DESIGN AND SYNTHESIS OF NOVEL INHIBITORS OF GOLGI α-MANNOSIDASE II

by

CHARLES JOHN STANTON III

(Under the Direction of Geert-Jan Boons)

ABSTRACT

In recent years, considerable effort has been devoted to unraveling the biological roles of oligosaccharides and the mechanism-of-action of the enzymes implicated in their biosynthesis. Cells that have undergone oncogenic transformation often display abnormal cell surface oligosacharides and these changes in gycosylation are important determinants of the stage, direction and fate of tumor progression. Inhibition of the mannose trimming enzyme human Golgi α–mannosidase II (HGMII), which acts late in the *N*-glycan processing pathway, provides one route to blocking the oncogene-induced changes in cell surface oligosaccharide structures. Potent inhibitors of glycosidases are thought to mimic oxocarbenium ion-like transition states. For example, the inhibitory activity of the natural product swainsonine has long been attributed to its five-membered ring resembling a flattened six-membered ring, and thus attaining an oxocarbenium-like structure.

In an effort to identify more effective inhibitors of Class 2 mannosidases, we have initiated a program to design novel synthetic inhibitors, which are selective for the Golgi α-mannosidase II enzyme. Amongst the new analogs that have been synthesized are various bicyclic sulfonuim salt- and azasugar analogs of swainsonine. This Thesis describes two novel classes of unnatural glycosidase inhibitors.

INDEX WORDS: oligosaccharide, oncogenic, glycosylation, Golgi α-mannosidase II (HGMII), oxocarbenium, swainsonine, mannosidase, sulfonium salt, azasugar, glycosidase

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CHARLES JOHN STANTON III

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CHARLES JOHN STANTON III

Major Professor:	Geert-Jan Boons
Committee:	John Stickney
	George Majetich

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

Nearly three years have passed since I first stepped foot in the laboratory of Dr. Geert-Jan Boons, and those years have been both the best of times and the worst of times for me. I have grown in ways that I never could have dreamed, although at times, life looked quite bleak for me. With that, I am completing the requirements for my Masters Degree, and plan to carry forth my knowledge and learned skills, first in the land of industry, followed by a successful career in academia in which I can explore my true love, teaching. I would like to thank Dr. Geert-Jan Boons, for helping me reach this goal. Without his support there would be no reason for anybody to read this thesis, and no reason for me to write it.

I would like to dedicate this work, first and foremost, to my grandparents, Nanny and Pop-Pop; without them, I would have no Mom to eventually thank, and no ambition for medicine. I would also like to thank my parents, specifically my Mother, who has taught me many things in my life, and I hope one day to be half the person she is. She has inspired me and made tremendous personal sacrifices to give me opportunities and chances in life. Thank you, Mom!

I would like to mention again that Athens has been both the best of times and the worst of times for me. In fact, I was ready to call it quits on a number of occasions, coming the closest to quitting last summer. However, after a brief leave of absence, I came back with the focus and determination to finish this chapter in my life, and prepare myself for the next. The last eight months have provided exactly that. With that said, I would like to thank the girl who has helped me open my eyes once again, and more importantly, help me dream once more. She has instilled

in me the hope and the fire of desire once lost. It is to her, Ms. Nicole Schwartz, that I not only dedicate this Thesis too, but the remainder of my years. I love you, and I express my thanks.

Finally, to all those not mentioned here, in particular friends, family and well-wishers, enjoy! I love you all, just am tired of typing.

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I would like to take the time to name a few individuals in my life that stand out and that have really made a difference in where I am today. The first and probably the most important is Dr. Tomas Hudlicky, a former professor and adviser of mine from the University of Florida. He helped open my eyes and my heart to the art of synthetic organic chemistry, and his love for it provided the fuel for mine to grow. He is truly a great man, and I hope our paths cross again one day.

Next, Dr. Geert-Jan Boons, for providing me the chance to earn this degree, and to further my knowledge and experience in a new and exciting field. I would like to thank him with my whole heart. Through trials and tribulations we have made it to this point, and I plan to make the most out of my life with the lessons I have learned and experienced here at the University of Georgia.

Unfortunately, this list could continue forever. Instead of not acknowledging everyone, I will do my best, in no particular order: Dr. George Majetich (who will probably ask for his name to be removed from this list, but at least I tried), Dr. Thomas Johnson, Dr. John Stickney, Mr. Emilio Sanchez, my brothers, my Father (which we wont get into here), my family, and last, but certainly not least, my friends, who provide me the passion necessary to make me a balanced person. Thank you to you all and I wish the best for you!

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LIST OF ABBREVIATIONS

Ac	Acetyl
Ac_2O	Acetic anhydride
Bn	Benzyl
Boc	benzocycarbonyl
Brs	broad singlet
dd	double of doublets
DCM	methylene chloride
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
EtOH	ethanol
FAB-MS	fast atom bombardment mass spectroscopy

gCOSY	gradient Correlation Homonuclear Spectroscopy
gHSQC	gradient Homonuclear Single Quantum Coherence
Glc	Glucose
GlcNAc	*N*-Acetyl glucosamine
HOBt	*N*-hydroxybezotriazole
HPLC	high performance liquid chromatography
Hz	Hertz
LacNAc	*N*-acetyl lactosamine
Le^x	Lewis x
Le^y	Lewis y
m	multiplet
MeOH	methanol
m.p.	melting point
m/z	mass to charge ratio
Me	methyl
Min	minute

mM	millimolar
mmol	millimole
MS	molecular Sieves
NMR	Nuclear Magnetic Resonance
Ph	phenyl
ppm	parts per milliom
q	quartet
R_f	retention factor
s	singlet
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TLC	thin layer chromatography
Ts	*p*-Toluenesulfonyl

CHAPTER ONE

INTRODUCTION

Overview

Protein-linked carbohydrates are among the most complex and diverse set of post-translationally modified structures found on intracellular and secreted proteins. Large numbers of proteins contain *N*-linked oligosaccharides, including enzymes, cell surface receptors, secreted proteins, hormones, immunoglobulins, and viral antigens[1,2]. At the cellular level, *N*- and *O*-glycan structures have been shown to contribute to several aspects of biological recognition, including cell adhesion events during immune surveillance, inflammatory reactions, hormone action, arthritis, and viral infections [2]. Although some of the roles of *N*-linked glycans in cell adhesion have been identified, many of the details pertaining to the dynamics of cell adhesion events and changes in cell surface carbohydrate structures remain unresolved [2-5]. The cell- and tissue-specific changes in cell surface oligosaccharides during development have indicated that these structures may be involved in cell adhesion and migration events during embryogenesis [2, 6-8].

Alterations in the branching and extensions of *N*-glycans have also been found on the surfaces of cells that have undergone oncogenic transformation, and these changes correlate with alterations in cell adhesion that contribute to the invasiveness and metastatic potential of malignant cells [7]. A model has been developed over the last decade linking oncogene activation to the induction of oligosaccharide branching and extension at the cell surface. These changes in oligosaccharide structure have a direct

influence on the cell adhesion characteristics of the transformed cells and contribute to the development of the metastatic phenotype.

α-Mannosidase inhibitors that act late in the *N*-glycan processing pathway provide one route to blocking the oncogene-induced changes in cell surface oligosaccharide structures [9]. By inducing the formation of altered structures, the acceptor for oligosaccharide branching is no longer available. Although these compounds have been shown to exhibit potent anti-tumor and anti-metastatic activities, they also lead to serious complications resulting in a phenocopy of a lysosomal storage disease [10, 11]. Using this knowledge, it has been decided to take a biochemical and organic synthetic chemistry approach for the development of selective inhibitors for *N*-glycan maturation that should inhibit tumor progression.

An Overview of *N*-Glycosylation

The enzymes involved in the maturation of mammalian N-linked glycans are found in the endoplasmic reticulum (ER) and Golgi complex, in which they act upon newly synthesized glycoproteins to generate an array of complex structures from a common oligosaccahride precursor (Figure 1.1) [12-14].

The synthesis and maturation of *N*-linked oligosaccahrides occurs in four stages in the membranes of the ER and Golgi complex. In the first stage, a dolichol-linked $Glc_3Man_9GlcNAc_2$ oligosaccharide precursor is synthesized and transferred *en bloc* to the Asn-X-Ser/Thr sequins on newly synthesized polypeptides through the action of the multi-subunit oligosaccharide transferase complex (Fig. 1.1) [15].

The second stage involves the trimming of the transferred oligosaccharide resulting in the removal of all three glucose residues and six of the nine original mannose residues to generate the $Man_3GlcNAc_2$ core structure present on complex type oligosaccharides (Fig.1.1) [13]. The third stage of maturation involves the synthesis of

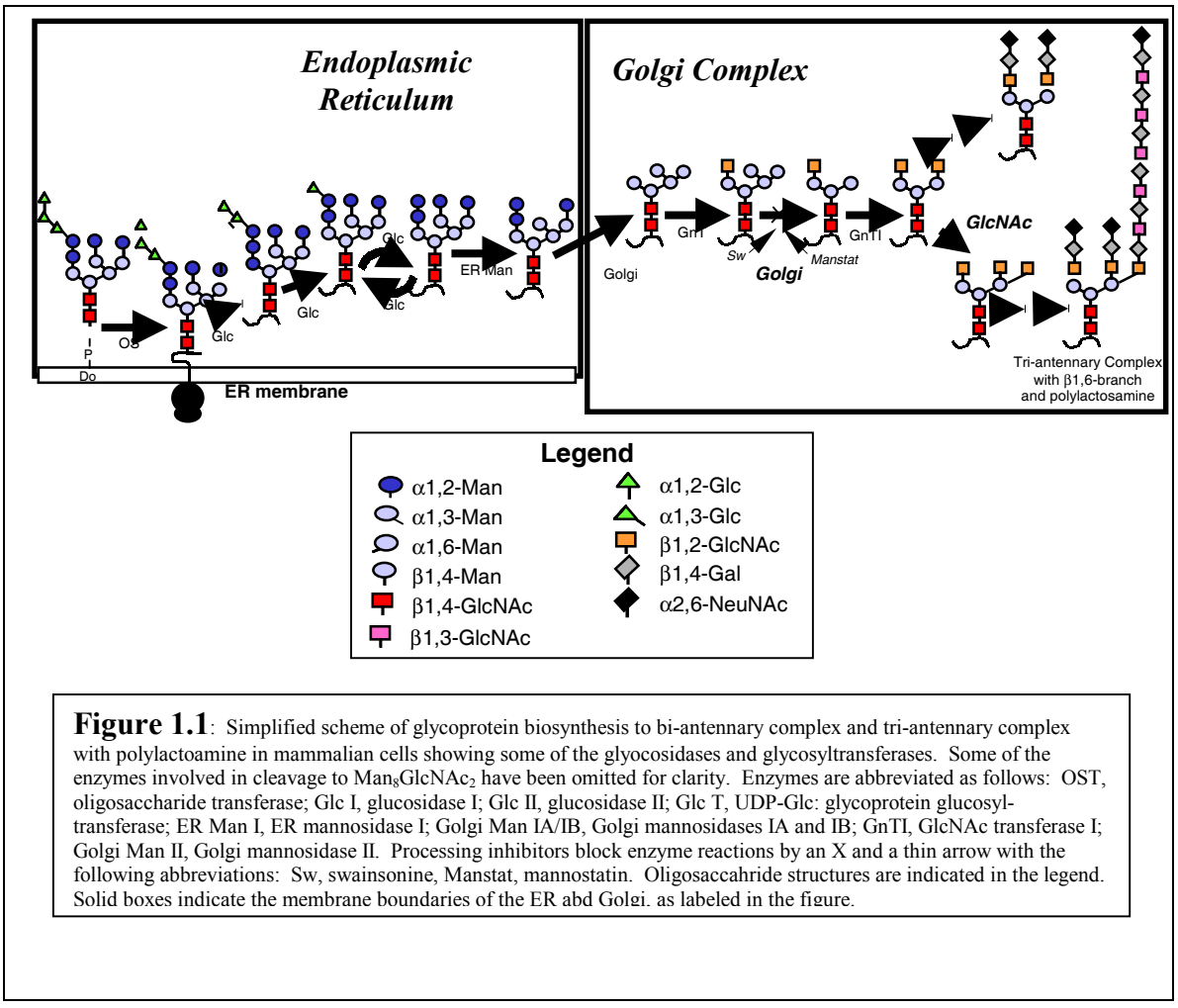


Figure 1.1: Simplified scheme of glycoprotein biosynthesis to bi-antennary complex and tri-antennary complex with polylactoamine in mammalian cells showing some of the glyocosidases and glycosyltransferases. Some of the enzymes involved in cleavage to $Man_8GlcNAc_2$ have been omitted for clarity. Enzymes are abbreviated as follows: OST, oligosaccharide transferase; Glc I, glucosidase I; Glc II, glucosidase II; Glc T, UDP-Glc: glycoprotein glucosyl-transferase; ER Man I, ER mannosidase I; Golgi Man IA/IB, Golgi mannosidases IA and IB; GnTI, GlcNAc transferase I; Golgi Man II, Golgi mannosidase II. Processing inhibitors block enzyme reactions by an X and a thin arrow with the following abbreviations: Sw, swainsonine, Manstat, mannostatin. Oligosaccahride structures are indicated in the legend. Solid boxes indicate the membrane boundaries of the ER abd Golgi, as labeled in the figure.

GlcNAc branches on the $Man_3GlcNAc_2$ core by the action of a collection of GlcNac transferases [16]. The final stage involves the elaboration of these branches with unique terminal capping structures characteristic of complex type oligosaccharides [2,17]. This final modification of *N*-glycans is the most complex stage in the biosynthesis of eukaryotic oligosaccharides. It incites the extreme diversity observed in mature *N*-glycan

structures, between organisms, between cells of like organisms, and between different glycoproteins of the same cell. Moreover, it is these branched and extended cell surface oligosaccharides that are implicated in the adhesion of cells, either by direct interactions with lectins associated with the extracellular matrix or the surfaces of other cells, or indirectly by influencing the protein-protein interactions of cell adhesion molecules at points of focal contact.

The complexity of *N*-glycan structures is largely based on the cell-specific expression of a collection of sugar transferases that specify the extension of oligosaccharide structures onto the trimmed $Man_3GlcNAc_2$ core structure derived from ER and Golgi α-mannosidase action [2]. Linkage-specific GlcNAc transferases establish the branching pattern of the $Man_3GlcNAc_2$ core and thereby generate the common bi-, tri-, or tetra-antennary complex type structures that are further extended with simple or highly complex branched or linear terminal capping structures. Among the most extended and complex termini are the polylactosamine-containing (repeating Galβ1,4GlcNAcβ1,3-) structures that are preferentially added to a single β1,6GlcNAc branch from the tri-mannosyl core (Figure 1.1). These structures are the high affinity ligands for extracellular lectins, such as galectin 1 and 3, and act as a scaffold for the elaboration of branched termini, such as Le^x, Le^y, and Sialyl-Le^x antigen sequences.

Association of the β1,6-Branch with Malignancy

One of the rate limiting factors in polylactosamine expression on *N*-glycans is the synthesis of the β1,6GlcNAc branch on the *N*-glycan $Man_3GlcNAc_2$ core. The reaction is catalyzed by the Golgi glycosyltransferase, GlcNAc transferase V (Gn TV) [28-30]. In

normal mammalian tissues β1,6GlcNAc-branched structures on the plasma membrane are largely restricted to cells that are capable of invasion. These cells include trophoblasts, endothelial cells, interstitial fibroblasts, and activated lymphocytes [18-20]. In transformed cells and human cancers such as carcinoma of the breast, colon, and skin, there is a significant increase in β1,6GlcNAc branching . Polyactosamine structures correlate with disease progression and higher concentrations of these structures are considered markers of malignancy in many tissues [23,24]. The binding of the cytotoxic lectin, L-phytohemagglutinin (L-PHA), is used to visualize β1,6-branches in malignant tissues.

Previous studies have demonstrated that β1,6-branching is induced upon transformation with polyoma [25,26] or Rous [27] sarcoma viruses, or by direct transfection with an activated form of Ras [28,29]. Similarly, the infection of cells with a temperature-sensitive form of RSV resulted in the appearance of larger oligosaccharides concurrent with the transformed morphology [30]. The cloning of the cDNA and the gene encoding of Gn TV (*Mgat5*) [31]has revealed signaling pathways leading to Gn TV gene activation[18,32,33]. Data has indicated that β1,6GlcNAc branching catatlyzed by Gn TV influences the migration of tumor cells and leukocytes, presumably by affecting their adhesion to the extracellular matrix. Data has also indicated that induction of β1,6-branching through oncogene signaling pathways contributes to the altered adhesion characteristics of metastaic cells. As there are no known inhibitors of Gn TV, the decision was made to block the β1,6-branch expression by targeting Golgi α-mannosidase II, an enzyme that acts upstream of Gn TV in *N*-glycan processing.

<u>Glycoprotein Processing Inhibitors as Anti-Tumor Agents</u>

Glycoprotein processing inhibitors have been isolated largely from natural sources. These inhibitors include the oligosaccharide transferase inhibitor, tunicamycin, and numerous α-glucosidase and α-mannosidase inhibitors that block the early trimming reactions [34]. To date, no natural or synthetic glycosyltransferase inhibitors have been identified that are both cell-permeable and effective in blocking oligosaccharide processing *in vivo*. Since selective and high affinity inhibition of Gn TV has not yet been achieved, inhibitors tend to be less than fully effective as a result of the multiple routes for early oligosaccharide processing in mammalian cells [13]. The α-mannosidase inhibitors can be divided into two groups based on their selective action toward the two classes of processing α-mannosidases in mammalian cells [12,13,35]. Class 1 mannosidases act early in the processing pathway and lead to the formation of a $Man_5GlcNAc_2$ processing intermediate. Aza-sugar manno-pyranose mimics, such as deoxymannojirimycin, inhibit these enzymes yielding extended high mannose structures [13,36,37].

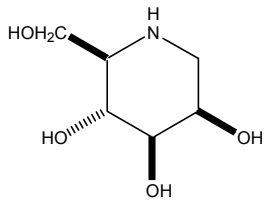


Figure 1.2. Chemical Structure of deoxymannojirimycin

In contrast the Class 2 mannosidases, Golgi α-mannosidase II and IIx, act after the GlcNAc transferase I step, and inhibition of these enzymes by the ring-flattened mannofuranose transition-state mimics and results in the formation of hybrid-type structures [36]. The resulting structures partially retain some of the character of complex

type oligosaccharides, but as a $Man_5GlcNAc_2$ core, they do not form the substrate structure necessary for recognition by Gn TV.

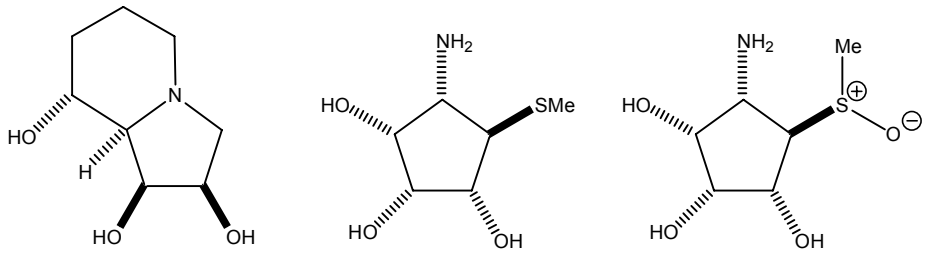


Figure 1.3. Chemical Structures of Swainsonine (left), Mannostatin A (middle), and Mannostatin B (right)

As glucosidase and Class 1 α-mannosidase inhibitors can block processing, they possess some potential for acting as inhibitors of β1,6-branch formation. However, accumulation of extended high mannose structures by these compounds can also affect the function and intracellular transport of certain glycoproteins [35,38-43]. The Class 2 mannosidase inhibitors, in contrast, do not appear to influence the function or transport of glycoproteins and exhibit low toxicity in tissue culture and in animals such as mice and humans [14,43].

Swainsonine, and all other known Golgi α-mannosidase II processing inhibitors, have an additional serious side effect that precludes their use as a therapeutic in that they also inhibit the broad-specificity lysosomal α-mannosidase, resulting in the accumulation of mannose containing oligosaccharides in tissues, serum, and urine as a phenocopy of the hereditary lysosomal storage disease, α-mannosidosis [10,11]. In addition to the inhibition of both the lysosomal α-mannosidase and Golgi α-mannosidase II by swainsonine, these two Class 2 α-mannosidases share several other features[13]. The inhibitory effects of swainsonine on both the lysosomal α-mannosidase and Golgi α-mannosidase II *in vivo* are rapidly reversible. Thus, studies of their effects on metastatic

potential rely upon chronic administration of the drug. A preferable approach for tumor growth inhibition therapy is the development of alternate lead compounds that specifically inhibit Golgi α-mannosidase II without affecting the lysosomal α-mannosidase and its corresponding complications. In order to understand the mechanism of α-mannosidase inhibition it is first necessary to summarize both the biochemistry of the α-mannosidases and the manner in which the various family members can be distinguished.

α-Mannosidases: Classification and Biochemical Characteristics

α-Mannosidases involved in glycoprotein maturation and catabolism have been divided into three broad families [13,37]. Two families termed Class 1 and Class 2 are mannosidases and exo-mannosidases respectively, as they excise mannose residues from the non-reducing terminus of the oligosaccharide. The third class is an endo-α-mannosidase that is involved in the early steps of glycoprotein maturation. The Class 1 mannosidases are distinguished from members of the other classes by several characteristics including similarities in sequence within a conserved 440-515 amino acid catalytic domain, specificity for cleaving α-1,2-mannose linkages, a required Ca^{2+} for catalytic activity, sensitivity to inhibition by the pyranose substrate mimics deoxymannojirimycin (dMNJ) and kifunensine (KIF), and cleavage of the glycosidic linkage by inversion of configuration of the released mannose residue. This classification contrasts with the more heterogeneous collection of processing and catabolic mannosidases, termed Class 2 mannosidases that are present in the ER, Golgi, lysosomes, and cytosol of mammalian cells, but have additional homologs in a widely diverse array

of organisms including eubacteria and archea. Class 2 mannosidases are larger (110-135 kDa), do not require cations for catalytic activity, are sensitive to inhibition by the furanose transition state analogs swainsonine, mannostatin, and 1,4-dideoxy-1,4-imino-D-mannitol, and cleave glycosidic linkages by a retention of anomeric configuration of the released monosaccharide [13,36,37,44-46]. Glycosyl hydrolases have also been independently classified into families based primarily on sequence similarity. In this scheme, Class 1 mannosidases correspond to the Famly 47 glycosyl hydrolases, while Class 2 enzymes are Family 38 glycosyl hydrolases [47-49].

The enzymology of the early trimming phases of the *N*-glycan pathway is surprisingly complex, employing members of all three mannosidase families in the conversion of $Man_9GlcNAc_2$ to distinct $Man_8GlcNac_2$ isomers. In contrast to the early processing events that generate $Man_8GlcNAc_2$ isomers, further cleavage to $Man_5GlcNAc_2$ in the Golgi is accomplished by a family of enzymes that are all Class 1mannosidases. While inhibition of these enzymes results in the accumulation of high mannose structures, they are not the focus of this research and are not affected by the inhibitors of the Class 2 α-mannosidases.

Golgi mannosidase II (Golgi Man II) is an enzyme that acts after the Class 1 Golgi mannosidase IA and IB and GlcNAc transferase I (Gn T I). As the final processing hydrolase in the *N*-glycan biosynthetic pathway, it is responsible for the removal of a α1,3- and a α-1,6-mannosyl residue to yield the Man_3GlcNA_2 core of complex type oligosaccharides [13]. The trimming and elongation phases of the processing pathway overlap at the GnT I/Golgi α-mannosidase II steps, with each reaction being obligatory for further maturation steps. The natural substrate specificity of the enzyme is

surprisingly restricted, recognizing only the two terminal non-reducing mannose residues on $GlcNAcMan_5GlcNAc_2$, but not $ManGlcNAc_2$ [50]. Golgi α-mannosidase is a 136 kDa disulfide-linked homodimer that has been purified and extensively characterized [51-58]. Despite the very precise specificity for the natural substrate $GlcNAcMan_5GlcNAc_2$, the enzyme also cleaves p-nitrophenyl- and 4-methylumbelliferyl-α-mannoside. A high yield but labor intensive purification procedure that was developed for the isolation of the catalytic domain of Golgi α-mannosidase II resulted in the purification of milligram quantities of this relatively low abundance enzyme from rat liver [54]. Peptide sequence data from the purified rat liver enzyme were used to clone the murine and human cDNAs in the Moremen lab [51,52]. Golgi mannosidase II has been shown to be a type II transmembrane protein with a small NH_2-terminal cytoplasmic tail, a single transmembrane domain, and a large luminal catalytic domain (~1097 amino acids) [52].

A deficiency in Golgi mannosidase II has been implicated in a human genetic disease. The disease, termed HEMPAS, is characterized by ineffective erythrpoiesis, bone marrow erythroid multinunclearity, and secondary tissue siderosis. There has been an identification of several HEMPAS patients that have reduced Golgi α-mannosidase II activity [58,59] and accumulate hybrid structures on erythrocyte glycoproteins consistent with the absence of Golgi α-mannosidase II activity. A mouse model for HEMPAS disease was also created by the inactivation of the Golgi mannosidase II gene in collaboration with others [60] and many of the characteristics of the human disease were reproduced. An activation of the Golgi α-mannosidase II gene resulted in viable animals exhibiting anemia, splenomegaly, and immature erythrocytes or reticulocytes in the peripheral blood similar to the pathology of HEMPAS patients.

In contrast to the trimming steps in the maturation of *N*-linked oligosaccharides, the catabolism of *N*-glycans predominately occurs through the action of broad specificity glycosidases in lysosomes [13,36]. High mannose and complex type oligosaccharides are broken down in lysosomes by a sequential exoglycosidase action requiring both sugar and anomeric configuration-specific glycosyl hydrolases. Thus, cleavage of α-linked mannosyl residues is catalyzed by a lysosomal α-mannosidase. Similar to other lysosomal glycosyl hydrolases, the lysosomal α-mannosidase has a broad linkage specificity, cleaving α1,2-, α-1,3, and α1,6-mannosyl linkages [13,36]. The cDNAs [61,62] and genes 13 encoding the lysosomal α-mannosidase have been cloned in the Moremen lab from a variety of mammalian and non-mammalian species. All of the genes encode a protein of similar size (~900-1000 amino acids), and the tested enzymes have a similar broad-specificity toward oligosaccharide substrates.

A human lysosomal storage disease characterized by a defect in the broad specificity lysosomal mannosidase, α-mannosidosis [63,64], results in varied clinical features such as coarse features, skeletal abnormalities, dilated cerebral ventricles, sever mental retardation, vacuolated lymphocytes in the bone marrow and blood, the creation of "storage" cells in bone marrow, and greatly elevated mannose-rich oligosaccharides in tissues, blood, and urine. α-Mannosidase patients exhibit a significant reduction in the levels of the lysosomal α-mannosidase in all tissue. The genetics of the disorder indicate an autosomal recessive inheritance of either a severe infantile form (type I), in which death is common before age 10, or a milder (type II) form that is recognized in juveniles and adults [64].

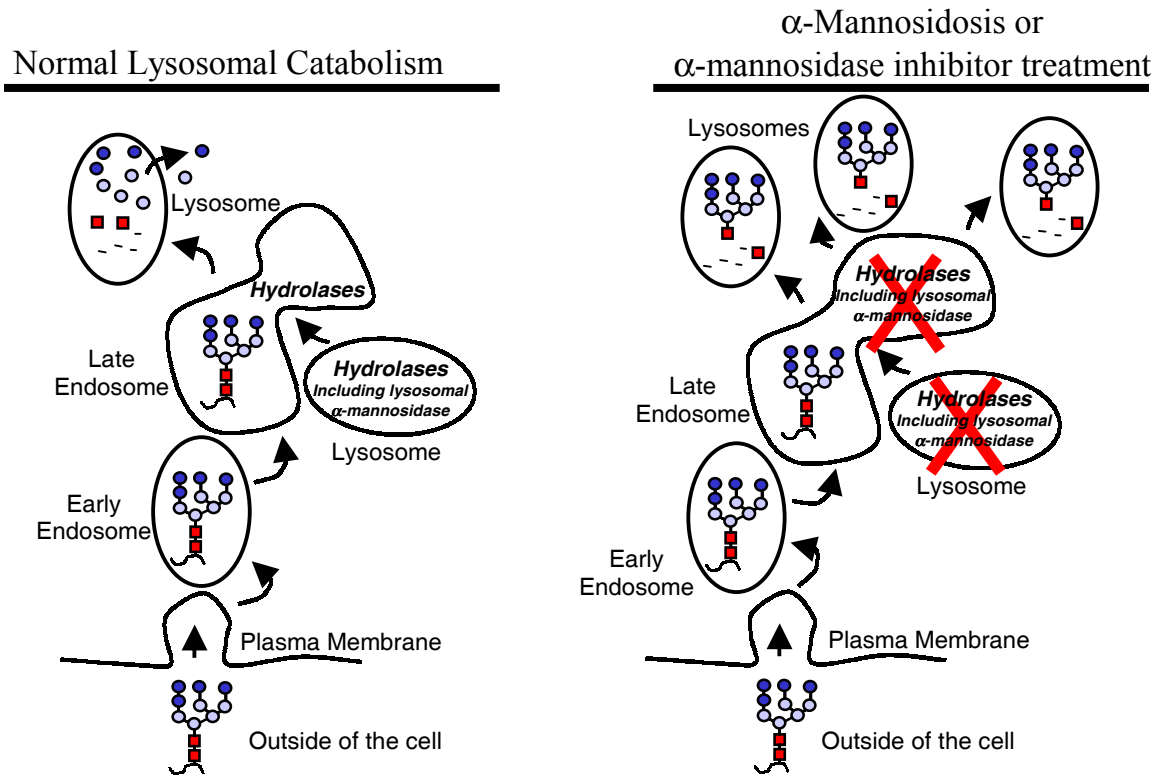


Figure 1.4. Catabolism of high mannoase oligosaccaharides in normal, α-mannosidosis, or α-mannosidase inhibitor treated cells. The catabolism of N-glycans occurs through the exoglycosidase cleavage from the reducing and non-reducing terminus. Blockage of the action of lysosomal α-mannosidase results in the accumulation of high mannose oligosaccharides containing a Manβ1,4-GlcNAc core and a proliferation of lysosomes containing undegraded oligosaccharides.

It should be mentioned that despite the similarities of the Class 2 mannosidases in their sequence, response to inhibitors, and catalytic mechanism as inverting enzymes, Golgi α-mannosidase II and the lysosomal α-mannosidase have significant differences in aglycone specificity that can be utilized for selective inhibitor design. Golgi α-mannosidase II has a highly selective substrate specificity as it recognizes $GlcNAcMan_5GlcNAc_2$ for cleavage to $GlcNAcMan_3GlcNAc_2$ [50]. The requirement for the prior action of GlcNAc transferase I prior to the Golgi α-mannosidase II action is

demonstrated by the inability of the enzyme to cleave $Man_5GlcNAc_2$, which is a >1000-fold less effective substrate. Thus, Golgi α-mannosidase II must have an extended aglycone recognition site to provide the considerably higher affinity interaction with the $GlcNAcMan_5GlcNAc_2$ substrate. In contrast, the lysosomal α-mannosidase appears to recognize the α-mannose glycone in a similar manner (it has a similar K_m for cleavage of the p-nitrophenyl-α-D-mannosidase substrate as Golgi α-mannosidase I)[61], but appears to have a considerably relaxed specificity for recognition of the aglycone [65]. The lysosomal α-mannosidase is able to cleave α-1,2-, α1,3-, and α1,6-mannose residues on a variety of aglycones during the catabolic degradation of $Man_9GlcNAc_2$. Thus, the Golgi and lysosomal α-mannosidases have significantly different aglycone binding sites that will be exploited to develop a selective Golgi α-mannosidase inhibitor.

Glycosidase Mechanisms and the Mechanism of Inhibitor Action

The distinctions in inhibition between the Class 1 and Class 2 α-mannosidases correlate with their mechanism of action. Class 1 α-mannosidases are inhibited by pyranose substrate mimics [36] that bind to the enzyme in a highly free energy $^1C_4$ conformation. In contrast, the Class 2 α-mannosidases are inhibited by an aza-sugar furanose ring-flattened transistion state analogs [36]. These differences in inhibitor profile are also related to their mechanism of action. Glycosidic bonds are hydrolysed enzymatically via general acid catalysis [66,67]. Two residues are critical for efficient rate acceleration of the reaction: a proton donor and a nucleophile/base. In addition, although this large family of enzymes comprises many different protein folds, the spatial relationship of their catalytic residues is found to show significant similarity. This is reflected in the fact that the majority of

glycosidases share related modes-of-action [36]. The enzymes may be usefully divided into two broad categories: those which catalyze hydrolysis of the glycosidic bond with net inversion of anomeric configuration (inverting enzymes), and those which cleave with net retention (retaining enzymes). In both cases of enzymes, the position of the proton donor is within hydrogen bonding distance from the oxygen. Retaining enzymes usually make use of the nucleophilic carboxylate group in a double displacement reaction to ensure stereoselectivity, whereas inverting enzymes usually use a water molecule – activated by a catalytic base – as the nucleophile, in a single step reaction. However, despite their simplistic appearance, the precise details of these mechanisms remain difficult to elucidate and key features continue to generate controversy.

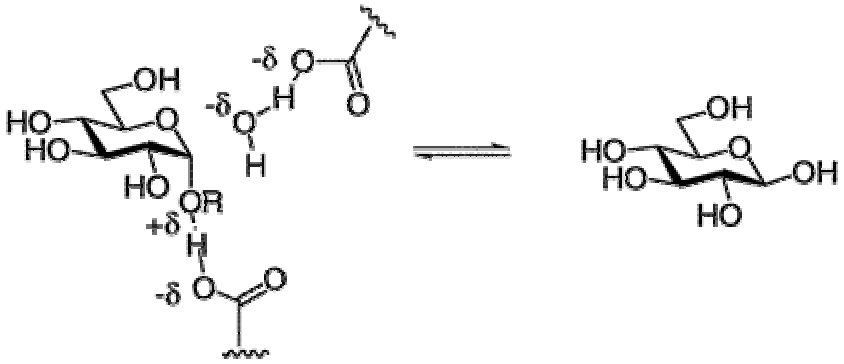


Figure 1.5. Mechanism for hydrolysis of the glycosidic bond with net inversion of anomeric configuration (inverting enzymes)

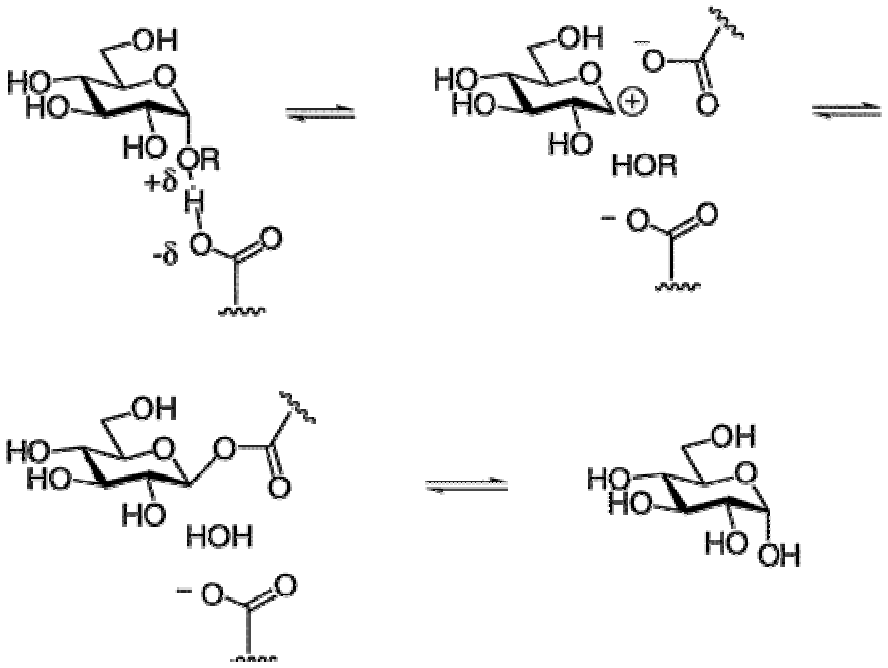


Figure 1.6. Mechanism for hydrolysis of the glycosidic bond with net retention (retaining enzymes).

Given the importance of glycosidases in the biosynthesis of oligosaccharides, it is not surprising that there exists a multitude of natural inhibitors for these enzymes. The structural diversity of these inhibitors is atypical and reflects the marked substrate specificity displayed by most glycosidases, and, perhaps more importantly, reflects the subtle and intriguing nuances in the modes-of-action of each enzyme in the class [68-72]. In fact, the structures of natural inhibitors have given valuable insights into the possible mode-of-action of the glycosidases they inhibit. The clues thus gleaned have served as an important guide in the design of synthetic analogs, some of which are very novel. Many of these synthetic and natural inhibitors can be considered as stable mimics of the short-lived intermediates stabilized at the transition states of the enzymatic hydrolysis reaction. For example, the inhibition by both mannostatin and the sulfonium salt might be reduced to the resemblance of their five-membered core structures to the flattened

shape of the putative carbocationic intermediates implicated in the enzymatic hydrolysis of mannosidases such as the proposed oxycarbonium ion. However, an in depth understanding of the mode-of-inhibition of the majority of inhibitors remains evasive and this has very often resulted in frustrated attempts to improve them.

Mannostatin

Mannostatins A and B were the first natural α-mannosidases inhibitors to be discovered that possess the cyclopentinol structure [73].

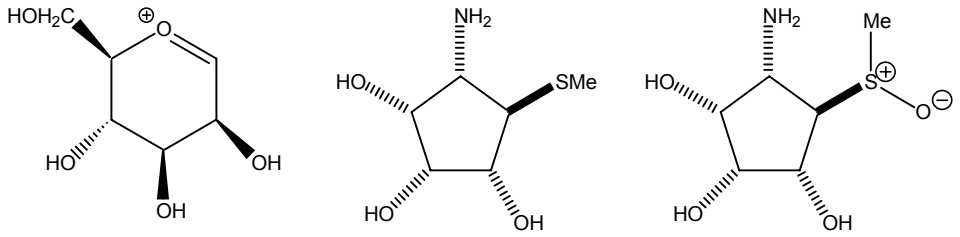


Figure 1.7. Chemical structures of the putative oxycarbonium ion (left), Mannostatin A (middle), and Mannostatin B (right)

In addition, they are the first non-azasugar type inhibitors of mannosidases. The inhibitors are of the reversible, competitive type and do not show the slow-binding phenomenon exhibited by other inhibitors such as swainsonine [74]. Despite their relative structural simplicity the mannostatins are nevertheless potent and selective. However, a straightforward correlation of their structures with their inhibitory properties has not proved simple when classical models were used. For example, the mode-of-inhibition of mannosidases by azasugars such as swainsonine [75], 1,4-dideoxy-1,4-imino-D-mannitol [76] and related analogs has been found to correlate with the degree to which they resemble

the oxycarbonium ion [77], a putative intermediate in the hydroylysis of mannosidases. A molecular graphics study intended to ratify the generality of this hypothesis with respect to mannosidase inhibitors has been somewhat successful in explaining the varying degrees of inhibition shown by a selection of azasugars [78]. Furthermore, the synthetic aminocyclopentitol has been designed as a mannosidase inhibitor using the same model [79]. Thus, the heteroatoms of the carbocycle superimpose well onto a 'flap-up' mannosyl oxycarbonium and as predicted by the transition state theory, this synthetic analog powerfully inhibits a number of mannosidases. Nevertheless, there has been argument as to whether the model is adequate in explaining the potent inhibition of the mannostatins, as neither can be superimposed in an unambiguous manner onto the 'flap-up' oxocarbenium ion.

An alternative mode-of-inhibition for mannostatins has been proposed on account of their resemblance to β-mannopyranosylamine: glycopyranosylamines have long been known to be competitive inhibitors of glycosidases [79]. If mannostatin and β-mannopyranosylamine sit similarly in a given active site, and the amino group of the inhibitor were to mimic the exocyclic oxygen of the substrate, the mannostatins would then simply owe their activity to their being substrate analogs. However, the substrates of α-mannosidases are α-glycosides, and the enzymatic reaction proceeds with retention of configuration. Preliminary evidence indicates that α-mannosidases adopt a retaining mechanism and are not inhibited by mannostatin A. This raises the possibility that the amino function of the mannostatins mimics the ring-oxygen of the substrate and that the mannaostatins are 'transition state' analogs. The amino group of the inhibitor would be protonated at physiological pH and expected to interact electrostatically with a catalytic

carboxylate group, thereby inhibiting the enzyme. The analog, wherein the amino group of mannostatin A is acetylated, shows no inhibitory activity whereas the synthetic analog, in which the amino group is monobenzylated, is an efficient mannosidase inhibitor.

The possible role of the methylthio and methylsulfenyl functions in mannostatins A and B respectively is intriguing, particularly because these features are unique to these natural glycosidase inhibitors. Thioether groups have been incorporated as structural features in synthetic mannosidase inhibitors even though the synthetic aminocyclopentitol which lacks this motif is nevertheless an efficient mannosidase inhibitor. It has been proposed that the methylthioester and methylsulfenyl groups lie near the 4-OH group of the mannosyl oxycarbonium ion, but it is also conceivable that these functions are mimic those of the hydroxymethyl group in the substrate. However, disagreement does exist regarding the importance of the hydroxymethyl interaction on the overall efficiency of a given inhibitor. If the thio functions play a role in the recognition and binding of the mannostatins by mannosidases, the extent of the importance of these structural elements remains unclear. It should be noted that mannostatins A and B, the epimer at sulfur of mannostatin B, and the unnatural sulfone analog all exert identical inhibitory activity against mannosidases.

The changes in *N*-linked oligosaccharides associated with the transformed and metastaic phenotypes have been demonstrated to be markers for malignancy in many cell types. Intervention in the glycoprotein processing pathway by inhibitor treatment has also demonstrated two overriding beneficial effects: a reduction of the β1,6-branching associated with the altered cell adhesion phenotype of transformed cells (reducing metastasis), and it causes an unanticipated acute anti-tumor effect by immune

stimulation. A significant drawback of the existing set of inhibitors and their corresponding analogs is that they also target lysosomal glycoprotein catabolism, thus causing a phenotype of a lysosomal storage disease. The human forms of the glycosidase targets for inhibitor design have been cloned, expressed, and well characterized. Based on their similarities in primary sequence, response to inhibitors, and mechanism of action, the Golgi and lysosomal α-mannosidases are predicted to possess similar glycone binding sites. In contrast, the aglycone binding sites for the two enzymes are predicted to be quite distinct due to the known differences in the aglycone substrate specificity of the two enzymes. Thus, more selective inhibitors could be designed to utilize these predicted structural differences in the aglycone binding sites between target enzymes.

Swainsonine

Swainsonine , the prototypical Class 2 α-mannosidase inhibitor, was first isolated from the Australian plant *Swainsona canescens* (Figure 1.8) and subsequently from additional plant species in America [36,80]. Although these plants were termed "locoweeds" as a result of the neurological symptoms induced by their ingestion, swainsonine is now known to be non-neurotoxic. The "loco" symptoms appear to result from the ingestion of contaminating alkaloids in the plants [80].

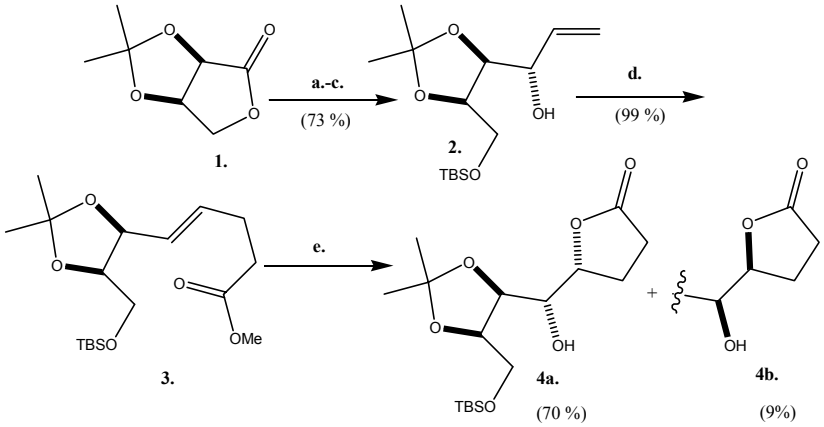
Figure 1.8. Swainsona canescens

Swainsonine influences the maturation of glycoproteins by causing the diversion of oligosaccharides to a hybrid type as opposed to complex structures. As such it would be anticipated that the compound might influence cell adhesion characteristics and thus the metastasis of transformed cells [9]. The low toxicity of the compound was revealed by the fact that no changes in cell growth rates were found in transformed cells in culture. Surprisingly, when transformed cells were introduced into animal models, swainsonine was found to have a strong immunostimulatory activity that resulted in a significant anti-cancer effect [9,81]. Swainsonine was shown to stimulate natural killer cells (NK cells) and lymphokine activated killer cells (LAK cells), and significantly blocked tumors in the lung, liver and spleen [81,82]. The drug was also shown to stimulate the anti-tumor activity of macrophages in a time- and dose-dependant fashion, even in immunocompromised

SCID and nude mice [83]. In addition, swainsonine stimulates bone marrow proliferation and has exhibited protective effects in mice against cytotoxic chemotherapy agents, thus raising the possibility that the drug may be used in patients along with traditional cytotoxic agents [84-86]. In mice, swainsonine causes enhanced bone marrow cellularity, engraftment efficiency, and colony forming units [9]. The immunostimulatory activity is believed to result from the observation that some lymphokines and growth factors (IL-1, IL-2, and TNF-α) have high mannose carbohydrate binding activity. It has been suggested that swainsonine plus IL-1 or TNF-α may act synergistically to enhance the direct killing of tumor cells by lymphocytes and the activation of host macrophages [7,9]. Although the specific mechanisms of these activities are not well understood, they are believed to be mediated through oligosaccharide structure. Swainsonine also induces the transcription of the tissue inhibitor of metalloproteinases (TIMP-1) [87] and suppresses the expression of MMP-2 [88], a proteinase that has an essential role in tumor invasion and metastasis [89]. Two phase I clinical trials have demonstrated that swainsonine has a low toxicity in humans and is well tolerated. Phase II clinical trials of swainsonine and modified analogs are presently underway [9,90].

In 1996, Pearson[91] reported a practical synthesis of (-)-Swainsonine. His synthesis of swainsonine (*schemes 1 and 2*) began with 2,3-*O*-isopropylidine-D-erythronolactone **1**, which is commercially available or may be prepared in large quantities from inexpensive D-isoascorbic acid. Reduction of **1** with diisobutylaluminum hydride provided 2,3-*O*-isopropylidene-D-erythrose. Addition of vinylmagnesium bromide followed by selective monoprotection of the resulting diol afforded the allylic alcohol **2** (97:3 anti/syn) in 73% yield from **1**. Separation of the diasteroemeric mixture

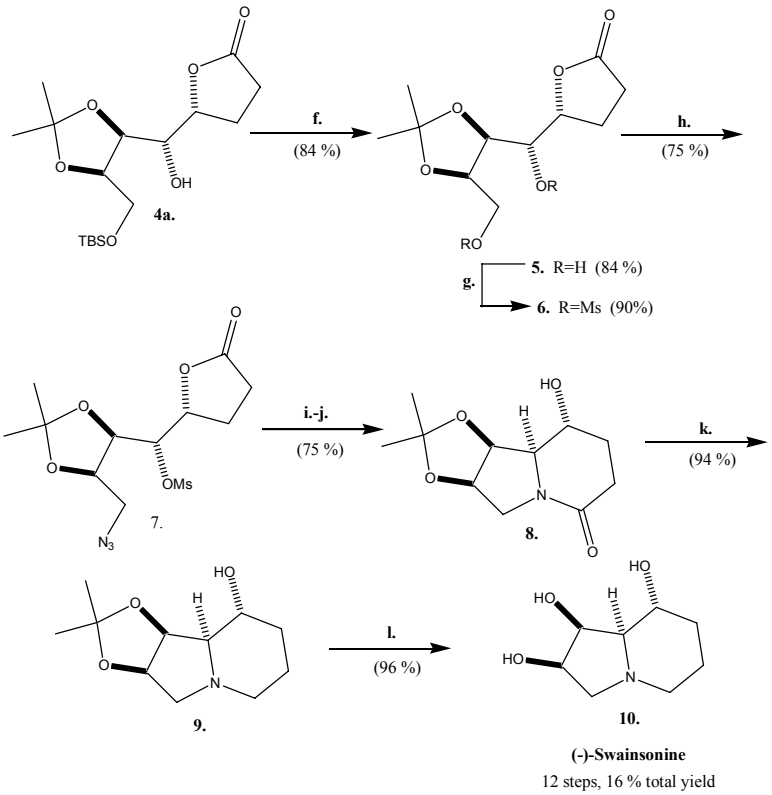
was possible; however, it was unnecessary, since both allylic alcohols **2** produce the same γ,δ-unsaturated ester **3** when subjected to Johnson orthoester Claisen rearrangement conditions. The rearrangement produced only the *E*-isomer within the limits of detection by high field ^{1}H NMR. Without purification, **3** was submitted to the Sharpless dihydroxylation procedure, affording the lactones **4a** and **4b** in 70% and 9% yields, respectively, after separation.



Scheme 1.1. (a) *i*-Bu_2AlH; (b) vinyl magnesium bromide; (c) TBDMS-Cl, imidazole; (d) $CH_3C(OMe)_3$, $EtCO_2H$, 110°C; (e) AD-Mix β

Removal of the silyl protecting group from **4a** gave the diol **5**, which was smoothly converted to the crystalline dimesylate **6**. Selective displacement of the less hindered mesylate of **6** with sodium azide afforded **7**. Palladium-catalyzed hydrogenolysis of **7** to the amine followed by filtration of the catalyst and treatment of the filtrate with sodium methoxide caused cyclization to the known crystalline bilactam **8** in 75% yield. Reduction of **8** with borane-methyl sulfide complex gave a 94% yield of crystalline **9**, also a known compound, which was hydrolyzed to swainsonine in 96%

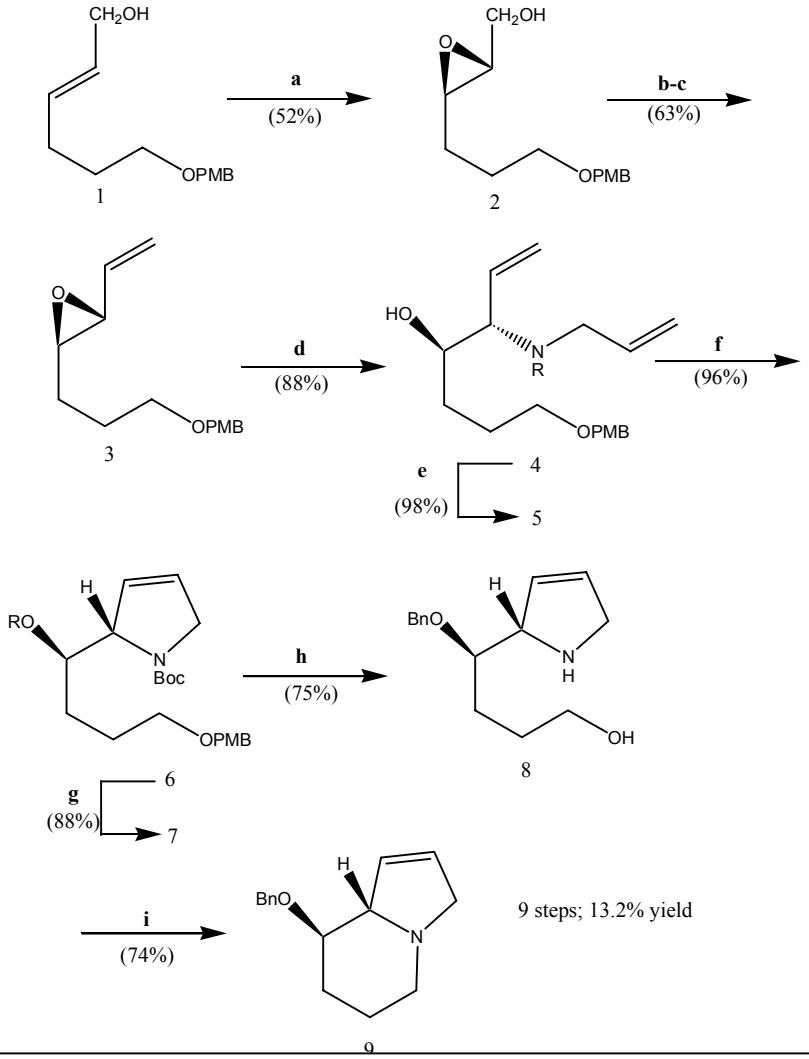
yield. While not the shortest synthesis, this route involves simple, reproducible steps that work well on a substantial scale.



Scheme1. 2. (f) $(n\text{-}Bu)_4NF$; (g) MsCl; (h) NaN_3;(i) H_2, $Pd(OH)_2$; (j) NaOMe; (k) $BH_3{\cdot}SMe_2$; (l) 6N HCl

A synthesis of (-)-Swainsonine as reported by Pyne[92] in 2002 can be seen in the following schemes (*schemes 3 and 4*). Commercially available 4-pentyl-1-ol was converted to the known trans-allylic alcohol **1**, in three high yielding steps. Catalytic asymmetric epoxidation of 1 using (-)-diisopropyl tartrate as the chiral ligand gave the (2*R*,3*R*)-epoxy alcohol **2** in 92% ee. Swern oxidation of the primary alcohol **2** gave the corresponding aldehyde that was converted to the vinyl epoxide **3** by a Wittig olefination reaction. Aminolysis of **3** was achieved by heating a solution of **3** in allylamine (10

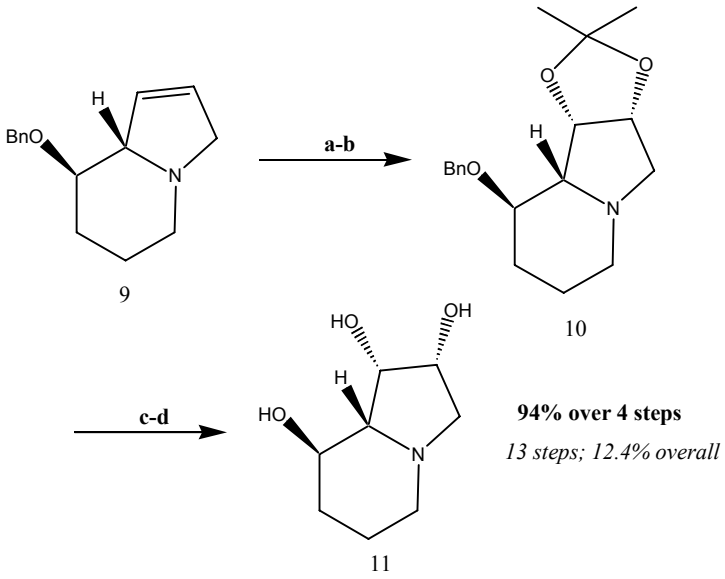
equiv) with *p*-TsOH·H_2O (0.1 equiv) as a catalyst in a sealed tube for 3 days at 105°C. The product anti-amino alcohol **4** was obtained as a single diastereoisomer in 88% yield with clean inversion of stereochemistry at the allylic carbon.



Scheme1. 3. (a) D-(-)-DIPT, $Ti(OPri)_4$, TBHP , CH_2Cl_2, 4A MS, -20°C; (b) (I) DMSO, $(COCl)_2$, CH_2Cl_2, -60°C, (ii) Et_3N; (c) $MePPh_3Br$, KHMDS, toluene; (d) allylamine, p-TsOH·H_2O (0.1 equiv); (e) $(Boc)_2O$, Et_3N, CH_2Cl_2; (f)$Cl_2(Cy_3P)_2Ru$=CHPh, CH_2Cl_2, reflux; (g) NaH, BnBr, Bu_4NI, THF, rt; (h) TFA/anisole, 0°C; (i) Ph_3P, CBr_3, Et_3N, 0°C

Protection of the amino group of **4** as its *N*-Boc derivative **5** followed by a ring-closing metathesis reaction at high dilution in refluxing dichloromethane solution gave the 2,5-dihydropyrrole derivative **6** in excellent overall yield (94%). The secondary hydroxyl function in **6** was then protected as its benzyl ether **7**. Treatment of **7** with

trifluoroacetic acid in the presence of anisole (10 equiv) as a carbocation scavenger gave, after base treatment, the amino alcohol **8** in 75% yield. Cyclization of **8** by activation of the primary hydroxyl and then intramolecular *N*-Alkylation (Ph_3P, CBr_4, Et_3N) gave the indolizidine derivative **9** in 74% yield. The relatively low yield in this reaction appears to be due to the co-formation of the pyrrole derivative of **9** as a minor (5-10%) product.



Scheme1. 4. (a) AD mix-α, $MeSO_4NH_2$, Bu^tOH/H_2O, 0^oC, 6d; (b) 2,2-dimethoxypropane, p-TsOH, 3h, rt; (c) $PdCl_2$, H_2, MeOH, rt, 1h; (d) 2M HCl, THF, rt, 20h; basic ion-exchange

Catalytic cis-dihydroxylation of **9** with osmium tetraoxide/*N*-methylmorpholine *N*-oxide, yielded a crude diol which was converted to the known acetonide derivative **10**. Compound **10** was then converted to (-)-swainsonine using literature procedures in 94% yield over the four steps. This procedure, as reported by Pyne, gave swainsonine in thirteen steps, with a 12.4% overall yield.

As stated earlier in this report, Swainsonine, and all other known Golgi α-mannosidase II processing inhibitors, have an additional serious side effect that precludes their use as a therapeutic in that they also inhibit the broad-specificity lysosomal α-

mannosidase, resulting in the accumulation of mannose containing oligosaccharides in tissues, serum, and urine as a phenocopy of the hereditary lysosomal storage disease, α-mannosidosis [10,11]. A preferable approach for tumor growth inhibition therapy is to develop alternate lead compounds that specifically inhibit Golgi α-mannosidase II without affecting the lysosomal α-mannosidase and its corresponding complications.

Aim of This Project

As one can easily see, the inhibitors that are presently known are inefficient to make, and those that have been made thus far lack the selectivity needed to actually be beneficial. The goal of this project is two-fold. The first goal is to overcome the selectivity issues which limit the use of Swainsonine. Swainsonine is a potent inhibitor of Golgi α-mannosidase II. However, it also inhibits the lysosomal α-mannosidase, a property that we plan to rectify. As mentioned before, despite the similarities of the Class 2 mannosidases in their sequence, response to inhibitors, and catalytic mechanism as inverting enzymes, Golgi α-mannosidase II and the lysosomal α-mannosidase have significant differences in aglycone specificity that can be utilized for selective inhibitor design. Golgi α-mannosidase II has a highly selective substrate specificity, recognizing $GlcNAcMan_5GlcNAc_2$ for cleavage to $GlcNAcMan_3GlcNAc_2$ [50]. The requirement for the prior action of GlcNAc transferase I prior to the Golgi α-mannosidase II action is demonstrated by the inability of the enzyme to cleave $Man_5GlcNAc_2$, which is a >1000-fold less effective substrate. Thus Golgi α-mannosidase II must have an extended aglycone recognition site to provide the considerably higher affinity interaction with the $GlcNAcMan_5GlcNAc_2$ substrate. In contrast, the lysosomal α-mannosidase appears to

recognize the α-mannose glycone in a similar manner (it has a similar K_m for cleavage of the *p*-nitrophenyl-α-D-mannosidase substrate as Golgi α-mannosidase I)[61], but appears to have a considerably relaxed specificity for recognition of the aglycone [65]. The lysosomal α-mannosidase is able to cleave α-1,2-, α1,3-, and α1,6-mannose residues on a variety of aglycones during the catabolic degradation of $Man_9GlcNAc_2$. Thus, the Golgi and lysosomal α-mannosidases have significantly different aglycone binding sites that will be exploited to develop a selective Golgi α-mannosidase inhibitor, which is something we plan to look into further.

The second goal deals with the intensive effort needed to construct these inhibitors. As seen already, it is far from a trival task, thirteen steps were necessary to build swainsonine. It would be much more beneficial to create these systems in a faster, more logical way. We have developed a novel class of lead compounds which will be tested for their effectiveness as Class 2 α-mannosidase inhibitors. The following sections give an introduction of the two "families" of compounds designed for this project, is discussed in the following sections.

Sulfonium Salts

Monocyclic amines such as 1-deoxynojrimycin or bicyclic derivatives such as swainsonine or castanospermine are postulated to bind to glycosidase enzymes by mimicking the shape and charge of the oxycarbenium ion intermediate for the hydrolysis reaction, as described earlier in this account. This requires that the nitrogen atom be protonated in the enzyme active site and that the interaction with the enzyme be dominated by stabilizing electrostatic interactions with active site carboxylate residues.

An alternative means of providing an inhibitor with the required charge-state would be to include in the structure an atom that carries a permanent positive charge at a suitable position. One obvious strategy would be to make this atom a positively charged sulfur, because sulfonium salts are known to be quite stable, as opposed to their highly unstable oxonium counterparts.

This reasoning was inspired by the pioneering work of the late B. Belleau who synthesized sulfonium-ion analogs of the morphinans, levorphanol and isolevorphanol, and showed that they were agonists or antagonists of morphine for the opiate receptor. More recently, the use of sulfonium salts as enzyme inhibitors has been elegantly demonstrated in the field of sterol biosynthesis. The cyclase enzyme that mediates the cyclization of the open-chain oxidosqualene precursor to sterols is inhibited by sulfonium salt mimics, either preformed or generated *in situ*, of the carbocation intermediates.

Recently a new class of glycosidase inhibitor with an intriguing inner-salt sulfonium-sulfate structure has been isolated from the roots and stems of the plant *Salacia reticulata*. Extracts of this plant have been traditionally used in the Ayurdevic method of Indian medicine as a treatment for diabetes. The most active ingredients of these extracts appear to be the sulfonium salts salacinol and kotalanol, both of which have an identical anhydro alditol structure to well-known imino-alditol inhibitors such as 1,4-dideoxy-1,4-imino-D-arabinitol.

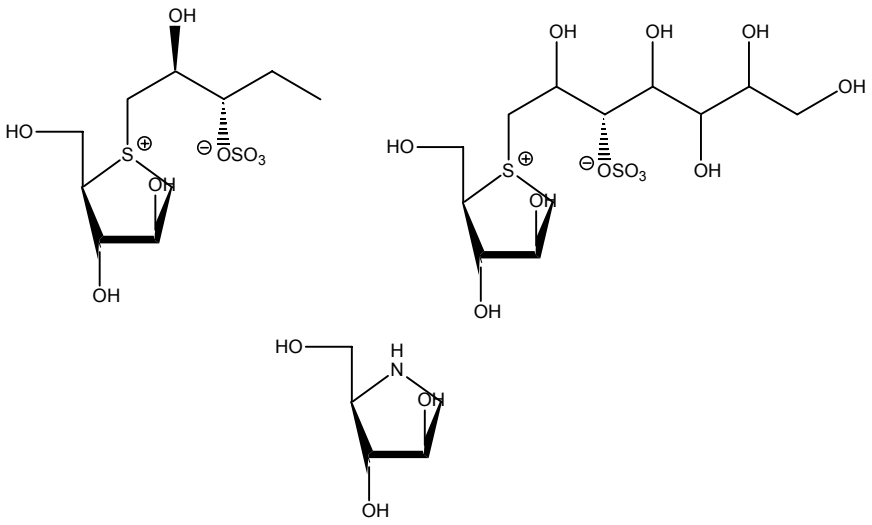


Figure1.9. Chemical Structures of salacinol (upper left), kotalanol (upper right), and 1,4-dideoxy-1,4-imino-D-arabinitol

The inhibition of glycosidase enzymes by these compounds rivals, or in some cases exceeds, the most potent amino sugar inhibitors, such as acarbose. It appears that the potential for glycosidase inhibition by sulfonium salts has not been neglected by nature. Thus, it is our contention that the synthesis of sulfur analogs of the known nitrogen-based glycosidase inhibitors may lead to glycosidase inhibitors exhibiting increased potency and utility.

"Swainsonine"-Type Analogs

As stated earlier in this Thesis, a major shortcoming of inhibitors of processing α-mannosidases such as swainsonine [6] is that they display poor selectivity for their target, human Golgi α-mannosidase II (HGMII). One of the most serious negative effects of this lack of selectivity is as result of the concurrent inhibition by azasugar (and it numerous synthetic analogs) of human lysosomal α-mannosidase (HLM), which leads to the

phencocopy of the inherited disease, mannosidosis, a rare autosomal recessive condition.[7] One of the major challenges in using azasugars inhibitors of glycosidase enzymes as biochemical tools or drugs is that they generally suffer from lack of specificity.

Goals of Project

A number of groups have attempted to design synthetic inhibitors of glycosidase in the aim of improving upon the activity of their natural counterparts. I have successfully synthesized a novel class of polyhydroxylated sulfonium salts intended to inhibit glycosidases [93]. Each has been designed to inhibit a particular enzyme activity, and all can be considered as analogs of swainsonine. However, the bicyclic sulfonium salts differ structurally from the designed azasugars in the crucial respect that the bridgehead nitrogen in the latter is replaced by a permanently charged positive sulfur.

The pronounced inhibition of certain α-mannosidases by the azasugar swainsonine is thought to reflect the resemblance of the inhibitor – in its protonated form – to the mannosyl oxocarbonium ion, supposedly formed as a transient intermediate during the enzymatic hydrolysis of α-mannosides. Although uncertainty remains as to the exact sequence of events and the precise nature of any intermediates in the mechanism of action of α-mannosidases, there is little doubt nevertheless that a transient species is generated at a crucial point on the reaction coordinate, a species which carries a considerable build-up of positive charge adjacent to the anomeric center. It is thus an interaction of the conjugate acid of the inhibitor (pKa = ca., 7.4) with the partially deprotonated enzymatic catalytic carboxyl group (pKa = ca. between 4.1 and 4.6) that is

presumed to be implicated in the observed tight binding of swainsonine by various α-D-mannosidases.

The novel compounds have been designed to mimic the mannoysyl cation both in terms of charges and shape. As anticipated, the compounds are potent inhibitors of α-mannosidase activity and as such are found to be markedly more selective than swainsonine itself.

CHAPTER TWO

POTENT AND SELECTIVE INHIBITION OF α-D-MANNOSIDASE ACTIVITY BY A BICYCLIC SULFONIUM SALT: A MANNOSYL CATION MIMIC

A sustained interest in the search for glycosidase inhibitors has been developed in recent years.[94-96] An important impetus for this interest has arisen from the recognition that the regulation of oligosaccharide metabolism using glycosidase inhibitors might offer a promising strategy in the treatment of diseases such as cancer, influenza and diabetes.[97] The use of such inhibitors as tools with which to further delineate the biosynthesis of oligosaccharides[98] and to clarify the catalytic mechanisms of glycosidases[95,96,99] has also provided an important stimulus in this area. The polyhydroxylated alkaloid swainsonine **1**,[100] for instance, has been particularly useful as a biochemical tool[98,99] and has also been instructive in the conception of novel synthetic α-D-mannosidase inhibitors.[101,102]

The pronounced inhibition of certain α–D-mannosidases by swainsonine[98,99] is thought to reflect the resemblance of the inhibitor - in its protonated form - to the mannosyl cation **2**.[103-105] Although there remain uncertainties as to the exact sequence of events and the precise nature of any intermediates in the mechanism of action of α–D-mannosidases such as the Golgi mannosidase II (GMII),[105-107] there is little doubt, that a transient species is generated at a crucial point on the reaction coordinate, a species which carries a considerable build-up of positive charge adjacent to the anomeric center of the substrate. Thus, it is an interaction of the conjugate acid of the inhibitor (pK_a= ~7.4), with the partially deprotonated enzymic catalytic carboxyl group (pK_a= between

4.1 and 4.6), that is presumed to be the origin of the observed tight binding of **1** by GMII and related enzymes. [108]

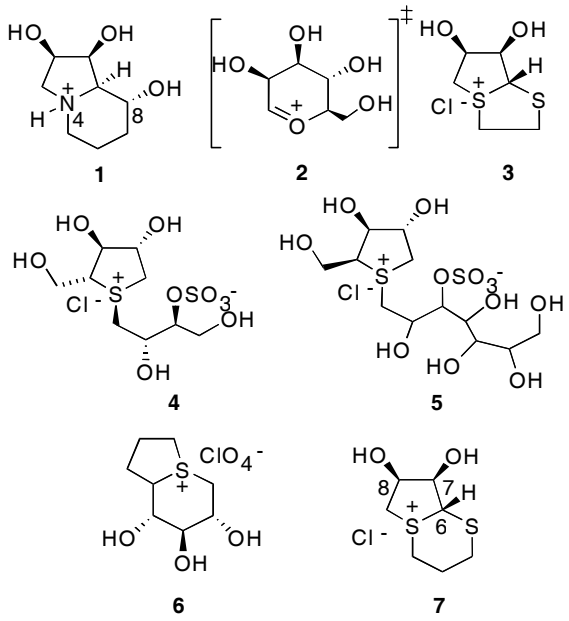
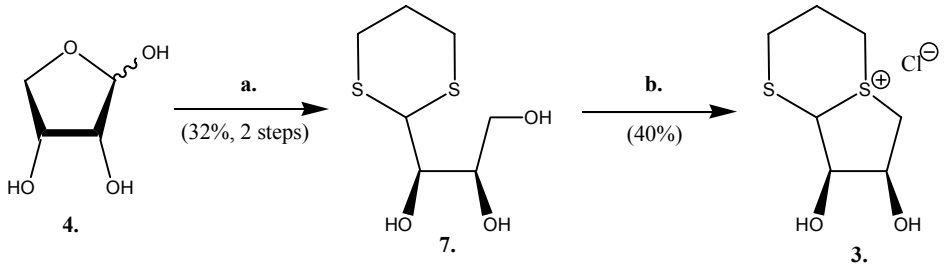


Figure 2.1.

Considerations such as these led us some time ago[109] to conjecture that properly designed sulfonium salts might be effective inhibitors of glycosidases. This indeed proved to be true with the pyrrolizidine analog **3**. The recent discovery of two natural polyhydroxy sufonium salts **4** and **5**,[110] and the demonstration that they too are potent inhibitors of glycosidases,[108,109] has firmly established our original conviction. In addition, a number of synthetic polyhydroxylated sulfonium salt analogs have been reported since our original discovery, several of which have also been shown to be weak inhibitors of their target glycosidases (e.g. **6**). [110,111]

This thesis describes the synthesis of the (1*R*,6*R*,7*R*,8*S*)-7,8-dihydroxy-5-thia-1-thioniabicyclo [4.3.0] nonane chloride **7** which can be considered as an analog of the natural compound **1**. Only two steps were required to prepare the bicyclic salt. Crude D-erythrose - obtained by controlled oxidative degradation [Pb(OAc)4-AcOH] of D-glucose - on reaction with 1,3-propanethiol and concentrated HCl gave, after flash chromatography on silica gel, the cyclic thioacetal **8** as a solid. Precursor **8** cyclized smoothly to the target salt **7** upon treatment with *p*-toluenesulphonyl chloride in pyridine (-10°C, 48h).[112] The intermediate γ-tosylate **9** was not isolated and examination of the crude reaction mixture by ^{1}H- and ^{13}C-NMR spectroscopy revealed it to comprise salt **7** as the only new sugar-derived product together with starting material. The product **7** was isolated as its chloride salt in ~40% yield by flash chromatography on silica gel.



Scheme 2.1 (a) 1,3 propanethiol, HCl ; (b) TsCl, pyridine, -10^{o}C

The 1*R*,6*R*,7*R*, 8*S* *cis*-fused structure ($^{4}C_{1}$ conformation, **7D**) of salt **7** has been elucidated by NMR spectroscopic techniques. The observed formation of only one sulfonium salt in the cyclization reaction is remarkable as four diastereomers are *a priori* possible since either sulfur atom can participate in tosylate displacement.[113] The proton spectrum of salt **7** shows a large three-bond scalar coupling of 10.6 Hz between H-6 (δ 4.736) and H-7 (δ 4.606), which indicates an almost anti-periplanar relationship,

consistent with the structure **7C**. The orientation of H-7 was confirmed by strong NOE's from H-7 to H-2_{axial} or H-4_{axial}. (These would not be present in structure **7D**). The equatorial orientation of H-6 in structure **7C** is consistent with the lack of strong NOE's from H-2_{axial} and H-4_{axial}, as would be expected, for example, in **7A**. This was confirmed by large three-bond proton-carbon coupling constants between H-6 and H-2 and H-6 and H-4, which, due to their trans-relationship, manifest sizable crosspeaks in the HMBC spectrum.[113] Contrary to the widely held notion that hydroxylated sulfonium salts are susceptible to facile decomposition[114] the bicyclic sulfonium salt **3** and recently described sulfonium salts [107-111] were seen to be relatively robust. We find this also to be true of sulfonium salt **7**. In fact, the latter compound shows no sign of decomposition (by ^{1}H-NMR spectroscopy), even after months at ambient temperature in methanol or aqueous solution.

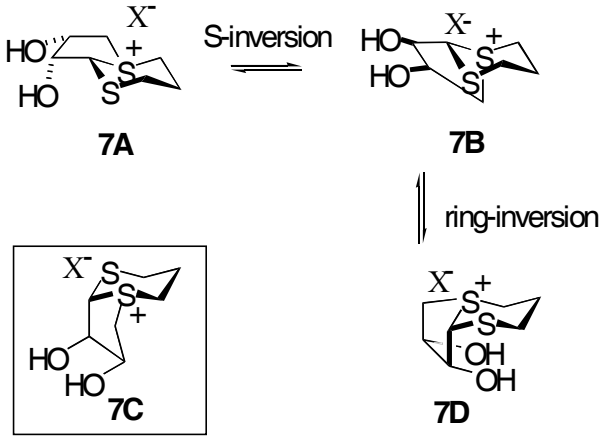


Figure 2.2

A preliminary study of the inhibition by salt **7** of a panel of twelve crude human liver glycosidases by salt **7** has been undertaken. Under standard assay conditions[102] (1mM

concentration) the analog **7** potently inhibits crude lysosomal-, Golgi- and cytososilic- α-D-mannosidase II activities in human liver (97%, pH 4; 100%, pH 6.5) but fails to inhibit the remaining crude liver enzymes tested (all of these were inhibited below 26%, at pH's 4 and 6.5). It is therefore seen to be markedly more selective than azasugar **1** in that, in addition to inhibiting human liver α–D-mannosidase activity (100%, pH 4; 93%, pH 6.5), it also inhibits α-D-glucosidase, β-D-galactosidase and α-D-arabinosidase activities to above 80% (1mM inhibitor concentration, pH 4).

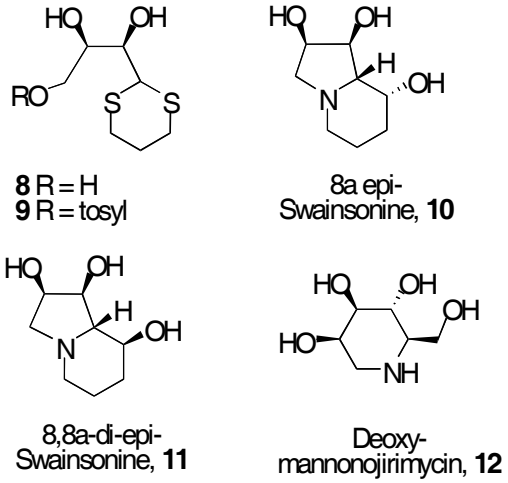


Figure 2.3

In order to more precisely define the remarkable selectivity of inhibitor **7,** more precisely, it was tested against both recombinant human lysosomal α-mannosidase (HLM)[115] and recombinant human GMII[115] using 4-methylumbelliferyl α-D-mannopyranoside as a fluorogenic substrate. K_i's were calculated from Dixon plots, exhibiting varying inhibitor concentrations from 160μM to 3mM. Inhibition of the

recombinant human GMII by compound **7** was found to be good (K_i=82.7μM), but the salt potently inhibited the recombinant HLM (K_i=6.1μM), and partially purified lysosomal enzyme from human liver (K_i=20μM).[102] Inhibition of the latter enzyme is competitive and increases with pH, reaching a maximum at pH 5.0-5.5 where upon it declines (data not shown). This is consistent with the positively charged inhibitor interacting with a group in the active site ionizing over the pH-range 4.0-5.5 (e.g. an aspartic or glutamic acid side chain).

The remarkable potency and selectivity of salt **7** is difficult to rationalize without evidence as to how the sulfonium salt might bind to its target proteins. It is nevertheless informative to compare the K_i values of salt **7** for inhibition of the human liver lysosomal α-D-mannosidase with those of 8a-*epi*-swainsonine **10** and 8,8a-di-*epi*-swainsonine **11** (respectively, 75μM and 2μM at pH 4),[102] as they share identical ring-junction and hydroxyl group stereochemistry. The salt **7** is seen to be 3 to 4 times more potent than 8a-*epi*-swainsonine **10** even though it bears one hydroxyl group fewer. However, is 10 times less active than the di-*epi* analog **11** which possesses a hydroxyl group at C-8 but has "incorrect" stereochemistry. The order of magnitude of these K_i values follow the trend[99] that lysosomal α-D-mannosidases are more susceptible to inhibition by mannofuranose- than mannopyranose analogs (e.g. deoxymannonojirimycin **12**, exhibits I_{50}=~1 mM at pH 4; K_i=75mM at pH 5.5).[102] The selectivity shown by salt **7** towards the panel of glycosidases might have been anticipated *a priori* to be less marked than that of the natural product **1** which itself possesses an "additional" hydroxyl group C-8 and the "correct" stereochemistry at H-8a (corresponding to centers S-5 and C-6 of salt **7**). In

addition, that the sulfonium salt **7** proves to be far more selective than **1** for the lysosomal mannosidase suggests that these latter features might also be detrimental to the activity of azasugar **1** towards that enzyme, and that characteristics particular to salt **7** (including for example, the replacement of a hydroxylmethyl moeity for a sulfur group and the ensuing changes expected in bond lengths and angles) may even accentuate this selectivity. A loss of 2-3 orders of magnitude in the inhibition of the HLM by salt **7** (K_i=6.1μM) relative to azasugar **1** (~K_i=70nM)[102] is the price of its improved selectivity.

General: Chemicals for synthesis were purchased from Aldrich, Acros, Sigma and Fluka and used without further purification. Molecular sieves were activated at 350°C in vacuo for 3 h. All solvents were distilled from the appropriate drying agents immediately before use. All reactions were performed under anhydrous conditions unless otherwise indicated. Reactions were monitored by thin-layer chromatography (Tlc) on Kieselgel 60 F_{524} (aluminium support, 0.2mm thickness) plates (Merck). Detection was by exmination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Flash chromatography[§] was done using silica gel (Merck, 70-230 mesh). Melting points (mp) were determined using a Reichert Thermovar apparatus and are uncorrected. NMR spectra were recorded on Varian Inova-300, -500, or -600 spectrometers equipped with Sun workstations. Spectra were recorded using deuterated solvents and chemical shift data (obtained using standard software provided by the manufacturer) is reported in parts per million (δ) where s, d, dd, t, q and m designate singlet, doublet, doublet of doublets, triplet, quartet and multiplet, respectively. Assignments were made, where required, with the aid of standard gCOSY, gHSQC, TOCSY, gHMBC and NOE experiments. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR Paragron 500. High resolution mass

spectra (HR MS) were obtained using an ESIQ-TOF High Resolution Mass Spectrometer [Medicinal Chemistry Department, University of Mississippi]. Optical rotation data was obtained at 23°C using a Perkin Elmer 241 Polarimeter.

(1', 3'-dithian-2'-yl)-D(-)-erythrose (8). - D-glucose (1.6g, 8.88mmol) was dissolved in water (2mL) and glacial acetic acid (95mL) and the solution cooled to ~18°C. Freshly crystallised lead tetraacetate (7.4g, 17.2mmol) was added in small portions for 10 min to a vigorously stirred mixture which was allowed to reach 23°C over 15 min. The reaction mixture was then treated with anhydrous oxalic acid (1.4g in 15mL acetic acid) and filtered through a short pad of celite. The filtrate was concentrated *in vacuo* and the residual syrup triturated with ethyl acetate (50mL). The organic phase was washed with ice water (2 x 3mL), dried (Na_2S0_4) and evaporated to give a colorless syrup (mainly di-*O*-formyl-D-erythrose)[§§] which was treated without further purification at 0°C with propananedithiol (3mL,) and conc. HCl (1mL). The reaction was allowed to reach 23°C and stirred vigorously for 1.5h after which it was cooled (ice-bath) and conc. NH_3 (aq) was added dropwise, until the mixture was slightly alkaline (litmus paper). Evaporation of this mixture *in vacuo* gave a solid which was extracted several times with hot THF. The THF extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to a pale yellow syrup. Flash chromatography[§] (silica gel; ethyl acetate :heptane, 5:1) gave the title compound (**8** as a colorless syrup (~600mg, 32% from D-glucose): $[\alpha]_D^{20}$ +7.6° (*c* =1.03, water); IR (neat): 3600, 1638, 1619, 1420, 1380, 1278, 1204, 1114, 1089, 1069, 1033, 892 cm^{-1}; ^1H NMR (MeOH-d_4): δ 1.86 and 2.09 (m, 2H, SCH_2CH_2), 2.89 (m, 4H, SCH_2 x 2), 3.59 (dd, *J* = 5.2 Hz, 1H, H-4), 3.75 (m, 3H, H-2, H-3, H-4'), 4.45 (d, *J* = 2.25

Hz, 1H, H-1); ^{13}C NMR (MeOH-d_4): δ 26.25 (SCH_2CH_2), 29.03 and 29.7 (SCH_2 x 2), 50.65 (C-1), 63.29 (C-4), 71.30 and 75.69 (C-2, C-3); Calcd for $C_7H_{14}O_3S_2$: 39.98; H, 6.71. Found: C, 40.43; H, 6.82.

(1*R*,6*R*,7*R*,8*S*)-7,8-Dihydroxy-5-Thia-1-Thioniabicyclo-[4.3.0]-Nonane Chloride (7). - Thioacetal **8**(100 mg, 0.5 mmol) was dissolved in dry pyridine (3 mL) and treated at -10°C with freshly recrystallised toluenesulfonyl chloride (117mg, 0.6mmol), in an argon atmosphere. The reaction mixture was left for 48 h at -10°C and then evaporated *in vacuo*. ^{13}C NMR and Tlc (CH_3CN:H_2O:AcOH = 20:4:1) showed the residual syrup (crude) to comprise starting material and the desired product (in the ratio ~3:2) as the only sugar-derived products. Flash chromatography (silica gel, elution with CH_2Cl_2:MeOH = 10:4 then 1:1 and finally MeOH alone) gave a solid on evaporation of the appropriate column fractions. This was reapplied to a short column of silica and eluted with CH_2Cl_2:MeOH = 10:1 to give the title compound **7** as a white solid (42mg, 38%): $[\alpha]_D^{20}$ -215.9° (*c* =1.10, H_2O); IR (KBr): 3600, 1636, 1624, 1417, 1405, 1385, 1334, 1307, 1294, 1244, 1181, 1171, 1157, 1120, 1098, 1042 cm^{-1}. See Table 2 for NMR data. HRMS (FAB): calc for $[C_7H_{13}O_2S_2]^+[X]^-$: *m/z* 193.03579. Found: 193.03559.

§ Still, W. C.; Khan, M.; Mitra, A. J. Org. Chem. **1978**, *43*, 2923

§§ For preparation of D-erythrose see: Perlin A. S. in *Methods in Carbohydr. Chem.* Whistler, R. L.; Wolfrom, M. L. *Eds*. Academic Press

Supplementary Material: NMR Instrumentation, Materials and Methods

Data for compound **7** : The sample was dissolved in DMSO and data were collected on a Varian Inova500 spectrometer at 35 degrees C. Proton and carbon assignments were obtained from one-dimensional proton and two-dimensional COSY, HSQC and HMBC spectra using standard Varian pulse sequences. Proton and carbon chemical shifts are reported in Table 2. One-dimensional NOE experiments were performed with the GOESY sequence (K. Stott, J. Stonehous, J. Keeler, T. -L. Hwang, and A. J.Shaka, *J. Am. Chem. Soc.,* **1995**, *117,* 199-4200). A mixing time of 200 msec was used.

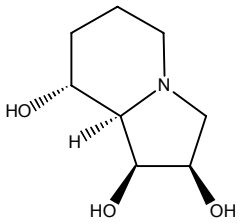
Table 2. Chemical shifts (600MHz) of compound **7** in DMSO at 35 degrees C.

	H4	H3	H2	H9	H8	H7	H6
^{1}H (ppm)	3.609(eq) 3.515(ax)	2.362(eq) 1.972(ax)	2.634(eq) 2.916(ax)	4.736	4.600	4.413	3.726 3.493
^{13}C (ppm)	48.1	21.6	20.3	50.6	72.8	69.5	36.8

CHAPTER THREE

SELECTIVE INHIBITION OF HUMAN GOLGI-α-MANNOSIDASE II BY A DIHYDROXYPERHYDRO[1,3]THIAZOLO[3,2-A]PYRIDINE: A NOVEL SWAINSONINE ANALOG.

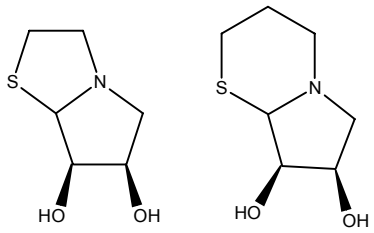
Inhibitors of glycosidases have long been identified as modulators of the biosynthesis of oligosaccharides.[1] Many of these oligosaccharides have been demonstrated to play key roles in a host of normal physiological[2a] processes and diseased states.[2b] Thus, it is not surprising that glycosidase inhibitors have also been targeted as agents for the treatment of conditions including diabetes,[3] cancer,[3b] and AIDS[3c] as well as for studying the details of these diseases at the molecular level.[4] The promise that potent and specific inhibition of 'trimming' enzymes might prove a viable strategy by which to counter viral infection and tumor progression has possibly generated the greatest interest in this family of compounds.[5] However, although a number of natural glycosidase inhibitors have been shown to exhibit potent anti-tumor and anti-metastatic activities, they also lead to serious complications due to unwanted side-effects. In fact, a major shortcoming of inhibitors of processing α-mannosidases such as swainsonine **1**[6] is that they display poor selectivity for their target, human Golgi α-mannosidase II (HGMII). One of the most serious negative effects of this lack of selectivity is that the concurrent inhibition by azasugar **1** (and it numerous synthetic analogs) of human lysosomal α-mannosidase (HLM) leads to the phencocopy of the inherited disease mannosidosis, a rare autosomal recessive condition.[7]



1.

Figure 3.1. Chemical Structure of Swainsonine

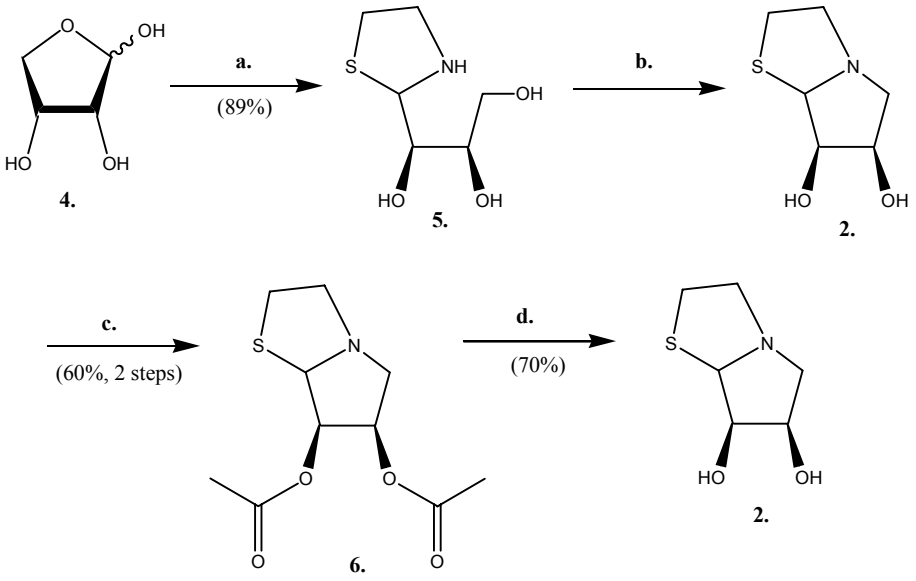
Modification of natural or synthetic leads in attempts to remedy this lack of selectivity has been relatively unsuccessful, especially considering the extensive research already invested in this area.[8] For example, although many hundreds of analogs of **1** have been successfully synthesized, most have been reported to be significantly worse than the parent compound itself, either in terms of *potency* or *selectivity* towards the target enzyme. Amongst the obstacles hampering efforts to improve glycosidase inhibitors is the fact that structure-activity relationships do not always prove straightforward. In addition, the modes-of-action of glycosidases themselves are not understood in sufficient detail as to be able to undertake rational inhibitor design with confidence.[9] The difficulties encountered thus far in attempts to accentuate the selectivity of **1** towards the inhibition of HGMII over HLM through chemical modification are a testament to these problems. We describe here the synthesis of two new heterocycles **2** and **3**, analogs of natural compound **1**, and show that analog **3**, in contrast to **1**, has a very accentuated selectivity as an inhibitor of HGMII over HLM, thus providing a rare opportunity to develop an α-mannosidase inhibitor as a cancer therapeutic.



2. **3.**

Figure 3.2. Chemical structures of proposed azasugar targets

The synthesis of the target molecules was achieved using an identical route.[10] In the particular case of the ring-contracted swainsonine analog **2**, reaction of D-erythrose (obtained by lead tetra-acetate oxidation of D-glucose) with 2-mercaptoethylamine hydrochloride in dry pyridine gave the desired cyclization precursor **4** quantitatively after ~24 hours at ambient temperature. The thiazolidine **5** was obtained as a yellow oil upon concentration of the reaction mixture, and the product precipitated out of ethanol solution as a white solid (a mixture of R/S epimers). This mixture (1:1 by ^{13}C-NMR) proved unstable to attempted silica gel purification and could not be separated, but its ^{13}C-NMR spectroscopic data was consistent with the proposed structure. The epimers, when subjected to our original cyclization conditions[10] – which serve to selectively activate the primary hydroxyl of precursor **4** - underwent an intramolecular nucleophilic displacement reaction. Thus, reaction of thiazolidine **4** with Ph_3P, CCl_4 and freshly distilled triethylamine in dry DMF gave, after silica gel purification, the target ring-contracted swainsonine analog **2** as a single compound.



Scheme 3.1. (a) $NH_2(CH_2)_2SH$, pyridine; (b) PPh_3, CCl_4, Et_3N / DMF (5 days); (c) acetic anhydride / pyridine; (d) NaOMe/MeOH

The diacetate derivative of **2** could be obtained under classical conditions (acetic anhydride/pyridine). The purified diacetate was deacetylated using sodium methoxide in methanol to give the corresponding deprotected analog that presented NMR spectroscopic data consistent with its expected structure. Of particular significance is the ~20ppm upfield displacement of the resonance assigned to C-4 in the starting material (δ=64.2 and 63.9ppm) upon cyclization to the target bicyclic system (δ=~58.8ppm). The ^{13}C- and ^{1}H-NMR spectra of **2** are well-resolved, and the peaks assigned using 2D COSY, HSQC and NOESY NMR experiments. Only two of the cyclization products, which are in theory are possible, can be reconciled with the small coupling observed for the downfield resonance corresponding to H-7a (δ=4.6ppm; $J_{7a,1}$=1Hz). Steric arguments are also seen to favor conformer **2a** over **2b**.

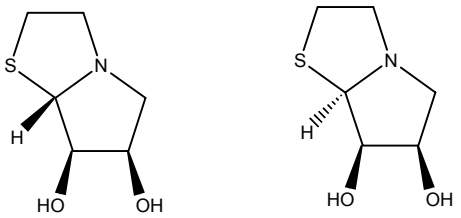
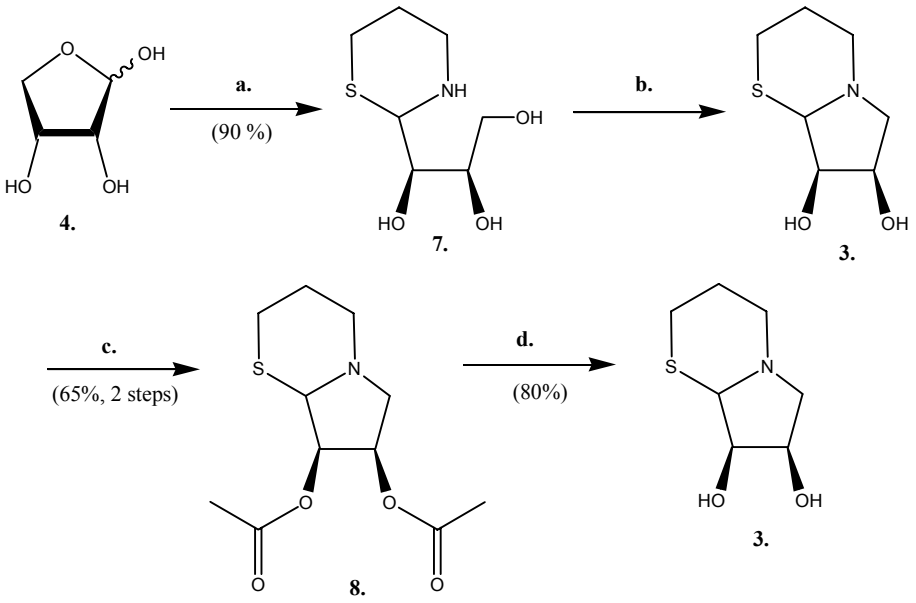


Figure 3.3.

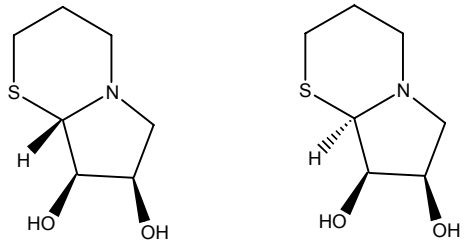
The swainsonine analog **3** was obtained by the reaction of D-erythrose with 3-amino-propanethiol hydrochloride in pyridine, and gave the cyclization precursor **7** as an R/S mixture (1:1 by ^{13}C-NMR). Although unstable to silica gel chromatography, the compounds crystallized from ethanol as a pure mixture. The ^{1}H- and ^{13}C-NMR spectroscopic data is consistent with the proposed structure.



Scheme 3.2. (a) $NH_2(CH_2)_2SH$, pyridine; (b) PPh_3, CCl_4, Et_3N / DMF (5 days); (c) acetic anhydride / pyridine; (d) NaOMe/MeOH

Cyclization under our reported conditions[10] again proceeded smoothly to give products **3** (3:2 mixture by ^{13}C-NMR). These could not be separated from one another or obtained free of contaminants such as DMF and triethylamine hydrochloride and PPh_3-

derived by-products, despite repeated silica gel chromatography. The product however, after acetylation under classical conditions, could be obtained pure by silica gel chromatography (3:1 by ^{13}C-NMR). The well-resolved ^{1}H-NMR spectrum confirmed the formation of just two cyclized components that, upon deacetylation, gave the target **3** as a *single* compound. The structure of the novel analog **3** was established by analysis of its well-resolved ^{1}H-NMR and ^{13}C-NMR spectra that were assigned with the aid of appropriate 2D-COSY and HSQC/HMBC experiments. Especially revealing are the characteristics of the resonance assigned to the bridgehead hydrogen H-8a (δ=2.8ppm; $J_{8a,1}$=6.8Hz). The large coupling constant observed is indicative of a trans relationship between protons H-1 and H-8a. In addition, the downfield chemical shift of this latter resonance indicates that it is antiperiplanar to the nitrogen lone pair. Furthermore, the lack of NOE's between H-2 and H-5_{ax} or H-2 and H-8a in the NOESY spectrum of **3** is consistent with it existing predominantly as conformer **3a** as opposed to **3b**. These could in theory interconvert by a simple ring-flip at room temperature, but the presence of a single set of resonances demonstrates that analog **3a** is the thermodynamically stable conformer and identical to that adopted by the natural compound **1** itself.[11]



3a. **3b.**

Figure 3.4. Chemicals structures of possible azasugar conformers

The analogs **2** and **3** have been assayed against both recombinant HLM[12] and recombinant HGMII,[13] using 4-methylumbelliferyl α-D-mannopyranoside as a fluorogenic substrate. IC_{50}'s were obtained by comparing measured fluorescence values relative to a those of a standard curve generated using 4-methylumbelliferone alone, and the data plotted as percentage inhibition versus inhibitor concentration (varied between 10nM and 1mM).[14] K_i determinations were calculated from Dixon plots and the data obtained under identical assay conditions except that inhibitor concentrations were varied from 160μM to 3mM. The ring-contracted analog **2** displays poor inhibition of the HGMII with K_i=278μM (IC_{50}=440μM) and moderate inhibition of the lysosomal enzyme with K_i=72μM (IC_{50}=170μM). Thus, analog **2** is seen to be just a four fold better inhibitor of the HLM compared with the HGMII. In striking contrast, the swainsonine mimic **3** displays good inhibition of the target enzyme HGMII with a K_i=17μM (IC_{50}=35μM) and, gratifyingly, only very poor inhibition of the lysosomal enzyme HLM with a K_i=>1mM (IC_{50}=>1mM). An analysis by ^{1}H-NMR spectroscopy of a solution of analog **3**, which had been subjected to the assay buffer for the duration of the assay, confirmed it to be stable in those conditions.

The origin of the selectivity observed for azasugar **3** is not clear to us at present. This bias for HGMII is all the more remarkable when one considers that the only difference between the natural compound **1** and analog **3** is that the equivalent of the hydroxymethyl group in swainsonine (at C-8 in **1)** is replaced by a sulfur atom in **3**. Analog **3** is seen to have one fewer hydroxyl function than azasugar **1** and, additionally, will present the increases in bond angles and lengths expected on replacing a ring

methylene group by a sulfur atom. The balance of features needed to ensure selectivity for HGMII is thus seen to be rather delicate: simply "contracting" the sulfur-containing ring by a carbon atom, as in analog **2**, also abolishes any preference completely. The possible role(s) in the mode of mannosidase inhibition of the hydroxylmethyl group (or its equivalent) in inhibitor-binding and transition-state stabilization has generated considerable comment but remains a matter of some conjecture.[15]

The discovery of analog **3** provides a very rare opportunity to develop an α-mannosidase inhibitor as a cancer therapeutic *without* the severe complications due to mannosidosis-like symptoms which invariably accompanies the use swainsonine and its analogs (which usually also act on HLM). The potential of analog **3** is further compounded when one considers that it has been obtained in two chemical steps from a commercially available sugar. We are currently exploring various modifications of the lead compound **3**, with a view to accentuate further its inherent selectivity and potency towards the HGMII.

General: Chemicals for synthesis were purchased from Aldrich, Acros, Sigma and Fluka and used without further purification. Molecular sieves were activated at 350°C in vacuo for 3 h. All solvents were distilled from the appropriate drying agents immediately before use. All reactions were performr under anhydrous conditions unless otherwise indicated. Reactions were monitored by thin-layer chromatography (Tlc) on Kieselgel 60 F_{524} (aluminium support, 0.2mm thickness) plates (Merck). Detection was by exmination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Flash chromatography[§] was done using silica gel (Merck, 70-230 mesh). Melting points (mp)

were determined using a Reichert Thermovar apparatus and are uncorrected. NMR spectra were recorded on Varian Inova-300, -500, or -600 spectrometers equipped with Sun workstations. Spectra were recorded using deuterated solvents and chemical shift data (obtained using standard software provided by the manufacturer) is reported in parts per million (δ) where s, d, dd, t, q and m designate singlet, doublet, doublet of doublets, triplet, quartet and multiplet, respectively. Assignments were made, where required, with the aid of standard gCOSY, gHSQC, TOCSY, gHMBC and NOE experiments. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR Paragron 500. High resolution mass spectra (HR MS) were obtained using an ESIQ-TOF High Resolution Mass Spectrometer [Medicinal Chemistry Department, University of Mississippi]. Optical rotation data was obtained at 23°C using a Perkin Elmer 241 Polarimeter.

Thiazolidine (4):

D-(-)-erythrose (60%, 1.0g, 5.0 mmol) was dissolved in pyridine (dry, 12 mL) under an inert atmosphere of Argon. Once dissolved, 2-mercaptoethylamine·HCl (0.95g, 8.36 mmol) was then added, and this solution was allowed to stir overnight (22h). This mixture was then dissolved in methanol (5.00 ml), and co-evaporated with toluene (4 x 20 mL), until the pyridine no longer persisted. The resulting yellow oil was then dissolved in MeOH, treated with activated Carbon, and concentrated under reduced pressure to afford a near colorless oil. This oil was then dissolved in a minimum amount of EtOH, and placed in the freezer to induce crystallization. The resulting off-white solid **4** was found to be a mixture of R/S epimers, which was used directly in the next step. (0.80g, 89%); ^{1}H NMR (300 MHz, CD_3OD) δ: 3.08-3.24 (m, 4H), 3.37-3.86 (m, 4H),

4.94-4.98 (m, 1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 30.04, 30.57, 50.54, 50.77, 63.96, 64.24, 66.87, 69.25, 71.21, 72.30, 74.47, 75.40.

(6R,7S,7aS)-6,7-diacetateperhydro[1,3]thiazolo[3,2-a]pyridine (5):

Thiazolidine **4** (0.80 g, 4.44mmol), triphenylphosphine (2.38 g, 9.07mmol), carbon tetrachloride (dry, 0.96 mL, 9.95mmol) and freshly distilled triethylamine (1.22 mL, 9.98mmol) were stirred together in DMF (dry, 8 mL) at 22°C for 5d under an inert Argon atmosphere, with exclusion of light. Methanol (20.00 mL) was then added and the mixture stirred for a further 30 min, and then concentrated *in vacuo* and partitioned between CH_2Cl_2 and H_2O. The aqueous layer was concentrated and the residue applied to a flash column (iatrobeads; CH_2Cl_2:MeOH; 5:2). The partially purified cyclized compound (0.20g, 0.815 mmol) was dissolved in pyridine (dry, 2 mL) under an inert atmosphere of Argon. Acetic anhydride (2 mL) was then added to this solution, and continued to stir overnight (22h). According to TLC (silica gel; EtOAc:Hexanes; 3:1), the reaction was complete. The solution was cooled to 0^{o}C, and treated with MeOH (5.00mL), and allowed to stir for an additional 30 min. This solution was then treated with H_2O, and extracted with CH_2Cl_2 (3x20mL). The organic layers were then combined, and co-evaporated with toluene, to produce a brown solid. This solid was applied to a silica gel column (flash; EtOAc:Hexanes; 3:1), to afford **5** as a light yellow oil. (0.120g, 59.80%) : $[\alpha]^{22}_{D}$ -235.3 (*c*1.0, $CHCl_3$) ^{1}H NMR (300 MHz, C_6D_6) δ:1.75 (d, 6H), 2.38-2.58 (m, 4H), 2.85 (dd, *J* = 9.0 Hz, *J* = 3.3 Hz, 1H), 2.99 (dd, *J* = 10.8 Hz, *J* = 3.9 Hz, 1H), 4.82 (s, 1H), 5.57 (q, 1H), 5.68 (d, *J*=5.1 Hz,1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 20.15, 20.20, 30.83, 54.76, 57.59, 71.65, 77.59, 78.12

(6R,7S,7aS)-6,7-dihydroxyperhydro[1,3]thiazolo[3,2-a]pyridine (2):

The acetate (0.059g, 0.241mmol) was dissolved in MeOH (dry, 2 mL), and once dissolved, was mixed with sodium methoxide (.20 mL), freshly prepared from and methanol and sodium, and stirred for 1 h under an inert Argon atmosphere. When the reaction was complete, the solution was treated with Dowex until neutral, after which the Dowex was filtered off, and the solution was the concentrated under reduced pressure yielding an orange-brown oil (0.027g, 70%), which proved to be the desired diol, **2**. ^{1}H NMR (300 MHz, CD_3OD) δ: 2.69-2.94 (m, 4H), 3.24 (s, 1H), 3.41 (d, J = 10.8 Hz, 1H), 4.15 (d, J = 4.8 Hz, 1H), 4.31 (q, 1H), 4.53 (s, 1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 31.23, 58.11, 58.80, 71.74, 79.31, 80.35

Thiazolidine (7):

D-(-)-erythrose (60%, 0.70 g, 3.5 mmol) was dissolved in pyridine (dry, 7.00mL) under an inert atmosphere of Argon. Once dissolved, 3-mercaptoethylamine (0.35 g, 3.9 mmol) was then added, and this solution was allowed to stir overnight (22h). This mixture was then dissolved in methanol (5 ml), and co-evaporated with toluene (4 x 20 mL), until the pyridine no longer persisted. The resulting yellow oil was then dissolved in MeOH, treated with activated Carbon, and concentrated under reduced pressure to afford a near colorless oil. This oil was then dissolved in a minimum amount of EtOH, and placed in the freezer to induce crystallization. The resulting off-white solid **7** was found to be a mixture of R/S epimers, which was used directly in the next step. (0.61 g, 90%); ^{1}H NMR (300 MHz, CD_3OD) δ: 1.52-1.72 (m, 2H), 2.68-2.82 (m, 2H), 2.93-3.12 (m, 1H). 3.46-3.61 (m, 4H), 3.66-3.75 (m, 1H), 4.38 (s, 1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 29.60, 29.75, 31.90, 32.30, 50.05, 50.19, 66.30, 66.70, 67.90, 69.50, 76.10, 76.35, 79.85, 79.82.

(7R,8S,8aS)-7,8-diacetateperhydro[1,4]thiazolo[4,2-a]pyridine (8):

Thiazolidine **7** (0.100g, 0.52mmol), triphenylphosphine (0.276g, 1.05mmol), carbon tetrachloride (dry, 0.103mL, 1.06mmol) and freshly distilled triethylamine (0.142mL, 1.02mmol) were stirred together in DMF (dry, 1.00mL) at 22°C for 5d under an inert Argon atmosphere, with exclusion of light. Methanol (5.00 mL) was then added and the mixture stirred for a further 30 min, and then concentrated *in vacuo* and partitioned between CH_2Cl_2 and H_2O. The aqueous layer was concentrated and the residue applied to a flash column (iatrobeads; CH_2Cl_2:MeOH; 5:2). The partially purified cyclized compound **3** (0.100g, 0.57 mmol) was dissolved in pyridine (dry, 1.25 mL) under an inert atmosphere of Argon. Acetic anhydride (1.25 mL) was then added to this solution, and continued to stir overnight (22h). According to TLC (silica gel; EtOAc:Hexanes; 3:1), the reaction was complete, so the solution was cooled to 0^oC, and treated with MeOH (5.00mL), and allowed to stir for an additional 30 min. This solution was then treated with H_2O, and extracted with CH_2Cl_2 (3x10mL). The organic layers were then combined, and co-evaporated with toluene, to produce a brown solid. This solid was applied to a silica gel column (flash; EtOAc:Hexanes; 3:1), to afford **8** as a light yellow oil (0.096g, 65%). ^1H NMR (300 MHz, C_6D_6) δ: 1.77-1.95 (m, 2H), 2.05 (d, J = 6.6 Hz, 6H), 2.59-2.86 (m, 4H), 3.11 (d, J = 13.2 Hz, 1H), 3.69 (dd, J = 10.2, 7.2 Hz, 1H), 4.34 (d, J = 4.5 Hz, 1H), 4.94 (dd, J = 6.6, 4.2 Hz, 1H), 5.35 (m, 1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 20.05, 20.15, 21.38, 28.25, 49.80, 55.57, 68.19, 69.79, 76.12.

(7R,8S,8aS)-7,8-dihydroxyperhydro[1,4]thiazolo[4,2-a]pyridine (3)

The acetate (0.06g, 0.23mmol) was dissolved in MeOH (dry, 2 mL), and once dissolved, was mixed with sodium methoxide (.20 mL), freshly prepared from and methanol and

sodium, and stirred for 1 h under an inert Argon atmosphere. When the reaction was complete, the solution was treated with Dowex until neutral, after which the Dowex was filtered off, and the solution was the concentrated under reduced pressure yielding an orange-brown oil (0.032g, 80%), which proved to be the desired diol, **2**. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.70-1.85 (m, 2H), 2.25 (dd, *J* = 9.0, 4.8 Hz, 1H), 2.35 (t, 1H), 2.71 (m, 2H), 3.02 (d, *J* = 12.6 Hz, 1H), 3.39 (dd, *J* = 9.0, 6.9 Hz, 1H), 3.55 (d, *J* = 6.6 Hz, 1H), 3.67 (t, 1H), 4.10 (m, 1H). ^{13}C NMR (300 MHz, CD_3OD) δ: 25.78, 28.90, 53.32, 61.04, 68.12, 71.61, 76.46

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