, X = NH_2
2.15 : R = Boc, X = $NHCOCH_3$

Chart 2.3. Parent iminoalditols (**2.10**-**2.15**).

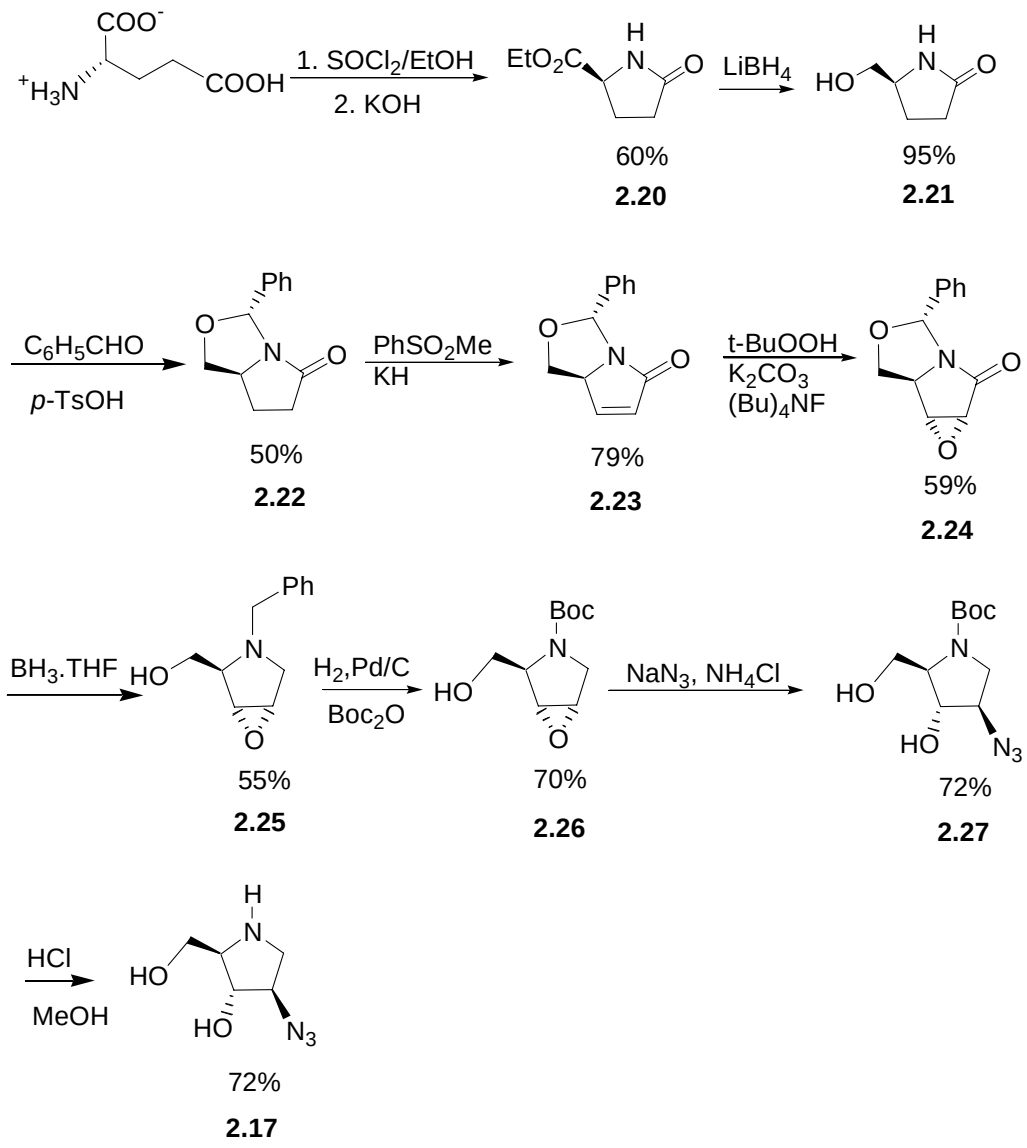
2.3 Results and discussion

Retrosynthetic analysis indicated that the target compounds **2.3**-**2.9** could be synthesized by reduction of the 2-azido function, its subsequent acylation, and removal of the benzylidene protecting group in compound **2.16**. Compound **2.16** could be synthesized, in turn, by nucleophilic attack of the azido compound **2.17** at the less hindered carbon of 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**2.18**). Compound **2.17** could be synthesized from the epoxide **2.19** that could be synthesized, in turn, from the commercially available L-glutamic acid (Scheme 2.3).



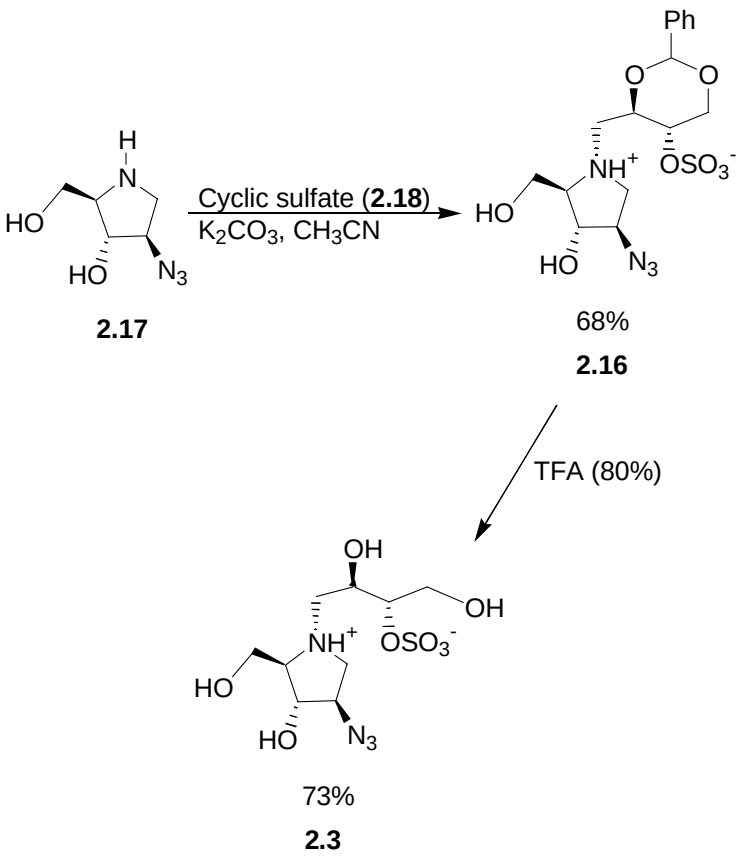
Scheme 2.3. Retrosynthetic analysis.

N-Boc-1,2,4-trideoxy-2-azido-1,4-imino-D-arabinitol **2.27** was synthesized from L-glutamic acid in 8 steps according to a literature procedure.[101-103] The Boc protecting group was then removed under acidic conditions to give the desired azido iminoalditol **2.17** in 72% yield (Scheme 2.4).



Scheme 2.4. Synthesis of compound **2.17**.

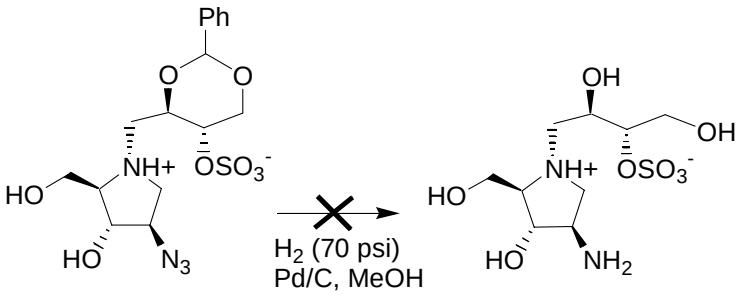
The alkylation reaction of **2.17** with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**2.18**) in acetonitrile in a sealed tube afforded the desired coupled product **2.16** in 68% yield. The benzylidene protecting group was then removed using trifluoroacetic acid (TFA, 80%) to afford the desired product **2.3** in 73% yield (Scheme 2.5).



Scheme 2.5. Coupling reaction.

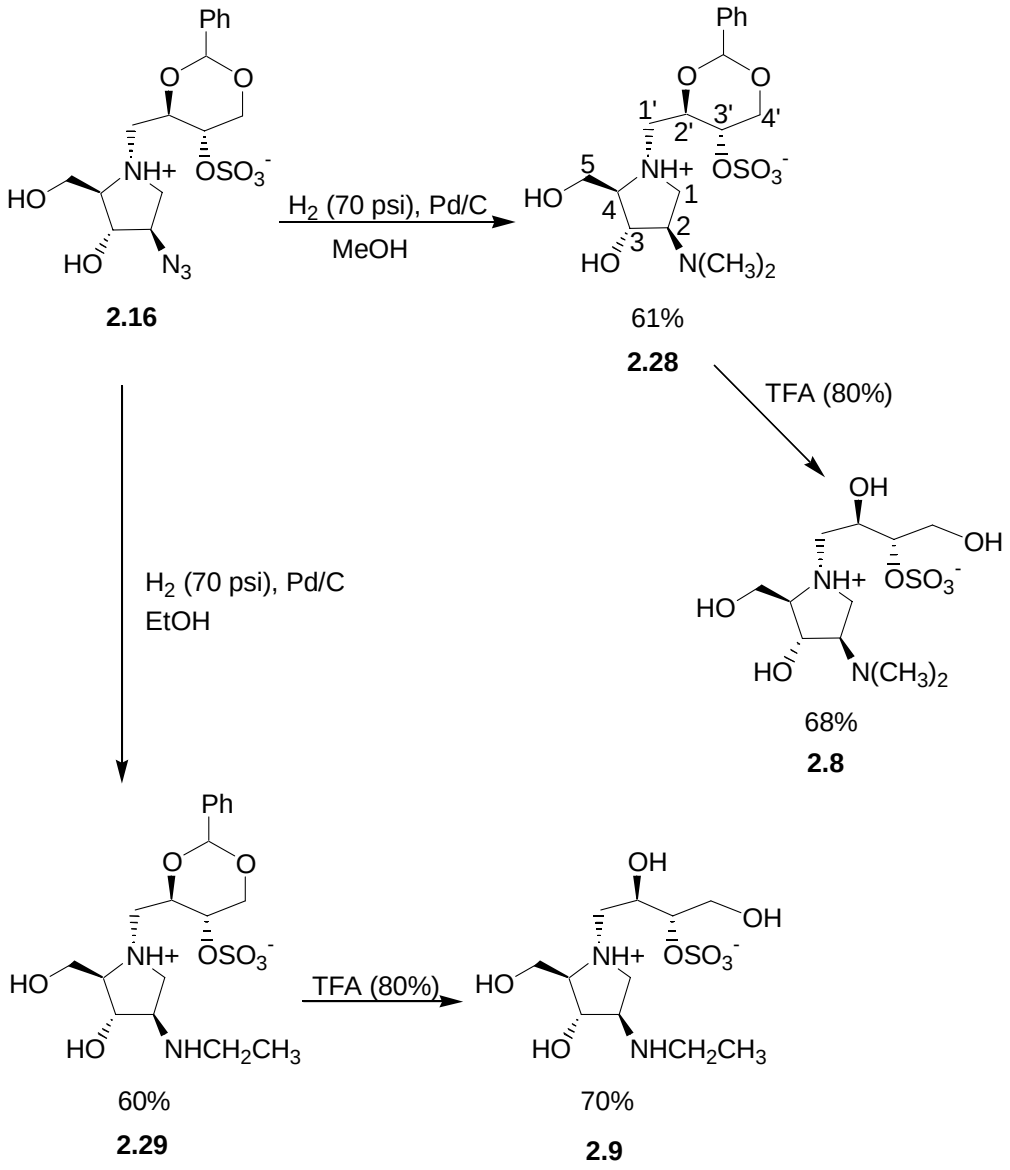
Hydrogenation of compound **2.16** in methanol at 70 psi did not yield the expected compound **2.4** (Scheme 2.6). Instead, we observed a very interesting result, namely the one-pot reduction of the azide to the amine followed by *N,N*-dimethylation, while the benzylidene protecting group remained intact (see **2.28**, Scheme 2.7). A 1-D NOESY experiment of compound **2.28** showed correlations between $N(CH_3)_2$ and H-2 and H-3 which confirmed that methylation of the amine had occurred. Also, correlations were observed between the H-1b resonance and H-1′b and H-4. Treatment of this compound with trifluoroacetic acid (80%) afforded the 2-*N,N*-dimethylamino analogue **2.8**. In order to explore this chemistry further, the hydrogenation reaction was then performed at 70 psi

H_2 in the presence of Pd/C in ethanol; the *N*-ethylamino derivative **2.29** was isolated. Treatment of this compound with trifluoroacetic acid (80%) afforded the 2-*N*-ethylamino analogue (**2.9**) (Scheme 2.7). These compounds presumably result from formation of formaldehyde or acetaldehyde on the catalyst surface. Reaction with the amines, after hydrogenation of the azides, would then give the imines which undergo further hydrogenation to yield the *N*-alkylamino derivatives.[104,105]



2.16 **2.4**

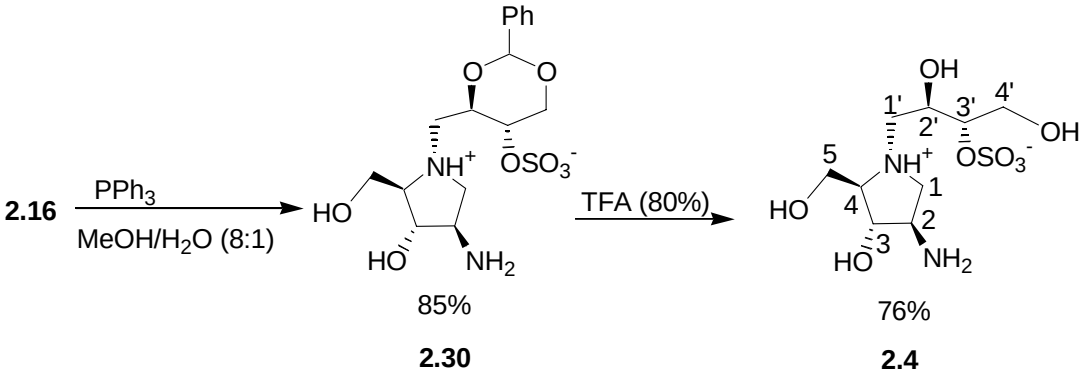
Scheme 2.6. Hydrogenation of compound **2.16**.



Scheme 2.7. Compounds **2.8** and **2.9** formed by hydrogenation of **2.16**.

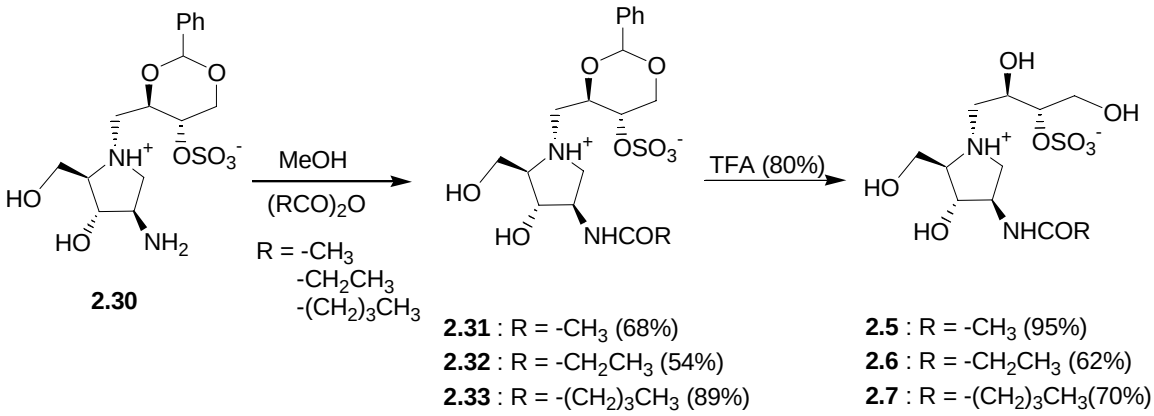
Reduction of the azido compound **2.16** to the amine **2.30** was accomplished using PPh_3, following a literature procedure.[106,107] Deprotection of the benzylidene protecting group afforded the desired 2-amino derivative **2.4** in 76% yield (Scheme 2.8). The stereochemistry at the ring nitrogen center of **2.4** was confirmed by means of a 1D-

NOESY experiment. Thus, irradiation of the H-1′b resonance showed correlations to H-4, H-1b, and a smaller correlation to H-1a. Irradiation of H-4 showed correlations to H-1′b, H-1b, and a smaller correlation to H-1′a. Correlations between H-1′b and H-4 indicated that H-1′b and H-4 are on the same face, and correlations between H-1 and H-4 indicated that the ring was intact and had not opened.



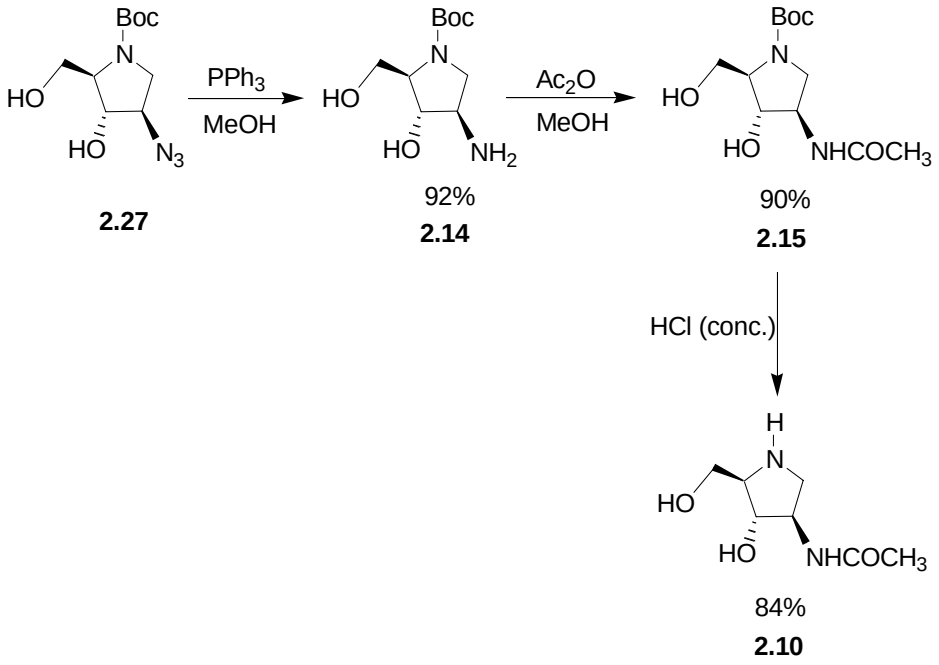
Scheme 2.8. Treatment of **2.16** with PPh_3.

Treatment of compound **2.30** with acetic, propionic, or valeric anhydride in methanol afforded the corresponding amides **2.31-2.33** in 54-89% yield. Removal of the benzylidene group in each of these compounds then afforded the corresponding ammonium salts **2.5-2.7** (Scheme 2.9).



Scheme 2.9. C-2 amido derivatives **2.5**, **2.6**, and **2.7**.

In order to obtain the parent iminoarabinitol, the azide **2.27** was first reduced using triphenylphosphine to afford the amine **2.14** in 92% yield. The ^{13}C NMR spectrum of this compound at ambient temperature showed two sets of peaks due to restricted rotation about the carbamate; the ^{13}C NMR spectrum at 70 °C using D_2O as the solvent revealed one set of peaks above coalescence. The amine **2.14** was then acetylated using acetic anhydride in methanol to afford the corresponding amide **2.15** in 90% yield; the ^{13}C NMR spectrum of this compound was also obtained at 70 °C in D_2O. Deprotection of **2.15** with HCl afforded, after neutralization, the desired 1,2,4-trideoxy-2-acetamido-1,4-imino-D-arabinitol (**2.10**) in 84% yield (Scheme 2.10).



2.27

92%
2.14

90%
2.15

84%
2.10

Scheme 2.10. Synthesis of 1,2,4-trideoxy-2-acetamido-1,4-imino-D-arabinitol (**2.10**).

Compounds **2.3**-**2.9**, their parent 2-acetamido (**2.10**) and 2-azido (**2.17**) iminoalditols, *N*-Boc protected-2-acetamido (**2.15**) and 2-amino (**2.14**) compounds were tested against two enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glycopyranosides. There are two main enzyme-catalyzed hydrolytic mechanisms known for cleaving this linkage. Examples of these two mechanistic classes include the *exo*-β-*N*-acetylglucosaminidases from family 3, and those from families 20 and 84 of the glycoside hydrolases, all of which are functionally related in that they catalyze the release of terminal 2-acetamido-2-deoxy-β-D-glycopyranose residues from glycoconjugates.[97,108-110] *exo*-β-*N*-Acetylglucosaminidases from family 3, of which *Vibrio cholerae* NagZ (VCNagZ) is a member, uses a catalytic mechanism involving the formation and

breakdown of a covalent glycosyl-enzyme intermediate.[109] The enzymes from family 20 [97,108] and more recently, 84,[111-113] of which human *O*-GlcNAcase is a member, have been shown to differ in that they use a catalytic mechanism involving assistance from the 2-acetamido group of the substrate.

The percent inhibition of enzyme activity in each case, at an inhibitor concentration of 250 μM, is shown in Chart 2.4. Among the ammonium ions, compounds **2.8** and **2.9** showed the highest activities, with **2.8** and **2.9** showing 33% and 27% inhibition of *O*-GlcNAcase, respectively, and 13% and 23% inhibition of NagZ, respectively. These results likely reflect better hydrophobic contacts within the active site of the enzyme by the non-polar methyl and ethyl groups on C-2; compound **2.4**, with the 2-amino group, showed the lowest (1%) activity. Among the parent iminoalditols, the 2-acetamido derivatives **2.10** and **2.15** showed the highest inhibition of the enzymes.

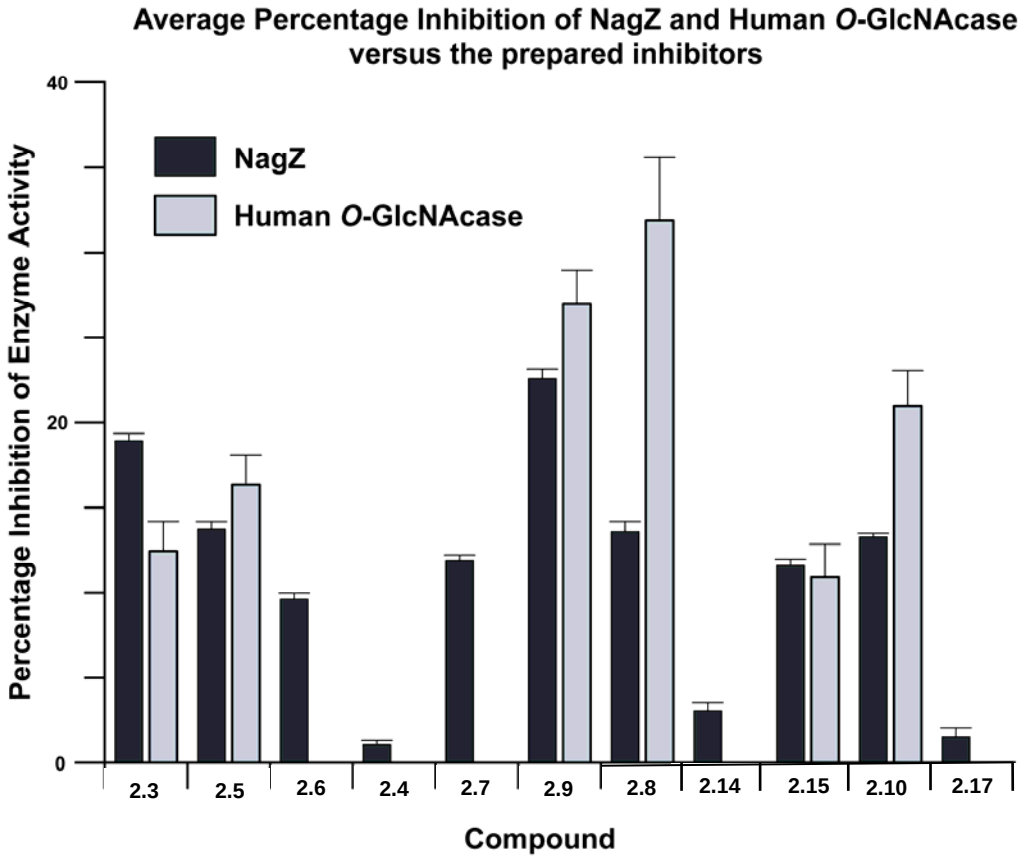


Chart 2.4. Percent inhibition of enzyme activity versus compound number.

2.4 Conclusions

Seven 2-*N*-substituted derivatives of the nitrogen analogue of the naturally occurring glucosidase inhibitor salacinol, were synthesized. Nucleophilic attack of 2-azido-1,4-iminoarabinitol at the less hindered carbon of a benzylidene-protected L-erythritol-1,3-cyclic sulfate proceeded smoothly and afforded the desired coupled products. These compounds are quite stable at room temperature in contrast to their sulfur congeners in which ring opening reactions were observed. The parent compound, 2-acetamido-1,2,4-trideoxy-1,4-imino-D-arabinitol, was also prepared from the *N*-Boc, 2-

azido derivative. A one-pot reduction and alkylation of the coupled azido derivatives was observed in hydrogenation reactions at 70 psi of the azide in methanol or ethanol containing Pd/C as a catalyst. The inhibitory activities of seven 2-substituted analogues of the nitrogen analogue of salacinol along with four of their iminoalditol parent compounds were tested against two enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glycopyranosides, namely *O*-GlcNAcase and NagZ; the compounds showed marginal inhibitory activity (<33% at 250μM).

2.5 Experimental

2.5.1 General methods

Optical rotations were measured at 21°C and reported in $deg dm^{-1} g^{-1} cm^3$. ^1H and ^{13}C NMR were recorded with frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY) and ^1H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the data was performed with MestRec software. 1D-NOESY experiments were recorded at 295 K on a 500 MHz spectrometer. For each 1D-NOESY spectrum, 512 scans were acquired with Q3 Gaussian Cascade pulse. A mixing time of 800 ms was used in all the 1D-NOESY experiments. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 3% ninhydrin in EtOH and heated. Column chromatography was performed with silica gel 60 (230-400 mesh). High resolution mass spectra were obtained by the electrospray ionization (ESI) technique, using a ZabSpec OA TOF mass spectrometer at 10000 RP.

2.5.2 Kinetic analysis of *O*-GlcNAcase and NagZ

All assays were carried out in triplicate at 37 °C for 30 min by using a stopped assay procedure in which the enzymatic reactions (50 μL) were quenched by the addition of a 4-fold excess (200 μL) of quenching buffer (200 mM glycine, pH 10.75). Assays were initiated by the addition, via syringe, of enzyme (5 μL), and in all cases the final pH of the resulting quenched solution was greater than 10. The time-dependent assay of human *O*-GlcNAcase[114] and *Vibrio cholerae* (VC) NagZ[115] enzymes revealed that both enzymes were stable over this period in the assay buffer: 50 mM NaH_2PO_4, 100 mM NaCl, 0.1% BSA, pH 7.4. The enzymes *O*-GlcNAcase and VCNagZ were used at a concentration of 0.16 μg μL^{-1} and 0.053 μg μL^{-1}, respectively in the assays using *p*-nitrophenyl GlcNAc as a substrate (0.25 mM for *O*-GlcNAcase, 0.5 mM for VCNagZ). All inhibitors were tested at a concentration of 250 μM. The progress of the reaction at the end of 30 min was determined by measuring the extent of 4-nitrophenolate liberated, as determined by absorbance measurements at 400 nm. The absorbance measurements were averaged to give a final result.

2.5.3 General procedure for trifluoroacetic acid treatment

The coupled compound (0.1 mmol) was dissolved in trifluoroacetic acid (2 mL, 80%) and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude product was collected.

2.5.4 General procedure for amide formation

To a solution of amine **2.30** (0.1 mmol) in MeOH (10 mL), desired anhydride (acetic, propionic, or valeric) (1.5 equiv.) was added. The mixture was stirred at room temperature for 4 h and the solvent was removed under reduced pressure to afford the crude product.

1′-((2-Azido-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzyl-idene-1′-deoxy-L-erythritol-3′-sulfate (2.16)

Compound **2.17** (200 mg, 1.0 mmol), 2,4-benzylidene-L-erythritol-1,3-cyclic sulfate (**2.18**) (413 mg, 1.2 equiv.), and K_2CO_3 (80 mg) were dissolved in CH_3CN: CH_3OH (5:1). The mixture was stirred in a sealed tube in an oil bath at 78° C for 16 h. K_2CO_3 was removed by filtration and the solvent was removed under reduced pressure. Flash chromatography of the crude product [EtOAc/MeOH, 10:1] afforded compound **2.16** as a solid (374 mg, 68%). Mp 110-112 °C; $[\alpha]_D$ +93 (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD) δ 7.41-7.30 (5H, m, Ar), 5.58 (1H, s, CHPh), 4.39 (1H, dd, $J_{4'b,4'a}$ = 11.0, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.09 (1H, ddd, $J_{2',3'}$ = 9.9 Hz, H-3′), 3.95 (1H, ddd, $J_{1'b,2'}$ = 8.4 Hz, H-2′), 3.84 (1H, dd, $J_{2,3}$ = 2.8 Hz, H-3), 3.82 (1H, ddd, $J_{1a,2}$ = 1.4, $J_{1b,2}$ = 5.8 Hz, H-2), 3.78 (1H, dd, $J_{3',4'}$ = 10.6 Hz, H-4′b), 3.58 (1H, dd, $J_{5b,5a}$ = 9.1, $J_{4,5a}$ = 7.2 Hz, H-5a), 3.57 (1H, dd, $J_{4,5b}$ = 6.4 Hz, H-5b), 3.29 (1H, dd, $J_{1'b,1'a}$ = 14.2 Hz, H-1′a), 3.08 (1H, dd, $J_{2,1a}$ = 1.4, $J_{1b,1a}$ = 11.6 Hz, H-1a), 2.79 (1H, dd, $J_{2,1b}$ = 5.8 Hz, H-1b), 2.54 (1H, dd, H-1′b), 2.50 (1H, ddd, $J_{3,4}$ = 3.4 Hz, H-4); ^{13}C NMR (CD_3OD) δ 138.0, 128.7, 127.9, 126.1 (6C, Ph), 100.9 (CHPh), 80.0 (C-3), 76.6 (C-2′), 73.3 (C-4), 69.3 (C-4′), 68.3 (C-3′), 66.2 (C-2), 59.7 (C-5), 57.7 (C-1), 54.9 (C-1′). Anal. Calcd. for $C_{16}H_{22}N_4O_8S$: C, 44.65; H, 5.15; N, 13.02. Found: C, 44.28; H, 4.94; N, 12.72.

1′-((2-Azido-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2.3)

Compound **2.16** (50 mg, 0.1 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 7:1] to afford compound **2.3** as a syrup (29 mg, 73%). $[\alpha]_D$ +50 (*c* 0.02, MeOH); ^{1}H NMR (CD_3OD) δ 4.35 (1H, ddd, $J_{2',3'}$ = 9.4, $J_{4'a,3'}$ = 4.65 Hz, H-3′), 3.99 (1H, ddd, $J_{1'a,2'}$ = 5.5 Hz, H-2′), 3.98 (1H, dd, $J_{3,2}$ = 5.1 Hz, H-2), 3.91 (1H, dd, $J_{4'b,4'a}$ = 12.0 Hz, H-4′a), 3.81 (1H, dd, $J_{4,3}$ = 2.2 Hz, H-3), 3.80 (1H, dd, $J_{3',4'b}$ = 5.0 Hz, H-4′b), 3.71 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 4.6 Hz, H-5a), 3.66 (1H, dd, $J_{4,5b}$ = 4.0 Hz, H-5b), 3.28 (1H, dd, $J_{1b,1a}$ = 11.1, $J_{2,1a}$ = 2.6 Hz, H-1a), 3.21 (1H, dd, $J_{1'b,1'a}$ = 13.2 Hz, H-1′a), 2.95 (1H, dd, $J_{2,1b}$ = 6.6 Hz, H-1b), 2.57 (1H, m, H-4), 2.56 (1H, dd, $J_{2',1'b}$ = 6.9 Hz, H-1′b); ^{13}C NMR (CD_3OD) δ 80.8 (C-3′), 76.8 (C-3), 73.3 (C-4), 69.8 (C-2′), 66.3 (C-2), 60.9 (C-5), 60.2 (C-4), 57.4 (C-1), 57.2 (C-1′). HRMS Calcd for $C_9H_{19}O_8N_4S$ (M+H): 343.0924. Found: 343.0972.

1′-((2-Amino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzyl-idene-1′-deoxy-L-erythritol-3′-sulfate (2.30)

To a solution of **2.16** (100 mg, 0.2 mmol) in MeOH : H_2O (15:1, 15 mL) was added PPh_3 (63 mg, 1.2 equiv.). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford **2.30** as a syrup (80 mg, 85%). $[\alpha]_D$ +21 (*c* 0.1, MeOH); ^{1}H NMR (D_2O) δ 7.41-7.29 (5H, m, Ar), 5.61 (1H, s, CHPh), 4.39 (1H, dd, $J_{3',4'a}$ = 5.4, $J_{4'b,4'a}$ = 11.0 Hz, H-4′a), 4.16 (1H, ddd, $J_{2',3'}$ = 9.8, $J_{4'b,3'}$ = 9.9 Hz, H-3′), 3.99 (1H, dd, $J_{2,3}$ = 2.8, $J_{4,3}$ = 4.8 Hz, H-3), 3.95 (1H, ddd, $J_{1'a,2'}$ = 1.9 Hz, H-2′), 3.77

(1H, dd, H-4′b), 3.67 (1H, dd, $J_{4,5a}$ = 3.1, $J_{5b,5a}$ = 12.3 Hz, H-5a), 3.55 (1H, dd, $J_{4,5b}$ = 2.4 Hz, H-5b), 3.43 (1H, m, H-2), 3.16 (1H, dd, $J_{1'b,1'a}$ = 14.4 Hz, H-1′a), 3.11 (1H, dd, $J_{2,1a}$ = 0.8, $J_{1b,1a}$ = 11.7 Hz, H-1a), 2.93 (1H, dd, $J_{2,1b}$ = 6.2 Hz, H-1b), 2.64 (1H, dd, $J_{2',1'b}$ = 7.5 Hz, H-1′b), 2.56 (1H, ddd, H-4)); ^{13}C NMR (D_2O) δ 136.4, 129.9, 128.9, 126.2 (6C, Ph), 101.1 (CHPh), 79.0 (C-2′), 75.1 (C-3), 71.9 (C-4), 68.9 (C-3′), 68.6 (C-4′), 58.7 (C-5), 56.0 (C-1), 55.8 (C-2), 53.4 (C-1′). HRMS Calcd for $C_{16}H_{25}O_8N_2S$ (M+H): 405.1331. Found: 405.1337.

1′-((2-Amino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol -3′-sulfate (2.4)

Compound **2.30** (130 mg, 0.3 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH/H_2O, 5:3:1] to afford compound **2.4** as a white solid (77 mg, 76%). Mp 210-212 °C (dec.); $[\alpha]_D$ +40 (*c* 0.1, MeOH); ^{1}H NMR (D_2O) δ 4.27(1H, ddd, $J_{2',3'}$ = 5.2 Hz, H-3′), 4.02 (1H, ddd, $J_{1'b,2'}$ = 6.9, $J_{1'a,2'}$ = 6.6 Hz, H-2′), 4.01 (1H, dd, $J_{4,3}$ = 4.6 Hz, H-3), 3.74 (1H, dd, $J_{4'b,4'a}$ = 12.6, $J_{3',4'a}$ = 3.4 Hz, H-4′a), 3.67 (1H, dd, $J_{3',4'b}$ = 5.6 Hz, H-4′b), 3.64 (1H, dd, $J_{5b,5a}$ = 12.2, $J_{4,5a}$ = 3.1 Hz, H-5a), 3.58 (1H, dd,$J_{4,5b}$ = 2.7 Hz, H-5b), 3.47 (1H, ddd, $J_{1a,2}$ = 2.0, $J_{3,2}$ = 2.0 Hz, H-2), 3.14 (1H, dd, $J_{1b,1a}$ = 11.5 Hz, H-1a), 2.92 (1H, dd, $J_{1'b,1'a}$ = 13.2 Hz, H-1′a), 2.83 (1H, dd, $J_{2,1b}$ = 5.9 Hz, H-1b), 2.51 (1H, ddd, H-4), 2.42 (1H, dd, H-1′b); ^{13}C NMR (D_2O) δ 81.2 (C-3′), 75.0 (C-3), 72.3 (C-4), 69.4 (C-2′), 59.5 (C-4′), 58.9 (C-5), 55.8 (C-2), 55.3 (C-1), 55.0 (C-1′). Anal. Calcd for $C_9H_{20}N_2O_8S$: C, 34.17; H, 6.37; N, 8.86. Found: C, 34.38; H, 6.41; N, 8.75.

1′-((2-Acetamido-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene -1′-deoxy-L-erythritol-3′-sulfate (2.31)

Compound **2.30** (34 mg, 0.08 mmol) was subjected to amide formation procedure using acetic anhydride and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford **2.31** as a syrup (25 mg, 68%). $[\alpha]_D$ -17 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD) δ 7.48-7.29 (5H, m, Ar), 5.58 (1H, s, CHPh), 4.52 (1H, dd, $J_{4'b,4'a}$ = 10.9, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.24 (1H, ddd, $J_{4'b,3'}$ = 9.8, $J_{2',3'}$ = 9.7 Hz, H-3′), 4.07 (1H, ddd, $J_{1'b,2'}$ = 7.6, $J_{1'a,2'}$ = 3.3 Hz, H-2′), 4.03 (1H, dd, $J_{4,3}$ = 8.5, $J_{2,3}$ = 3.1 Hz, H-3), 4.01 (1H, ddd, $J_{1a,2}$ = 3.0 Hz, H-2), 3.83 (1H, dd, $J_{5b,5a}$ = 12.1, $J_{4,5a}$ = 3.3 Hz, H-5a), 3.77 (1H, dd, H-4′b), 3.69 (1H, dd, $J_{4,5b}$ = 2.8 Hz, H-5b), 3.58 (1H, dd, $J_{1'b,1'a}$ = 12.4 Hz, H-1′a), 3.31 (1H, dd, H-1a), 3.21 (1H, dd, $J_{1a,1b}$ = 11.9, $J_{2,1b}$ = 6.2 Hz, H-1b), 2.92 (1H, dd, H-1′b), 2.80 (1H, dd, H-4), 1.96 (1H, s, CH_3); ^{13}C NMR (D_2O) δ 174.8 (CO), 136.0, 130.1, 128.9, 126.3 (6C, Ph), 101.0 (CHPh), 75.3 (C-3′), 73.6 (C-3), 72.5 (C-4), 68.4 (C-4′), 68.1 (C-2′), 57.8 (C-1), 56.1 (C-5), 54.7 (C-1′), 54.3 (C-2). Anal. Calcd for $C_{18}H_{26}N_2O_9S$: C, 48.42; H, 5.87; N, 6.27. Found: C, 48.20; H, 5.96; N, 6.19.

1′-((2-Acetamido-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2.5)

Compound **2.31** (26 mg, 0.06 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 2:1] to afford compound **2.5** as a syrup (20 mg, 95%). $[\alpha]_D$ +50 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD) δ 4.48 (1H, ddd, $J_{2',3'}$ = 4.6 Hz, H-3′), 4.06 (1H, ddd, $J_{1'b,2'}$ = 6.3 Hz, H-2′), 3.96 (1H, ddd, $J_{3,2}$ = 4.9 Hz, H-2), 3.93 (1H, dd, $J_{3',4'a}$ = 4.1 Hz, H-4′a), 3.91 (1H, dd, $J_{4,3}$ = 3.4

Hz, H-3), 3.82 (1H, dd, $J_{4'a,4'b}$ = 12.0, $J_{3',4'b}$ = 5.1 Hz, H-4′b), 3.72 (1H, dd, $J_{5b,5a}$ = 11.8, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.63 (1H, dd, $J_{4,5b}$ = 3.3 Hz, H-5b), 3.15 (1H, dd, $J_{1b,1a}$ = 10.1 Hz, H-1a), 3.11 (1H, dd, $J_{1'b,1'a}$ = 12.7, $J_{2',1'a}$ = 7.5 Hz, H-1′a), 2.85 (1H, dd, $J_{2,1b}$ = 6.1 Hz, H-1b), 2.53 (1H, dd, H-1′b), 2.45 (1H, ddd, H-4), 1.96 (3H, s, CH_3); ^{13}C NMR (CD_3OD) δ 172.0 (CO), 80.9 (C-3′), 78.0 (C-3), 73.7 (C-1′), 69.7 (C-2′), 60.9 (C-4′), 60.5 (C-5), 57.3 (C-4), 57.1 (C-1), 56.5 (C-2), 21.4 (CH_3). HRMS Calcd for $C_{11}H_{23}O_9N_2S$ (M+H): 359.1124. Found: 359.1122.

1′-((1,4-Imino-2-propionamido-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene -1′-deoxy-L-erythritol-3′-sulfate (2.32)

Compound **2.30** (65 mg, 0.16 mmol) was subjected to amide formation procedure using propionic anhydride and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford **2.32** as a syrup (40 mg, 54%). $[\alpha]_D$ +250 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD) δ 7.51-7.31 (5H, m, Ar), 5.58 (1H, s, CHPh), 4.57 (1H, dd, $J_{4'b,4'a}$ = 10.9, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.27 (1H, ddd, $J_{4'b,3'}$ = 9.8, $J_{2',3'}$ = 9.7 Hz, H-3′), 4.01 (1H, ddd, $J_{1'b,2'}$ = 6.7 Hz, H-2′), 3.98 (1H, ddd, $J_{3,2}$ = 5.4 Hz, H-2), 3.95 (1H, dd, $J_{4,3}$ = 3.8 Hz, H-3), 3.79 (1H, dd, H-4′b), 3.76 (1H, dd, $J_{5b,5a}$ = 11.5, $J_{4,5a}$ = 3.8 Hz, H-5a), 3.64 (1H, dd, $J_{4,5b}$ = 2.6 Hz, H-5b), 3.37 (1H, dd, $J_{1b,1a}$ = 13.6, $J_{2,1a}$ = 4.1 Hz, H-1a), 3.16 (1H, dd, $J_{1'b,1'a}$ = 10.2 Hz, H-1′a), 2.92 (1H, dd, H-1′b), 2.68 (1H, dd, $J_{2,1b}$ = 6.7 Hz, H-1b), 2.49 (1H, ddd, H-4), 2.22 (2H, q, $J_{CH3,CH2}$ = 7.6 Hz, CH_2), 1.11 (3H, t, CH_3)); ^{13}C NMR (CD_3OD) δ 175.7 (CO), 138.0, 128.7, 127.9, 126.1 (6C, Ph), 100.9 (CHPh), 78.7 (C-2′), 77.9 (C-3), 73.9 (C-4), 69.4 (C-3′), 69.2 (C-4′), 59.5 (C-5), 57.9 (C-1′), 56.3 (C-2), 55.8 (C-1), 28.9 (CH_2), 9.2 (CH_3). HRMS Calcd for $C_{19}H_{29}O_9N_2S$ (M+H): 461.1594. Found: 461.1599.

1′-((1,4-Imino-2-propionamido-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2.6)

Compound **2.32** (20 mg, 0.08 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.6** as a syrup (10 mg, 62%). $[\alpha]_D$ +100 (*c* 0.02 , MeOH); ^{1}H NMR (CD_3OD) δ 4.49 (1H, ddd, $J_{2',3'}$ = 4.7 Hz, H-3′), 4.05 (1H, ddd, H-2′), 3.95 (1H, ddd, H-2), 3.92 (1H, dd, $J_{4'b,4'a}$ = 11.9, $J_{3',4'a}$ = 4.2 Hz, H-4′a), 3.89 (1H, dd, $J_{4,3}$ = 3.5 Hz, H-3), 3.82 (1H, dd, $J_{3',4'b}$ = 5.1 Hz, H-4′b), 3.69 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 4.1 Hz, H-5a), 3.61 (1H, dd, $J_{4,5b}$ = 3.5 Hz, H-5b), 3.13 (1H, dd, $J_{1b,1a}$ = 10.0 Hz, H-1a), 3.08 (1H, dd, $J_{1'b,1'a}$ = 12.8, $J_{2',1'a}$ = 7.8 Hz, H-1′a), 2.81 (1H, dd, $J_{2,1b}$ = 5.9 Hz, H-1b), 2.49 (1H, dd, $J_{2',1'b}$ = 5.9 Hz, H-1′b), 2.39 (1H, ddd, H-4), 2.23 (2H, q, $J_{CH3,CH2}$ = 7.6 Hz, CH_2), 1.12 (3H, t, CH_3); ^{13}C NMR (CD_3OD) δ 175.7 (CO), 80.9 (C-3′), 78.2 (C-3), 73.8 (C-4), 69.9 (C-2′), 60.9 (C-4′), 60.7 (C-5), 57.3 (C-1), 57.2 (C-1′), 56.4 (C-2), 28.9 (CH_2), 9.3 (CH_3). HRMS Calcd for $C_{12}H_{25}O_9N_2S$ (M+H): 373.1281. Found: 373.1295.

1′-((1,4-Imino-2-pentanamido-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene-1′-deoxy-L-erythritol-3′-sulfate (2.33)

Compound **2.30** (13 mg, 0.03 mmol) was subjected to amide formation procedure using valeric anhydride and the crude product was purified by flash chromatography [EtOAc/MeOH, 6:1] to afford **2.33** as a syrup (14 mg, 89%). $[\alpha]_D$ +85 (*c* 0.04, MeOH); ^{1}H NMR (CD_3OD) δ 7.39-7.20 (5H, m, Ar), 5.47 (1H, s, CHPh), 4.46 (1H, dd, $J_{4'b,4'a}$ = 10.9, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.16 (1H, ddd, $J_{4'b,3'}$ = 9.8, $J_{2',3'}$ = 5.5 Hz, H-3′), 3.89 (1H, ddd, H-2′), 3.86 (1H, ddd, $J_{3,2}$ = 5.5 Hz, H-2), 3.83 (1H, dd, H-3), 3.68 (1H, dd, H-4′b),

3.64 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 6.1 Hz, H-5a), 3.52 (1H, dd, $J_{4,5b}$ = 1.5 Hz, H-5b), 3.24 (1H, dd, $J_{1'b,1'a}$ = 13.7, $J_{2',1'a}$ = 5.1 Hz, H-1′a), 3.04 (1H, dd, $J_{1b,1a}$ = 9.8 Hz, H-1a), 2.79 (1H, dd, $J_{2,1b}$ = 5.9 Hz, H-1b), 2.54 (1H, dd, $J_{2',1'b}$ = 6.8 Hz, H-1′b), 2.34 (1H, ddd, $J_{3,4}$ = 2.0 Hz, H-4), 2.10 (2H, dt, $J_{CH2,CH2}$ = 7.3, $J_{NH,\ CH2}$ = 2.6 Hz, CH_2), 1.47 (2H, m, CH_2), 1.23 (2H, m, CH_2), 0.82 (3H, t, $J_{CH2,CH3}$ = 7.3 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 174.9 (CO), 138.1, 128.6, 127.9, 126.1 (4C, Ph), 100.9 (CHPh), 78.9 (C-2′), 78.0 (C-3), 73.9 (C-4), 69.4 (C-3′), 69.2 (C-4′), 59.7 (C-5), 58.1 (C-1), 56.4 (C-2), 55.8 (C-4′), 35.5, 28.0, 22.2 (3×CH_2), 12.9 (CH_3). HRMS Calcd for $C_{21}H_{33}O_9N_2S$ (M+H): 489.1907. Found: 489.1923.

1′-((1,4-Imino-2-pentanamido-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy- L-erythritol-3′-sulfate (2.7)

Compound **2.33** (14 mg, 0.03 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.7** as a syrup (8 mg, 70%). $[\alpha]_D$ +60 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD) δ 4.47 (1H, ddd, $J_{2',3'}$ = 9.3 Hz, H-3′), 4.05 (1H, ddd, H-2′), 3.97 (1H, ddd, $J_{1b,2}$ = 6.0 Hz, H-2), 3.92 (1H, dd, $J_{4'b,4'a}$ = 12.0, $J_{3',4'a}$ = 4.2 Hz, H-4′a), 3.90 (1H, dd, $J_{4,3}$ = 3.6, $J_{2,3}$ =1.9 Hz, H-3), 3.82 (1H, dd, $J_{3',4'b}$ = 5.1 Hz, H-4′b), 3.71 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 4.2 Hz, H-5a), 3.63 (1H, dd, $J_{4,5b}$ = 3.6 Hz, H-5b), 3.15 (1H, dd, $J_{1b,1a}$ = 10.0 Hz, H-1a), 3.12 (1H, dd, $J_{1'b,1'a}$ = 12.9, $J_{2',1'a}$ = 7.3 Hz, H-1′a), 2.87 (1H, dd, H-1b), 2.54 (1H, dd, $J_{2',1'b}$ = 6.2 Hz, H-1′b), 2.46 (1H, ddd, H-4), 2.22 (2H, dt, $J_{CH2,CH2}$ = 7.3, $J_{NH,\ CH2}$ = 0.8 Hz, CH_2), 1.59 (2H, m, CH_2), 1.36 (2H, m, CH_2), 0.93 (3H, t, $J_{CH2,CH3}$ = 7.3 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 175.0 (CO), 80.9 (C-3′), 78.1 (C-3), 73.8 (C-4), 69.7 (C-2′), 60.9 (C-

4′), 60.6 (C-5), 57.4 (C-1′), 57.2 (C-1), 56.4 (C-2), 35.6, 28.0, 22.2 (3×CH_2), 12.9 (CH_3). HRMS Calcd for $C_{14}H_{29}O_9N_2S$ (M+H): 401.1594. Found: 401.1617.

1′-((2-*N*,*N*-Dimethylamino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*- benzylidene-1′-deoxy-L-erythritol-3′-sulfate (2.28)

Compound **2.16** (100 mg, 0.23 mmol) was dissolved in MeOH (20 mL), Pd/C (100 mg) was added, and the mixture was stirred under H_2 (70 psi) overnight. The catalyst was removed by filtration and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.28** as a syrup (61 mg, 61%). $[\alpha]_D$ +80 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD) δ 7.48-7.32 (5H, m, Ar), 5.59 (1H, s, CHPh), 4.59 (1H, dd, $J_{4'b,4'a}$ = 10.9, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.30 (1H, ddd, $J_{4'b,3'}$ = 10.0, $J_{2',3'}$ = 9.9 Hz, H-3′), 4.23 (1H, dd, $J_{4,3}$ = 5.8, $J_{2,3}$ = 4.0 Hz, H-3), 3.94 (1H, ddd, $J_{1'a,2'}$ = 3.2 Hz, H-2′), 3.85 (1H, dd, $J_{5b,5a}$ = 12.1, $J_{4,5a}$ = 2.7 Hz, H-5a), 3.78 (1H, ddd, H-4′b), 3.63 (1H, dd, $J_{4,5b}$ = 2.2 Hz, H-5b), 3.49 (1H, dd, $J_{1b,1a}$ = 11.6, $J_{2,1a}$ = 2.2 Hz, H-1a), 3.30 (1H, dd, H-1′a), 3.18 (1H, ddd, H-2), 2.92 (1H, dd, $J_{2,1b}$ = 7.3 Hz, H-1b), 2.72 (6H, s, 2×CH_3), 2.68 (1H, dd, $J_{2',1'b}$ = 6.1 Hz, H-1′b), 2.51 (1H, ddd, H-4); ^{13}C NMR (CD_3OD) 137.9, 128.7, 127.9, 126.1 (4C, Ph), 101.1 (CHPh), 79.5 (C-2′), 72.7 (C-3), 72.5 (C-4), 71.5 (C-2), 69.3 (C-3′), 68.7 (C-4′), 58.3 (C-5), 54.9 (C-1), 53.7 (C-1′), 41.3 (CH_3). Anal. Calcd. for $C_{18}H_{28}N_2O_8S$: C, 49.99; H, 6.52; N, 6.48. Found: C, 50.35; H, 6.48; N, 6.53. HRMS Calcd for $C_{18}H_{29}O_8N_2S$ (M+H): 433.1645. Found: 433.1645.

1′-((2-*N*,*N*-Dimethylamino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2.8)

Compound **2.28** (28 mg, 0.06 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.8** as a syrup (15 mg, 68%). $[\alpha]_D$ -9 (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD) δ 4.43 (1H, ddd, $J_{2',3'}$ = 4.7 Hz, H-3′), 4.11 (1H, dd, $J_{4,3}$ = 4.5 Hz, H-3), 4.06 (1H, ddd, H-2′), 3.94 (1H, dd, $J_{4'b,4'a}$ = 11.9, $J_{3',4'a}$ = 4.5 Hz, H-4′a), 3.80 (1H, dd, $J_{3',4'b}$ = 4.6 Hz, H-4′b), 3.79 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.65 (1H,dd, $J_{4,5b}$ = 3.0 Hz, H-5b), 3.42 (1H, dd, $J_{1b,1a}$ = 11.3, $J_{2,1a}$ = 3.0 Hz, H-1a), 3.11 (1H, dd, $J_{2',1'a}$ = 7.1 Hz, H-1′a), 2.99 (1H, ddd, $J_{3,2}$ = 0.3 Hz, H-2), 2.76 (1H, dd, $J_{2,1b}$ = 7.9 Hz, H-1b), 2.56 (6H, s, 2×CH_3), 2.43 (1H, ddd, H-4), 2.42 (1H, dd, $J_{1'a,1'b}$ = 12.6, $J_{2',1'b}$ = 5.9 Hz, H-1′b); ^{13}C NMR (CD_3OD) 80.2 (C-3′), 73.3 (C-3), 72.9 (C-4), 71.2 (C-2), 69.6 (C-2′), 60.6 (C-4′), 58.9 (C-5), 55.9 (C-1′), 54.7 (C-1), 41.7 (CH_3). HRMS Calcd for $C_{11}H_{24}O_8N_2SNa$ (M+Na): 367.1151. Found: 367.1152.

1′-((2-*N*-Ethylamino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene-1′-deoxy-L-erythritol-3′-sulfate (2.29)

Compound **2.16** (16.5 mg, 0.04 mmol) was dissolved in EtOH (15 mL), Pd/C (60 mg) was added, and the mixture was stirred under H_2 (70 psi) overnight. The catalyst was removed by filtration, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.29** as a syrup (10 mg, 60%). $[\alpha]_D$ +43 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD) δ 7.56-7.29

(5H, m, Ar), 5.58 (1H, s, CHPh), 4.57 (1H, dd, $J_{4'b,4'a}$ = 10.9, $J_{3',4'a}$ = 5.4 Hz, H-4′a), 4.37 (1H, ddd, $J_{4'b,3'}$ = 9.8, $J_{2',3'}$ = 9.7 Hz, H-3′), 4.22 (1H, dd, $J_{4,3}$ = 3.6, $J_{2,3}$ = 1.8 Hz, H-3), 3.94 (1H, ddd, $J_{1'b,2'}$ = 5.7, $J_{1'a,2'}$ = 4.3 Hz, H-2′), 3.88 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 2.3 Hz, H-5a), 3.78 (1H, dd, H-4′b), 3.62 (1H, dd, $J_{4,5b}$ = 1.9 Hz, H-5b), 3.41 (1H, dd, $J_{1b,1a}$ = 11.2 Hz, H-1a), 3.33 (1H, ddd, $J_{1a,2}$ = 1.0 Hz, H-2), 3.26 (1H, dd, $J_{1'b,1'a}$ = 13.8 Hz, H-1′a), 3.11 (2H, m, CH_2), 2.97 (1H, dd, $J_{2,1b}$ = 5.2 Hz, H-1b), 2.78 (1H, dd, H-1′b), 2.56 (1H, ddd, H-4), 1.29 (3H, t, $J_{CH2,CH3}$ = 7.3 Hz, CH_3); ^{13}C NMR (CD_3OD) 138.0, 128.7, 127.9, 126.1 (6C, Ph), 101.1 (CHPh), 78.8 (C-2′), 73.8 (C-3), 73.4 (C-4), 69.3 (C-2), 69.2 (C-3′), 62.9 (C-4′), 58.7 (C-1), 55.2 (C-5), 54.4 (C-1′), 41.1 (CH_2), 10.5 (CH_3). HRMS Calcd for $C_{18}H_{29}O_8N_2S$ (M+H): 433.1645. Found: 433.1638.

1′-((2-*N*-Ethylamino-1,4-imino-1,2,4-trideoxy-D-arabinitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2.9)

Compound **2.29** (10 mg, 0.02 mmol) was subjected to trifluoroacetic acid treatment and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.9** as a syrup (5.6 mg, 70%). $[\alpha]_D$ +29 (*c* 0.04, MeOH); ^{1}H NMR (CD_3OD) δ 4.37 (1H, ddd, $J_{2',3'}$ = 4.7 Hz, H-3′), 4.04 (1H, dd, H-3), 3.98 (1H, ddd, $J_{1'a,2'}$ = 8.3, $J_{1'b,2'}$ = 5.2 Hz, H-2′), 3.82 (1H, dd, $J_{4'b,4'a}$ = 11.9, $J_{3',4'a}$ = 4.2 Hz, H-4′a), 3.71 (1H, dd, $J_{3',4'b}$ = 5.1 Hz, H-4′b), 3.69 (1H, dd, $J_{5b,5a}$ = 11.7, $J_{4,5a}$ = 2.4 Hz, H-5a), 3.54 (1H, dd, $J_{4,5b}$ = 2.3 Hz, H-5b), 3.27 (1H, dd, $J_{1b,1a}$ = 11.0 Hz, H-1a), 3.18 (1H, ddd, $J_{1b,2}$ = 5.3 Hz, H-2), 2.96 (2H, m, CH_2), 2.94 (1H, dd, $J_{1'b,1'a}$ = 12.7 Hz, H-1′a), 2.72 (1H,dd, H-1b), 2.41 (1H, dd, H-1′b), 2.36 (1H, ddd, $J_{3,4}$ = 4.5 Hz, H-4), 1.19 (3H, t, $J_{CH2,CH3}$ = 7.2

Hz, CH_3); ^{13}C NMR (CD_3OD) 80.6 (C-3′), 74.3 (C-3), 73.4 (C-4), 69.3 (C-2′), 63.1 (C-2), 60.6 (C-4′), 58.9 (C-5), 55.8 (C-1′), 54.6 (C-1), 41.2 (CH_2), 10.9 (CH_3). HRMS Calcd for $C_{11}H_{24}O_8N_2SNa$ (M+Na): 367.1151. Found: 367.1193.

_N_-Benzyl-2,3-epoxy-1,4-imino-1,2,3,4-tetradeoxy-D-ribitol (2.25)

To a solution of compound **2.24** (16.7 g, 76.7 mmol) in dry THF (100 ml) was added BH_3.THF (160 mL, 1M solution) at 0° C. The mixture was stirred under N_2 overnight. The reaction was quenched by slowly adding methanol until gas evolution had stopped. Additional methanol (50 mL) was added and the solvents were removed under reduced pressure. The residue was dissolved in methanol (200 mL), the mixture was heated to reflux for 1 h, and the solvent was removed under reduced pressure. Methanol (100 mL) was added and evaporated under reduced pressure. This procedure was repeated twice. Diethyl ether (250 mL) and HCl (250 mL, 0.5 M) were added and the layers were separated. The organic layer was extracted with HCl (250 mL, 0.5 M). The aqueous layers were combined and adjusted to pH 11-12 with NaOH (10 M), and extracted with dichloromethane (300 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography [Hexanes/EtOAc, 2:1] to afford **2.25** as a syrup (11.0 g, 70%). $[\alpha]_D$ -18 (*c* 2.9, MeOH); ^1H NMR ($CDCl_3$) δ 7.34-7.22 (5H, m, Ph), 3.86, 3.79 (2H, d, J_{gem} = 13.4 Hz, CH_2Ph), 3.65 (1H, dd, $J_{2,3}$ = 2.8, $J_{2,1b}$ = 0.5 Hz, H-2), 3.57 (1H, ddd, H-3), 3.48 (1H, dd, $J_{5b,5a}$ = 10.8, $J_{4,5a}$ = 5.1 Hz, H-5a), 3.37 (1H, dd, $J_{4,5b}$ = 6.1 Hz, H-5b), 3.24 (1H, ddd, H-4), 3.15 (1H, dd, $J_{1b,1a}$ = 13.2, $J_{2,1a}$ = 1.2 Hz, H-1a), 2.93 (1H, dd, H-1b); ^{13}C NMR ($CDCl_3$) 140.0, 128.76, 128.72, 127.4 (6C,

Ph), 66.8 (C-4), 63.1 (CH_2Ph), 61.5 (C-5), 60.2 (C-3), 57.9 (C-2), 54.4 (C-1). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 69.99; H, 7.33; N, 6.65.

2-Azido-1,4-imino-1,2,4-trideoxy-D-arabinitol (2.17)

To a solution of compound **2.27** (1.67 g, 6.5 mmol) in methanol (100 mL) was added concentrated HCl (1.2 mL). The mixture was stirred overnight. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **2.17** as a syrup (0.73 g, 72%). $[\alpha]_D$ +1 (*c* 0.7, MeOH); ^{1}H NMR (CD_3OD) δ 4.23 (1H, ddd, $J_{3,2}$ = 4.5 Hz, H-2), 4.12 (1H, dd, H-3), 3.89 (1H, dd, $J_{5b,5a}$ = 11.8, $J_{4,5a}$ = 4.2 Hz, H-5a), 3.76 (1H, dd, $J_{4,5b}$ = 7.5 Hz, H-5b), 3.64 (1H, dd, $J_{1b,1a}$ = 12.4, $J_{2,1a}$ = 6.5 Hz, H-1a), 3.52 (1H, ddd, $J_{3,4}$ = 5.4 Hz, H-4), 3.25 (1H, dd, $J_{2,1b}$ = 4.6 Hz, H-1b); ^{13}C NMR (CD_3OD) δ 74.5 (C-3), 66.7 (C-4), 65.3 (C-2), 58.4 (C-5), 47.2 (C-1). HRMS Calcd for $C_5H_{11}N_4O_2$ (M+H): 159.0882. Found: 159.0884.

***N*-Boc-2-amino-1,4-imino-1,2,4-trideoxy-D-arabinitol (2.14)**

To a solution of **2.27** (270 mg, 1.0 mmol) in MeOH : H_2O (20:1, 30 mL) was added triphenylphosphine (329 mg, 1.25 mmol). The mixture was stirred at room temperature overnight, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography [EtOAc/MeOH/H_2O, 5:3:1] to afford **2.14** as an amorphous solid (223 mg, 92%). $[\alpha]_D$ -58.82 (*c* 2.55, H_2O); ^{1}H NMR (CD_3OD) δ

4.01-3.95 (1H, m, H-5a), 3.95-3.85 (1H, m, H-3), 3.77 (1H, m, H-1a), 3.67-3.60 (2H, m, H-5b, H-4), 3.26 (1H, m, H-2), 3.14 (1H, dd, $J_{1b,1a}$ = 11.4, $J_{2,1b}$ = 4.4 Hz, H-1b), 1.48 (9H, s, $C(CH_3)_3$); ^{13}C NMR (at 70 °C, D_2O) δ 156.8 (CO), 82.4 ($C(CH_3)_3$), 79.5 (C-3), 66.1 (C-4), 60.9 (C-5), 55.6 (C-5), 52.3 (C-2), 52.3 (C-1), 28.5 (CH_3). HRMS Calcd for $C_{10}H_{21}O_4N_2$ (M+H): 233.1501. Found: 233.1490.

_N_-Boc-2-acetamido-1,4-imino-1,2,4-trideoxy-D-arabinitol (2.15)

To a solution of **2.14** (50 mg, 0.2 mmol) in methanol (15 mL) was added acetic anhydride (0.2 mL, 10 equiv.) and the mixture was stirred for 2 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 5:1] to afford **2.15** as a syrup (53 mg, 90%). $[\alpha]_D$ +320 (*c* 0.125, H_2O); ^1H NMR (CD_3OD) δ 4.26-4.06 (1H, m, H-3), 4.11 (1H, m, H-2), 3.92-3.79 (2H, m, H-1a, H-5a), 3.72 (1H, dd, $J_{5a,5b}$ = 11.0 Hz, H-5b), 3.57 (1H, m, H-4), 3.12 (1H, dd, $J_{1a,1b}$ = 11.7, $J_{2,1b}$ = 4.6 Hz, H-1b), 1.95 (3H, s, CH_3), 1.48 (9H, s, $C(CH_3)_3$); ^{13}C NMR (at 70 °C, D_2O) δ 174.9 (CO), 156.8 (CO), 82.7 ($C(CH_3)_3$), 76.6 (C-3), 65.5 (C-4), 60.8 (C-5), 54.9 (C-2), 49.9 (C-1), 28.4 (CH_3), 22.7 (CH_3). HRMS Calcd for $C_{12}H_{23}O_5N_2$ (M+H): 275.1607. Found: 275.1610.

2-Acetamido-1,4-imino-1,2,4-trideoxy-D-arabinitol (2.10)

To a solution of **2.15** (37 mg, 0.1 mmol) in methanol (5 mL) was added HCl (0.03 mL) and the mixture was stirred for 3.5 h. The solvent was removed under reduced

pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 2:1] to afford **2.10** as a syrup (24 mg, 84%). $[\alpha]_D$ +4 (*c 0.17*, MeOH); ^{1}H NMR (D_2O) δ 4.15 (1H, ddd, H-2), 4.07 (1H, dd, $J_{2,3}$ = 7.7 Hz, H-3), 3.81 (1H, dd, $J_{5b,5a}$ = 12.7, $J_{4,5a}$ = 3.2 Hz, H-5a), 3.71 (1H, dd, $J_{4,5b}$ = 5.7 Hz, H-5b), 3.60 (1H, dd, $J_{1b,1a}$ = 12.3, $J_{2,1a}$ = 8.6 Hz, H-1a), 3.45 (1H, ddd, $J_{3,4}$ = 8.1 Hz, H-4), 3.09 (1H, dd, $J_{2,1b}$ = 7.6 Hz, H-1b), 1.86 (3H, s, CH_3); ^{13}C NMR (D_2O) δ 174.8 (CO), 73.1 (C-3), 63.9 (C-4), 57.7 (C-5), 54.5 (C-2), 46.4 (C-1), 22.0 (CH_3). HRMS Calcd for $C_7H_{15}O_3N_2$ (M+H): 175.1083. Found: 175.1081.

CHAPTER 3. ATTEMPTED SYNTHESIS OF 2-ACETAMIDO AND 2-AMINO DERIVATIVES OF SALACINOL—RING OPENING REACTIONS.

Reproduced in part from "Attempted Synthesis of 2-Acetamido and 2-Amino Derivatives of Salacinol. Ring Opening Reactions." *Journal of Organic Chemistry*, **2006**, *71*, 4671-4674.

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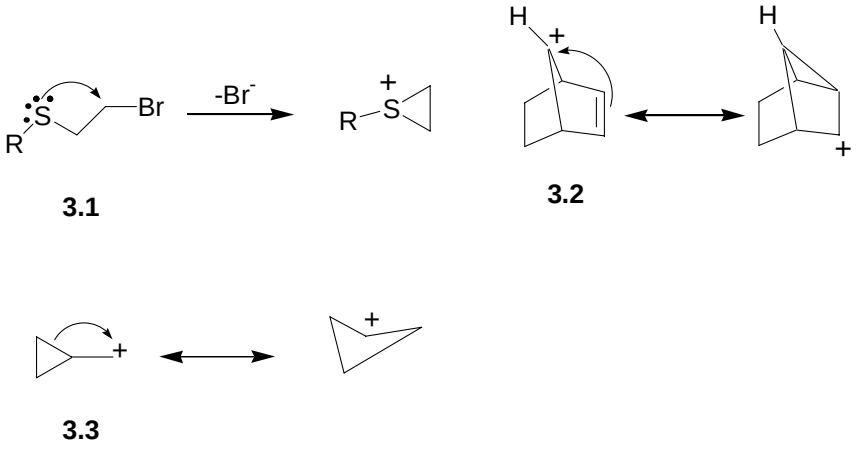
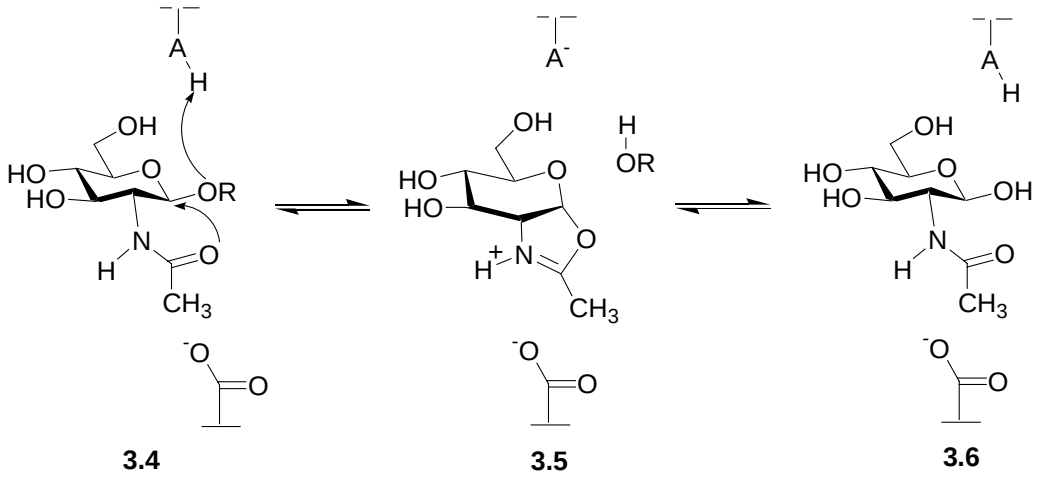
3.1 Neighboring group participation

Neighboring group participation in organic chemistry has been defined as the interaction of a reaction center with a lone pair of electrons in an atom (**3.1**) or the electrons present in a π bond (**3.2**) or σ bond (**3.3**) (Scheme 3.1).[116] When neighboring group participation occurs, it is possible that the stereochemical outcome of the reaction is unexpected compared to that in a normal reaction.



Scheme 3.1. Neighboring group participation in compounds **3.1**, **3.2**, and **3.3**.

Neighboring group participation has been widely studied in carbohydrate chemistry.[117-120] One example of this effect is the participation of the *N*-acetyl group of the *N*-acetyl-β-hexosaminidase inhibitor (**3.4**) to attack the anomeric carbon of the sugar moiety to form the intermediate **3.5** (Scheme 3.2).[97,109,110,121]

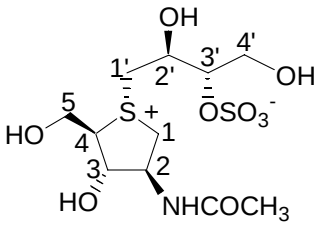


Scheme 3.2. Neighboring group participation of the *N*-acetyl group, proposed in the mechanism of β-hexosaminidases.[110]

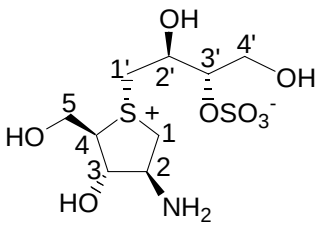
3.2 Introduction

We and others have reported the synthesis of salacinol and its diastereomers,[122-129] the nitrogen and selenium analogues of salacinol,[87,89,130-135] chain-extended analogues,[136-140] and other analogues.[136-140] Some of these derivatives exhibited interesting biological properties.[90,139,140]

It was of interest to investigate the effect of introducing an acetamido or an amine function at the C-2 position of salacinol to broaden the scope of glycosidase inhibitory activities, in particular, to target the hexosaminidase enzymes. Therefore, we report herein the attempted synthesis of the 2-acetamido- (**3.7**) and 2-amino- (**3.8**) substituted analogues of salacinol (Chart 3.1). These derivatives were found to undergo ring opening reactions by nucleophilic participation of the amide or amine moieties to give acyclic amido or ammonium sulfates, respectively.



3.7

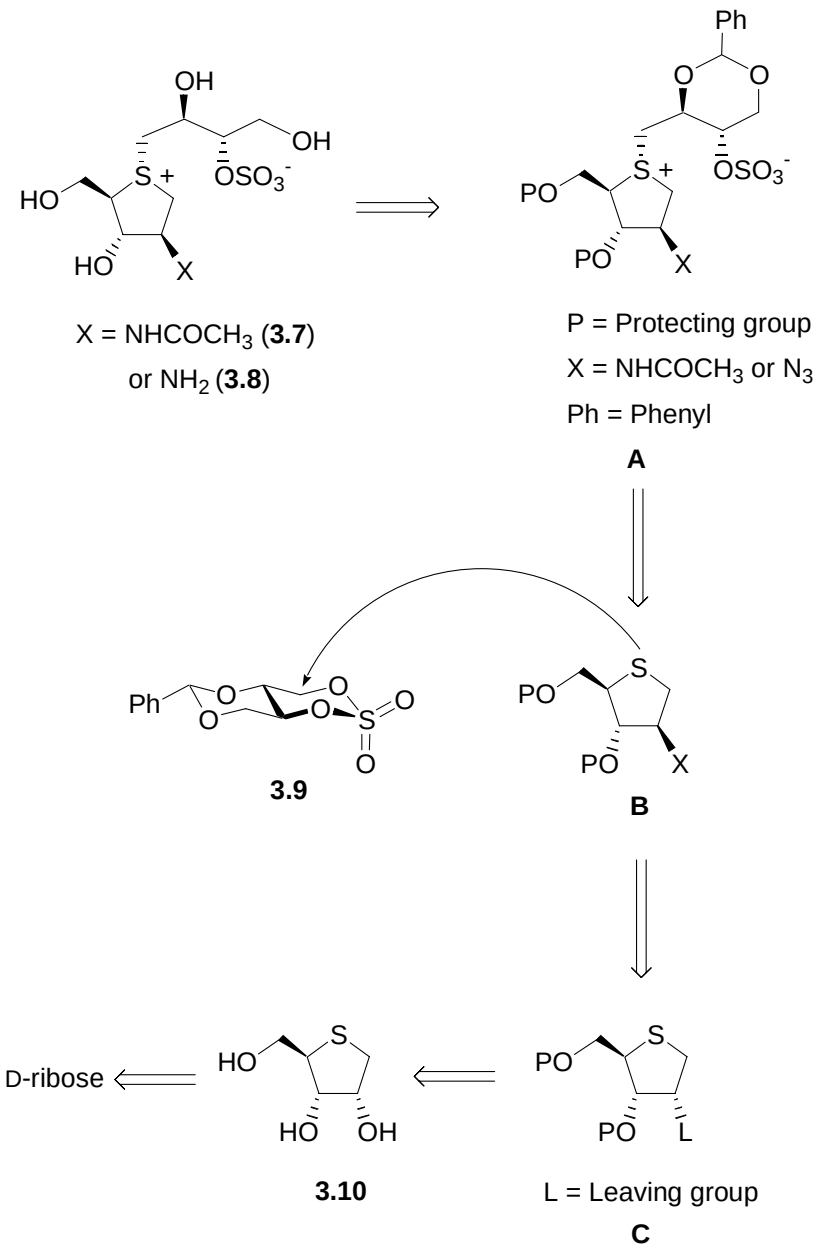


3.8

Chart 3.1. Target compounds **3.7** and **3.8**.

3.3 Results and discussion

The target compounds **3.7** and **3.8** could be obtained by hydrogenolysis of the coupled product **A**, which has an acetamide or an azide group at C-2 (Scheme 3.3). Compound **A** could be synthesized, in turn, by the alkylation of a 2-azido- or 2-acetamido-1,4-anhydro-4-thio-D-arabinitol derivative (**B**) with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**). Compound **B** could, in turn, be synthesized from the reaction of sodium azide with a selectively protected 1,4-anhydro-4-thio-D-ribose (**C**) unit in which C-2 bears a good leaving group. Compound **C** could be obtained from 1,4-anhydro-4-thio-D-ribose (**3.10**) which could be synthesized, in turn, from commercially available D-ribose.[141]

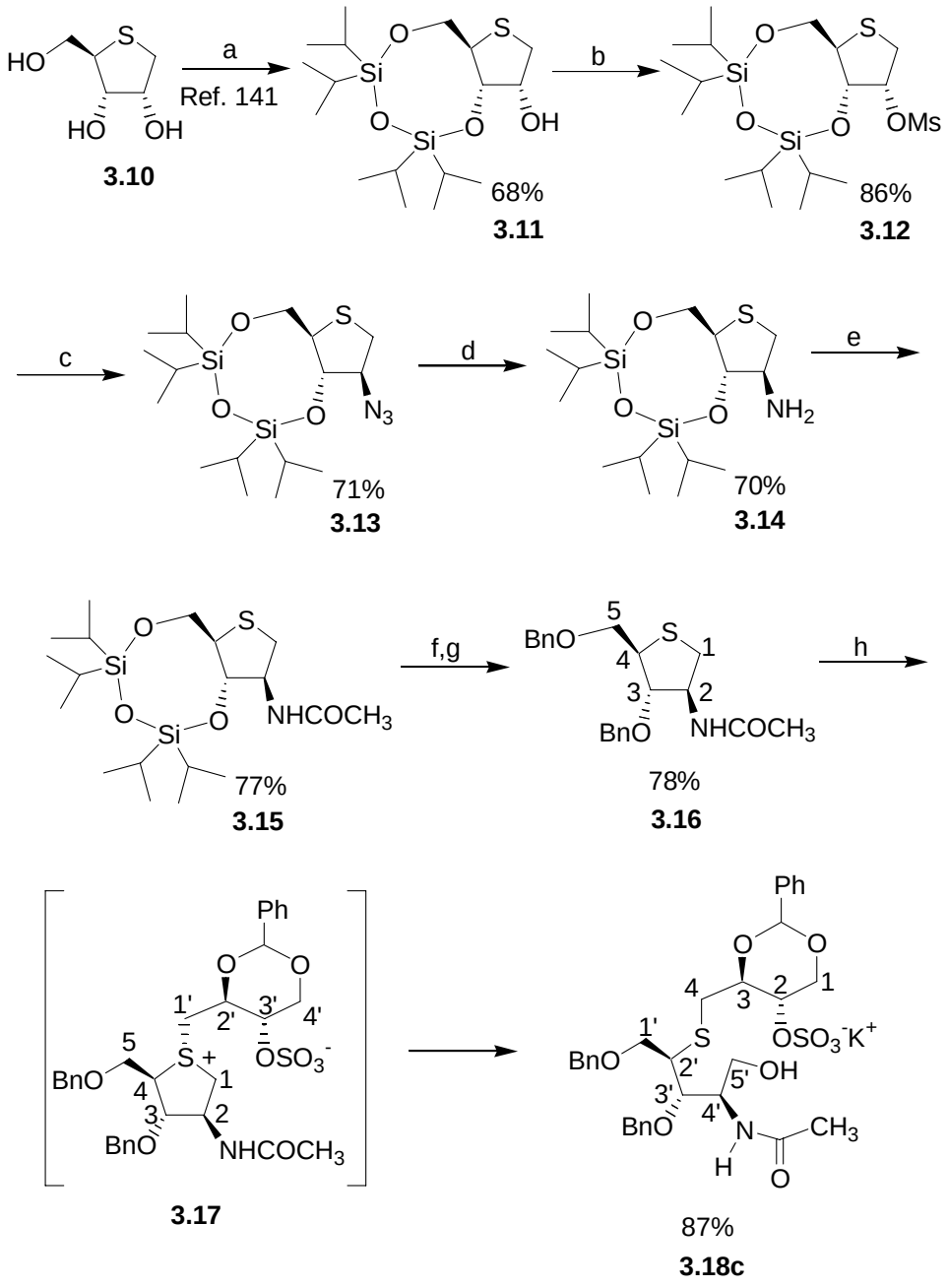


Scheme 3.3. Retrosynthetic analysis.

1,4-Anhydro-4-thio-D-ribose (**3.10**) was synthesized from commercially available D-ribose in 11 steps according to a literature procedure.[141] In order to introduce an azide group selectively at C-2 of compound **3.10**, the 3-OH and 5-OH groups were first protected using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to give **3.11**, and the 2-OH group of compound **3.11** was then mesylated (**3.12**). The substitution of the mesylate in **3.12** by an azide group did not proceed in MeOH; however, at 75 °C in DMF, the substitution proceeded with inversion of configuration at C-2 (**3.13**). The azide group in compound **3.13** was then reduced to an amine using triphenylphosphine (PPh_3) in dioxane:MeOH:H_2O (10:3:1) to afford compound **3.14** in 70% yield; acetylation then afforded compound **3.15** in 77% yield.

The alkylation reaction of compound **3.15** with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**) did not proceed as expected. Instead, the silyl protecting group was removed under the reaction conditions. Therefore, we chose to replace this group by benzyl ethers. The silyl protecting group in **3.15** was thus removed using tetra-n-butylammonium fluoride (TBAF), and the crude product obtained was then subjected to benzylation using benzyl bromide and a mixture of BaO and $Ba(OH)_2.8H_2O$ to afford compound **3.16** in 78% yield. A 1D-NOESY experiment performed on compound **3.16** confirmed the stereochemistry at C-2 by showing a correlation between H-2 and H-4 of the five membered ring. The alkylation reaction between compound **3.16** and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**) gave compound **3.18c** in 87% yield. A 1D-NOESY experiment performed on the coupled product showed no NOE correlation between H-1' and H-1 (as would be expected for the target compound **3.17**); the latter result also confirms that the five membered ring had been opened to give an acyclic

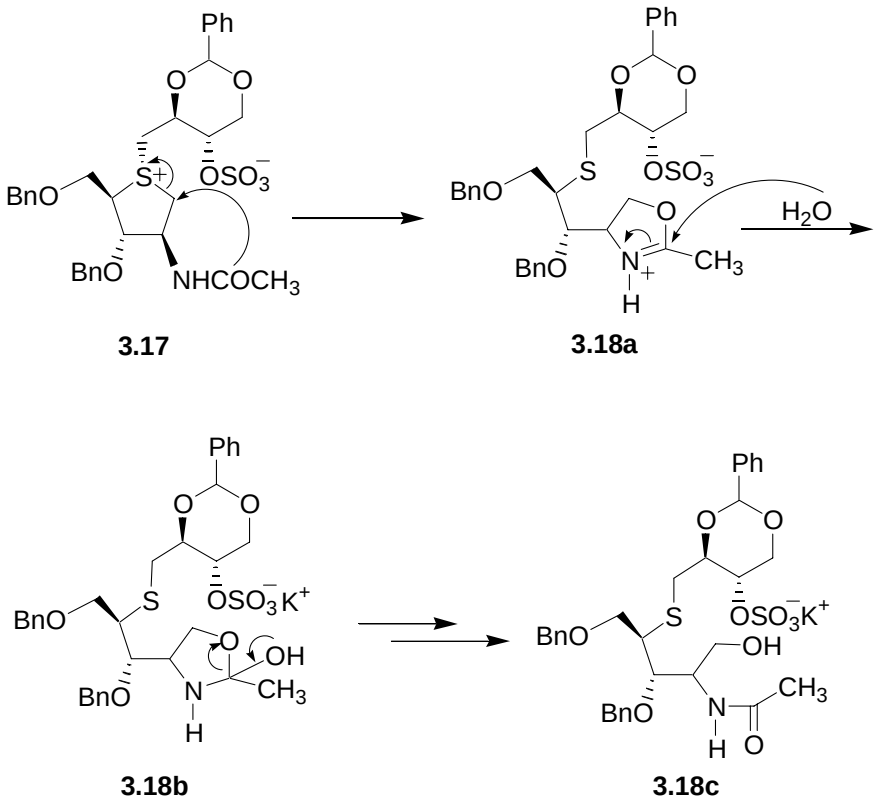
thioether. The ^{13}C NMR spectrum of the coupled product indicated the presence of a carbonyl group, and a g-HMBC spectrum showed a correlation between this carbonyl carbon and H-2, and no correlation between the carbonyl carbon and H-1, thus suggesting **3.18c** as the product of the reaction. The IR spectrum of this product suggested the presence of an OH group as well as an amide carbonyl group, further corroboration of the structure of **3.18c**. The high resolution mass spectrum confirmed the molecular formula of compound **3.18c**. The microanalysis of compound **3.18c** also confirmed the presence of potassium as the counter ion (Scheme 3.4).



Scheme 3.4. Synthesis of the 2-acetamido derivative. Reagents and conditions: (a) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine ($TIPSCl_2$), rt; (b) methanesulfonyl chloride, pyridine; (c) NaN_3, DMF, 85 °C; (d) PPh_3, dioxane:MeOH:H_2O (10:3:1), 55 °C; (e) Ac_2O, pyridine (f) tetra-*n*-butylammonium fluoride (TBAF), THF; (g) BnBr, BaO

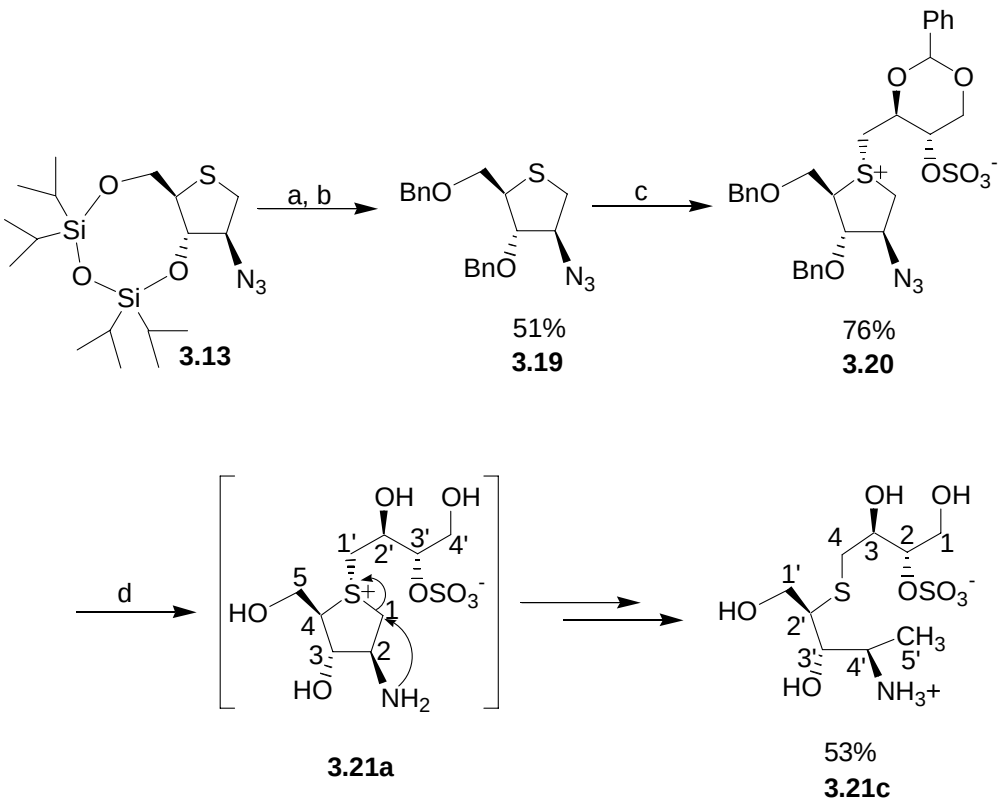
(9.3 equiv), $Ba(OH)_2.8H_2O$ (1.75 equiv), DMF; (h) 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate, K_2CO_3, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 60 °C.

Scheme 3.5 shows the proposed mechanism for the formation of **3.18c**. Nucleophilic attack of the amide oxygen on C-1 of compound **3.17** would result in opening of the five-membered ring to give (**3.18a**) which could then react with water during the processing and purification to give **3.18b**. Further rearrangement of the orthoaminal (**3.18b**) would result in **3.18c**.



Scheme 3.5. Proposed mechanism for rearrangement of **3.18a**.

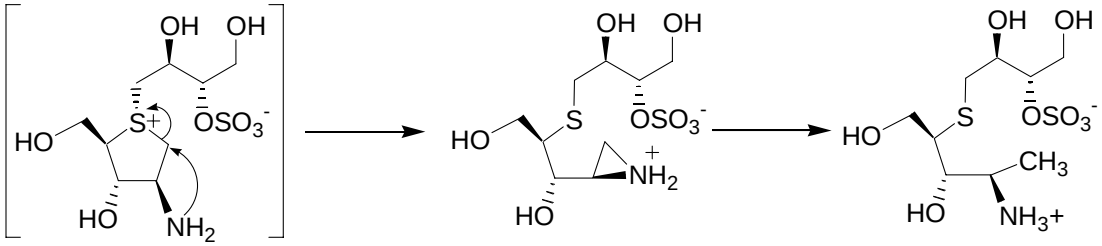
In order to prevent the intramolecular nucleophilic participation of the amide group observed in compound **3.18a**, we next examined the alkylation reaction of a 2-azido arabinitol derivative (Scheme 3.6). Starting from compound **3.13**, the silyl protecting group was substituted by benzyl protecting groups (**3.19**). The alkylation reaction of compound **3.19** with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**) gave the coupled product **3.20** in 76% yield. A 1D-NOESY experiment of the coupled product **3.20** confirmed the formation of the sulfonium salt by showing a correlation between H-1, H-1' and H-4 in this product. Hydrogenolysis of compound **3.20** afforded the acyclic thioether **3.21c** in 53% yield, and not the expected compound **3.21a** (Scheme 3.6). A 1D-NOESY experiment showed no correlation between H-1 and H-1' as one would have expected for compound **3.21a**. g-HMBC data confirmed that compound **3.21c** was the likely product of the reaction. Thus, no correlation between H-5' and C-2' or between H-5' and C-4 was observed. In addition, no correlation between H-2' and C-5' or H-4 and C-5' was observed. These results confirm that the ring is opened. Correlations between H-2' and C-4 and also between H-4 and C-2' (alternative numbering; see Scheme 3.6) were observed, indicating that the side chain is on C-2'. A DEPT experiment showed the existence of a CH_3 group which corroborated the structure **3.21c**, and the high resolution mass spectral data were also consistent with the molecular formula of compound **3.21c**.



Scheme 3.6. Synthesis of the 2-amino derivative. Reagents and conditions: (a) tetra-*n*-butylammonium fluoride (TBAF), THF; (b) BnBr, NaH, DMF; (c) 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 64 °C; (d) H_2, Pd/C.

Scheme 3.7 shows the proposed mechanism for the formation of compound **3.21c** from **3.21a**. Nucleophilic participation of the free amine (**3.21a**) formed during the hydrogenolysis reaction would result in the formation of the aziridinium compound

3.21b. Further reduction of compound **3.21b** during the hydrogenolysis reaction would then afford **3.21c**.

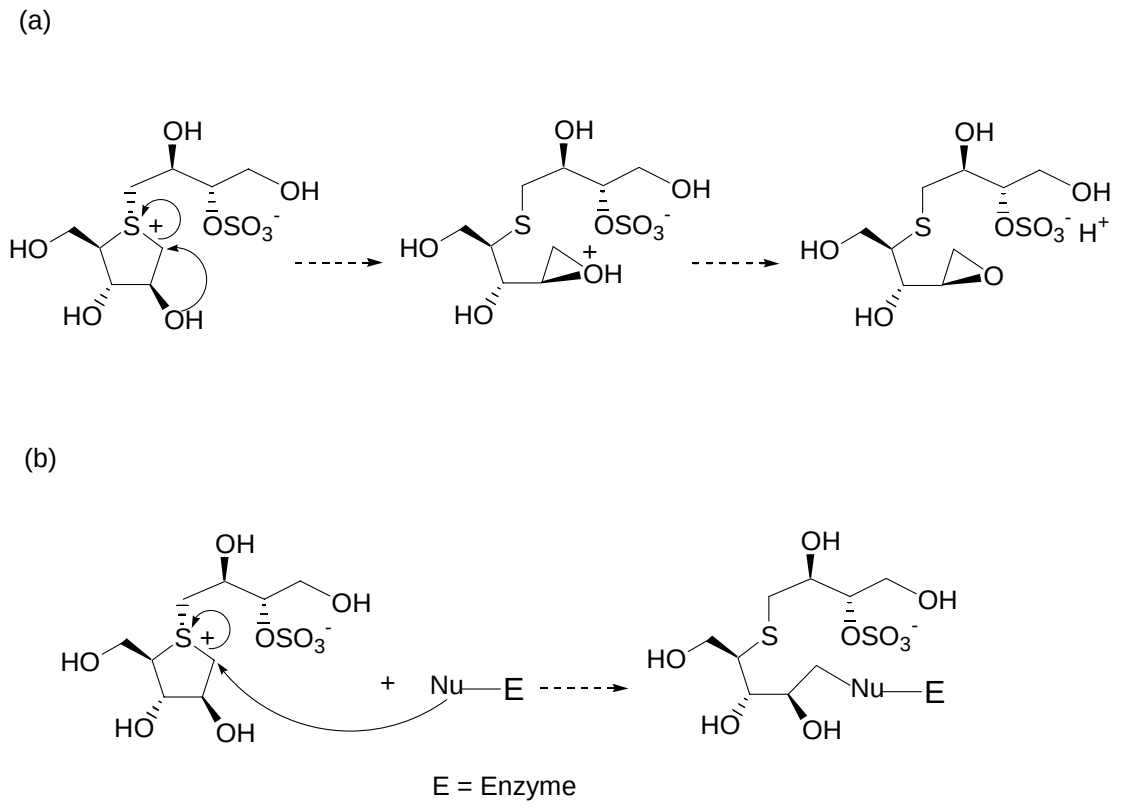


3.21a **3.21b** **3.21c**

Scheme 3.7. Proposed mechanism for the formation of compound **3.21c**.

3.4 Conclusions

In conclusion, the synthesis of the 2-acetamido- and 2-amino- derivatives of salacinol was attempted. However, the 2-acetamido analogue underwent ring opening of the five membered ring by nucleophilic participation of the amide. In the case of the 2-amino derivative, the amine group participated to open the five membered ring via formation of an aziridinium ion. These are the first examples of which we are aware of the instability of salacinol analogues. Our synthetic efforts to date have not revealed any ring-opened, epoxide products stemming from the participation of O-2 in salacinol analogues (Scheme 3.8a). Furthermore, enzyme inhibition studies with a variety of glycosidase enzymes have been consistent with a model of reversible, competitive inhibition, and have not involved ring-opening reactions by nucleophilic groups in the enzyme active sites (Scheme 3.8b).



Scheme 3.8. Potential nucleophilic participation of (a) O-2, (b) enzyme active site nucleophile.

3.5 Experimental

3.5.1 General methods

Optical rotations were measured at 20°C and reported in $deg dm^{-1} g^{-1} cm^3$. ^1H and ^{13}C NMR were recorded with frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY) and ^1H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the data was performed with MestRec software. 1D-NOESY experiments were recorded at 295 K

on a 500 MHz spectrometer. For each 1D-NOESY spectrum, 512 scans were acquired with Q3 Gaussian Cascade pulse. A mixing time of 800 ms was used in all the 1D-NOESY experiments. Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Column chromatography was performed with silica gel 60 (230-400 mesh). High resolution mass spectra were obtained by the electrospray ionization (ESI) technique, using a ZabSpec OA TOF mass spectrometer at 10000 RP.

1,4-Anhydro-2-*O*-(methanesulfonyl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-ribitol (3.12)

To a solution of compound **3.11** (354 mg, 0.9 mmol) in pyridine (10 mL) was added methanesulfonyl chloride (0.1 mL, 1.3 equiv). The mixture was stirred at rt under N_2 for 2 h. The reaction mixture was then quenched by addition of ice, and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL) and brine (50 mL).The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography [Hexanes/EtOAc, 10:1] to afford **3.12** (363 mg, 86%) as a solid. Mp 53-55 °C. $[\alpha]_D$ +42.69 (c 5.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 5.26 (1H, dd, $J_{3,2}$ = 3.7 Hz, H-2), 4.26 (1H, dd, $J_{4,3}$ = 10.0 Hz, H-3), 4.04 (1H, dd, $J_{5b,5a}$ = 12.7, $J_{4,5a}$ = 2.8 Hz, H-5a), 3.91 (1H, dd,

$J_{4,5b}$ = 1.9 Hz, H-5b), 3.49 (1H, brd, $J_{3,4}$ = 9.7 Hz, H-4), 3.19 (1H, brd, $J_{1b,1a}$ = 12.7, $J_{2,1a}$ = 3.1 Hz, H-1a), 2.97 (1H, brd, $J_{2,1b}$ = 1.2 Hz, H-1b), 1.10 – 1.00 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$): δ 84.0 (C-2), 75.0 (C-3), 58.5 (C-5), 48.9 (C-4), 39.2 (CH_3), 31.6 (C-1), 17.6, 17.5, 17.5, 17.5, 17.4, 17.4, 17.3, 17.2 (8×CH_3 of TIPDS), 13.7, 13.5, 13.0, 12.9 (4×CH of TIPDS); MALDI-TOF MS m/e 471.55 (M^+), 493.52 (M^+ + Na). Anal. Calcd. for $C_{18}H_{38}O_6S_2Si_2$: C, 45.92; H, 8.13. Found: C, 46.05; H, 7.98.

1,4-Anhydro-2-deoxy-2-azido-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol (3.13)

To a solution of compound **3.12** (1.2 g, 2.5 mmol) in dry DMF (50 mL) was added sodium azide (0.6 g, 4 equiv). The mixture was stirred at 85 °C for 12 h and then cooled to room temperature. The reaction was quenched by addition of ice. The residue was diluted with ether (100 mL) and washed with H_2O (200 mL) and brine (200 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was then purified by flash chromatography [Hexanes/EtOAc, 20:1] to afford **3.13** (0.8 g, 71%) as a colorless syrup. $[\alpha]_D$ +33.33 (c 1.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 3.99 (1H, t, $J_{4,3}$ = 8.7 Hz, H-3), 3.93 (1H, dd, $J_{5b,5a}$ = 12.4 Hz, H-5a), 3.87 (1H, m, H-2), 3.72 (1H, dd, $J_{5a,5b}$ = 12.4 Hz, H-5b), 3.20 (1H, ddd, $J_{5a,4}$ = 3.1, $J_{5b,4}$ = 5.4, $J_{3,4}$ = 8.4 Hz, H-4), 2.87 (1H, dd, $J_{2,1a}$ = 7.4, $J_{1b,1a}$ = 10.8 Hz, H-1a), 2.61 (1H, dd, $J_{2,1b}$ = 10.4 Hz, H-1b), 1.08-0.95 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$): δ 79.3 (C-3), 68.5 (C-2), 62.8 (C-5), 49.2 (C-4), 28.4 (C-1), 17.6, 17.56, 17.54, 17.5, 17.4, 17.3, 17.29, 17.2 (8×CH_3 of TIPDS), 13.9, 13.5, 13.0, 12.9 (4×CH of TIPDS); MALDI-TOF MS m/e 418.57 (M^+ + H). Anal. Calcd. for $C_{17}H_{35}SO_3N_3Si_2$: C, 48.88; H, 8.44; N, 10.06. Found: C, 49.19; H, 8.65; N, 9.89.

1,4-Anhydro-2-deoxy-2-amino-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol (3.14)

To a solution of compound **3.13** (290 mg, 0.7 mmol) in dioxane:MeOH:H_2O (10:3:1) (28 mL) was added PPh_3 (237 mg, 1.3 equiv). The mixture was stirred at 55 °C for 14 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 6:1] to afford **3.14** (190 mg, 70%) as a colorless syrup. $[\alpha]_D$ -6.98 (c .32, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 4.01 (1H, dd, $J_{5b,5a}$ = 12.4, $J_{4,5a}$ = 3.1 Hz, H-5a), 3.80 (1H, dd, $J_{4,5b}$ = 5.2, H-5b), 3.78 (1H, dd, $J_{2,3}$ = 6.2 Hz, H-3), 3.36 (1H, m, H-2), 3.26 (1H, ddd, $J_{3,4}$ = 8.2, $J_{5a,4}$ = 3.1, $J_{5b,4}$ = 4.9 Hz, H-4), 2.89 (1H, dd, $J_{1b,1a}$ = 10.3, $J_{2,1a}$ = 7.0 Hz, H-1a), 2.56 (1H, dd, $J_{2,1b}$ = 10.3 Hz, H-1b), 1.14-0.94 (28H, m, TIPDS); ^{13}C NMR: δ 81.4 (C-3), 62.6(C-5),60.2 (C-2), 50.4 (C-4), 31.6 (C-1), 17.7, 17.64, 17.63, 17.62, 17.6, 17.5, 17.44, 17,43 (8×CH_3 of TIPDS), 14.1, 13.6, 13.05, 13.04 (4×CH of TIPDS); MALDI-TOF MS m/e 391.77 (M^+), 414.34 (M^+ + Na). Anal. Calcd. for $C_{17}H_{37}SO_3NSi_2$: C, 52.13; H, 9.52, N, 3.58. Found: C, 52.25; H, 9.47; N, 3.74.

1,4-Anhydro-2-deoxy-2-acetamido-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol (3.15)

To a solution of compound **3.14** (188 mg, 0.5 mmol) in pyridine (15 mL) was added Ac_2O (0.9 mL, 20 equiv). The mixture was stirred at rt under N_2 for 7 h and then quenched by addition of ice. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 5:1] to afford **3.15** (160 mg, 77%) as a white solid. Mp 169-170 °C; $[\alpha]_D$ +9.60 (c 0.49, CH_2Cl_2); ^{1}H NMR

($CDCl_3$): δ 5.47 (1H, d, $J_{2,NH}$ = 7.2 Hz, NH), 4.33 (1H, m, $J_{3,2}$ = 0.8 Hz, H-2), 4.09 (1H, dd, $J_{4,3}$ = 7.4 Hz, H-3), 3.96 (1H, dd, $J_{4,5a}$ = 3.2, $J_{5b,5a}$ = 12.3 Hz, H-5a), 3.69 (1H, dd, $J_{4,5b}$ = 5.9 Hz, H-5b), 3.26 (1H, ddd, H-4), 3.12 (1h, dd, $J_{1b,1a}$ = 10.9, $J_{2,1a}$ = 6.9 Hz, H-1a), 2.53 (1H, dd, $J_{2,1b}$ = 8.3 Hz, H-1b), 1.05-0.95 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$): δ 169.8 (CO), 77.9 (C-2), 62.5 (C-5), 57.8 (C-2), 50.9 (C-4), 30.5 (C-1), 23.5 (CH_3), 17.4, 17.39, 17.37, 17.34, 17.33, 17.2, 17.1, 17.0 (8×CH_3 of TIPDS), 14.1, 13.4, 12.9, 12.6 (4×CH of TIPDS); MALDI-TOF MS m/e 433.78 (M^+), 456.52 (M^+ + Na). Anal. Calcd. for $C_{19}H_{39}SO_4NSi_2$: C, 52.61; H, 9.06; N, 3.22. Found: C, 52.27; H, 8.84; N, 2.98.

1,4-Anhydro-2-deoxy-2-acetamido-3,5-di-*O*-benzyl-4-thio-D-arabinitol (3.16)

To a solution of compound **3.15** (150 mg, 0.3 mmol) in distilled THF at 0 °C was added 1M THF solution of TBAF (0.7 mL, 2 equiv). The mixture was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure and the crude product was dissolved in dry DMF (20 mL). Benzyl bromide (0.2 mL, 5 equiv), barium oxide (0.49 g, 9.3 equiv) and barium hydroxide octahydrate (0.2 g, 1.75 equiv) were added at 0 °C. The mixture was stirred at rt for 12 h and then quenched by adding ice. The crude product was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (3×200 mL) and brine (200 mL), filtered, and concentrated. The crude product was purified by flash chromatography [Hexanes/EtOAc, 10:1] to afford **3.16** (100 mg, 78%) as a solid. Mp 88-90 °C. $[\alpha]_D$ +34.28 (c 0.14, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.37-7.25 (10 H, m, Ar), 6.72 (1H, d, $J_{H2,NH}$ = 8.1 Hz, NH), 4.70 (1H, m, $J_{1a,2}$ = 4.4, $J_{3,2}$ = 2.2 Hz, H-2), 4.64 and 4.51 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.56 and 4.54 (2H, 2d, $J_{A,B}$ = 8.3 Hz, CH_2Ph), 3.92 (1H, m, H-3), 3.73 (1H, dd, $J_{5b,5a}$ = 9.7, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.69 (1H, m, H-4), 3.53 (1H, dd, $J_{4,5b}$ =

3.7 Hz, H-5b), 3.31 (1H, dd, $J_{1b,1a}$ = 11.5 Hz, H-1a), 2.74 (1H, d, H-1b), 1.48 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): δ 128.8, 128.7, 128.6, 128.5,128.4, 128.1, 128.0, 127.9, 126.3 (12 C, Ph), 88.1 (C-3), 74.2, 71.8 (2×CH_2Ph), 71.7 (C-5), 56.3 (C-2), 53.4 (C-4), 37.8 (C-1), 23.0 (CH_3); MALDI-TOF MS m/e 371.95 (M^+), 394.16 (M^+ + Na). Anal. Calcd. for $C_{21}H_{25}SNO_3$: C, 67.89; H, 6.78; N, 3.77. Found: C, 67.78; H, 6.73; N, 3.52.

Potassium(2S,3S)-1,3-benzylidenedioxy-4-[(2R,3S,4R)-4'-acetamido-1',3'-bisbenzyl-oxy-5'-hydroxy-2'-pentylthio]butane-2-sulfate (3.18c)

A mixture of compound **3.16** (66.6 mg, 0.2 mmol), 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**) (63.5 mg, 1.3 equiv) and K_2CO_3 (40 mg) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (1.5 mL). The mixture was stirred in a sealed tube at 60 °C for 13 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [CH_2Cl_2/MeOH, 10:1] to afford **3.18c** (100 mg, 87%) as a syrup. $[\alpha]_D$ -28 (c 0.03, MeOH). IR : ν 3200 cm^{-1} (OH) and 1700 cm^{-1} (CO). ^1H NMR (CD_3OD): δ 7.4-7.1 (15H, m, Ar), 5.46 (1H, s, CHPh), 4.58, 4.45 (2H, 2d, $J_{A,B}$ = 10.9 Hz, CH_2Ph), 4.51 (1H, ddd, $J_{5'b,4'}$ = 7.5, $J_{5'a,4'}$ = 6.8, $J_{3',4'}$ = 1.5 Hz, H-4'), 4.48 (1H, dd, $J_{2,1}$ = 5.4 Hz, H-1a), 4.42, 4.39 (2H, 2d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.26 (1H, ddd, $J_{3,2}$ = 9.8 Hz, H-2), 3.88 (1H, dd, $J_{2',3'}$ = 8.7 Hz, H-3'), 3.84 (1H, ddd, $J_{4,3}$ = 1.9 Hz, H-3), 3.78 (1H, dd, $J_{1'b,1'a}$ = 9.8, $J_{2',1'a}$ = 4.1 Hz, H-1'a), 3.70 (1H, dd, $J_{2',1'b}$ = 4.5 Hz, H-1'b), 3.68 (1H, dd, $J_{2,1b}$ = 1.4, $J_{1a,1b}$ = 10.8 Hz, H-1b), 3.6 (1H, dd, $J_{5'b,5'a}$ = 10.7, $J_{4',5'a}$ = 6.9 Hz, H-5'a), 3.4 (1H, dd, $J_{4',5'b}$ = 7.6 Hz, H-5'b), 3.2 (1H, ddd, H-2'), 3.16 (1H, dd, $J_{4b,4a}$ = 15.3, $J_{3,4a}$ = 1.9 Hz, H-4a), 2.93 (1H, s, CH_3), 2.74 (1H, dd, $J_{3,4b}$ = 8.1 Hz, H-4b); ^{13}C NMR (CD_3OD): δ 172.9 (CO), 138.4, 138.3, 137.9, 128.7, 128.2, 128.1, 127.9,

127.8, 127.6, 127.5, 126.2 (18C, Ph), 101.3 (CHPh), 81.6 (C-3), 77.2 (C-3'), 74.4, 73.0 (2×CH_2Ph), 70.3 (C-1'), 69.9 (C-2), 69.0 (C-1), 61.8 (C-5'), 52.3 (C-4'), 47.9 (C-2'), 34.8 (C-4), 21.8 (CH_3). Anal. Calcd. for $C_{32}H_{38}NO_{10}S_2K$: C, 54.91; H, 5.48; N, 2.00. Found: C, 54.89; H, 5.50; N, 2.10. HRMS Calcd. for $C_{32}H_{38}NO_{10}S_2$ (M-(H+K)): 660.19426. Found: 660.19407.

1,4-Anhydro-2-deoxy-2-azido-3,5-di-*O*-benzyl-4-thio-D-arabinitol (3.19)

To a solution of compound **3.13** (145 mg, 0.4 mmol) in distilled THF at 0 °C was added 1M THF solution of TBAF (0.7 mL, 2 equiv). The mixture was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure and the crude product was dissolved in dry DMF (15 mL). The mixture was cooled to 0 °C and NaH (67 mg, 8 equiv) was added followed by dropwise addition of benzyl bromide (0.2 mL, 4 equiv). The mixture was stirred at rt for 15 h and quenched by the addition of ice. The crude product was diluted with ether (100 mL) and washed with brine (200 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated and the crude product was purified by flash chromatography [Hexanes/EtOAc, 30:1] to afford **3.19** (63 mg, 51%) as a colorless syrup. $[\alpha]_D$ +16.52 (c 0.12, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.37-7.27 (10 H, m, Ar), 4.63 (2H, s, CH_2Ph), 4.54 (2H, s, CH_2Ph), 4.17 (1H, ddd, $J_{1a,2}$ = 5.9 Hz, H-2), 3.93 (1H, dd, $J_{2,3}$ = $J_{4,3}$ = 4.9 Hz, H-3), 3.62 (1H, ddd, $J_{5b,5a}$ = 8.9, $J_{4,5a}$ = 6.9 Hz, H-5a), 3.56 (1H, ddd, $J_{5b,4}$ = 5.8 Hz, H-4), 3.50 (1H, dd, H-5b), 3.08 (1H, dd, $J_{1b,1a}$ = 11.4 Hz, H-1a), 2.82 (1H, dd, H-1b); ^{13}C NMR ($CDCl_3$): δ 128.6, 128.5, 128.1, 127.8 (12C, Ph), 86.0 (C-3), 73.3, 72.7 (2×CH_2Ph), 72.1 (C-5), 67.8 (C-2), 49.0 (C-4), 31.9 (C-1); MALDI-TOF

MS m/e 378.44 (M^+ + Na). Anal. Calcd. for $C_{19}H_{21}SN_3O_2$: C, 64.20; H, 5.96; N, 11.82. Found: C, 64.48; H, 6.13; N, 11.48.

2-Azido-3,5-di-*O*-benzyl-1,4-dideoxy-1,4-[[(*2'S,3'S*)-2',4'-*O*-benzylidene-3'-(sulfo-oxy)-butyl]-(*S*)-episulfoniumylidene]-D-arabinitol inner salt (3.20)

Compound **3.19** (148 mg, 0.4 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**3.9**) (170 mg, 1.5 equiv) was dissolved in HFIP (2 mL) and the mixture was stirred in a sealed tube at 64 °C for 24 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [CH_2Cl_2/MeOH, 50:1] to afford compound **3.20** (198 mg, 76%) as a solid. $[\alpha]_D$ +41.69 (c 0.30, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.38-7.1 (15H, m, Ar), 5.51 (1H, S, CHPh), 4.87 (1H, ddd, $J_{1b,2}$ = 7.4, $J_{1a,2} = J_{2,3}$ = 5.9 Hz, H-2), 4.68 and 4.48 (2H, 2d, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.61 (1H, ddd, $J_{2',3'}$ = 9.7, $J_{4'a,3'}$ = 5.3, $J_{4'b,3'}$ = 9.6 Hz, H-3'), 4.57 (1H, dd, $J_{3',4'a}$ = 5.3, $J_{4'b,4'a}$ = 10.5 Hz, H-4'a), 4.45 (1H, dd, $J_{2',1'a}$ = 2.4, $J_{1'b,1'a}$ = 14.2 Hz, H-1'a), 4.38 (1H, ddd, H-2'), 4.24 and 4.18 (2H, 2d, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.19 (1H, dd, $J_{2',1'b}$ = 3.6 Hz, H-1'b), 4.12 (1H, dd, H-3), 4.11 (1H, dd, $J_{2,1a}$ = 5.9 Hz, H-1a), 3.82 (1H, ddd, $J_{5a,4} = J_{3,4}$ = 5.3, $J_{5b,4}$ = 4.1 Hz, H-4), 3.77 (1H, dd, $J_{3',4'b}$ = 10.1 Hz, H-4'b), 3.50 (1H, dd, $J_{2,1b}$ = 7.4, $J_{1b,1a}$ = 13.7 Hz, H-1b), 3.46 (1H, dd, $J_{4,5a}$ = 5.3, $J_{5b,5a}$ = 10.4 Hz, H-5a), 3.29 (1H, dd, $J_{4,5b}$ = 4.2 Hz, H-5b); ^{13}C NMR ($CDCl_3$): δ 136.7, 136.5, 136.3, 129.6, 128.9, 128.8, 128.7, 128.6, 128.5, 128.2 (18C, Ph), 101.56 (CHPh), 83.68 (C-3), 76.21 (C-2'), 73.73, 73.58 (2×CH_2Ph), 69.18 (C-4'), 66.67 (C-3'), 65.53 (C-5), 65.42 (C-2), 64.41 (C-4), 48.76 (C-1'), 43.37 (C-1); MALDI-TOF MS m/e 628.38 (M^+). Anal. Calcd. for $C_{30}H_{33}S_2N_3O_8$: C, 57.40; H, 5.30; N, 6.69. Found: C, 57.17; H, 5.41; N, 6.53.

(2S,3S)-1,3-dihydroxy-4-[(2R,3S,4S)-4'-amino-1',3'-dihydroxy-2'-pentylthio]butane-2-sulfate (3.21c)

Compound **3.20** (178 mg, 0.3 mmol) was dissolved in AcOH:H_2O (4:1, 10mL) and the solution was stirred under H_2 (90 Psi) with palladium hydroxide catalyst on carbon (100 mg). The mixture was filtered after 72 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH/H_2O, 10:3:1] to afford compound **3.21c** (50 mg, 53%) as a colorless syrup. $[\alpha]_D$ -0.05 (c 0.22, MeOH); ^{1}H NMR (D_2O): δ 4.23 (1H, ddd, $J_{3,2}$ = 6.9 Hz, H-2), 3.93 (1H, ddd, H-3), 3.80 (1H, dd, $J_{1b,1a}$ = 12.7, $J_{2,1a}$ = 3.2 Hz, H-1a), 3.74 (1H, dd, $J_{1'b,1'a}$ = 12.0, $J_{2',1'a}$ = 5.5 Hz, H-1'a), 3.70 (1H, dd, H-1b), 3.67 (1H, dd, $J_{2',3'}$ = 5.8 Hz, H-3'), 3.63 (1H, dd, $J_{2',1'b}$ = 5.9 Hz, H-1'b), 3.45 (1H, dt, $J_{3',4'}$ = 6.1 Hz, H-4'), 2.92 (1H, ddd, H-2'), 2.88 (1H, dd, $J_{3,4a}$ = 3.8 Hz, H-4a), 2.64 (1H, dd, $J_{4a,4b}$ = 14.0, $J_{3,4b}$ = 8.2 Hz, H-4b), 1.15 (3H, d, H-5'); ^{13}C NMR (D_2O): δ 81.2 (C-2), 73.7 (C-3'), 69.4 (C-3), 60.4 (C-1'), 59.8 (C-1), 49.8 (C-2'), 49.5 (C-4'), 34.3 (C-4), 16.1 (C-5'). HRMS Calcd. for $C_9H_{20}NO_8S_2$ (M-H): 334.06249. Found: 334.06248.

CHAPTER 4. SYNTHESIS OF 2-DEOXY-2-FLUORO AND 1,2-ENE- DERIVATIVES OF THE NATURALLY OCCURRING GLYCOSIDASE INHIBITOR, SALACINOL, AND THEIR INHIBITORY ACTIVITIES AGAINST RECOMBINANT HUMAN MALTASE GLUCOAMYLASE

Reproduced in part from "Synthesis of 2-deoxy-2-fluoro and 1,2-ene derivatives of the naturally occurring glycosidase inhibitor, salacinol, and their inhibitory activities against recombinant human maltase glucoamylase" *Carbohydrate Research*, **2008**, *343*, 951-956.

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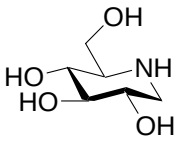
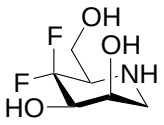
4.1 Introduction to glycosyl fluorides

Glycosyl fluorides have considerable importance as substrates and inhibitors in enzymatic reactions. Their unusual combination of stability and reactivity has enabled their use as glycosyl donors with a variety of carbohydrate processing enzymes. Moreover, the installation of fluorine on a carbohydrate scaffold commonly modifies the properties of the glycosyl fluoride such that the resultant compounds act as slow substrates or even inhibitors of enzyme action.[142]

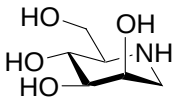
As seen in chapters 1 and 2, iminosugars play an important role in acting as strong and specific inhibitors of carbohydrate-processing enzymes such as glycosidases and glycosyltransferases. A well-known iminosugar known for its glycosidase inhibitory activity is 1-deoxynojirimycin (**1.16**, Chapter 1). Interestingly, the gem-difluorinated mannonojirimycin analogue (**4.1**) was found to be a stronger inhibitor than its corresponding iminosugar **4.2** and 1-deoxynojirimycin (**1.16**) at pH 6.8 and 5.0 suggesting the beneficial influence of a fluorine atom on the iminosugar. It is proposed that the unprotonated iminosugar is the species preferably bound by β-glucosidase, due to the lower pK_a value of iminosugar (**4.1**) than of **1.16** or **4.2**, leaving iminosugar **1.16** and **4.2** mostly protonated at pH 5.0, unlike iminosugar **4.1** (Chart 4.1). Table 4.1 shows the inhibitory activities (K_i) of compounds **4.1**, **4.2**, and **1.16** against β-glucosidases. Compound **4.1** shows better inhibitory activity against almond β-glucosidases relative to its non fluorinated analogue **4.2** (Table 4.1). The inhibitory activity of compound **4.1** is even better than that of 1-deoxynojirimycin **1.16** (Table 4.1).[143]



1.16



4.1



4.2

Chart 4.1. 1-Deoxynojirimycin (**1.16**), gem-difluorinated mannonojirimycin (**4.1**), and 1-deoxymannonojirimycin (**4.2**).

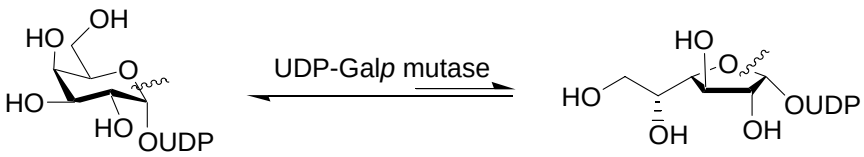
Table 4.1. K_i (μM) of compounds **4.1**, **4.2**, and **1.16**.[143]

Iminosugars	K_i (μM)	
	β-glucosidase (almond) pH = 6.8	β-glucosidase (almond) pH = 5.0
Compound **4.1**	45 ± 5	92 ± 7
Compound **4.2**	300	1400
Compound **1.16**	47	300

Glycosyl fluorides can be applied as substrates for mechanistic probes. They have proven particularly useful as substrates for glycosidases. Glycosyl fluorides were first shown to act as substrates for glycosidases with the β-galactosidase of *Escherichia coli* in 1961.[144] Then, it was realized that the rapid hydrolysis of glycosyl fluorides by the

corresponding glycosidases was a general phenomenon. Typically, glycosyl fluorides are excellent substrates with high k_{cat}/ K_m values.[142] High values of k_{cat}/ K_m are of particular use for the determination of enzymatic reaction rates with small amounts of enzyme and for the determination of K_i values for tight-binding inhibitors. Moreover, the generality of these compounds as substrates for glycosidases and their high k_{cat}/ K_m is reflected in their common usage to determine the stereochemical outcome of enzyme-catalyzed glycosidase hydrolysis.[145,146]

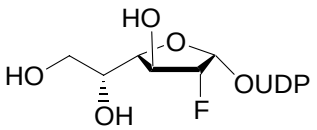
Fluorinated compounds have been used to study the mechanism of a carbohydrate processing enzyme. Thus, UDP-Gal*f* (**4.4**) is the compound derived from UDP-Gal*p* (**4.3**) with catalysis by UDP-galactopyranose mutase (Scheme 4.1).[147,148]



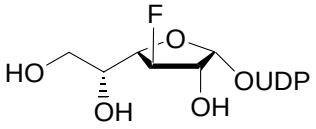
4.3 **4.4**

Scheme 4.1. Conversion of UDP-Gal*p* (**4.3**) to UDP-Gal*f* (**4.4**).

To understand the mechanism of this transformation, UDP-2-deoxy-2-fluorogalactofuranose (UDP-[2-F]Gal*f*) (**4.5**) and UDP-3-deoxy-3-fluorogalactofuranose (UDP-[3-F]Gal*f*) (**4.6**) were used to examine the competence of these two compounds as substrates and/or inhibitors for UDP-Gal*p* mutase.[149]



4.5



4.6

Chart 4.2. UDP-[2-F]Gal*f* (**4.5**) and UDP-[3-F]Gal*f* (**4.6**).

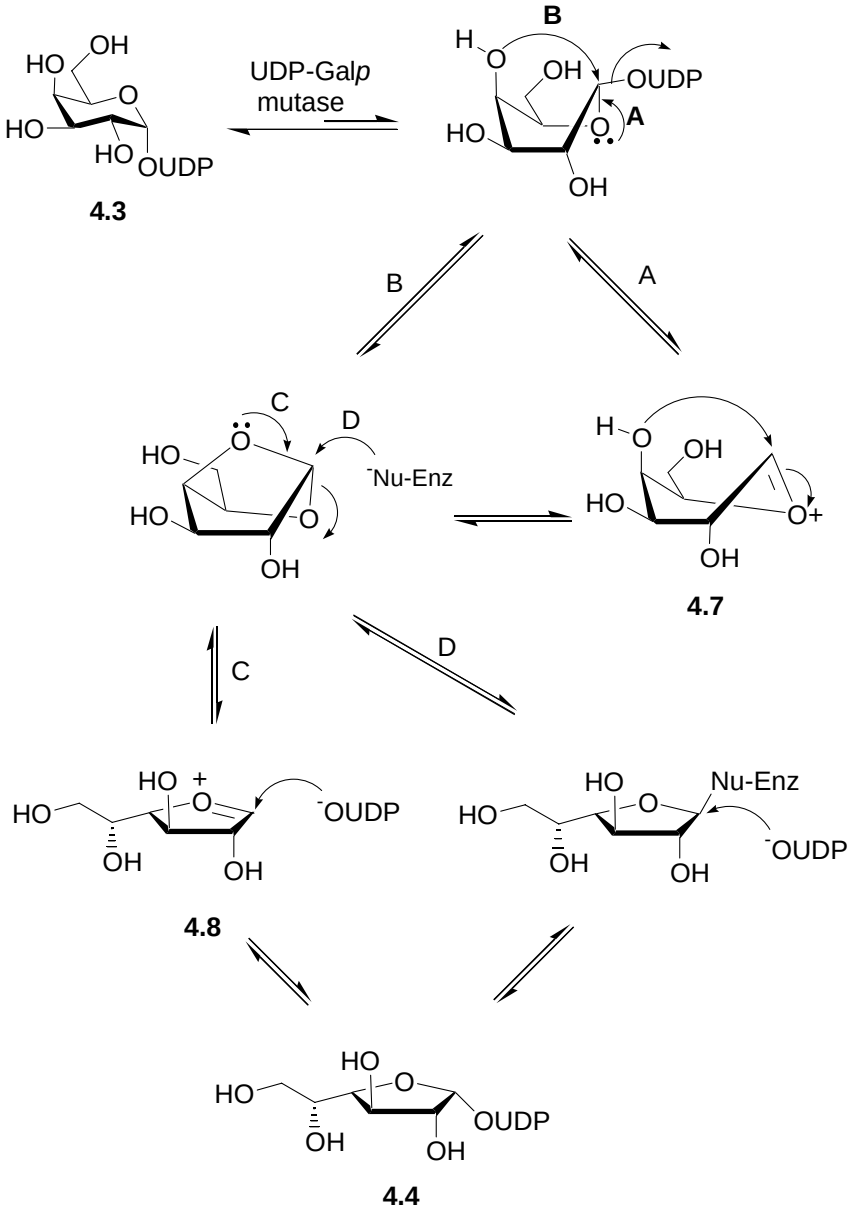
Kinetic parameters have been calculated for reduced UDP-Gal*p* mutase (Table 4.2). The kinetic parameters for turnover of **4.5** and **4.6** in the reverse direction under reducing conditions were determined by fitting the initial reaction rates to the Michaelis-Menten equation based on nonlinear regression. The K_m values were determined to be 65 and 861 μM for UDP-[2-F]Gal*f* (**4.5**) and UDP-[3-F]Gal*f* (**4.6**), respectively. The corresponding k_{cat} values were estimated to be 0.033 and 5.7 s^{-1} (Table 4.2). In comparison to that of UDP-Gal*f* (**4.4**), the catalytic efficiencies (k_{cat}/K_m) for UDP-[2-F]Gal*f* and UDP-[3-F]Gal*f* have decreased by approximately 4 and 3 orders of magnitude, respectively. Since the K_m values for UDP-[2-F]Gal*f* (**4.5**) and UDP-Gal*f* (**4.4**) are comparable, the substitution of 2-OH with a fluorine atom appears to have little effect on the interactions in the Michaelis complex. In contrast, the large K_m value for UDP-[3-F]Gal*f* (**4.6**) implicates the 3-hydroxyl group of the galactose unit in binding to the reduced enzyme to form the Michaelis complex. Replacing the 3-OH with a fluorine atom may have altered the hydrogen-bonding network essential for the interactions in the Michaelis complex.[149]

The reduced catalytic efficiency for UDP-[3-F]Gal*f* has been attributed, by STD-NMR and molecular dynamics, to the partial population of a binding mode of UDP-[3-F]Gal*f* that is non-productive with respect to reaction.[150]

Table 4.2. Kinetic parameters for compounds **4.4**, **4.5**, and **4.6**.[149]

substrates	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m	ratio
UDP-Gal*f* (**4.4**)	22	27	1.2273	1
UDP-[2-F]Gal*f* (**4.5**)	65	0.033	0.00051	4.1×10^{-4}
UDP-[3-F]Gal*f* (**4.6**)	861	5.7	0.0066	5.4×10^{-3}

As indicated by k_{cat} values, both **4.5** and **4.6** are poorer substrates than UDP-Gal*f* (**4.4**). The rate reduction is especially profound for **4.5**, whose fluorine substituent is immediately adjacent to the anomeric center. Such rate retardation is likely due to destabilization of the oxacarbenium ion-like intermediates or transition states by the electron-withdrawing fluorine group. This effect is expected to be distance-dependent and should result in suppression of the anomeric C─O bond cleavage. Thus, the above results support a mechanism for the catalysis by UDP-Gal*p* mutase involving oxacarbenium ions such as **4.7** and/or **4.8**, as intermediates and/or transition states (Scheme 4.2).[149]



Scheme 4.2. Proposed mechanism of conversion of UDP-Gal*p* (**4.3**) to UDP-Gal*f* (**4.4**).

Theoretical and experimental studies on nucleotides containing a 1,2,4-trideoxy-2-fluoro-1,4-anhydro-4-thio-D-arabinose sugar ring had indicated an unusual conformational preference due to steric and stereoelectronic effects.[151] In addition, the substitution of a fluorine atom for a hydroxyl group has proven advantageous in the

design of carbohydrate-based enzyme inhibitors. Therefore, it was of interest to synthesize a salacinol analogue with a fluorine atom at the C-2 position (**4.9**) to probe its glycosidase inhibitory activity (Chart 4.3).

We report herein the synthesis of **4.9** and its elimination product **4.10** (Chart 4.3), together with their inhibitory activities against human maltase glucoamylase (MGA), a key intestinal enzyme involved in the breakdown of maltooligosaccharides into glucose itself.

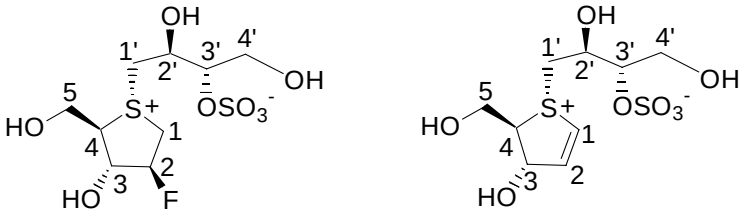
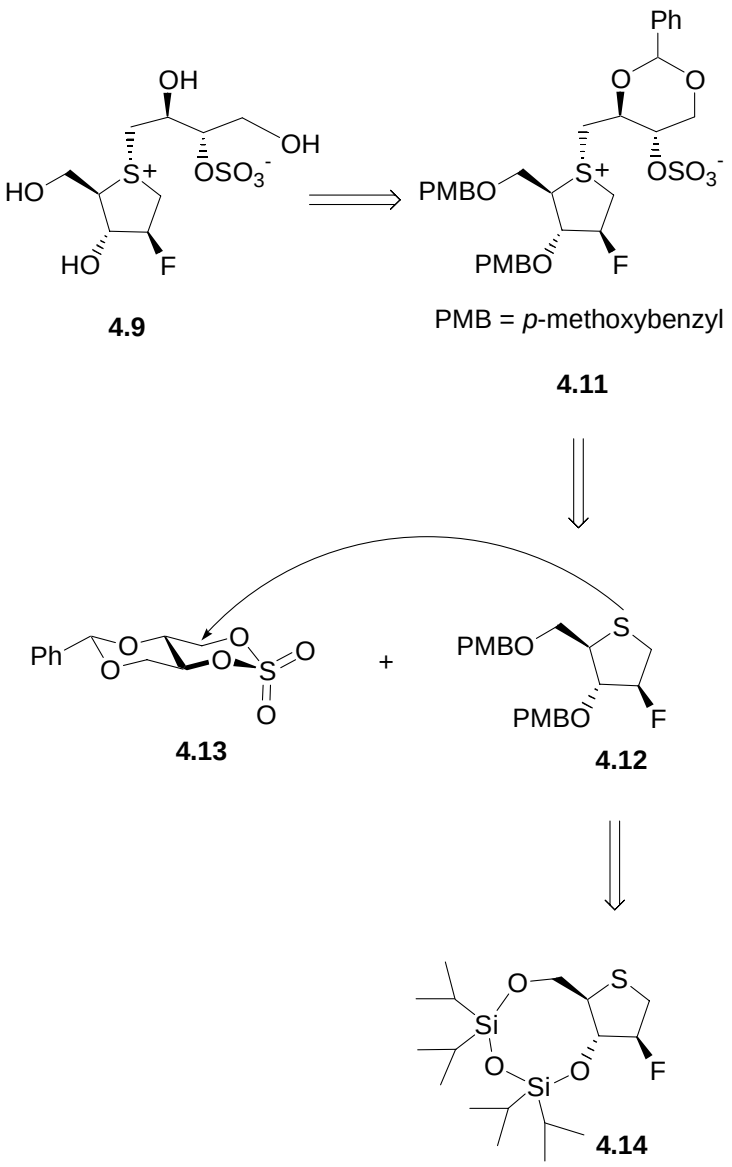


Chart 4.3. Target compounds **4.9** and **4.10**.

4.2 Results and discussion

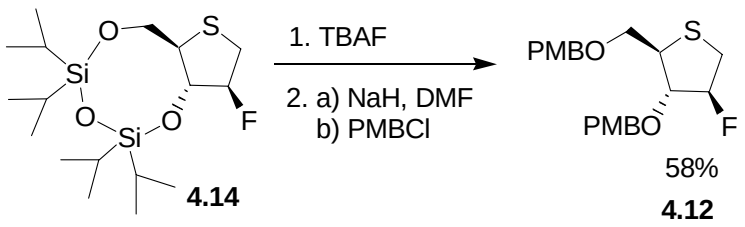
Retrosynthetic analysis indicated that the target compound **4.9** could be synthesized by removal of the benzylidene and *p*-methoxybenzyl (PMB) protecting groups in compound **4.11**, which could be synthesized, in turn, by nucleophilic attack of compound **4.12** at the less hindered carbon of 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate **4.13**. Compound **4.12** could be synthesized from 1,4-anhydro-2-deoxy-2-fluoro-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol **4.14** (Scheme 4.3).[141,151]



Scheme 4.3. Retrosynthetic analysis.

Compound **4.14** was synthesized from 1,4-anhydro-4-thio-D-arabinitol according to literature procedures.[141,151] The silyl protecting group in **4.14** was removed using tetrabutylammonium fluoride (TBAF), and the resulting diol was protected with the acid

labile protecting group, *p*-methoxybenzyl (PMB), to afford compound **4.12** in 58% yield (Scheme 4.4).



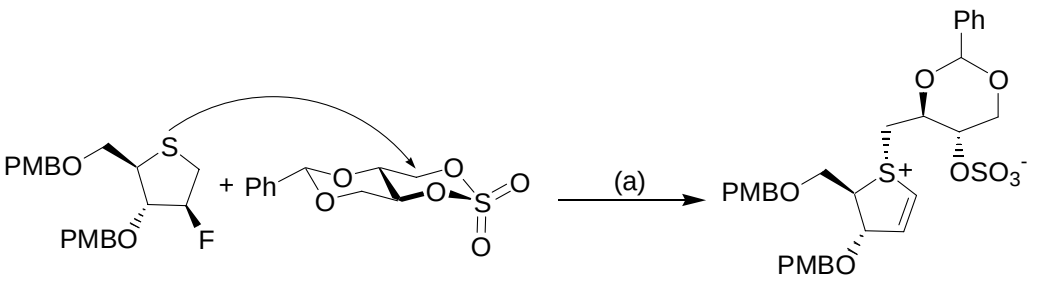
Scheme 4.4. Synthesis of compound **4.12**.

The alkylation reaction of **4.12** with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate **4.13**[122,123] in hexafluoroisopropanol (HFIP) as the solvent containing an excess of K_2CO_3 in a sealed tube did not yield the desired compound **4.11**, but the elimination product **4.15**. Presumably elimination follows alkylation owing to the increased acidity of the protons adjacent to the sulfonium-ion center. Compound **4.15** was deprotected using trifluoroacetic acid (TFA, 90%) to afford compound **4.10** for testing as a potential glycosidase inhibitor (Scheme 4.5).

The alkylation reaction of **4.12** with the cyclic sulfate **4.13** in HFIP did not proceed in the absence of K_2CO_3 at 50-70 °C, and at higher temperatures, the starting materials started to decompose. The alkylation reaction of **4.12** with the cyclic sulfate **4.13** in acetonitrile containing K_2CO_3 was attempted but the reaction did not proceed and

starting compounds were recovered. This result suggested that HFIP might be a better choice of solvent for this type of reaction.

Interestingly, the alkylation reaction of **4.12** with the cyclic sulfate **4.13** in HFIP containing 0.3 M equiv of K_2CO_3 in a sealed tube at 68 °C proceeded smoothly to give the desired coupled product **4.11**. The benzylidene and *p*-methoxybenzyl protecting groups were then removed using trifluoroacetic acid (90%) to afford the desired product **4.9** (Scheme 4.5).

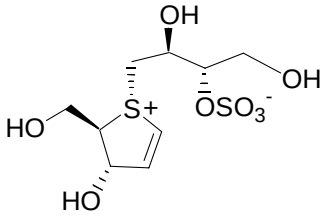


4.12 **4.13**

62%

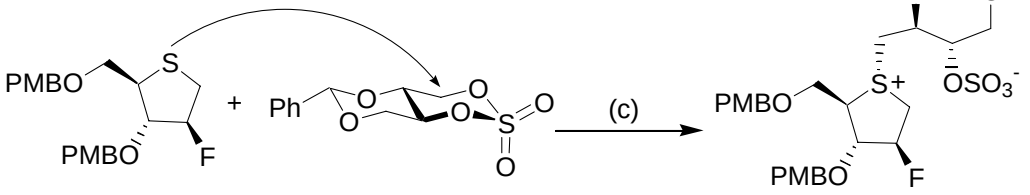
4.15

(b)



74%

4.10

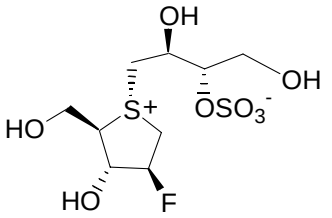


4.12 **4.13**

70%

4.11

(b)



75%

4.9

Scheme 4.5. Reaction of compound **4.12** with compound **4.13**, with different amounts of K_2CO_3. Reagent and conditions: (a) K_2CO_3 (excess), HFIP, 55-70 °C; (b) TFA (90%); (c) K_2CO_3 (0.3 M equiv), HFIP, 68 °C.

Finally, we comment on the inhibitory activities of the compounds **4.9** and **4.10** against recombinant human maltase glucoamylase (MGA). Compound **4.10** inhibited MGA with an IC_{50} = 150 μM, whereas compound **4.9** showed greater inhibition with K_i = 6 ± 1 μM. Because salacinol itself shows a K_i = 0.2 ± 0.02 μM,[5] it would appear that the OH group on C-2 of salacinol is critical as a hydrogen-bond donor with functional groups in the active site of MGA.

4.3 Conclusions

2-Deoxy-2-fluoro and 1,2-ene derivatives of salacinol, a naturally occurring glycosidase inhibitor, were synthesized. Nucleophilic attack of 2-deoxy-2-fluoro-3,5-di-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-D-arabinitol (**4.12**) at the least hindered carbon of a benzylidene protected L-erythritol-1,3-cyclic sulfate (**4.13**) in the presence of excess K_2CO_3 proceeded to an alkene derivative of the sulfonium salt by releasing one HF molecule from the product. Coupling reaction did not proceed to the desired product by removal of the K_2CO_3 from the reaction mixture and after some time starting compounds started to decompose. In presence of 0.3 M eqiv of K_2CO_3 in the reaction mixture, the reaction proceeded to the desired 2-deoxy-2-fluorosalacinol. This allowed us to conclude that little amount of K_2CO_3 is necessary for the reaction to proceed. Solvent change from HFIP to acetonitrile did not help the reaction to proceed. This confirms that HFIP might

be the best choice of solvent for this type of reaction. Enzyme kinetic studies revealed that compound **4.9** is a marginal inhibitor of MGA.

4.4 Experimental

4.4.1 General methods

Optical rotations were measured at 21°C and reported in $degdm^{-1}g^{-1}cm^3$. ^1H and ^{13}C NMR were recorded with frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY) and ^1H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the data was performed with MestRec software. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Column chromatography was performed with silica gel 60 (230-400 mesh). High resolution mass spectra were obtained by the electrospray ionization (ESI) technique, using a ZabSpec OA TOF mass spectrometer at 10000 RP.

4.4.2 Enzyme assays

Enzyme assays of MGA with compounds **4.9** and **4.10** were determined using a *p*NP-glucoside assay to follow the production of *p*-nitrophenol upon addition of enzyme (500 nM). Initially, IC_{50} values were determined; in the case of **4.10**, a high value (150 μM) dictated that further detailed kinetic analysis was not justified, whereas in the case of

4.9, further kinetic analysis was pursued. The latter assay was carried out in 96-well microtiter plates containing 100 mM MES buffer pH 6.5, inhibitor (at three different concentrations), and *p*-nitrophenyl-α-D-glucopyranoside (*p*NP-glucoside) as substrate (2.5, 3.5, 5, 7.5, 15, and 30 mM) with a final volume of 50 μL. Reactions were incubated at 37 °C for 35 min and terminated by addition of 50 μL of 0.5 M sodium carbonate. The absorbance of the reaction product was measured at 405 nm in a microtiter plate reader. All reactions were performed in triplicate, and absorbance measurements were averaged to give a final result. Reactions were linear within this time frame. The program GraFit 4.0.14 was used to fit the data to the Michaelis-Menten equation and estimate the kinetic parameters, K_m, K_{mobs} (K_m in the presence of inhibitor), and V_{max} of the enzyme. K_i value for the inhibitor was determined by the equation $K_i = [I]/((K_{mobs}/K_m) - 1)$. The K_i reported for the inhibitor was determined by averaging the K_i values obtained from three different inhibitor concentrations.

1,4-Anhydro-2-deoxy-2-fluoro-3,5-di-*O*-(*p*-methoxybenzyl)-4-thio-D-arabinitol (4.12)

To a solution of compound **4.14** (1.9 g, 4.9 mmol) in distilled THF (25 mL) was added tetrabutylammonium fluoride (TBAF) in THF (9.8 mL, 1M). The mixture was stirred at room temperature for 2 h and then quenched by addition of ice. The organic phase was separated and the solvent was removed under reduced pressure. The crude product was dissolved in DMF (20 mL) and the solution was added dropwise to a suspension of sodium hydride (0.6 g, 26 mmol) in DMF (50 mL) at 0 °C. After addition was complete, the temperature was brought to room temperature and the mixture was

stirred at room temperature for 1 h. PMBCl (2.3 g, 15 mmol) was added dropwise to the mixture, the mixture was stirred at room temperature overnight, and then the reaction was quenched by addition of ice. The mixture was extracted with ether (150 mL), the organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography [Hexanes/EtOAc, 5:1] to afford **4.12** as a syrup (1.1 g, 58%). $[\alpha]_D$ 21 (*c* 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.27-7.22, 6.90-6.85 (8H, m, Ar), 5.15 (1H, m, $J_{F,2}$ = 50.4, $J_{3,2}$ = 3.2, $J_{1a,2}$ = 7.4 Hz, H-2), 4.59-4.50 (2H, d, J_{AB} = 11.6 Hz, CH_2Ph), 4.49-4.43 (2H, d, CH_2Ph), 4.21 (1H, m, $J_{F,3}$ = 11.4 Hz, H-3), 3.82, 3.81 (6H, s, OMe), 3.61-3.56 (1H, m, H-4), 3.57 (1H, m, H-5a), 3.47 (1H, m, H-5b), 3.39-2.83 (2H, m, H-1a, H-1b); ^{13}C NMR ($CDCl_3$): δ 159.6, 159.4, 130.3, 129.9, 129.7, 129.6, 114.1, 113.9 (8C, Ar), 97.7 (d, $J_{F,2}$ = 181.9 Hz, C-2), 84.2 (d, $J_{F,3}$ = 24.2 Hz, C-3), 73.0 (CH_2Ph), 71.8 (C-5), 71.7 (CH_2), 55.5 (2×OMe), 49.5 (C-4), 34.5 (d, $J_{F,1}$ = 22.3 Hz, C-1). HRMS Calcd for $C_{21}H_{25}O_4SFNa$ (M+Na): 415.1349. Found: 415.1348.

1,4-Dideoxy-1,2-ene-3,5-di-*O*-*p*-methoxybenzyl-1,4-[[(2S,3S)-2,4-*O*-benzylidene-3-(sulfooxy)butyl]-episulfoniumylidene]-D-arabinitol Inner Salt (4.15)

Compound **4.12** (50 mg, 0.1 mmol), 2,4-benzylidene-L-erythritol-1,3-cyclic sulfate (**4.13**) (46 mg, 1.3 equiv), and K_2CO_3 (40 mg, 0.3 mmol) were dissolved in HFIP. The mixture was stirred in a sealed tube in an oil bath at 70 °C for 20 h. K_2CO_3 was removed by filtration and the solvent was removed under reduced pressure. Flash chromatography of the crude product [EtOAc/MeOH, 5:1] afforded compound **4.15** as a syrup (51 mg, 62%). $[\alpha]_D$ +93 (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD): δ 7.40-7.32 (5H, m,

Ar), 7.20, 7.13, 6.90, 6.84 (8H, dd, Ar), 7.00 (1H, dd, $J_{1,2}$ = 5.7, $J_{3,2}$ = 2.7 Hz, H-2), 6.57 (1H, d, H-1), 5.54 (1H, s, CHPh), 4.88 (1H, dd, H-3), 4.59 (1H, d, J_{AB} = 11.6 Hz, CH_2Ph), 4.50 (1H, d, CH_2Ph), 4.44 (1H, dd, $J_{4'b,4'a}$ = 10.6, $J_{3',4'a}$ = 5.3 Hz, H-4′a), 4.43 (1H, m, $J_{2',3'}$ = 4.1 Hz, H-3′), 4.32 (1H, m, H-2′), 4.30, 4.28 (2H, d, J_{AB} = 11.7 Hz, CH_2Ph), 4.17 (1H, m, H-4), 4.11 (1H, d, $J_{1'b,1'a}$ = 13.9, $J_{2',1'a}$ = 4.1 Hz, H-1′a), 3.88 (1H, dd, $J_{2',1'b}$ = 3.4 Hz, H-1′b), 3.80-3.73 (1H, m, H-4′b), 3.77, 3.76 (6H, s, 2×OMe), 3.53 (1H, dd, $J_{5b,5a}$ = 10.7, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.36 (1H, dd, $J_{4,5b}$ = 4.5 Hz, H-5b); ^{13}C NMR (CD_3OD): δ 160.1, 160.0 (2C, Ar), 146.5 (C-2), 137.8, 136.9 (2C, Ar), 130.2, 129.7, 128.2, 127.9 (4C, Ar), 129.3, 129.0, 128.9, 128.7, 126.2, 126.1 (6C, Ph), 119.4 (C-1), 114.0, 113.9 (4C, Ar), 101.1 (CHPh), 85.9 (C-3), 75.3 (C-2′), 72.8, 72.7 (2C, CH_2Ph), 68.6 (C-4′), 67.6 (C-3′), 66.2 (C-4), 65.3 (C-5), 54.6 (OMe), 49.4 (C-1′). HRMS Calcd for $C_{32}H_{37}O_{10}S_2$ (M+1): 645.1823. Found: 645.1828.

1,4-Dideoxy-1,2-ene-1,4-[[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylid-ene]-D-arabinitol Inner Salt (4.10)

Compound **4.15** (50 mg, 0.1 mmol) was dissolved in trifluoroacetic acid (2 mL, 90%) and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **4.10** as a syrup (18.1 mg, 74%). $[\alpha]_D$ +50 (*c* 0.02, MeOH); ^{1}H NMR (CD_3OD): δ 6.93 (1H, dd, $J_{1,2}$ = 5.7, $J_{3,2}$ = 2.6 Hz, H-2), 6.61 (1H, d, H-1), 5.18 (1H, m, $J_{4,3}$ = 1.0 Hz, H-3), 4.39 (1H, m, $J_{3',2'}$ = 8.4, $J_{1'b,2'}$ = 4.1 Hz, H-2′), 4.32 (1H, m, $J_{4'b,3'}$ = 3.2 Hz, H-3′), 4.16 (1H, m, H-4), 4.15 (1H, dd, $J_{5b,5a}$ = 12.7, $J_{4,5a}$ = 3.6 Hz, H-5a), 4.07 (1H, dd, $J_{1'b,1'a}$ = 13.4, $J_{2',1'a}$ = 4.2 Hz, H-1′a), 4.01 (1H, dd, $J_{4,5b}$ =

5.2 Hz, H-5b), 3.95 (1H, dd, $J_{4'b,4'a}$ = 12.2, $J_{3',4'a}$ = 3.4 Hz, H-4′a), 3.86 (1H, dd, H-1′b), 3.84 (1H, dd, H-4′b)); ^{13}C NMR (CD_3OD): δ 147.6 (C-2), 119.3 (C-1), 79.3 (C-3), 78.6 (C-3′), 70.9 (C-4), 65.7 (C-2′), 60.4 (C-4′), 58.7 (C-5), 53.6 (C-1′). HRMS Calcd for $C_9H_{17}O_8S_2$ (M+1): 317.0359. Found: 317.0362.

1,2,4-Trideoxy-2-fluoro-3,5-di-*O*-*p*-methoxybenzyl-1,4-[[(2S,3S)-2,4-*O*-benzylidene-3-(sulfooxy)butyl]-episulfoniumylidene]-D-arabinitol Inner Salt (4.11)

Compound **4.12** (53 mg, 0.1 mmol), 2,4-benzylidene-L-erythritol-1,3-cyclic sulfate (**4.13**) (60 mg, 1.7 equiv), and K_2CO_3 (5 mg, 0.04 mmol) were dissolved in HFIP. The mixture was stirred in a sealed tube in an oil bath at 68 °C overnight. K_2CO_3 was removed by filtration and the solvent was removed under reduced pressure. Flash chromatography of the crude product [EtOAc/MeOH, 5:1] afforded compound **4.11** as a syrup (63 mg, 70%). $[\alpha]_D$ +180 (*c* 0.008, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.46-7.43, 7.38-7.35 (5H, m, Ar), 7.21, 7.07 (4H, dd, Ar), 6.88-6.83 (4H, dd, Ar), 5.57 (1H, d, $J_{F,2}$ = 48.1 Hz, H-2), 5.51 (1H, s, CHPh), 4.69 (1H, m, $J_{2',3'}$ = 9.8, $J_{4'a,3'}$ = 5.4 Hz, H-3′), 4.62 (1H, d, J_{AB} = 11.9 Hz, CH_2Ph), 4.60 (1H, dd, H-4′a), 4.53 (1H, d, CH_2Ph), 4.46 (1H, dd, $J_{5b,5a}$ = 13.9, $J_{4,5a}$ = 3.0 Hz, H-5a), 4.42 (1H, m, $J_{F,3}$ = 8.3 Hz, H-3), 4.38 (1H, ddd, $J_{3,4}$ = 9.6 Hz, H-4), 4.28 (1H, dd, $J_{4,5b}$ = 2.9 Hz, H-5b), 4.26-4.19 (1H, m, H-1a), 4.23, 4.17 (2H, J_{AB} = 11.5 Hz, CH_2Ph), 4.12 (1H, m, H-2′), 4.10 (1H, m, $J_{1a,1b}$ = 9.9 Hz, H-1b), 3.82 (1H, m, $J_{3',4'b}$ = 6.1 Hz, H-4′b), 3.81, 3.78 (6H, s, OMe), 3.52 (1H, dd, $J_{1'b,1'a}$ = 10.1 Hz, H-1′a), 3.48 (ddd, $J_{2',1'}$ = 7.1, $J_{F,1'}$ = 2.2 Hz, H-1′b); ^{13}C NMR ($CDCl_3$): 160.1, 159.8, 136.8, 130.6, 130.2, 129.8 (6C, Ar), 129.6, 128.8, 128.6, 127.8, 126.4 (5C, Ph), 114.4, 114.2 (2C, Ar), 101.8 (CHPh), 95.6 (d, $J_{F,2}$ = 185.2 Hz, C-2), 82.9 (d, $J_{F,3}$ = 25.5 Hz, C-3), 76.5

(C-4), 73.4, 72.5 (2C, CH_2Ph), 69.3 (C-4′), 66.8 (C-3′), 65.8 (d, $J_{F,1'}$ = 6.8 Hz, C-1′), 65.2 (C-2′), 55.5(2×OMe), 49.7 (C-5), 47.9 (d, $J_{F,1}$ = 23.8 Hz, C-1). HRMS Calcd for $C_{32}H_{37}O_{10}S_2FNa$ (M+Na): 687.1704. Found: 687.1709.

1,2,4-Trideoxy-2-fluoro-1,4-[[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfonium-ylidene]-D-arabinitol Inner Salt (4.9)

Compound **4.11** (50 mg, 0.1 mmol) was dissolved in trifluoroacetic acid (2 mL, 90%) and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [EtOAc/MeOH, 3:1] to afford compound **4.9** as a syrup (19 mg, 75%). $[\alpha]_D$ +16 (*c* 0.24, MeOH); ^{1}H NMR (CD_3OD): δ 5.51 (1H, d, $J_{F,2}$ = 48.4 Hz, H-2), 4.77 (1H, d, $J_{F,3}$ = 4.8 Hz, H-3), 4.38 (1H, ddd, $J_{2',3'}$ = 7.6, $J_{3',4'b}$ = 3.7 Hz, H-3′), 4.32 (1H, ddd, H-2′), 4.19 (1H, dd, H-5a), 4.18 (1H, m, H-4), 4.12 (1H, dd, $J_{1b,1a}$ = 13.2, $J_{2,1a}$ = 3.6 Hz, H-1a), 4.09 (1H, dd, $J_{5a,5b}$ = 14.0, $J_{4,5b}$ = 4.0 Hz, H-5b), 4.01 (1H, dd, $J_{2,1b}$ = 6.0 Hz, H-1b), 3.97 (1H, dd, $J_{4'b,4'a}$ = 11.9, $J_{3',4'a}$ = 6.1 Hz, H-4′a), 3.96 (1H, dd, $J_{1'b,1'a}$ = 12.2, $J_{2',1'a}$ = 3.5 Hz, H-1′a), 3.85 (1H, dd, $J_{2',1'b}$ = 1.9 Hz, H-1′b), 3.83 (1H, dd, H-4′b); ^{13}C NMR (CD_3OD): δ 97.7 (d, $J_{F,2}$ = 184 Hz, C-2), 79.1 (C-2′), 76.5 (d, $J_{F,3}$ = 25 Hz, C-3), 70.7 (C-4), 66.3 (C-3′), 60.5(C-4′), 58.7(d, $J_{F,1'}$ = 6.9 Hz, C-1′), 51.9 (C-1), 47.2 (C-5). HRMS Calcd for $C_9H_{17}O_8S_2FNa$ (M+Na): 359.0241. Found: 359.0247.

CHAPTER 5. SYNTHESIS OF A SUITABLE PRECURSOR OF 4′-THIONUCLEOSIDES AND 4'-THIOOLIGONUCLEOTIDES

Reproduced in part from "Synthesis and Conformational Analysis of 2'-Fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU)" Jonathan K. Watts, Kashinath Sadalapure, Niloufar Choubdar, B. Mario Pinto, and Masad J. Damha *Journal of Organic Chemistry*, **2006**, *71*, 921-925;

"2'-Fluoro-4'-thioarabino-modified oligonucleotides conformational switches linked to siRNA activity" Jonathan K. Watts, Niloufar Choubdar, Kashinath Sadalapure, Francis Robert, Alexander S. Wahba, Jerry Pelletier, B. Mario Pinto and Masad J. Damha *Nucleic Acid Research*, **2007**, *35*, No.5, 1441-1451.

5.1 Introduction

Nucleoside analogues constitute an important class of biologically active compounds, especially as antiviral and anticancer agents. For example, 1-β-D-arabinosylcytosine (araC, **5.1**) is clinically used for the treatment of acute myeloblastic leukemia,[152] and 2′-deoxy-2′,2′-difluorocytidine (gemcitabine, **5.2**) has been introduced as a chemotherapeutic agent for solid tumors (Chart 5.1).[153,154]

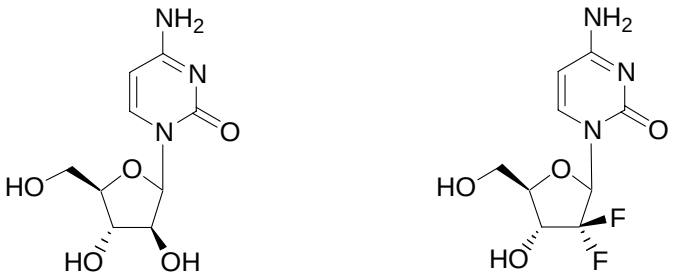
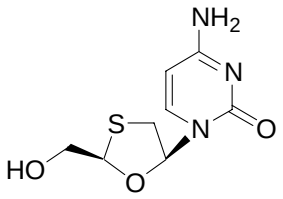


Chart 5.1. 1-β-D-Arabinosylcytosine (araC, **5.1**), 2′-deoxy-2′,2′-difluorocytidine (gemcitabine, **5.2**).

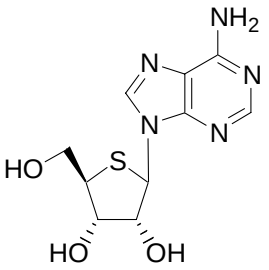
Some nucleoside analogues are also used to inhibit reverse transcriptase coded by human immunodeficiency virus (HIV), a causative agent of acquired immunodeficiency syndrome (AIDS).[155,156] Of particular importance, 3′-thiacytidine (3TC, **5.3**), has recently been approved as an anti-HIV agent by the United States Food and Drug Administration (Chart 5.2).[157-160]



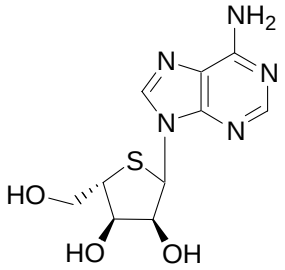
5.3

Chart 5.2. 3′-Thiacytidine (3TC, **5.3**).

Another class of nucleosides are 4′-thionucleosides. The first example of this class was reported in 1964 by Reist et al., who synthesized the 4′-thio counterparts of naturally occurring adenosine (**5.4, 5.5**) (Chart 5.3).[161]



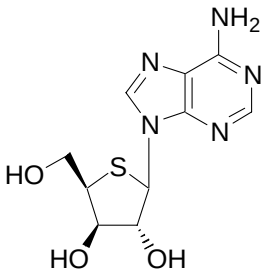
5.4



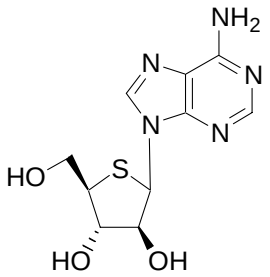
5.5

Chart 5.3. 4-Thio-D- (**5.4**) and -L-ribofuranose (**5.5**) adenine nucleosides.

In 1968, 9-(4-thio-D-xylofuranosyl)adenine (**5.6**) and 9-(4-thio-D-arabinofuranosyl)adenine (**5.**7) were synthesized.[162]



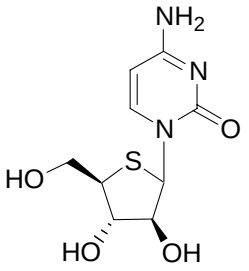
5.6



5.7

Chart 5.4. 9-(4-Thio-β-D-xylofuranosyl)adenine (**5.6**), 9-(4-thio-β-D-arabinofuranosyl)adenine (**5.7**).

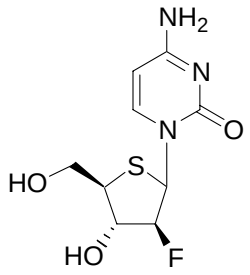
Since then, several examples of 4′-thionucleotides have been reported including 4′-thio-araC (**5.8**) which is as active as araC (**5.1**) itself (Chart 5.5).[163]



5.8

Chart 5.5. 4′-Thio-araC (**5.8**).

Several 4′-thionucleosides have also been reported for their therapeutic activities. Among them, 1-(2-deoxy-2-fluoro-β-D-4-thio-arabinofuranosyl)cytosine (4′-thioFAC) (**5.9**) has shown broad antitumor activities against various human tumor cell lines *in vitro* as well as *in vivo*.[164]



5.9

Chart 5.6. 1-(2-Deoxy-2-fluoro-β-D-4-thio-arabinofuranosyl)cytosine (4′-thioFAC) (**5.9**).

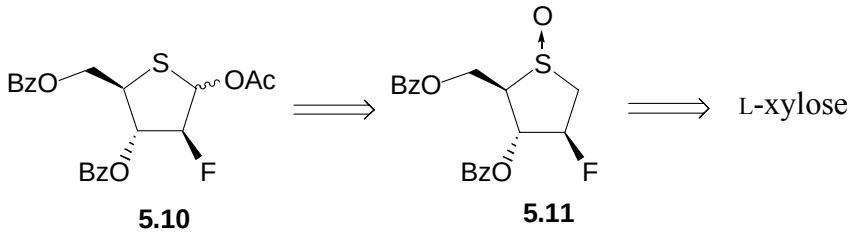
In 1991, Walker[165,166] and Secrist[167] independently reported the synthesis of pyrimidine 2′-deoxy-4′-thionucleotides. The method developed by Walker which included a new and efficient synthesis of 2-deoxy-4-thioribose, was particularly useful.[168]

A major advantage of 4'-thionucleosides is that these compounds are resistant to hydrolytic cleavage of the glycosyl linkage catalyzed by nucleoside phosphorylase.[169] This is a major advantage since several 4′-oxy antivirals have fatal drawbacks with regard to their metabolic stability.[170,171] In addition, the potent antiviral activity and cytotoxicity of 4′-thionucleosides suggest that they are well-recognized as substrates by both viral and host cell kinases. Thus, 4′-thionucleosides have received considerable attention as potential antiviral agents.[170-176]

Thus, we embarked on the synthesis of 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (**5.10**) as a precursor for the synthesis of nucleosides and nucleotides (Scheme 5.1).

5.2 Results and discussion

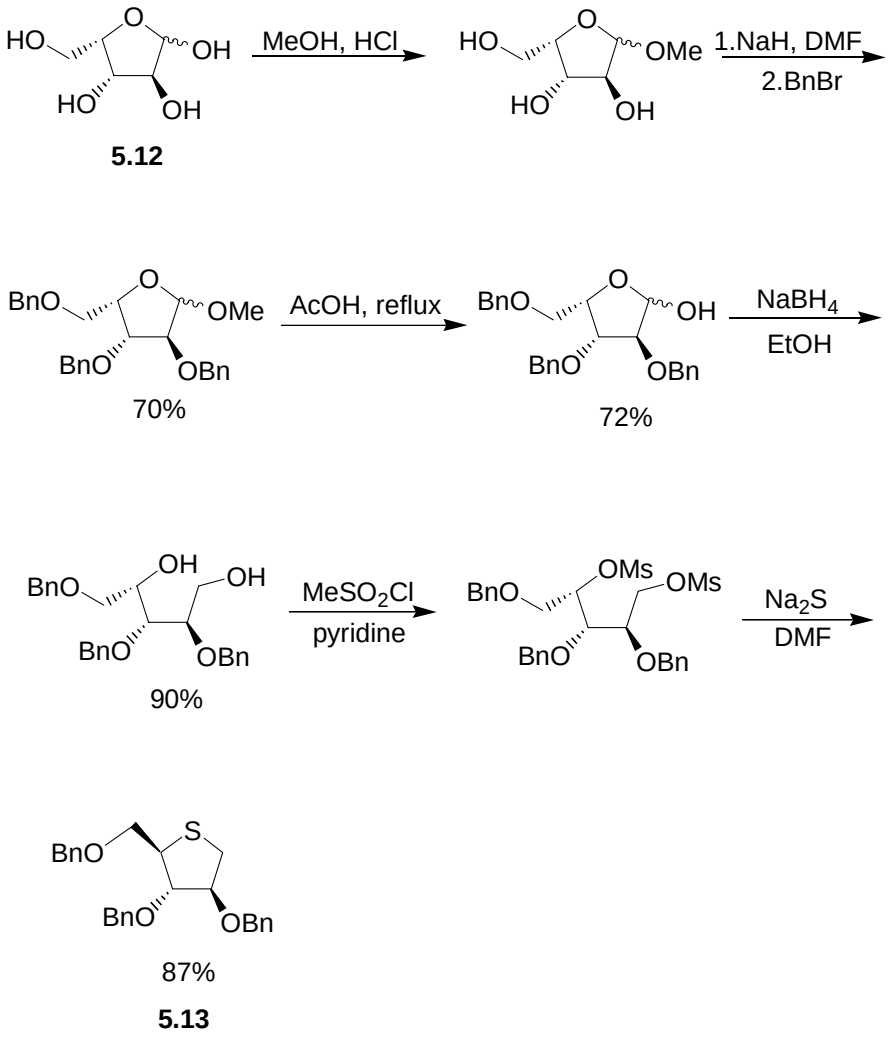
Retrosynthetic analysis shows that the target compound **5.10,** can be synthesized from the Pummerer rearrangement of the sulfoxide **5.11,** which can be obtained from L-xylose in 12 steps (Scheme 5.1).



5.10 **5.11**

Scheme 5.1. Retrosynthetic analysis.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-arabinitol (**5.13**) was synthesized from L-xylose (**5.12**) following a similar procedure developed by Satoh et al. (Scheme 5.2).[177]

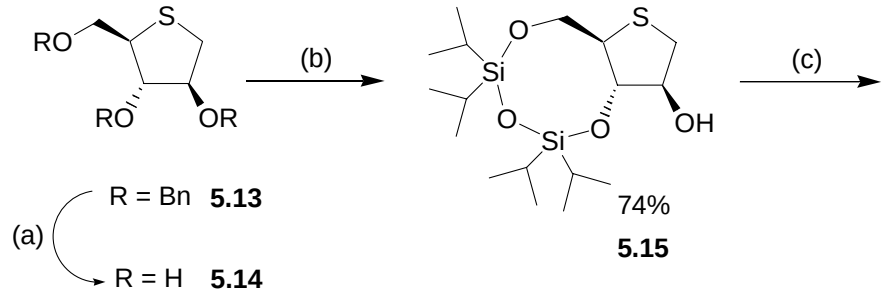


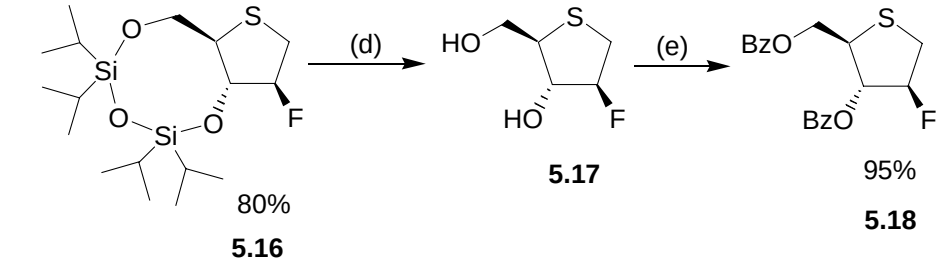
Scheme 5.2. Synthesis of 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-arabinitol (**5.13**).

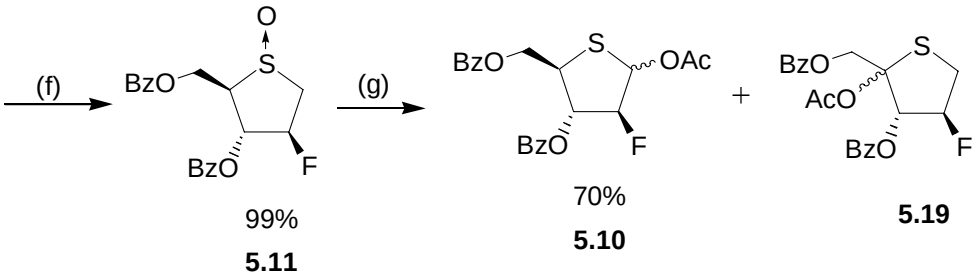
Compound **5.13** was then deprotected using Birch reduction (Li/liq.NH_3) to afford the triol **5.14** (Scheme 5.3).[75] In order to introduce a fluorine substituent at C-2 selectively, it was first necessary to protect the 3-OH and 5-OH groups. Treatment of the triol **5.14** with equimolar ratios of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine afforded the desired compound **5.15**.

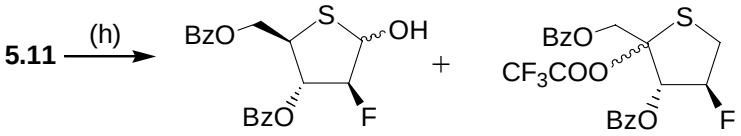
We then attempted a direct nucleophilic displacement of the 2-OH group using diethylamino sulfur trifluoride (DAST). Although fluoride ions have been used to cleave silyl protecting group,[141] the reaction proceeded within 10 minutes to give the desired 2-fluoro derivative (**5.16**) in 80% yield without significant cleavage of the silyl protecting group. Moreover, the reaction proceeded with retention of configuration at C-2, presumably through an epi-sulfonium ion intermediate.[178,179] A similar observation was made by Yoshimura et al. in the synthesis of 2'-modified-2'-deoxy-4'-thiocytidines.[180]

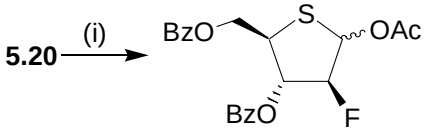
In order to introduce various bases at C-1 to synthesize nucleosides, we chose to functionalize C-1 as an *O*-acetate derivative through the Pummerer reaction, as used by Naka et al.[141] The sulfide **5.16** was subjected to ozonization and then to Pummerer rearrangement but several product spots were observed on the TLC of the reaction mixture probably due to partial cleavage of the silyl protecting group. Therefore, we decided to replace the silyl protecting group with benzoyl (Bz) protecting groups. Therefore, sulfide **5.16** was treated with tetrabutylammonium fluoride (Bu_4NF) followed by BzCl in pyridine to afford compound **5.18**. Sulfide **5.18** was then subjected to ozonization at –78 °C to afford the sulfoxide **5.11**. Reaction of the sulfoxide with Ac_2O at 110 °C afforded the desired 1-*O*-acetyl derivative **5.10** but as a 1:1 inseparable mixture with the 4-*O*-acetate isomer (**5.19**). In order to avoid this problem we performed the Pummerer rearrangement using trifluoroacetic anhydride ($(CF_3CO)_2O$). Pummerer rearrangement using $(CF_3CO)_2O$ proceeded in mild condition (0 °C) and the side product (**5.21**) resulting from the rearrangement to the C-4 was easily separated from the hemiacetal product **5.20** using flash chromatography. Acetylation of **5.20** afforded the desired product **5.10** (Scheme 5.3).


RO
RO
OR
S
(b)
O
Si
O
Si
O
OH
S
(c)
(a)
R = Bn
5.13
R = H
5.14
74%
5.15


O
Si
O
Si
O
F
S
(d)
HO
HO
F
S
(e)
BzO
BzO
F
S
80%
5.16
5.17
95%
5.18

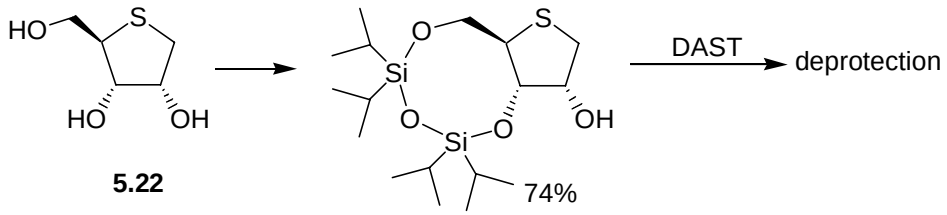

(f)
O
S
BzO
BzO
F
(g)
BzO
OAc
BzO
F
+
BzO
AcO
BzO
F
S
99%
5.11
70%
5.10
5.19


5.11
(h)
BzO
OH
BzO
F
+
BzO
CF3COO
BzO
F
S
54%
5.20
5.21


5.20
(i)
BzO
OAc
BzO
F
S
73%
5.10

Scheme 5.3. Synthesis of 1-*O*-Acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (**5.10**). Reagents and conditions: a) Na, liq. NH_3, -78 °C, b) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, rt., 3h, c) DAST, CH_2Cl_2, -18 °C, 15 min, d) Bu_4NF, THF, rt., 30 min, e) BzCl, pyridine, rt., 6h, f) O_3, CH_2Cl_2, -78 °C, 30 min, g) Ac_2O, 110 °C, 3h, h) $(CF_3CO)_2O$, 0 °C, 30 min, i) Ac_2O, pyridine, rt., 1h.

We were also interested in synthesizing the analogues of compound **5.10** with the opposite stereochemistry at C-2. Hence, 1,4-anhydro-4-thio-D-ribitol (**5.22**) was synthesized according to a literature procedure.[141] The 3-OH and 5-OH were protected by the silyl protecting group to afford **5.23**. Unfortunately, the silyl protecting group was removed during the reaction with DAST and did not proceed to the fluorination at C-2 (Scheme 5.4). This might be due to the attack of the fluoride ion to the silyl protecting group that are in the same face during the reaction progression resulting in the silyl deprotection.



Scheme 5.4. Reaction of compound **5.23** with DAST.

5.3 Conclusions

1-*O*-Acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (**5.10**), a suitable precursor for the synthesis of nucleosides, was synthesized starting from the commercially available L-xylose. 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-arabinitol (**5.13**) was synthesized from L-xylose following a literature procedure. The 3-OH and 5-OH were first protected by the silyl protecting group which then replaced with benzoyl protecting group. Fluorine atom was introduced to the compound using DAST reagent with 80% yield. Ozonization afforded the desired sulfoxide with quantitative yield. The first Pummerer rearrangement was performed on the sulfoxide using acetic anhydride and heating 110 °C that resulted in the inseparable mixture of 1-*O*-acetate and 4-*O*-acetate products. In order to obtain the pure 1-*O*-acetate, Pummerer rearrangement was performed using trifluoroacetic anhydride at 0 °C to afford the correspong hemiacetal. Acetylation of the hemiacetal afforded the desired 1-*O*-acetate. Using trifluoroacetic anhydride in the Pummere rearrangement allowed us to separate the undesired 4-*O*-trifluoroacetate (**5.21**) using flash chromatography due to different polarity of the 4-*O*-trifluoroacetate.

Compound **5.10** was used by our collaborators at McGill University for the synthesis of 2'-deoxy-2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU).[151] Conformational analysis performed by our collaborators on the nucleoside (4'S-FMAU) revealed that this compound adopts predominantly a northern conformation. This nucleoside (4'S-FMAU) was also incorporated into oligonucleotides by our collaborators.[181] They showed that oligonucleotides containing 4'S-FMAU were unable

to elicit *E.coli* or human RNase H activity, thus corroborating the hypothesis that RNase H prefers duplexes containing oligonucleotides that can adopt eastern conformations.[181]

5.4 Experimental

5.4.1 General methods

Optical rotations were measured at 23°C and reported in $degdm^{-1}g^{-1}cm^3$. ^1H NMR were recorded with frequencies of 500 and 400 MHz, ^{13}C NMR with 100.6 MHz and ^{19}F NMR with 282.3 MHz. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY) and ^1H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the data was performed with MestRec software. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Column chromatography was performed with silica gel 60 (230-400 mesh). Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix.

1,4-Anhydro-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol (5.15)

To a solution of 1,4-anhydro-4-thio-D-arabinitol **5.14** (2.1 g, 14 mmol) in anhydrous pyridine (10 ml) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (5.3 g, 16.8 mmol). The mixture was stirred at room temperature for 3h and then quenched by

addition of ice. The solvent was removed under reduced pressure and the crude product was dissolved in ethyl acetate (30 ml) and washed with ice cold 1% aqueous HCl (3x15 ml), followed by brine. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 2:1] to afford compound **5.15** (3.4g, 8.6 mmol, 61%) as a syrup. $[\alpha]_D$: – 4 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.17 (br ddd, 1H, $J_{1a,2}$ = 6.7, $J_{1b,2}$ = 8.8, $J_{2,3}$ = 7.8 Hz, H-2), 4.02 (dd, 1H, $J_{3,4}$ = 7.9, H-3), 3.99 (dd, 1H, $J_{4,5a}$ = 3.2, $J_{5a,5b}$ =12.3 Hz, H-5a), 3.78 (dd, 1H, $J_{4,5b}$ = 5.8 Hz, H-5b), 3.24 (ddd, 1H, H-4), 2.95 (dd, 1H, $J_{1a,1b}$ = 10.4 Hz, H-1a), 2.73 (dd, 1H, H-1b), 2.20 (br s, 1H, OH), 1.20-0.90 (m, 28H, 4 × $SiCH(CH_3)_2$); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 79.9 (C-2), 77.6 (C-3), 63.0 (C-5), 48.6 (C-4), 30.5 (C-1), 17.4, 17.3, 17.2, 17.1, 17.0 (CH_3), 13.6, 13.3, 12.8, 12.7 (SiCH); MALDI MS: *m*/*e* 415.13 (M^++Na). Anal. Calcd for $C_{17}H_{36}O_4SSi_2$: C, 51.99; H, 9.24. Found: C, 51.83; H, 9.32.

1,4-Anhydro-2-deoxy-2-fluoro-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabinitol (5.16)

A solution of DAST (1.2 g, 9.5 mmol) in anhydrous CH_2Cl_2 (5 ml) was added dropwise to a solution of **5.15** (3.1 g, 7.9 mmol) in anhydrous CH_2Cl_2 (15 ml) while cooling to -20 ºC. After 15 min at –20 ºC, the reaction was quenched by addition of ice and the mixture was partitioned between CH_2Cl_2 and water. The separated organic layer was washed with saturated $NaHCO_3$ followed by brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 6:1] to afford **5.16** (2.5 g, 6.3 mmol, 80%) as a

colorless syrup. $[\alpha]_D$: − 14 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.98 (dddd, 1H, $J_{2,F}$ = 53.4, $J_{1b,2}$ = 7.8, $J_{2,3}$ = 7.3, $J_{1a,2}$ = 6.9 Hz, H-2), 4.34 (ddd, 1H, $J_{3,F}$ = 15.3, $J_{3,4}$ = 8.2 Hz, H-3), 3.99 (ddd, 1H, $J_{5a,5b}$ = 12.3, $J_{4,5a}$ = 3.3, $J_{5a,F}$ = 0.5 Hz, H-5a), 3.79 (ddd, 1H, $J_{4,5b}$ = 5.6, $J_{5b,F}$ = 1.9 Hz, H-5b), 3.21 (dddd, 1H, $J_{4,F}$ = 1.1 Hz, H-4), 3.05 (ddd, 1H, $J_{1a,1b}$ = 11.1, $J_{1a,F}$ = 6.9Hz, H-1a), 2.90 (ddd, 1H, $J_{1b,F}$ = 16.9 Hz,H-1b), 1.30-0.85 (m, 28H, 4 × SiCH(CH_3)$_2$); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 96.6 (d, $J_{2,F}$ = 189.2 Hz, C-2), 77.8 (d, $J_{3,F}$ = 22.9 Hz, C-3), 62.5 (C-5), 47.6 (d, $J_{4,F}$ = 7.6 Hz, C-4), 28.7 (d, $J_{1,F}$ = 22.1 Hz, C-1), 17.5, 17.4, 17.3, 17.1, 17.0 (CH_3), 13.7, 13.4, 13.1, 12.9 (SiCH). MALDI MS: *m*/*e* 374.94 (M^+-F). Anal. Calcd for $C_{17}H_{35}FO_3SSi_2$: C, 51.73; H, 8.94. Found: C, 51.76; H, 8.93.

1,4-Anhydro-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-D-arabinitol (5.18)

To a solution of **5.16** (2.5 g, 6.2 mmol) in THF (10 ml) was added a 1M solution of tetra-*n*-butylammonium fluoride in THF (3 ml, 3 mmol). The mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure with temperature below 30 ºC. The crude product was dissolved in ethyl acetate (50 ml) and washed with small volumes of water and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to afford crude 1,4-anhydro-2-deoxy-2-fluoro-4-thio-D-arabinitol (**5.17**) as a pale-yellow syrup.

The crude diol **5.17** (1.0 g) was dissolved in anhydrous pyridine (10 ml) and the mixture was cooled in an ice bath. Benzoyl chloride (4.0 ml, 34 mmol) was added and the reaction mixture was stirred at room temperature for 6 h. The reaction was quenched by addition of ice. The solvent was removed under reduced pressure, the crude product was dissolved in ethyl acetate (30 ml) and washed with ice cold 1% aqueous HCl (3×15 ml),

followed by brine. The organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 2:1] to afford **5.18** (2.1 g, 5.9 mmol, 95%) as a syrup. $[\alpha]_D$: + 41 (*c* 0.78, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (m, 4H, Ar), 7.62 (m, 2H, Ar), 7.44 (m, 4H, Ar), 5.84 (ddd, 1H, $J_{3,F}$ = 9.6, $J_{2,3}$ = 2.6, $J_{3,4}$ = 2.6 Hz, H-3), 5.38 (dddd, 1H, $J_{2,F}$ = 49.4, $J_{1a,2}$ = 4.3, $J_{1b,2}$ = 2.9 Hz, H-2), 4.55 (ddd, $J_{5a,5b}$ = 11.1, $J_{4,5a}$ = 7.1, $J_{5a,F}$ = 1.7 Hz, H-5a), 4.49 (dd, 1H, $J_{4,5b}$ = 8.5, $J_{5b,F}$ < 1 Hz, H-5b), 3.88 (br dd, 1H, $J_{4,F}$ < 1 Hz, H-4), 3.36 (ddd, 1H, $J_{1a,F}$ = 30.5, $J_{1a,1b}$ = 12.6 Hz, H-1a), 3.31 (ddd, 1H, $J_{1b,F}$ = 19.3 Hz,H-1b); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 166.0, 164.9 (C=O), 133.6, 133.1 (Ar), 129.8, 129.7 (Ar), 128.5, 128.4 (Ar), 96.2 (d, $J_{2,F}$ = 183.1 Hz, C-2), 78.9 (d, $J_{3,F}$ = 28.9 Hz, C-3), 65.5 (d, $J_{5,F}$ = 4.5 Hz, C-5), 48.9 (C-4), 34.6 (d, $J_{1,F}$ = 22.8 Hz, C-1). MALDI MS: *m*/*e* 383.20 (M^++Na). Anal. Calcd for $C_{19}H_{17}FO_4S$: C, 63.32; H, 4.75. Found: C, 63.60; H, 4.80.

1,4-Anhydro-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-sulfinyl-D-arabinitol (5.11)

Ozone gas was bubbled through a clear solution of **5.17** (2.1 g, 5.8 mmol) in CH_2Cl_2 (15 ml) at -78 ºC. The reaction was completed in 30 min. as indicated by persistence of a blue color. Nitrogen gas was bubbled through the solution to remove excess ozone until the blue color vanished. The mixture was allowed to warm to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography [Hexanes/EtOAc, 2:1] to afford **5.11** (2.2 g, 5.8 mmol, 99%) as a white solid. Mp. 141-142 °C; ^{1}H NMR (500 MHz, $CDCl_3$) for the α-isomer: δ 8.05 (m, 4H, Ar), 7.60 (m, 2H, Ar), 7.45 (m, 4H, Ar), 5.80 (dddd, 1H, $J_{2,F}$ = 49.6, $J_{1a,2}$ = 5.3, $J_{1b,2}$ = 4.8, $J_{2,3}$ = 3.9 Hz, H-2), 5.74 (ddd, 1H, $J_{3,F}$ = 13.2, $J_{3,4}$ = 3.9 Hz, H-3), 4.89

(dd, $J_{5a,5b}$ = 11.9, $J_{4,5a}$ = 4.9, $J_{5a,F}$ ~ 0 Hz, H-5a), 4.74 (dd, 1H, $J_{4,5b}$ = 7.5, $J_{5b,F}$ ~0 Hz, H-5b), 3.65 (ddd, 1H, $J_{4,F}$ ~ 0 Hz, H-4), 3.75 (ddd, 1H, $J_{1a,F}$ = 14.9, $J_{1a,1b}$ = 14.1 Hz, H-1a), 3.45 (ddd, 1H, $J_{1b,F}$ = 25.7 Hz, H-1b); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 165.7, 165.3 (C=O), 134.0, 133.5, 130.1, 129.7, 128.6, 128.5 (Ar) 95.44 (d, $J_{2,F}$ = 184.6 Hz, C-2), 77.3 (d, $J_{3,F}$ = 29.0 Hz, C-3), 71.6 (C-4), 61.1 (d, $J_{5,F}$ = 3.0 Hz, C-5), 55.8 (d, $J_{1,F}$ = 19.8 Hz, C-1). MALDI MS: *m*/*e* 377.20 (M^++H), 399.15 (M^++Na). Anal. Calcd for $C_{19}H_{17}FO_5S$: C, 60.63; H, 4.55. Found: C, 60.79; H, 4.53.

1-*O*-Acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (5.10)

A mixture of **5.11** (2.2 g, 2.4 mmol) and Ac_2O (6.0 ml) were heated at 110 ºC for 3 h. The reaction was quenched by addition of ice after cooling to room temperature. The mixture was partitioned between EtOAc (10 ml) and water (10 ml) and further stirred for 2h at ambient temperature. The separated organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 19:1] to afford an inseprable mixture of α,β-anomers (1:1) of **5.10** (1.67g, 4 mmol, 70%) and 2-*O*-acetyl-4-deoxy-4-fluoro-1,3-di-*O*-benzoyl-5-thio-α/β-thereo-pentofuranose (**5.19**) as a white solid.

2-Fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (5.20)

Solid sulfoxide **5.11** (107 mg, 284 mmol) was cooled to 0°C. Trifluoroacetic anhydride (3 ml) added and the reaction was stirred for 30 min at 0°C. Trifluoroacetic anhydride was removed under reduced pressure and the crude product was purified by

flash chromatography [Hexanes/EtOAc, 24:1→2:1] to afford an inseparable mixture of the α,β-anomers of **5.20** (58 mg, 153.5 mmol, 54%) as a white solid. Mp. 112-113°C; ^{1}H NMR (500 MHz, $CDCl_3$) for the major β-isomer: δ 8.10-7.97 (m, 4H, Ar), 7.62-7.32 (m, 6H, Ar), 6.08 (ddd, 1H, $J_{3,F}$ = 11.9, $J_{3,2}$ = 7.2, $J_{3,4}$ = 5.6 Hz, H-3), 5.49 (ddd, 1H, $J_{1,OH} \approx J_{1,F} \approx$ 4.0 Hz, H-1), 5.20 (ddd, 1H, $J_{2,F}$ = 51.2, $J_{2,1}$ = 3.9 Hz, H-2), 4.67 (dd, 1H, $J_{5a,5b}$ = 11.4, $J_{5a,4}$ = 7.0, H-5a), 4.60 (ddd, 1H, $J_{5b,4}$ = 6.5, $J_{5b,F}$ = 1.1 Hz, H-5b), 3.75 (td, 1H, H-4), 2.85 (d, 1H, O<u>H</u>); ^{13}C NMR (500 MHz, $CDCl_3$): δ 166.1, 165.3 (<u>C</u>OPh), 133.6, 133.1 (Ar) 129.8, 129.7, 128.5, 128.3 (Ar), 95.1 (d, $J_{2,F}$ = 198.4 Hz, C-2), 76.5 (d, C-1), 76.5 (d, C-3), 66.6 (C-5), 44.5 (d, $J_{4,F}$ = 5.8 Hz, C-4).

^{1}H NMR (500 MHz, $CDCl_3$) for the minor α-isomer: δ 8.10-7.97 (m, 4H, Ar), 7.62-7.32 (m, 6H, Ar), 5.83 (dddd, 1H, $J_{3,F}$ = 12.1, $J_{3,4}$ =3.0, $J_{3,2}$ = 2.1, $J_{3,1}$ = 0.9 Hz, H-3), 5.63 (dddd, 1H, $J_{1,F}$ = 8.9 Hz, H-1), 5.32 (ddd, 1H, $J_{2,F}$ = 47.2, $J_{2,1}$ = 1.8 Hz, H-2), 4.53-4.46 (m, 2H, $J_{5b,4}$ = 7.1, $J_{5a,5b}$ = 7.9, $J_{5b,F}$ = 1.4, $J_{5a,F}$ = 0.9 Hz, H-5a and H-5b), 4.21 (dddd, 1H, $J_{4,F}$ = 1.5, $J_{4,5a}$ = 6.9 Hz, H-4), 2.46 (d, 1H, $J_{1,OH}$ = 8.2 Hz, O<u>H</u>) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 166.0, 164.9 (<u>C</u>OPh), 133.8, 133.2 (Ar), 129.8, 129.7, 128.6, 128.4 (Ar), 100.4 (d, $J_{2,F}$ = 186.3 Hz, C-2), 83.4 (d, $J_{1,F}$ = 30.9 Hz, C-1), 78.7 (d, $J_{3,F}$ = 29.7 Hz, C-3), 65.4 (C-5), 50.9 (S, C-4). For the α,β mixture: MALDI MS: m/e 399.9 (M^{+}+Na). Anal. Calcd for $C_{19}H_{17}O_5SF$: C, 60.63; H, 4.55. Found: C, 60.36; H, 4.67.

1-*O*-Acetyl-2-deoxy-2-fluoro-3, 5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (5.10)

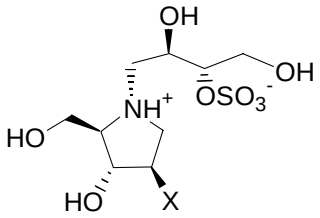
Solid hemiacetal **5.20** (445 mg, 1.2 mmol) was dissolved in pyridine (15 ml) and acetic anhydride (2 ml) was added. The reaction mixture was stirred at room temperature for 1h. The solvent was removed under reduced preesure and the crude product was

dissolved in EtOAc (70 ml) and washed with water and brine. EtOAc was removed under reduced pressure and the crude product was purified by flash chromatography [Hexanes/EtOAc, 10:1→ 4:1] to afford a mixture of α,β-anomers of **5.10** (363 mg, 0.868 mmol, 73%) as a white solid (β:α = 2:1). Mp. 141-142 °C. ^{1}H NMR (400 MHz, $CDCl_3$) NMR data for the major isomer **5.10β**: δ 8.10-7.90 (m, 4H, Ar), 7.60-7.25 (m, 6H, Ar), 6.17 (d, 1H, $J_{1,2}$ = 4.4, $J_{1,F}$ ~ 0 Hz, H-1a), 6.08 (ddd, 1H, $J_{3,F}$ = 11.7, $J_{3,4}$ = 7.3, $J_{2,3}$ = 9.0 Hz, H-3), 5.31 (ddd, 1H, $J_{2,F}$ = 50.7 Hz, H-2), 4.68 (dd, $J_{5a,5b}$ = 11.4, $J_{4,5a}$ = 6.1, $J_{5a,F}$ ~ 0 Hz, H-5a), 4.49 (ddd, 1H, $J_{4,5b}$ = 6.4, $J_{5b,F}$ = 0.5 Hz, H-5b), 3.74 (ddd, 1H, $J_{4,F}$ = 6.3 Hz, H-4), 2.12 (s, 3H, CH_3); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 169.7 ($\underline{C}OCH_3$), 165.8, 165.4 ($\underline{C}OPh$), 133.6, 133.2, 129.9, 129.7, 128.5, 128.3 (Ar), 92.4 (d, $J_{2,F}$ = 206.8 Hz, C-2), 75.7 (d, $J_{3,F}$ = 22.9 Hz, C-3), 73.9 (d, $J_{1,F}$ = 16.8 Hz, C-1), 66.0 (C-5), 42.4 (d, $J_{4,F}$ = 6.9 Hz, C-4), 21.0 (CH_3). ^{19}F NMR (282.3 MHz, $CDCl_3$): δ -191.75 (dd, J = 9 Hz, 51 Hz).

^{1}H NMR (400 MHz, $CDCl_3$) for the minor isomer **5.10α**: δ 8.10-7.90 (m, 4H, Ar), 7.60-7.25 (m, 6H, Ar), 6.23 (ddd, 1H, $J_{1,F}$ = 13.9, $J_{1,2}$ = 2.2, $J_{1,4}$ = 0.7 Hz, H-1a), 5.88 (ddd, 1H, $J_{3,F}$ = 12.3, $J_{3,4}$ = 3.5, $J_{2,3}$ = 3.7 Hz, H-3), 5.39 (ddd, 1H, $J_{2,F}$ = 47.6 Hz, H-2), 4.55 (dd, $J_{5a,5b}$ = 11.4, $J_{4,5a}$ = 7.8, $J_{5a,F}$ = 0.6 Hz, H-5a), 4.47 (ddd, 1H, $J_{4,5b}$ = 6.6, $J_{5b,F}$ = 1.5 Hz, H-5b), 4.10 (ddd, 1H, $J_{4,F}$ = 4.4 Hz, H-4), 2.12 (s, 3H, CH_3); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 169.4 ($\underline{C}OCH_3$), 165.9, 165.0 ($\underline{C}OPh$), 133.7, 133.2, 129.7, 129.4, 128.8, 128.4 (Ar), 98.4 (d, $J_{2,F}$ = 187.7 Hz, C-2), 81.5 (d, $J_{1,F}$ = 32.8 Hz, C-1), 77.5 (C-3), 64.9 (C-5), 49.2 (C-4), 20.8 (CH_3); ^{19}F NMR (282.3 MHz, $CDCl_3$) -186.9 (ddd, J = 12 Hz, 48 Hz). For the α,β mixture: MALDI MS: *m*/*e* 441.25 (M^++Na); 457.18 (M^++Ka). Anal. Calcd for $C_{21}H_{19}FO_6S$: C, 60.28; H, 4.58. Found: C, 60.45; H, 4.60.

CHAPTER 6. GENERAL CONCLUSIONS

In chapter two of this thesis the synthesis of seven 2-substituted derivatives of the nitrogen analogue of salacinol (**2.3-2.9**), a naturally occurring glycosidase inhibitor, was described for the purpose of structure-activity studies with hexosaminidase enzymes (Chart 6.1).



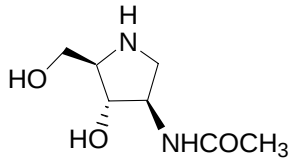
2.3 : X = N_3
2.4 : X = NH_2
2.5 : X = $NHCOCH_3$
2.6 : X = $NHCOCH_2CH_3$
2.7 : X = $NHCOCH_2CH_2CH_2CH_3$
2.8 : X = $N(CH_3)_2$
2.9 : X = $NHCH_2CH_3$

Chart 6.1. Target compounds **2.3**-**2.9**.

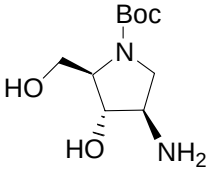
Curiously, hydrogenation of the azido zwitterionic compound (**2.3**) in methanol resulted in reduction of the azide and subsequent methylation of the resulting amine in one pot. Similarly, a hydrogenation reaction with ethanol as the solvent, followed by trifluoroacetic acid treatment afforded the *N*-ethyl derivative **2.9** (Chart 6.1). The 2-amino analogue **2.4** (Chart 6.1) was finally obtained by reduction of the azide function using triphenylphosphine.

Unlike their sulfonium ion counterparts (Chapter 3), compounds **2.4**-**2.7** (Chart 6.1) were stable and did not undergo ring opening reactions.

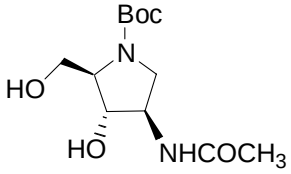
The parent nitrogen heterocycles, 1,2,4-trideoxy-2-acetamido-1,4-imino-D-arabinitol (**2.10**), its corresponding *N*-Boc-protected compound **2.15**, *N*-Boc-1,2,4-trideoxy-2-amino-1,4-imino-D-arabinitol (**2.14**), and the parent iminoalditol (**2.17**) (Chart 6.2) were synthesized and were tested along with the 2-substituted analogues (**2.3**-**2.9**, Chart 6.1) against two enzymes that cleave the β-glycosidic linkage of 2-acetamido-2-deoxy-β-D-glycopyranosides, namely *O*-GlcNAcase and *Vibrio cholerae* NagZ; the compounds showed marginal inhibitory activity (<33% at 250μM).



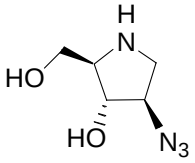
2.10



2.14



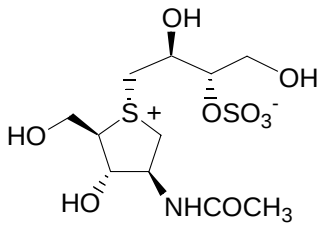
2.15



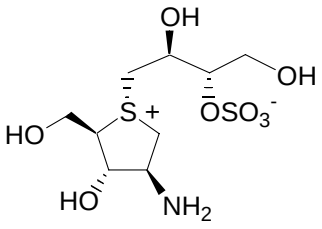
2.17

Chart 6.2. 1,2,4-Trideoxy-2-acetamido-1,4-imino-D-arabinitol (**2.10**), its corresponding *N*-Boc-protected compound (**2.15**), *N*-Boc-1,2,4-trideoxy-2-amino-1,4-imino-D-arabinitol (**2.14**), and compound **2.17**.

In chapter three of this thesis we described the attempted synthesis of 2-acetamido (**3.7**) and 2-amino (**3.8**) derivatives of salacinol (Chart 6.3).



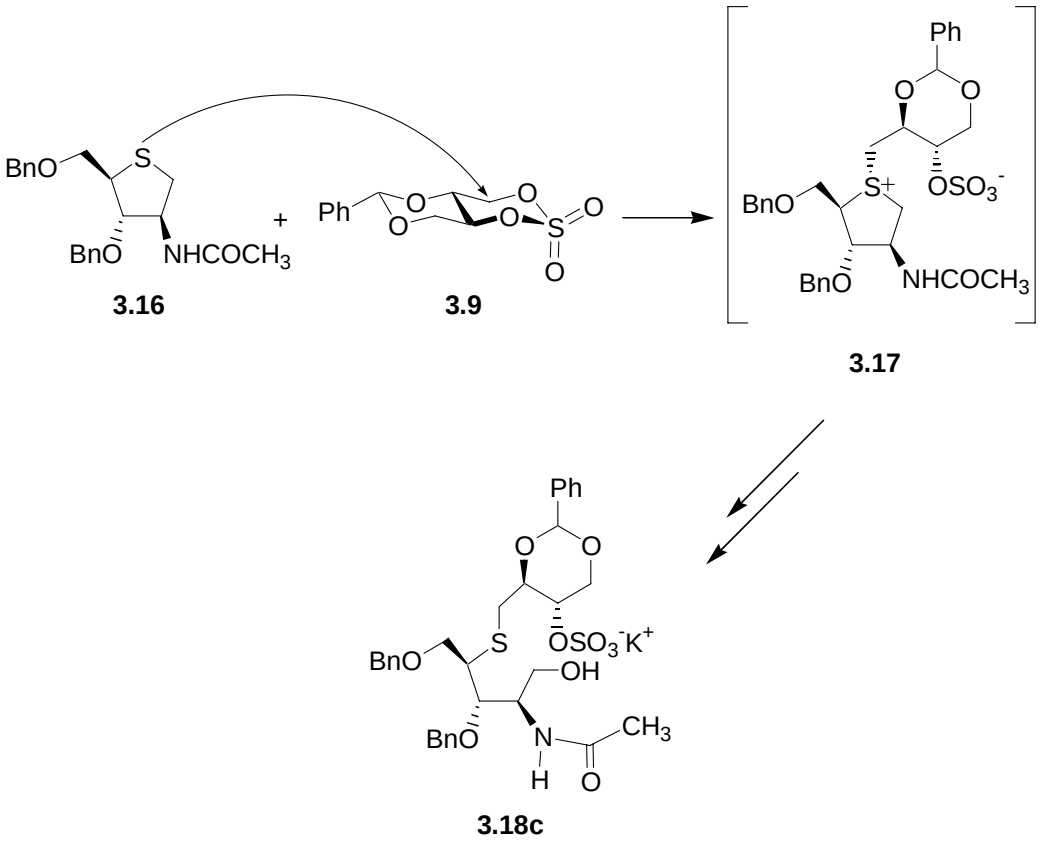
3.7



3.8

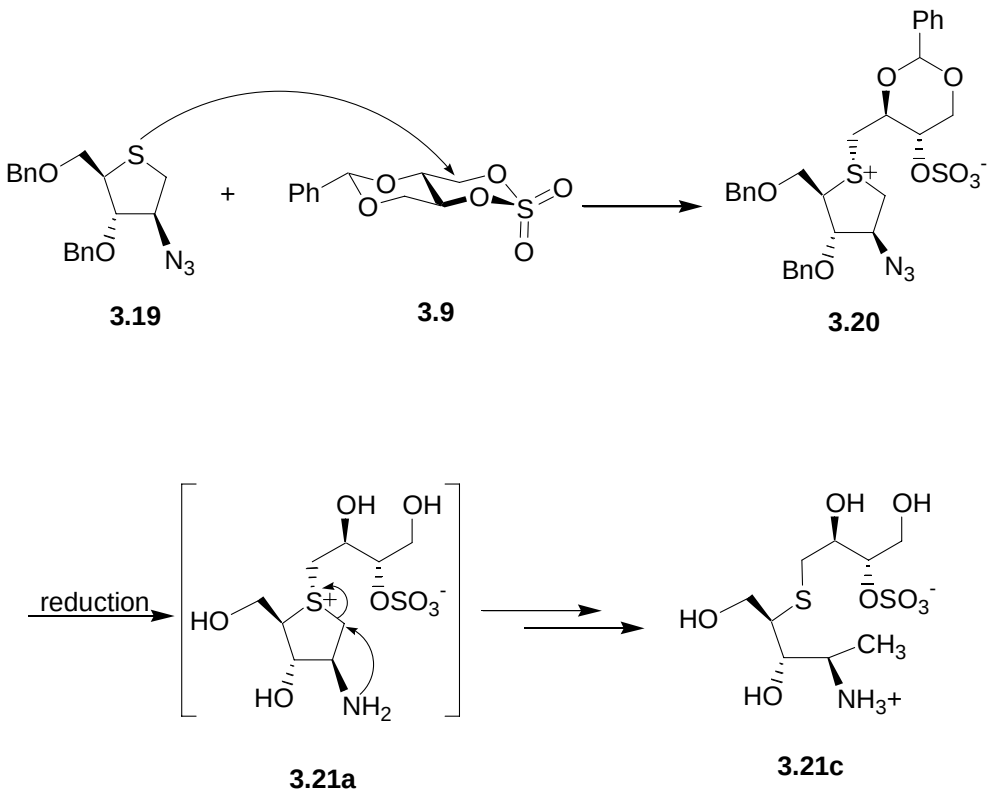
Chart 6.3. Target compounds **3.7** and **3.8**.

However, reaction of the protected acetamidothioarabinitol **3.16** unit with the cyclic sulfate derived from L-erythritol (**3.9**) gave the corresponding sulfonium sulfate **3.17** which underwent ring opening to give an acyclic amido sulfate **3.18c** (Scheme 6.1).



Scheme 6.1. Coupling reaction of 2-acetamidothioarabinitol **3.16** with the L-cyclic sulfate **3.9**.

Reaction of the protected azidothioarabinitol **3.19** with the cyclic sulfate **3.9** proceeded to afford the sulfonium sulfate **3.20**. However, upon reduction of the azido function to an amine (**3.21a**) it formed an acyclic ammonium sulfate (**3.21c**) (Scheme 6.2).

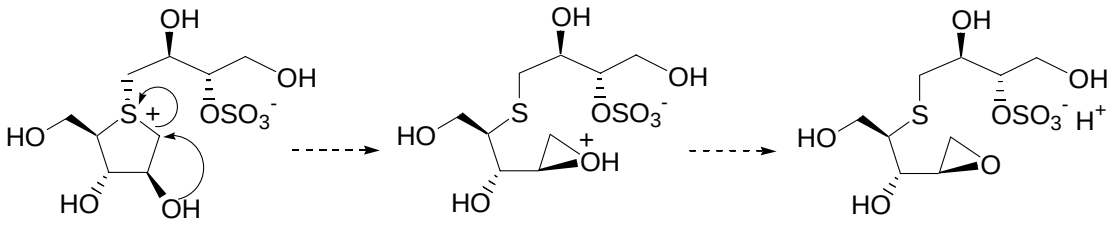


3.19 **3.9** **3.20**

3.21a **3.21c**

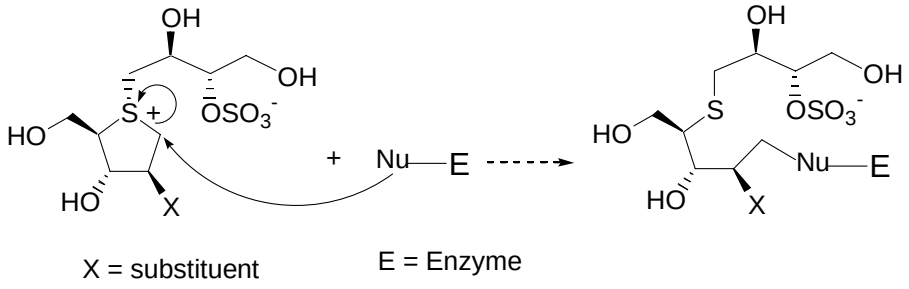
Scheme 6.2. Coupling reaction of azidothioarabinitol **3.19** with the L-cyclic sulfate **3.9**.

These are the first examples of which we are aware of the instability of salacinol analogues. Our synthetic efforts to date have not revealed any ring-opened, epoxide products stemming from the neighboring group participation of O-2 in salacinol analogues (Scheme 6.3).



Scheme 6.3. Neighboring group participation of O-2 in salacinol.

Furthermore, enzyme inhibition studies with a variety of glycosidase enzymes have been consistent with a model of reversible, competitive inhibition and have not involved ring-opening reactions by nucleophilic groups in the enzyme active sites. These results suggest that certain compounds of this class might function as irreversible inhibitors by reaction with nucleophilic groups in the enzyme active site (Scheme 6.4).



Scheme 6.4. Nucleophilic participation within the enzyme active site.

In chapter four of this thesis we described the synthesis of 2-deoxy-2-fluorosalacinol **4.9** and the 2-deoxy-1,2-ene **4.10** derivative of salacinol for structure activity studies with human maltase glucoamylase (MGA) (Chart 6.4).

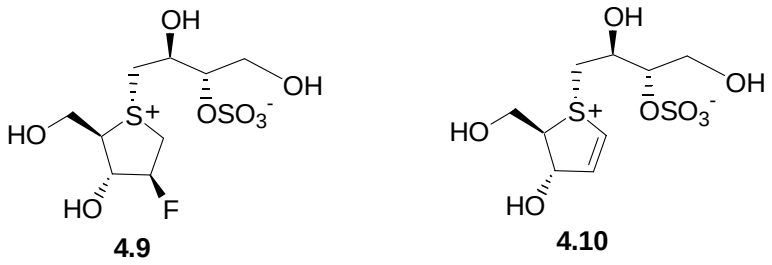
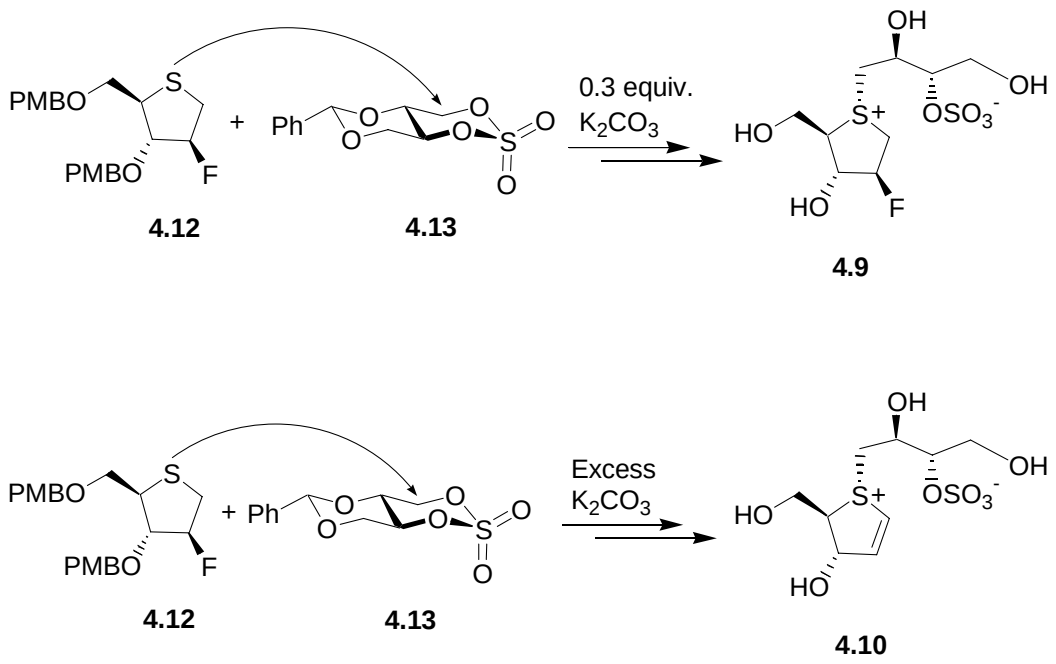


Chart 6.4. 2-Deoxy-2-fluorosalacinol **4.9** and the 2-deoxy-1,2-ene derivative **4.10**.

2-Deoxy-2-fluorosalacinol **4.9** was synthesized through the coupling reaction of 2-deoxy-2-fluoro-3,5-di-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-D-arabinitol (**4.12**) with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**4.13**) in hexafluoroisopropanol (HFIP) containing 0.3 equivalents of K_2CO_3 (Scheme 6.5). Excess K_2CO_3 resulted in elimination of HF from the coupled product, and formation of the alkene derivative of salacinol (**4.10**) (Scheme 6.5).



Scheme 6.5. Coupling reactions of 2-deoxy-2-fluoro-3,5-di-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-D-arabinitol (**4.12**) with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**4.13**).

The 2-deoxy-1,2-ene- derivative of salacinol (**4.10**) and 2-deoxy-2-fluorosalacinol **4.9** inhibited recombinant human maltase glucoamylase with an IC_{50} value of 150 μM and a K_i value of 6 ± 1 μM, respectively. Since salacinol itself shows a K_i = 0.2 ± 0.02 μM, it would appear that the OH group on C-2 of salacinol is critical as a hydrogen-bond donor with functional groups in the active site of MGA.

In chapter five of this thesis we described the synthesis of 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-4-thio-α/β-D-arabinofuranose (**5.10**) from the Pummerer rearrangement of the sulfoxide **5.11**, which was synthesized , in turn, from the thioether **5.14** (Chart 6.5).

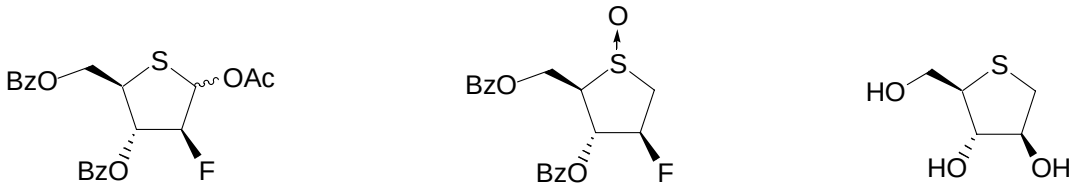
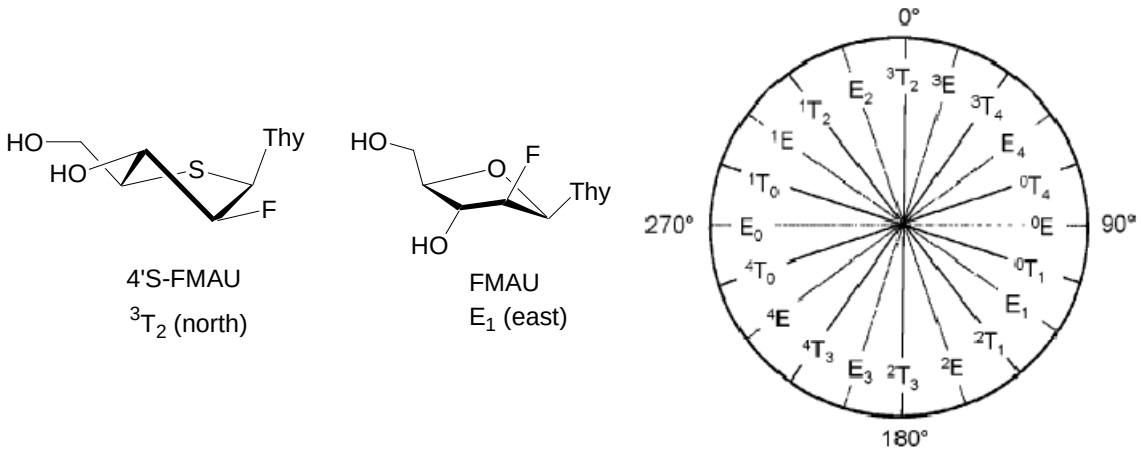


Chart 6.5. Target compound **5.10**, the sulfoxide **5.11**, and the thioether **5.14**.

Compound **5.10** was used by my collaborator, J.K. Watts, at McGill University for the synthesis of 2'-deoxy-2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU, Chart 6.6a).[151] Conformational analysis performed by J.K. Watts and Dr. B. Mario Pinto on the nucleoside (4'S-FMAU) revealed that this compound adopts predominantly a northern conformation, in contrast to the oxygen congener which adopts an eastern conformation (Chart 6.6a). The designation of the northern conformation is based on a pseudorotational wheel that has been used to describe the conformations of nucleosides (Chart 6.6b).[182]



(**a**) (**b**)

Chart 6.6. a) 2'-Deoxy-2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU); b) pseudorotational wheel; E = envelope, T = twist.

This nucleoside (4'S-FMAU) was also incorporated into oligonucleotides by our collaborators (Table 6.1).[181]

Table 6.1. Oligonucleotide sequences.

I	5'-UGA CAU ttt ttt UCA CGU-3'
II	5'-UGA CAU TTT TTT UCA CGU-3'
III	5'-UGA CAU *TTT TTT* UCA CGU-3'
IV	5'-tga cat ttt ttt tca cgt-3'
V	5'-UGA CAU UUU UUU UCA CGU-3'

They showed that oligonucleotides containing 4'S-FMAU were unable to elicit *E.coli* or human RNase H activity, thus corroborating the hypothesis that RNase H prefers duplexes containing oligonucleotides that can adopt eastern conformations.[181] The RNase H family of enzymes recognize and cleave the RNA strand of AON:RNA (AON: antisense oligonucleotides) hybrids having a conformation that is intermediate between the pure A- or B-form conformations adopted by dsRNA and dsDNA, respectively. Sugar geometries that fall within the eastern (O4'-endo) range within the AON have been postulated to actively induce RNase H-assisted RNA strand cleavage.

2'-Deoxy-2'-fluoro-4'-thioarabinouridine (4'S-FAU) was also incorporated into a 21-mer small interfering RNA (siRNA) by our collaborators and the resulting siRNA molecules were able to trigger RNA interference with good efficiency. Small interfering RNAs (siRNA) are one of the most recent additions to the wide repertoire of nucleic acid molecules used to silence gene expression.

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