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STEREOSELECTIVE OXYGENATION OF A DOUBLE BOND: DESIGN AND SYNTHESIS OF AZASUGAR INHIBITORS OF GLYCOCONJUGATE PROCESSING ENZYMES

by

Dalibor Sames

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF CHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1996
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Dalibor Sames entitled "Stereoselective Oxygenation of a Double Bond: Design and Synthesis of Azasugar Inhibitors of Glycoconjugate Processing Enzymes" and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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I also owe my gratitude to my co-workers Dr. Lajos Szabo, Scott Mitchell, Caroline Kriss, Jason Chruma and my friends Lawrence Williams, Jimbo Johnson, Christopher Johnson and Sidney Edsall.
DEDICATION

This dissertation is dedicated to my dearest mother Eva, father Arnost,
brothers Martin and Arnost and girlfriend Sidney.
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ABSTRACT

A novel methodology for the synthesis of amino sugars and aza-sugars, important inhibitors of glycosidases, is described. This is accomplished via the intermediacy of N-diphenylmethylene-protected \( \alpha \)-amino esters. This methodology can be divided into three major phases. First, the reduction-alkenylation reaction with DIBAL-TRIBAL and oxygenated alkenyllithium nucleophiles provides unsaturated 1,2-amino alcohols with excellent threo-selectivity (>20:1). Second, optimization of catalytic osmylation conditions with regards to chemical yield, stereoselectivity, and osmium content is described. A mixture of 1.2 mol% of osmium tetroxide in the presence of 3 eq. of potassium ferricyanide \((K_3Fe(CN)_6)\) as an oxidant, 3 eq. of \( K_2CO_3 \) and 3 mol% of \((DHQ)_2PHAL \) chiral ligand represents optimized dihydroxylation conditions. Depending upon the substrate, good to excellent anti-selectivities are observed. The separation of diastereomeric mixtures of protected amino tetrols is accomplished by flash chromatography on silica gel. Thus, four contiguous chiral centers are established efficiently in two steps. The third stage involves manipulation of the protecting groups and subsequent cyclization. Deprotection of the silyl protecting group in the presence of acyl groups is complicated by migration. The cyclization is accomplished via the reductive amination of acyclic amino aldehydes with NaBH\(_3\)CN.

D-N-methyl-fucosamine is prepared from protected L-serine. Depending on the order of osmylation and cyclization steps, this methodology provides either L-azaafucose or L-azagulose from protected L-alanine in 8 steps. Furthermore, 1-cyano-L-azaafucose is prepared via treatment of acyclic amino aldehydes with HCN (Strecker reaction).
CHAPTER ONE

INTRODUCTION TO GLYCOCONJUGATES
1.1 Biooligomers as the Carriers of Biological Information.

The integrity of living cells hinges on the storage, maintenance, translation and transfer of biological information with high fidelity. It may be stated with a high dose of confidence that all biological information is stored in the form of chemical messages ("static form"), or in the form of chemical interactions and transformations ("dynamic form"). Obvious candidates for the storage of information are biopolymers, where particular sequences of the building units represent corresponding codes. Discovery of deoxyribonucleic acid structure provided a crucial entry into understanding the molecular details of a cellular alphabet. The process of transcription and translation provided a link between the "static" and the "dynamic form" of information. Nucleic acids and proteins are biopolymers that are synthesized via a template mechanism. The formation of crucial linkages does not involve the generation of stereocenters. The third class of biopolymers, namely oligosaccharides, differ from the two aforementioned classes in two ways. First, oligosaccharides occur in branched forms which precludes a template mode of biosynthesis. Second, there are numerous regio- and stereochemical possibilities for linking two monosaccharide units. Such differences obviously preclude the possibility of a simple code for translating genetic information into a new "glycoform" ("Second order translation"). Such sequences of saccharides reflect the whole physiological state of the cell, rather than a particular piece of a message stored as a gene (DNA sequence).

Since glycoconjugates found on the cell surface in the form of glycolipids or glycoproteins play crucial roles in cellular communication processes, a great deal of attention has been devoted to the field which has been named "glycobiology." Regulation of the metabolism of glycoconjugates and their involvement in cell growth regulation remains for the most part a mystery. Furthermore, an extensive crosstalk
between the glycosylation pathways, and the protein and nucleic acid metabolism at different levels of regulation has been uncovered, which opens a whole new perspective on the complexity of the cellular machinery.

As frequently encountered, various natural products have been isolated and found to interfere with glycoconjugate metabolism. Such compounds provide valuable insights into the biochemical details and significance of glycoconjugates, and offer opportunities for the development of novel biochemical tools and therapeutics. Particularly, aza-saccharides and amino carbocycles have proved to be very potent inhibitors of glycosidases, and in some cases glycosyltransferase inhibitors. Metabolism, the biological roles of glycoconjugates, design and chemical synthesis of glycosidase inhibitors will be discussed in following chapters.
1.2 Introduction to Glycoconjugates.

Glycoconjugates represent a class of naturally occurring molecules that consists of the glycan portion (saccharide part) and the protein or lipid part (aglycone). Depending on the character of the linkage or the type of aglycone this class is subdivided to N-linked glycoproteins, O-linked glycoproteins, glycosphingolipids, etc. This chapter will mainly focus on glycosphingolipids (GSL).2

Glycosphingolipids consist of the ceramide backbone and the glycan. Ceramide is composed of D-erythro-sphingosine (2R, 3S) and the fatty acid. Glycosylation of the primary hydroxy group of the ceramide yields glycosphingolipids (Figure 1). Glucose (β-linkage) is the first residue in most cases. In the mammalian brain galactosylceramide (Gal-β-Cer) is found. N-Deacylation provides lyso-GSL which are physiological intermediates.

Based on the saccharide structure, mammalian glycoconjugates can be classified into four major groups.3 All four groups can be derived from the lactosyl ceramide (Scheme 1.1). Gal-β-1,3-GlcNAc-β-1,3-Lactosyl ceramide represents the core structure of the Lacto series (LcCer). The regio-isomeric Neolacto series (nLcCer) possesses the galactose linked via β-1,4-linkage to N-acetylglucosamine (GlcNAc). GalNAc-β-1,4-LacCer is characteristic for the ganglio series (GgCer) and Gal-α-1,4-LacCer constitutes the core of the Globoseries (GbCer). Lower organisms (c.f. marine organisms) express other structural types (e.g. isoglobo, moll) which do not necessarily contain a glucose as the first residue.4 Also, qualitative and quantitative expression of GSL is species specific.

Gangliosides, sialylated GSL with the ganglio core structure form a very important subgroup. Gangliosides play important regulatory roles as will be discussed
in the following section. In Figure 2 is an example of ganglioside GM2. Such designation has been suggested by Svennerholm and is commonly used.

**Figure 1**

Sphingosine (D-erythro-sphingosine, 2S, 3R)

Ceramide (C₁₆ Ceramide)

Glucosylceramide (β-Glucosylceramide, Glc-Cer)

Galactosylceramide (β-Galactosylceramide, Gal-Cer)

Mannosylceramide (β-Mannosylceramide, Man-Cer)

LactosylCeramide (LacCer)
IUPAC-IUB abbreviated name is Il³-NeuNAc-α-Gg3Cer. The Roman numeral Iı with the superscript 3 indicates that the second residue of the gangliotriose (counted from the ceramide) is substituted at position 3 with sialic acid via an α linkage.

Scheme 1.1

Lacto Series

Neolacto Series

Ganglio Series

Globo Series
The discovery of blood group epitopes in 1900 first pointed out carbohydrates as carriers of biological information. At present there are over 200 erythrocyte antigens assigned to 22 blood groups. Blood groups with carbohydrate epitopes have been studied most, namely, ABH, Lewis (Le), ii, P-related, P1, T and Tn. The human blood groups with the peptide epitopes are specific to erythrocytes, whereas the carbohydrate antigens, carried by sphingolipids and/or proteins, are also found in tissues and body fluids. The role of these structures on erythrocytes is not clear at present. LeX, sialyl-LeX and sialyl-Lea have been implicated in leukocyte recruitment to a site of damaged tissue.

The carbohydrate-based blood groups can be divided into two basic groups known as Type I and Type II (Figure 3). Note that the Type I is derived from Lacto series (Lacto I) and Type II from Neolacto (Lacto II) series. Lewis antigens differ by the extent and position of L-fucosylation. Fuc-α-1,2-Gal disaccharide defines the H (0) serological blood group. α-1,3-GalNAcT(N-Acetyl-galactosyl transferase) is expressed in A blood group individuals and α-1,3-GalT in B blood group individuals.
The ABO carbohydrate epitopes are found on various carbohydrate core structures carried by lipids and proteins.\textsuperscript{10}

Figure 3

\begin{center}
\begin{tabular}{c c}
\textbf{TYPE I} & \textbf{TYPE II} \\
\end{tabular}
\end{center}

\begin{center}
\begin{tabular}{c c c c}
Le\textsuperscript{a} & R: H & Le\textsuperscript{x} & R: H \\
Le\textsuperscript{b} & R: & Le\textsuperscript{y} & R: \\

Blood Group Type: & A & B & H \\
R: & & & H (O)
\end{tabular}
\end{center}
1.3 Sphingolipids. Metabolism.

The lipophilic backbone in GSL's consists of sphingosine and fatty acid residue. Both components vary in length and functionality (c.f. degree of unsaturation and oxidation), although sphingosine itself is found most often as C18 in mammals. This variability coupled with the large information capacity of oligosaccharides provide GSL's with an enormous capacity to carry biological information. The ceramide backbone is embedded in the membrane and influences organization of GSL's on the cell surface. Hydroxy substitution on either a sphingosine (c.f. phytosphingosine), or fatty acid significantly enhances the antigenicity of the carbohydrate portion.11 A recent review by Grant discusses the substituent effect and spectroscopic methods (EPR, 2D NMR) employed in studying these phenomena.12 Some evidence (molecular modeling and electron spectroscopy) suggests the perpendicular orientation of the glycosyl moiety and the ceramide. Thus, in contrast to a common belief, one face of the glycoepitope is interacting with the membrane while the opposite side of the carbohydrate is exposed. Also, ceramide and sphingosine have recently emerged as important second messengers in cell signaling.13

Metabolic pathways for biosynthesis and biodegradation are outlined in Schemes 1.2 and 1.3. This subject has been reviewed extensively.14 The first step of ceramide biosynthesis is the nucleophilic addition of L-serine to palmitoyl-SCoA catalyzed by serine palmitoyltransferase. The ketone group of 3-dehydrosphinganine is reduced by 3-dehydrosphinganine reductase to form dihydrosphingosine. Acylation of the amino group and dehydrogenation catalyzed by sphinganine N-acyltransferase and dihydroceramide desaturase provides ceramide.15
Scheme 1.2

\[ \text{L-Serine} + \text{CH}_3(\text{CH}_2)_{14}\text{COSCoA} \]

\[ \text{Palmitoyl-SCoA} \]

\[ \text{Serine Palmitoyltransferase} \]

\[ \text{3-Dehydro sphinganine} \]

\[ \text{3-Dehydro sphinganine Reductase (NADPH)} \]

\[ \text{Sphinganine N-acyltransferase} \]

\[ \text{Dihydroceramide Desaturase} \]
Scheme 1.3

\[
\text{ethanolamine-phosphate} + \text{Palmitaldehyde} \rightarrow \text{Sphingosine-phosphate}
\]

\[\text{Sphingosine - Phosphate Lyase}\]

\[
\text{Sphingosine - Phosphate Phosphatase} \rightarrow \text{Sphingosine Kinase}
\]

\[
\text{Sphingosine} \rightarrow \text{N,N-Dimethylspingosine}
\]

\[
\text{Ceramide}
\]

\[
\text{Sphingosine Kinase}
\]
Metabolism of GSL's and phosphosphingolipids is linked via the sphingomyelin cycle (SM cycle, Scheme 1.4). Ceramide can undergo several metabolic pathways: it can be converted to sphingomyelin by phosphocholine transferase; it can be glycosylated to enter GSL metabolism; or it can be converted to sphingosine. Sphingosine is transformed to sphingosine phosphate with sphingosine kinase. The sphingosine phosphate lyase facilitates the "retroaldol" cleavage to give ethanolamine phosphate and palmitaldehyde as the products of sphingolipid biodegradation. All enzymatic steps involved in biosynthesis of ceramide occur on the cytosolic face of the endoplasmic reticulum. Biochemical assays are now available for all of the biosynthetic enzymes. It should be pointed out that sphingosine is not generated through de novo synthesis, but only via degradation of GSL's and phospholipids. On the other hand, ceramide can be generated via both of these pathways. The regulation of these processes is not understood at present. The subject is now being intensively studied because of the important role of sphingolipids in cell signaling.
1.4 Biological Role of Sphingolipids.

Degradation products of GSL's and sphingomyelin, namely, ceramide, sphingosine, sphingosine phosphate and dimethylsphingosine have been shown to play important roles in the regulation of cellular processes.\textsuperscript{21} GSL and sphingomyelin, which are constituents of membranes, are cleaved by glucosidase and sphingomyelinase to produce ceramide, a secondary messenger in the signal transduction. An analogy can be seen between the sphingomyelin cycle and phosphatidylinositol diphosphate (PIP\textsubscript{2}) metabolism. Phospholipase C (PLC), a cytoplasmic membrane-associated enzyme, hydrolyzes PIP\textsubscript{2} (phosphatidylinositol diphosphate) and produces two signaling molecules IP\textsubscript{3} (inositol triphosphate) and DAG (diacylglycerol). PLC is activated by a ligand-bound receptor.\textsuperscript{22} Sphingomyelinase\textsuperscript{23} resides in the cytoplasmic membrane while glucosidase and ceramidase\textsuperscript{24} have been localized in lysosomes. Biological roles of ceramide, sphingosine and sphingosine phosphate are summarized in Table 1.

Ceramide has been shown to mediate the α-TNF (tumor necrosis factor) induced apoptosis,\textsuperscript{25} and to inhibit leukemia cell growth.\textsuperscript{26} Although the mechanism has not been fully deciphered, it has been reported that ceramide activates mitogen-activated protein kinase (MAPK)\textsuperscript{27} and a serine/threonine phosphatase named ceramide-activated protein phosphatase (CAPP).\textsuperscript{28} It also activates PKC\textsubscript{ζ} (protein kinase C). These studies have implicated CAPP and PKC\textsubscript{ζ} as mediators of cellular activities of ceramide, including apoptosis and c-Myc oncogene regulation. Ceramide also appears to be involved in the regulation of vesicular transport. It retards the vesicular movement in cells (exo- and endocytosis, endosome-lysosome movement).\textsuperscript{29}

The report on sphingosine-induced inhibition of PKC has received a great deal of interest in biochemical and pharmaceutical circles.\textsuperscript{30} Sphingosine and lyso-GSL are potent inhibitors of PKC.
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<th>Biochemical Mechanism</th>
<th>Cellular Response</th>
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<td></td>
<td>Release of Ca(^{2+}) from ER DNA binding of AP-1 PA, MAP kinase</td>
<td>Cell motility Mitogenic 3T3 Fibroblast</td>
</tr>
<tr>
<td>N,N-Dimethyl- sphingosine</td>
<td>PKC inhibition</td>
<td></td>
</tr>
<tr>
<td>Sphingosine</td>
<td>PKC inhibition (in vitro, human platelets)</td>
<td>Proliferation (leukemia MOLT-4 cell)</td>
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<td>Ceramide</td>
<td>Protein phosphatase (CAPP) Protein kinase (MAPK) PKCζ</td>
<td>Apoptosis Growth (HL-60, leukemia U937)</td>
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Recently, sphingosine-induced (PKC independent) inhibition of cell proliferation has been demonstrated. Sphingosine inhibits retinoblastoma gene product protein dephosphorylation in vivo and RNA primase in vitro.\(^{31}\) Retinoblastoma gene is an important tumor suppressor gene. Sphingosine phosphate (SP) has also emerged as an important signaling molecule. It inhibits cell motility and tumor cell invasiveness.\(^{32}\) Exogenous addition of sphingosine phosphate to cultured 3T3 fibroblasts induced proliferation. Such cellular activities of SP may be explain by the following biochemical activities: 1) SP releases Ca\(^{2+}\) from ER,\(^{33}\) 2) SP enhances the DNA
binding of transcription activator AP-1, SP activates MAP (by a G protein-
dependent mechanism) and epidermal growth factor receptor (EGFr)
phosphorylation. N,N-Dimethylsphingosine, a known sphingosine metabolite, also
exhibits strong inhibition of PKC.

A number of naturally-occurring compounds structurally related to sphingosine
have been identified (Figure 4). Phytosphingosine, 4-hydroxysphinganine, is a
naturally occurring sphingoid. It has been identified in fungi, plants, yeasts, microorganisms, and marine organisms. Also, its widespread occurrence in
mammalian tissues has been demonstrated. Phytosphingosine occurs mainly as a
constituent of GSL, as well as glycophasphatidylinositol (GPI) anchors of proteins.

It accumulates in some tumors. Tetrahydroxy-sphingosine structures with a Z double bond, named
tetrahydroxy-LCB (long chain base) have been isolated as either free bases or their
 corresponding cerebrosides from human brain, algae and plants. Fuminisins
form a class of tumorgenic mycotoxins. Fuminisins bear a remarkable structural
similarity to sphingosine (Fumonisin B, Figure 4) and have been found to inhibit de
 novo sphingolipid biosynthesis at the level of sphinganine acyltransferase. A family
of antifungal agents, the sphingofungins, has been identified and certain members of
this family proved to be potent and specific inhibitors of serine palmitoyltransferase. Their absolute stereochemistry has been established.

The sphingoid amino acid ISP-1 (thermozymocidine) has been discovered to
possess immunosuppressive activity comparable to that of cyclosporine. ISP-1
inhibits serine palmitoyltransferase, as reported recently.
Figure 4

Sphingosine

Phytosphingosine

Tetrahydroxy-LCB

Fuminsin B1 $R = COCH_2(COOH)CH_2COOH$

ISP-1 (myriocin, thermozymocidin)
1.5 Metabolism of Glycosphingolipids.

Metabolism of sphingolipids and GSL is linked by the first glycosylation step. In the biosynthetic pathway ceramide is transported to the early Golgi apparatus where it is glycosylated. In most cases the first step is the formation of GlcCer catalyzed by glucosylceramide synthase (ceramide: β-glucosyltransferase). This enzyme is located on the cytoplasmic face of the Golgi. Since the GalT which catalyzes LacCer synthesis is located on the lumenal side of the Golgi an inverting protein which facilitates a flip of GlcCer must be present. Trafficking and topology of GSL metabolism has been the subject of several review articles. Sequential addition of monosaccharide units and/or sialic acid yields higher GSL's and gangliosides, as schematically shown (Scheme 1.5). Several dozens of glycosyltransferases have been cloned. However X-ray crystal structures are not available at present.

The exact mechanism of how the genetic information is "translated" into oligosaccharide sequences is unknown at present. It is obvious that the regulation of GT gene expression plays a crucial role. However, more complex factors, such as membrane organization of the Golgi, membrane trafficking, availability and transport of glycosyl donors, and the role of endogenous GT inhibitors must be considered.

In catabolism the cell surface GSL's are endocytosed and fully degraded in lysosomes or they can be recycled back to the Golgi at intermediate stages. Lysosomal degradation is accomplished in most cases stepwise with particular glycosidases. In addition to glycosidases, sphingolipid activator proteins (SAP) are required for degradation of lower GSL's (up to GM2). Presumably, SAPs act as "lifase" proteins and thus make the terminal sugar unit of shorter GSL's accessible to glycosidases. A number of lipid storage diseases caused by glycosidase and SAP deficiencies have been characterized.
Scheme 1.5

![Scheme diagram showing the conversion of ceramide to various glycolipids through enzymatic reactions involving β-glucosidase, Glucosyl-Ceramide Synthase, β-1,4-GalT, β-1,3-GlcNAcT, α-2,3-SialT I, α-2,3-SialT II, α-2,3-SiaT, α-1,3-FucT, GM3, GM2, GM1a, GD3, GD2, GD1b, SialylLe^x, and Le^x.]
1.6. Biological Role of Glycosphingolipids.

GSLs and other glycoconjugates have been identified as tissue specific differentiation and tumor markers (c.f. Le$^X$ is also referred to as the stage specific embryonic antigen, SSEA). Most GSL's and gangliosides were identified immunologically long before their structures were known. However, the dramatic increase in the number of reports made it clear that GSL's do not simply reflect physiological changes of the cell, but actively participate in cellular events such as cell adhesion, and cell signaling. It is now widely recognized that the glycosylation patterns of GSLs change distinctly and specifically in all known tumors.

Gangliosides have attracted a great deal of attention as modulators of membrane functional proteins. Gangliosides inhibit cell growth and induce differentiation in a number of cell lines. Gangliosides induce neuritogenesis in mouse neuroblastoma cells and stimulate neuronal repair.

Recent reports provide greater insights into the possible mechanism of GSL action. Ganglioside GM3 inhibits tyrosine phosphorylation directly whereas lyso-GM3 enhances serine phosphorylation of epidermal growth factor receptor. Gangliosides interact with IL-4 and inhibit IL-4-stimulated helper T-cell proliferation. Another recent report strongly suggests a close link between ganglioside metabolism and the phosphorylation machinery. GM1 inhibits DNA synthesis in isolated nuclei, and as reported later, sulfate- and sialic acid-containing GSLs (SO$_3$-3-GalCer, NeuAc-2,3-paragloboside, GM3, GM1, GD1a) inhibit DNA polymerase $\alpha$.

Plasmalopcytosine (Figure 5), isolated from the white matter of human brain, proved to be a very potent endogenous nerve growth receptor. Plasmalopcytosines are 4,6 and 3,4 cyclic palmitals of psychosine (lyso-GalCer).
Although the exact mechanism of early events at the membrane is not known at present, its action results in activation of MAP kinases.\textsuperscript{76}

\textbf{Figure 5}

\begin{center}
\includegraphics[width=\textwidth]{plasmalopsychosine}
\end{center}

Plasmalopsychosine (GalCer 3,4-Palmitol)
CHAPTER TWO
GLYCOCONJUGATE PROCESSING ENZYMES: MECHANISTIC DISCUSSION AND INHIBITION
2.1 Glycosidases.

Glycosidases are ubiquitous enzymes which are involved in the degradation of carbohydrate foodstuffs, the processing of eucaryotic glycoproteins and glycolipids. They are essential enzymes for normal development of all organisms. Glycosidases can be classified according to their function, location in the organism, or according to their mechanistic characteristics. Exoglycosidase enzymes remove the terminal monosaccharide of the non-reducing end while endoglycosidases cleave glycosidic linkages within oligosaccharide chains.

The glycosidic linkage is an acetal functional group, and therefore a number of mechanistic possibilities for the hydrolysis can be envisioned. As with any acetal linkage, the cleavage of a glycoside can proceed by at least two mechanistic routes. The "exo" route involves the cleavage of the exocyclic C-O bond which can take place at some point between the SN₂ and SN₁ limits. Analogously, the "endo" path is initiated by the endocyclic C-O bond cleavage. Since both of these mechanisms have been proposed for enzymatic hydrolysis, the available data must be examined in detail in order to arrive at the best possible explanations. The knowledge of the intimate mechanisms is indispensable for the development of potent and selective inhibitors. Based on the stereochemical outcome of hydrolysis, glycosidases can be classified as "retaining" or "inverting". This mechanistic discussion will focus on retaining glycosidases.

The exact mechanism of enzymatic glycosidic cleavage has been a matter of considerable debate. Crystallographic studies on lysozyme, and lysozyme-inhibitor complex by Phillips and Dwek implicate two carboxyl groups as catalytic residues. Involvement of aspartate (position 52 in lysozyme) and
glutamate (position 35 in lysozyme) has been confirmed by mutagenic studies. Based on this information, two mechanisms have been proposed (Scheme 2.3).

Scheme 2.1

In the exo mechanism, the glutamate protonates the exocyclic oxygen which triggers the cleavage process. The flattening of the pyranose ring, a necessary requirement for the stereoelectronic assistance of the ring oxygen lone pair, imposes a kinetic barrier. Thus, this point of the reaction coordinate represents the transition
state, and the point of maximal assistance of an enzyme. Cordes and Bull proposed a fully charged oxonium intermediate, while Sinnott suggested the assistance of the aspartate residue in the rate determining step, and the existence of a covalently bound acyl intermediate (Scheme 2.3). 

### Scheme 2.2

Whereas the notion of a considerable charge buildup in the transition state was strengthened by the strong inhibition of glycosidases by nojirimycins (azasugars), secondary deuterium isotope effect studies demonstrated little charge development. Recently, a report by Ganem on novel glycosidase inhibitors also supports the significance of the flattening of the ring, and discounts the significance of charge buildup in the transition state. Although the examination of inhibitors provide important mechanistic insights into enzymatic reactions in general, in this case interpretation of the azasugar inhibition as compelling evidence for a charged species in the transition state may be misleading. The inhibitor-enzyme complex does not necessarily follow the substrate route, and may halt the enzyme at various positions of the reaction coordinate. Furthermore, the inhibitor may "steer" the complex off the "normal" substrate-enzyme path.
Scheme 2.3 Retaining Glycosidase - Mechanism

Phillips, Dwek

Cordes and Bull

Sinott
Scheme 2.5  Retaining Glycosidase - Endo Mechanism

Fleet 1985, Post & Karplus 1986
Kinetic data on various substrates should be considered as the major indicators of the mechanism. Further evidence for the direct involvement of a glutamate residue as a nucleophile in the mechanism of a retaining glucosidase (Agrobacterium β-glucosidase) was provided by trapping the glycosyl-enzyme intermediate with 2-deoxy-2-fluoroglucosyl fluoride.\(^89\)

An alternative mechanism suggesting the endocyclic bond cleavage was put forth by Fleet\(^90\) and was supported by a subsequent molecular dynamics simulation of the lysozyme-(GlcNAc)\(^6\) complex by Karplus (Scheme 2.5).\(^91\) It should be pointed out that according to this scenario the initial bond cleavage is not the rate determining step. Stereoelcronics of this step are favorable and no significant kinetic barrier is present. Rotation about the 1,2 C-C bond brings the substrate into a conformation that leads to reclosure of the ring and departure of the aglycone. According to Karplus and Frank,\(^92\) the ring distortion in the exo mechanism is replaced here with bond rotation. Although both mechanisms provide the same product and can explain most of the kinetic data, the cardinal evidence which rules out the endo mechanism comes from hydrolysis studies of pyridinium glucoside and glucosylfluoride (Scheme 2.6).\(^93\) Clearly, these compounds cannot provide the required positive charge stabilization from the exocyclic substituents, yet are hydrolyzed rapidly. Even though alternative pathways may take place with different enzymes and substrates, the available experimental evidence favors the exo mechanism for the enzymes which have been studied. It also should be noted that most of the structural evidence comes from relatively small extracellular glycosidases (hen egg lysozyme, saliva amylase, intestinal amylase) which bind and cleave large substrates, while eukaryotic Golgi or lysosomal enzymes are larger and membrane bound and therefore much harder to characterize.
Scheme 2.6
2.2 Glycosidase Inhibitors.

Inhibitors of glycoside hydrolases not only provided mechanistic insights into these enzymes, but also proved to be important tools for the elucidation of the new metabolic pathways of glycoproteins. An exciting story began with the isolation of gluconojirimycin (nojirimycin) in 1966 (Figure 6). 1-Deoxynojirimycin was first obtained from nojirimycin by catalytic hydrogenation with platinum, or by reduction with sodium borohydride in the course of structural elucidation. Subsequently, nojirimycin and 1-deoxy-nojirimycin were isolated from other microorganisms and plants.

Originally, gluconojirimycin was found to be effective against rice plant infection. In 1970 nojirimycin and 1-deoxynojirimycin were reported to possess glucosidase inhibitory activity. Significant inhibition of glucoamylase, microbial α- and β-glucosidases, mammalian intestinal oligo- and disaccharidases were reported. Initial efforts focused on chemical modification and development of antihyperglycemic drugs.

In 1982, Saunier et al. reported the inhibition of Golgi glucosidase I and II by 1-deoxy-gluconojirimycin. Glucosidase I and II are important trimming enzymes which remove glucose residues from N-linked glycoproteins (Glc3Man9GlcNAc2), which is a critical step for further biosynthetic processing of these glycoconjugates.

Gluconojirimycin has become an important model for further development of glycosidase inhibitors. A number of nojirimycin cogeners such as manojirimycin or siastatin (Figure 6) have been isolated or synthesized. Also, bicyclic polyhydroxylated alkaloids have been found in various plants. Castanospermine, a potent glucosidase inhibitor, and swainsonine, a very potent inhibitor of a number of mannosidases, are examples of bicyclic indolizidine alkaloids.
Figure 6

Gluconojirimycin (GNJ)

1-deoxy-GNJ (azaglucose)

Mannojirimycin (Azamannose)

AchiN

COOH

Siastatine B

Castanospermine

Swainsonine

Australine

DMDP

L-Azafucose

Five-membered Azafucose
As shown in Figure 6, castanospermine can be viewed as a conformationally restricted azaglucose. Australine and DMDP (2R,5R-Dihydroxymethyl-3R,4R-dihydroxypyrrolidine) are examples of pyrrolizidine and pyrrolidine alkaloids, respectively. Australine, a ring-contracted isomer of castanospermine, is an inhibitor of α-glucosidases. DMDP is a potent inhibitor of yeast α-glucosidases, almond emulsin β-glucosidase and other enzymes. In analogy to naturally occurring azasugars, azafucose was designed and synthesized by Fleet.\textsuperscript{105} It is the most potent inhibitor of α-L-fucosidases known. The five-membered ring azasugars were introduced by Wong (Figure 6).\textsuperscript{106}

A second major class of naturally occurring glycosidase inhibitors are amino-carbocyclic alcohols (aminocyclitols or conduritolamines).\textsuperscript{107} Valienamine is an example of aminoconduritol (Figure 7). Valienamine does not possess any significant activity by itself, but when incorporated into pseudo-oligosaccharides known as acarbose homologs, these compounds show inhibition of various mammalian and pancreatic α-glucosidases. Trehalozin is an interesting example of a diglycoside homolog which inhibits trehalase.\textsuperscript{108} In 1989, an unusual pentasubstituted cyclopentane named mannostatin A was isolated. Mannostatin A is a potent inhibitor of a number of mannosidases (rat epididymal α-mannosidase, $\text{K}_i= 48\text{nM}$; jack bean α-mannosidase, $\text{IC}_{50}= 70\text{nM}$; rat liver lysosomal α-mannosidase, $\text{IC}_{50}= 160 \text{nM}$). Mannostatin A also inhibits Golgi processing mannosidase II but is inactive against mannosidase I.\textsuperscript{109}
The antitumor and anti-HIV activity of some azasugars has resulted in a great deal of attention from biological and chemical circles.\textsuperscript{110} The use of azasugar analogs to prevent the formation of the aberrant asparagine-linked glycans and to inhibit catabolic glycosidases is being actively pursued as a therapeutic strategy for cancer. Pulmonary colonization by murine melanoma cells in mice was reduced (80\%) by treatment with swainsonine and castanospermine. The treated cells carried more endoglycosidase-H-sensitive and concanavalin-A binding glycans on the cell surface, indicating that the inhibitors had prevented the formation of complex glycans which have been implicated in metastasis. Furthermore, there is much evidence that swainsonine stimulates the immune system's response to tumors. Swainsonine induces
an increase in natural killer T-cells. It also enhances IL-2 (interleukin 2) receptor expression, IL-2-induced proliferation, and induces tumorcidal activity of macrophages. Such exciting results, together with swainsonine's lack of cytotoxicity, suggest potential uses in the therapies for tumors and immunodeficiencies.\(^{111}\) Although some possible explanations for swainsonine's activities have been suggested, the mechanism is not known at present. Its effect on cell surface glycosylation to explain the stimulation of immune cell proliferation and activation seems likely.

Castanospermine, 1-deoxyglucoojirimycin and DMDP all affect HIV infectivity at concentrations which are not cytotoxic to lymphocytes. After a systematic survey of 47 natural and synthetic amino-sugar analogs, N-methyl, -ethyl and -butyl derivatives of deoxynojirimycin proved to be the most potent.\(^{112}\) In particular, N-butyl-DNJ resulted in the eventual elimination of HIV virus from cultures after a prolonged time. This compound is in clinical trials for the treatment of AIDS. A recent report by Platt provided the first definitive evidence that glucosidase I inhibition occurs at the antiviral concentration of N-butyl-DNJ.\(^{113}\) The interaction between the viral envelope glycoprotein gp 120 (MW 120 kDa) and the lymphocyte glycoprotein CD4 is a crucial step for viral entry into a host cell. This study demonstrated that the glycosylation of gp 120 was significantly altered in the presence of N-butyl-DNJ, and thus strongly indicates a candidate mechanism for the anti-viral activity of this compound.

6-O-Butanoyl-castanospermine was 20 times more active than castanospermine and 50 times more active than N-butyl-DNJ. Obviously, physicochemical properties of azasugars affect the cellular distribution and may enhance activity in vivo. It has been shown that the lipophilic character of the butyl group targets the inhibitor to membrane associated enzymes of the Golgi and/or
lysosomes. Interestingly, N-butyl-DNJ also inhibits glucosylceramide synthase, a glycosyltransferase which attaches the first glucose residue to ceramide. The galactose analog N-butyl-azagalactose was found to be a selective inhibitor of glycolipid biosynthesis with no effect on the maturation of N-linked glycoproteins or lysosomal glucocerebrosidase.

Very real prospect of applications of amino-sugar analogs as cellular probes and therapeutics reinforced the interest in the development of more potent and specific glycosidase inhibitors. The aspect of potency has been pursued by several laboratories. The concept of pyranose ring flattening in the transition state led to the development of a series of pyranose analogs with an sp$^2$ hybridized C1 positions (Figure 8).

**Figure 8**

![Chemical structures](image)
Gluconolactone oxime has been introduced by Vasella.\textsuperscript{116} Gluco-amidine, amidrazone and amidoxime analogs have been designed and synthesized by Ganem.\textsuperscript{117} Ganem reported structures having an endocyclic double bond which would represent the best mimic of the putative flattened transition state. However, Vasella synthesized the gluco-amidoxime and showed by \textsuperscript{15}N NMR and X-ray data that the correct tautomer is the glucohydroximo-lactam with the double bond in the exocyclic position (Figure 8).\textsuperscript{118} Although Ganem's study provided important data, conclusions drawn from these studies require careful interpretation. Since Vasella elucidated the structure of only the oxime, the amidine and amidrazone compounds are drawn according to Ganem. Table 2 shows a comparison of several types of glucosidase inhibitors. It should be noted that azaglucose, glucoamidine, and gluco-amidoxime are inhibitors which differ in pK\textsubscript{a} by several orders of magnitude, yet possess comparable glucosidase activities. According to Ganem, this suggests the relative importance of a ring flattening over charge development in the rate determining step. However, this argument is not general, as shown in Table 3. A chair-like azafucose is 170 times more potent than fucoamidrazone.\textsuperscript{119} Also, the five membered azafucose analogs are less potent than azafucose.

While the resemblance of inhibitors to a pyranose or a transition state form of a monosaccharide can explain the observed data in the case of glucosidase hydrolases, it is not possible to formulate general guidelines for the development of potent inhibitors. Every class and every enzyme should be treated separately, as can be seen from the available data.
Table 2

<table>
<thead>
<tr>
<th>INHIBITOR</th>
<th>Ki [μM] Almond β-glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Inhibitor 1" /></td>
<td>18</td>
</tr>
<tr>
<td><img src="image2" alt="Inhibitor 2" /></td>
<td>7.8</td>
</tr>
<tr>
<td><img src="image3" alt="Inhibitor 3" /></td>
<td>52</td>
</tr>
<tr>
<td><img src="image4" alt="Inhibitor 4" /></td>
<td>10 (Ganem) pK_a 10.6</td>
</tr>
<tr>
<td><img src="image5" alt="Inhibitor 5" /></td>
<td>13.8 (Ganem) pK_a 5.6</td>
</tr>
<tr>
<td><img src="image6" alt="Inhibitor 6" /></td>
<td>16 (Vasella)</td>
</tr>
<tr>
<td><img src="image7" alt="Inhibitor 7" /></td>
<td>50 000</td>
</tr>
<tr>
<td>INHIBITOR</td>
<td>Ki [nM] Bovine α-fucosidase</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><img src="image1" alt="Inhibitor structure 1" /></td>
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<tr>
<td><img src="image2" alt="Inhibitor structure 2" /></td>
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</tr>
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<td>2000</td>
</tr>
<tr>
<td><img src="image5" alt="Inhibitor structure 5" /></td>
<td>820 (recombinant α-fucosidase)</td>
</tr>
</tbody>
</table>
The mechanism of binding and inhibition of mannosidase inhibitors is especially unclear. The "parent" azamannose inhibits α-mannosidases relatively weakly. Based on modeling studies, it was suggested that the mannosyl oxonium ion flips to a more stable "axial" form (Figure 9). Based on this prediction a novel amino-cyclopentenol ("Merrell Dow amino-cyclitol") was synthesized, and proved to be very potent for certain α-mannosidases (jack bean α-mannosidase, IC50 = 62 nM; swainsonine 87 nM). However, this compound showed relatively modest activity for other α-mannosidases (mung bean glycoprotein processing α-mannosidase, IC50 = 1μM; swainsonine 40 nM). This model also does not sufficiently explain the potency of mannostatin A. Mannostatin A cannot be superimposed with either a pyranose form of mannose, nor with the proposed oxonium intermediates. Presumably, an unusual mode of inhibition takes place in the case of mannostatin A, and therefore an understanding of its activity may lead to new insights into glycosidase function.

Figure 9

Mannosyl oxonium cation

Azamannose
(Ki = 68 μM)
(jack bean α-mannosidase)

"Axial" oxonium cation

Merrell Dow amino-cyclitol
IC50 = 62 nM; jack bean α-mannosidase
Schmidt's amino-C-glucosides represent an interesting examples of novel inhibitors (Figure 10). The two isomers differ by two orders of magnitude in inhibition potency against almond β-glucosidase.123

![Figure 10](image_url)

| Ki     | 70 μM | 7.6 mM |

Sweet almond β-glucosidase inhibitor

Some exoglycosidases do not possess any selectivity beyond the monosaccharide unit they cleave. On the other hand, some exoglycosidases and most endoglycosidases possess various degrees of sequence specificity. Acarbose analogs and trehalozin are examples of natural endoglycosidase inhibitors. Note that in both cases the amino-sugar analog is linked via a covalent C-N bond. Glc-α1,3-aza-mannose and Glc-α1,3-swainsonine have been synthesized and both showed potent inhibition against Golgi endomannosidase (Figure 11).124 Trehalozin served as an inspiration for the design of trehazoloid inhibitor of yeast α-glucosidase (Ki = 9.3 μM).125 Its high activity is attributable to the aglycone portion. Following the same approach, azafucosyl-disaccharide has been synthesized as a potential sequence specific α-L-fucosidase inhibitor.126 Its activity has not been reported yet.
Figure 11

Glc-α1,3-azaglucose

Glc-α1,3-swainsonine

Trehazolin

Trehazoloid inhibitor (yeast α-glucosidase)

Aza-diglycoside (Wong)
As discussed earlier in this section, initial efforts toward the development of glycosidase inhibitors have laid the groundwork for the rational design of glycosidase inhibitors. Both potency and selectivity have been addressed to some extent. Thus, the convergence of both approaches may provide potent inhibitors with tunable specificities.
CHAPTER THREE

SYNTHESIS OF AZASUGAR INHIBITORS: BACKGROUND
3.1 Synthesis of Azasugars from Sugar Precursors.

As was presented in the previous chapter, the inhibition of glycosidases showed unexpected biological effects, and a considerable amount of synthetic effort has been devoted toward azasugars and other inhibitors.

The first synthesis of D-gluconojirimycin and 1-deoxy-D-gluconojirimycin appeared in 1968 by Inouye et al. (Scheme 3.1). 6-O-Trityl-glucofuranose intermediate I was prepared from D-glucose in four steps following known procedures. The free hydroxyl was oxidized with DMSO and acetic anhydride. Reductive amination with hydroxylamine and Raney nickel gave a 9:1 mixture of secondary amines II and III. Major amine II was the desired product. The reductive removal of trityl and benzyl groups with lithium metal in liquid ammonia and subsequent mild acetal deprotection with SO$_2$ provided gluconojirimycin sulfite. Treatment with basic resin provided a product which was identical with the natural compound. It should be noted that this synthesis was a part of the structural confirmation of D-gluconojirimycin.

The striking resemblance of nojirimycins with their parent sugars can explain the large number of synthetic approaches which employ sugars as the starting point. An excellent review by Nishimura comprehensively summarized the synthetic approaches.

Ganem et al. synthesized azaglucose and azamannose from their corresponding monosaccharides via a straightforward methodology (Scheme 3.2). Tri-O-benzyl-6-bromopyranose IV was prepared from methyl-α-D-glucopyranoside in 73% yield via several known steps. When bromoether IV was refluxed with a mixture of zinc dust, benzylamine and NaBH3CN in 1-propanol and water, reductive ring opening occurred with concomitant reductive amination to afford aminoalkene VI in
91% yield. Intramolecular aminomercuration provided cyclic bromomercurials VII and VIII after neutralization, ligand exchange and flash chromatography.

Scheme 3.1

D-Glucose → 4 steps → 46%

1. DMSO, Ac₂O 73%
2. NH₂OH.HCl
3. RaNi, MeOH 94%

Dowex 1X2 (OH⁻) → 1. Li/NH₃; 74%
2. SO₂, H₂O 96%

Gluconojirimicin (GNJ) → Gluconojirimicin sulfite
Scheme 3.2

1. O₂, NaBH₄, DMF 70%

2. H₂/Pd-C

28% overall yield

Nojirimicin (Azaglucose)
Organomercury isomer VII was converted to nojirimycin with via radical trapping with oxygen in the presence of NaBH4/DMF/O2 in 70% yield and subsequent benzyl group deprotection. The overall yield from IV was 28%. Ganem also synthesized C-azaglycosides via the same synthetic strategy. In that work the intermediate aldehyde V was isolated and subsequently treated with the appropriate Grignard reagent. The resulting secondary alcohol was oxidized, converted to the amine, and cyclized with mercury trifluoroacetate. However, low stereoselectivity was a major shortcoming of this approach.

Interestingly, azafucose has not been found as a natural product. Because of the immunogenic nature of L-fucose and its high occurrence in tumors, azafucose has been synthesized by Fleet (Scheme 3.3). Intermediate IX was synthesized in five steps from methyl-α-D-glucopyranoside. This sequence involved double inversion of positions 2 and 3, followed by protection of the free hydroxyl groups as benzyl ethers. Selective deprotection of the benzyl acetal, followed by reduction of the 6 position via tosylation of the primary hydroxyl group and hydride displacement, provided 6-deoxy-altrose X. In four steps which include hydrolysis and reduction of the anomic center, double mesylation, and the displacement of the primary mesylate with azide afforded intermediate XI. Since direct hydrogenolysis yielded a mixture of products, sodium hydrogen telluride treatment was applied followed by subsequent deprotection of three benzyl groups with hydrogen. This protocol yielded the first synthesis of azafucose.

Professor Fleet's laboratory produced a large number of natural and synthetic azasugar analogs, and became one of the most important contributors to the field. Recent efforts of this laboratory have focused on the synthesis of iminoheptinols, one carbon richer homologs of nojiri-sugars (Scheme 3.4). The additional
hydroxymethyl group provides an opportunity for linking the azazugar moiety to other saccharides.

Scheme 3.3
Scheme 3.4

The few examples of carbohydrate syntheses presented here demonstrates that such approaches may, in some cases, become very lengthy and require repetitive protection, inversion and deprotection (cf. 16 step synthesis of azafucose). Therefore, alternative approaches starting from chiral acyclic precursors have been developed.
3.2 Synthesis of Azasugars from Acyclic Precursors

Kibayashi's synthesis of D-azaglucose is outlined in Scheme 3.5. Epoxide intermediate XII was obtained from diethyl tartrate in six steps. The nitrogen was introduced via an oxirane opening with sodium azide. The manipulation of protecting groups, which involved MOM protection and t-butyldimethylsilyl group deprotection, was followed by conversion of the primary alcohol to the mesylate ester. Reduction of azide XIV gave an amine which cyclized in the presence of triethylamine to afford the protected D-azaglucose. Reflux with hydrochloric acid in methanol yielded the hydrochloride salt of D-azaglucose.

Recently, Johnson et al. reported the synthesis of D-tallo-nojirimycin and D-manno-nojirimycin. The crucial step in this strategy is the enzymatic resolution of the cyclopentenone-diol which provides enantiomerically pure diol XV (Scheme 3.6). Compound XV was iodinated at the α-keto-position and the ketone was reduced under Lueche conditions. Introduction of the required 6-hydroxymethyl group was managed by palladium-catalyzed CO insertion and reduction. Ozonolysis and reductive amination provided N-benzyl-D-azatalose in good yield and stereoselectivity. Hydrogenolysis of the N-benzyl protected compound XVII yielded D-azatalose.

An extensive series of azasugars (approx. 30) was prepared in Wong's laboratory via chemo-enzymatic methodology. Aldolase catalyzed aldol reaction of dihydroxyacetone phosphate (DHAP) and various aldehydes set two new chiral centers with excellent stereoselectivity. Aldolases accepted a variety of chiral aldehydes as substrates. As shown in Scheme 3.7, starting from a chiral aldehyde, three contiguous chiral centers were established. All four diastereomers at position 3 and 4 were synthesized by choosing from four different aldolase enzymes. The 3,4-threo or
3,4-erythro intermediates were prepared with fuculose-1-phosphate aldolase and fructose-1,6-diphosphate aldolase, respectively (Scheme 3.8).

**Scheme 3.5**

Dicetyl L-tartrate → 6 steps → XII → 1. NaN₃; 75% → 2. MOMCl → XIII → 1. TBAF/THF → 2. MsCl, Py → 89% → XIV

Nojirimycin (Azaglucose)
Scheme 3.6

1. I₂, Py, CCl₄
2. NaBH₄, CeCl₃, MeOH
3. TBSCI, Im, DMF
94%

1. CO, Bu₃SnH
   Pd(PPh₃)₄, THF
2. NaBH₄, CeCl₃
76%

1. O₃, MeOH; DMS
2. BnNH₂Cl, NaBH₃CN
   MeOH; 62%

H₂/Pd·C
81%

D-Ta-to-nojirimycin
(Azatalose)
The phosphate ester was removed with acidic phosphatase. Note that although these steps are 100% stereoselective, they proceed in poor yield. Reductive cyclization with hydrogen at 50 psi provided azafucose analog XIX and pyrrolidine analog XX. The major shortcoming of this otherwise elegant approach is the low yielding enzymatic aldol condensation. The chemical synthesis of XIX from D-ribose was reported recently by the same author.\textsuperscript{136}

 Scheme 3.7

\[
\text{DHAP} \xrightarrow{\text{Aldolase}}
\]
Scheme 3.8

1. DHAP/Fructose-1-phosphate aldolase
2. acid phosphatase; 10%

1. DHAP/Fructose-diphosphate aldolase
2. acid phosphatase; 31%

H$_2$/Pd-C, 50 psi
76%

8% overall yield

3,4-erythro

(S)-2-Azidopropanal

3,4-threo

24% overall yield

26% overall yield
CHAPTER FOUR

TANDEM C-C/ C-O BOND FORMATION:
ENTRY INTO AMINO SUGAR ANALOGS
4.1 Synthetic Strategy

We have chosen protected $\alpha$-amino acids as starting materials for the synthesis of amino sugars based on the encouraging preliminary results from our laboratory. It has been demonstrated that diphenylmethylene imine protected $\alpha$-amino acid esters can be converted to unsaturated amino alcohols with various aliphatic side chains. These results formed the basis for our synthetic strategy towards amino sugar analogs with the ultimate goal of developing a general synthetic methodology which would allow us to access both known and newly designed inhibitors of glycosidases and glycosyltransferases. The general synthetic strategy is outlined in Scheme 4.1. Schiff base protected serine ester can be converted to unsaturated amino alcohols. Oxygenation of the double bond can provide acyclic aminoalcohols with four contiguous chiral centers. Selection of suitable protecting groups is crucial for the last stage of our methodology, which is the manipulation of protecting groups and cyclization. It is clear that such an approach may lead to a variety of potentially desirable products such as amino sugars, polyhydroxypiperidines, polyhydroxypyrrolidines and aminocyclitols.

Matt Peterson in our laboratory developed a highly selective method for the synthesis of threo-1,2 amino alcohols from diphenylmethylene-protected $\alpha$-amino esters (Scheme 4.2). Treatment of a protected $\alpha$-amino ester with a 1:1 mixture of DIBAL:TRIBAL at -78°C in CH$_2$Cl$_2$, followed by addition of an alkenyllithium provided 1,2-amino alcohols. Alkenyllithiums with varying lengths of the aliphatic side chain were generated from the corresponding alkenyliodides in hexane. This turned out to be a crucial modification since the conventional generation of alkenyllithiums in polar solvents resulted in significant decrease in selectivity (c.f. $>20:1$ threo : erythro in hexane, 8:1 in ether, 3:1 in THF). It was shown that under these conditions,
significant reduction of the ester does not occur until after the carbon nucleophile is added, which presumably generates an aluminum "ate" species which is a stronger reducing agent than iBu₂AlH itself. Thus, a one-pot reaction provides access to threo-1,2-amino alcohols which can be elaborated in a number of ways.

Scheme 4.1

I → C-C Bond formation

II → Oxygenation

III → Protection Manipulation and Cyclization
Schiff base protected serine esters can be converted to unsaturated amino alcohols with either E or Z stereochemistry, based on the selection of appropriate haloalkene (Scheme 4.3). These amino alcohols can in principal be converted to eight diastereomeric series in either enantiomeric form (Scheme 4.4).

Scheme 4.2
Thus, the E-amino alcohol can yield galacto- and ido-isomers via the cis-dihydroxylation (c.f. osmylation). The same pair can be prepared from the Z-isomer by the trans-dihydroxylation (c.f. epoxidation and ring opening). Analogously, the gluco- and altro-isomers can be constructed via the trans-dihydroxylation of the E-amino alcohol or cis-dihydroxylation of the Z-amino alcohol. Also, if the erythro-amino alcohols could be prepared, this approach would provide a direct entry to all sixteen isomers of amino hexoses.

In order to apply our methodology, three major problems had to be addressed (I-III, Scheme 4.1). First, it had to be shown that the existing reduction-alkenylation method can be extended to functionalized lithio-alkene nucleophiles. This step also required the efficient preparation of haloalkenes and subsequent metallation protocol in nonpolar solvents. Second, efficient catalytic osmylation had to be effected, and the stereoselectivity had to be investigated. The third stage required the manipulation of the protecting groups and the cyclization protocol.
Scheme 4.4

cis-dihydroxylation

trans-dihydroxylation

Galacto

Gluco

Ido

Altro

trans-dihydroxylation

cis-dihydroxylation
Scheme 4.5

I

C-C Bond Formation

1. DIBAL : TRIBAL / hexane
   -78°C

2. Li\(\rightarrow\)OR

II

Stereoselective Oxygenation

OsO\(_4\)

III

Protection Manipulation and Cyclization

N-Methyl-D-fucosamine

L - Azafucose
The simplicity of the propenyllithium nucleophile and the predicted outcome of the OsO₄-catalyzed dihydroxylation step were the two major reasons for selecting N-methyl-D(+)−fucosamine as our first target, which is also of biological interest (Scheme 4.5). The total synthesis of this naturally occurring unusual sugar proved the stereochemical outcome of our methodology. L-Azafucose was selected as our second target for its interesting biological activity, and served as a starting point of our approach to the design and synthesis of fucosidase and fucosyltransferase inhibitors. Thus, L-serine with E-propenyl lithium provides D-fucosamine, while L-alanine with a protected 3-lithio-allylic alcohol yields L-azafucose.
4.2 Preparation of 1-Haloalkenes

E-1-Bromopropene was prepared according to a published procedure. The commercially available mixture of 1-bromopropenes (30% E) was refluxed with sodium hydroxide in n-BuOH for five days. Pure E isomer 3 was recovered in 30% yield (Scheme 4.6).

\[
\text{Scheme 4.6}
\]

\[
\begin{align*}
\text{Br-} & \quad \text{NaOH, nBuOH} \\
30\% \text{ E} & \quad \rightarrow \\
\text{Br-} & \quad 3 \\
30\% \text{ recovered}
\end{align*}
\]

1) NBS (0.5 eq.), CCl₄, benzoylperoxide; 58%
2) Na₂CO₃, H₂O, 90°C; 80%

\[
\begin{align*}
\text{Br-} & \quad \text{OH} \\
\text{3 : 2 Z/E} & \quad 4a/4b
\end{align*}
\]

Inseparable by vacuum distillation

3-Bromo-propene-1-ol can be prepared in several ways. In the first route, a mixture of 1-bromopropene was treated with N-bromo-succimide in the presence of benzoylperoxide. Hydrolysis of the allylic bromide gave a 2:3 mixture of 4a and 4b. Although careful fractional distillation resulted in partial separation, several rounds of distillation did not accomplish any significant improvement.

Despite their well deserved reputation for explosive decomposition, a method based on 1-haloacetylenes worked quite well in our hands. Bromination of distilled propargyl alcohol with sodium hypobromite at -5°C gave 1-bromo-propargyl alcohol.
5 which was subjected without purification to reduction with chloroalanes generated in situ from LAH and AlCl₃ (Scheme 4.7). After minor optimization, only E-isomer 4a was obtained in 70% yield on one hundred gram scale. Standard protection protocols yielded benzyl-protected bromoalkene 7 and t-butyldimethylsilyl-protected bromoalkene 8. A similar LAH/AlCl₃ reduction of 1-iodo-propynol 9 at various temperatures resulted in reductive removal of iodine (Scheme 4.8).

**Scheme 4.7**

![Scheme 4.7 diagram]

- 7: R = Bn; 88%
- 8: R = TBS; 85%

**Scheme 4.8**

![Scheme 4.8 diagram]

- Major product
Hydrometallation of alkynes have been utilized for the synthesis of functionalized alkenes. Formal addition of the metal-hydrogen bond across the triple C-C bond yields a vinylmetal species that can be manipulated in various ways. While hydroalumination of alkylacetylenes provides E-vinyl-alane species, propargyl alcohol is known to yield 10 in only 23% yield.\textsuperscript{141} In our hands less than 10% of vinylalane 10 was obtained (Scheme 4.9). Judging by $^1$H NMR spectroscopic analysis, the major product was di-aluminum species 11 resulting from the double addition to the triple bond.

Another published procedure for the preparation of vinyl iodides involved hydroboration of the triple bond with catechol borane (Scheme 4.10).\textsuperscript{142} According to this procedure, the vinylborane can be hydrolyzed with water and the resulting vinylboronic acid can be replaced with iodine to yield only the E-iodoalkenes. Only acetylenes with alkyl or cycloalkyl chains were tested, and in our hands propargyl alcohol, either free or protected, has not yielded any identifiable products. Black syrup was obtained (Scheme 4.11).
Therefore, we decided to explore hydrostannylation which has been a popular method for the preparation of various vinylmetal or vinyl-halogen species. Vinylstannanes are versatile intermediates which allow for tin-metal or tin-halogen exchange. They can also be used in the Stille reaction for sp\(^2\)-sp\(^2\) C-C bond formation. Seebach's group and Jung's group reported contradicting results on the stereoselectivity of tributyltin hydride addition to propargyl alcohol. Therefore, we have tested various conditions with four different substrates. The results are summarized in Scheme 4.12. The reactions were run without solvent at 110°C with a catalytic amount of AIBN. When excess propynol was used, the Z-isomer was the major product (entry 1 and 2, Scheme 4.12). The E-isomer and the terminal isomer were the side products. When an excess of nBu\(_3\)SnH was utilized (1.3 equiv.), E-vinylstannane became the predominant product. A larger excess of nBu\(_3\)SnH did not improve the selectivity (entry 4, Scheme 4.12). The protecting group had a very minor effect on selectivity and no effect on the yield. The excess of
nBu₃SnH can be distilled, collected and recycled. Thus, the use of excess nBu₃SnH does not pose any problems with regard to safety or cost.

**Scheme 4.12**

\[
\begin{array}{c}
nBu₃SnH + \text{AIBN} \\
\rightarrow \\
100-110°C
\end{array}
\]

<table>
<thead>
<tr>
<th>nBu₃SnH</th>
<th>( \equiv \text{OR} )</th>
<th>Bu₃Sn</th>
<th>Bu₃Sn \equiv \text{OR}</th>
<th>\equiv \text{SnBu₃}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 (H)</td>
<td>70%</td>
<td>20%</td>
<td>10%</td>
</tr>
<tr>
<td>1</td>
<td>2 (TMS)</td>
<td>69%</td>
<td>19%</td>
<td>12%</td>
</tr>
<tr>
<td>1.3</td>
<td>1 (TBS)</td>
<td>16%</td>
<td>69%</td>
<td>15%</td>
</tr>
<tr>
<td>2</td>
<td>1 (TBS)</td>
<td>16%</td>
<td>69%</td>
<td>15%</td>
</tr>
<tr>
<td>1.3</td>
<td>1 (Bn)</td>
<td>15%</td>
<td>70%</td>
<td>15%</td>
</tr>
</tbody>
</table>

**Scheme 4.13**

\[
\begin{array}{c}
\text{Bu₃Sn} \equiv \text{OR} + \text{I₂/CH₂Cl₂} \\
\rightarrow \\
\text{Bu₃Sn} \equiv \text{OR}
\end{array}
\]

- **R = Bn**
- **R = TBS**

- distilled R = Bn; 80%, 12a/12b 8:1 E/Z (105°C, 0.5 mm)
- R = TBS; 75%, 13a/13b/13c 8:1:1 E/Z/Terminal (61-65°C, 0.4 mm)
Vinylstannanes can be distilled *in vacuo*, but no separation was accomplished in this case. Iodine-tin exchange with vinylstannanes was easily accomplished with iodine in CH₂Cl₂ at room temperature. This exchange proceeded with complete retention of configuration. A mixture of iodoalkenes 12a/12b and 13a/13b was purified by vacuum distillation. The E-enriched fractions were separated and used in the following steps (Scheme 4.13).

In summary, efficient routes for the preparation of E-bromo and E-iodoalkenes (3,4a,7,8,12a,13a) have been established and hundred gram quantities of these compounds are readily available.

For the sake of completeness, it should be mentioned that hydrozirconation of the triple bond with Cp₂Zr(H)Cl (Schwartz reagent) provides, according to literature precedent, only the E-vinylzirconium species (Scheme 4.14). Intermediates of this type can be converted to iodoalkenes in the usual way with iodine. However, this route was not pursued, mainly due to the high cost of Schwartz's reagent. It has been recently reported that Cp₂Zr(H)Cl can be generated *in situ* from Cp₂ZrCl₂ and NaBH₃Et₃.

**Scheme 4.14**

\[ \text{Scheme 4.14} \]

\[ \text{Cp}_2\text{Zr}(\text{H})\text{Cl} \text{ OPhBr} \]

\[ \text{Cp}_2\text{Zr}(\text{Cl}) \text{ OPhBr} \]

\[ \text{D}_2\text{O} \]

\[ \text{Cp}_2\text{Zr} \]
4.3 Halogen-Lithium Exchange. Metallation and Alkenylation Studies.

The first major problem that had to be addressed was the preparation of the desired lithio-alkenes in nonpolar solvents such as hexane. Such a prerequisite was crucial for preserving the chelation control in the DIBAL-TRIBAL/alkenyllithium nucleophile addition sequence. Addition of a nucleophile in ether or THF resulted in a dramatic decrease in the stereoselectivity of this step.\textsuperscript{149} It has been reported by our laboratory that vinyllithiums with long aliphatic chains can be prepared in hexane from the corresponding 1-iodoalkenes with two equivalents of t-BuLi.\textsuperscript{150} E-1-Propenyllithium has been previously generated from 1-bromopropene with lithium metal in only 40\% yield.\textsuperscript{151} We attempted the bromine-lithium exchange with 2 equivalents of t-BuLi (solution in pentane) in hexane. Propenyllithium was formed efficiently as judged by trapping with benzaldehyde at room temperature. However, cannulation of a heterogeneous solution into a reaction mixture of Schiff base protected amino acid ester with DIBAL:TRIBAL at -78°C gave a poor yield (28\%) of the desired alcohol 14 (Scheme 4.15, entry 1). The major product was alcohol 15, a result of overreduction. When a heterogeneous solution of propenyllithium was filtered under anhydrous conditions prior to the addition, no desired 14 was detected. This experiment clearly demonstrated that propenyllithium is poorly soluble in hexane. As an alternative solvent we selected toluene (low freezing point, $\pi$-chelation of Li$^+$). Because of possible deprotonation of toluene we performed the exchange reaction with t-BuLi in hexane at 0°C. After one hour the precipitate was allowed to settle, the top hexane layer was syringed out and the same volume of toluene was added. After vigorous stirring, the mixture was cannulated into the reaction vessel. As shown in Scheme 4.15 (entry 2), this modification resulted in a dramatic increase in the yield of the desired alcohol 14.
Scheme 4.15

\[ \text{R}^1 = \text{H} \quad \text{Br} \quad 14 \quad 28\% \quad 15 \quad 60\% \]

2 eq. tBuLi / hexane, 0°C

\[ \text{R}^1 = \text{H} \quad \text{Br} \quad 60 \text{-} 70\% \quad 20\% \]

2 eq. tBuLi, 0°C
hexane → toluene

\[ \text{R}^1 = \text{OTBS} \quad // \quad 16 \quad 70\% \quad 17 \quad 20\% \]

\[ \text{R}^1 = \text{H} \quad \text{Br} \quad \text{OR} \quad 18 \quad \text{R} = \text{Bn} \quad 20\% \quad 60\% \]

2 eq. tBuLi / hexane, 0°C

19a R = TBS 17%

\[ \text{R}^1 = \text{H} \quad \text{I} \quad \text{OR} \quad 18 \quad \text{R} = \text{Bn} \quad 60\% \quad 20\% \]

2 eq. tBuLi / hexane, 0°C

19a R = TBS 70%
Gilman titration\(^{152}\) showed that trans-propenyllithium is four times more soluble in toluene than in hexane. Alcohol 16a (Scheme 4.15, entry 3) was prepared in the same way starting with protected serine 1.

Protected bromoalkenes 7 and 8, when treated with tBuLi in the same fashion and subsequently added to the reaction vessel, afforded poor yields (20%) of the corresponding alcohols 18 and 19a. Overreduction predominated, indicating a small amount of alkenyllithium nucleophile was present in the hexane solution. On the other hand, iodoalkenes 12a and 13a underwent rapid metallation and yielded the corresponding amino alcohols 18 and 19a in very good yield and with excellent selectivity. These results can be explained in terms of the different reactivity of alkenylhalides toward t-BuLi. Iodoalkenes are known to be much more reactive than bromoalkenes. Bromoalkenes are, in turn, more reactive than chloroalkenes (Scheme 4.16).

Scheme 4.16

Reactivity of Haloalkenes toward Lithium Metallation

Seebach reported that bromoallylic alcohols in THF undergo deprotonation at the \(\alpha\)-position to the bromine which is facilitated by the electronegative character of bromine and the anchimeric assistance of the allylic oxygen (Scheme 4.17).\(^{153}\) Geminal elimination of LiBr leads to a vinylcarbene which rearranges to propargyl alcohol. In the presence of an excess of tBuLi 1-propynyllithium is formed. 1-Propynyllithiums are poor nucleophiles in the reduction-alkenylation reaction, as has been demonstrated previously in our laboratory. Thus, it was concluded that the same
side reaction takes place in hexane. According to Seebach, deprotonation was suppressed below -110°C temperatures. Such low temperature requires the use of a Trapp-mixture (THF:Ether:Pentane 4:1:1) as solvent.\textsuperscript{154} The use of the corresponding iodides proved to be a much more practical solution in our laboratory.

**Scheme 4.17**

\[ \text{Br} = \text{Bn} / \text{TBDMS} \]
\[ 2 \text{eq. tBuLi} / \text{hexane} \]
\[ 0^\circ \text{C} \]

7: \( R = \text{TBS} \)
8: \( R = \text{Bn} \)

Li\text{Br} elimination
rearrangement

\[ \text{excess t-BuLi} \]

In summary, efficient conditions have been found for metallation of alkenylhalides and for addition of \textit{in situ} generated alkenyllithium nucleophiles. The desired Schiff base protected amino alcohols (14, 16a, 18, 19a) were prepared in multigram quantities.
4.4 Rapid Configurational Assignment of Schiff Base Protected 1,2-Amino Alcohols

The issue of stereoselectivity of the C-C bond forming step was addressed. Originally, the stereoconfiguration of several amino alcohols was determined following the standard method, which involved removal of the Schiff base with aqueous HCl, followed by cyclization to the carbamate with carbonyl-diimidazole. Compound 14 gave the free amino alcohol 20, whose amino and hydroxy groups were engaged in a cyclic carbamate to form compound 21. Comparison with analogous sphingosines confirmed the threo-configuration (Scheme 4.18). The $J_{2,3}$ coupling constant reflects the specific coupling between protons 4 and 5 which was determined by a decoupling experiment.

Scheme 4.18

\[
\begin{align*}
1) \text{HCl} & \quad 2) \text{Na}_2\text{CO}_3 & \text{ImdhCO} \\
14 & \quad 20 & \quad 21 \\
4.41 \text{ ppm, } J_{2,3} = 7.5 \text{ Hz}
\end{align*}
\]

It has been previously shown in our laboratory that Schiff base protected 1,2-amino alcohols exist in solution in both acyclic and cyclic form. The equilibrium constant depends on the configuration. The cyclic form of the threo-isomer is more stable than the acyclic tautomer because of the trans configuration of the two substituents. On the other hand, the erythro-isomer forms the cis-oxazolidine which proved less stable due to the steric repulsion of the two substituents. The existence of two tautomers in solution complicates the threo/erythro ratio assignment of the crude
mixtures from reduction-alkenylation reaction. We trapped the cyclic form with the very reactive trichloroacetyl-isocyanate (TAI). As outlined in Scheme 4.19, the model compound 14 was cyclized with TAI to give the trichloroacetyl-urea derivative 22. This reaction is instantaneous and quantitative, and is normally performed in the NMR tube just prior to the NMR experiment. Compound 23 was prepared in the same way and the corresponding coupling constants were determined. Also, integration of erythro/threo mixtures could be measured without the complication of the Schiff base-oxazolidine tautomerism. Thus, a new and rapid method was developed for the assignment of stereoselectivity of newly prepared amino alcohol Schiff bases. The isolation and purification steps in the previous method were eliminated.

Scheme 4.19

![Scheme 4.19](image-url)
As determined by $^1$H NMR of crude TAI-mixtures, the reduction-alkenylation method provided amino alcohols 14, 16a, 18, 19a with excellent stereoselectivity (>20:1 threo : erythro).
4.5 Dihydroxylation of a Double Bond via Catalytic Osmylation.

Osmium tetroxide is one of the most selective and frequently used oxidant in organic synthetic chemistry. It delivers two oxygen atoms to the double bond in a cis manner. Osmium tetroxide is highly selective for olefins and converts them to diols in very good yields.\(^{156}\) The exact mechanism of this reaction has been the subject of debate.\(^{157}\) Sharpless proposed the [2+2] cycloaddition as the early event which forms a metallaoxetane intermediate XXI which rearranges to osmate ester XXII (Scheme 4.20).\(^{158}\) The previously accepted mechanism invokes a direct [3+2] addition which leads to the same ester XXII. Hydrolysis of the osmate ester yields the diol product and an osmate salt (Os VI). Reoxidation of the osmium (VI) species has been accomplished with variety of oxidizing agents (cf. tBuOOH, NMO, K\(_3\)Fe(CN)\(_6\)). Thus, the catalytic protocol minimizes the two major shortcomings of osmium tetroxide as a stoichiometric reagent, which is its high toxicity and its high cost. It should be noted that the hydrolysis of osmate esters with a higher degree of substitution is the slowest step of the catalytic cycle.\(^{159}\)

It is well known that tertiary amines increase the rate of osmium tetroxide addition to olefins. It is illustrated schematically in Scheme 4.21 that amine complexation to the osmium tetroxide dramatically changes the symmetry of this reagent. The equality of all four oxygen atoms in tetrahedral tetroxide is destroyed by this complexation event. It was proposed that the axial and equatorial oxygens of the trigonal bipyramidal complex differ in electron density and thus render the complex more reactive.\(^{160}\) Complexation of tertiary amines was exploited in the development of the asymmetric dihydroxylation protocol.\(^{161}\)
Scheme 4.20

Chemical reactions and structures are depicted as follows:

- [2+2] reaction with an oxidant to form XXI
- [2+3] reaction with an oxidant to form XXII
- Oxidant: OsO$_2$(OH)$_4$$^{2-}$
- More reactive

Scheme 4.21

Chemical reactions and structures are depicted as follows:

- More reactive
The stereoselectivity of osmylation of chiral substrates has also been studied. Two seminal reports by Kishi and Stork appeared in 1983. Kishi studied an extensive set of allylic alcohols, ethers and esters under stoichiometric and catalytic conditions. It was observed that the majority of substrates of the type XXIII give the anti-compounds as the major products (Scheme 4.22). The degree of stereoselectivity depends primarily on the character of the R₂ group. It was found that acyl protected allylic alcohols typically yield lower selectivity (~3:1 vs 6:1). Kishi proposed a reactant-like model. According to this model, the allylic hydrogen is eclipsed by the olefin and osmium tetroxide approaches from the face that is anti to the allylic oxygen (Scheme 4.22).

**Scheme 4.22**

\[
\text{OsO}_4 \quad \begin{array}{c}
  \text{R} \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \\
  \text{R}_4 \\
  \text{R}_3 \quad \text{R}_1 \quad \text{R}_2 \\
  \text{R}_4 \\
\end{array}
\]

Kishi's Model

Stork reported osmylation of chiral α,β-unsaturated esters (Scheme 4.23). It was found that the E and Z isomers gave opposite results. The Z-isomer yielded the syn-product as the major diastereomer, and this result could not be
explained with Kishi's model. Stork proposed that in case of the E isomer, the hydroxyl group is coplanar with the double bond and that osmium tetroxide approaches anti to the ethyl group (bulky group). In the Z-isomer $A^1,3$ steric interactions become dominant which will force the allylic hydrogen into coplanarity and the transition state depicted in Scheme 4.23 provides the syn product.

Scheme 4.23

**Anti**

$\text{OsO}_4$

NMO, aq. acetone

**Syn**

$\text{OsO}_4$

NMO, aq. acetone

R = Me, H

Stork's Model

**Anti**

**Syn**

The exact origin of such $\pi$-facial differentiation has been debated extensively. Both electronic and steric arguments have been invoked. Houk suggested $\sigma$-acceptors would occupy antiperiplanar ("outside") or eclipsed ("inside") position and the best
α-donor would be anti to the electrophile to facilitate olefin HOMO-electrophile LUMO interactions. Houk also concluded that sterics dominate the energetics of the transition state formation. Scheme 4.24 clearly demonstrates that, at least with cyclic substrates, the hydroxyl group does not exert any strong electronic effect on the double bond which would result in preference for one face. Complexation directed osmylation has also been reported (Scheme 4.25).

Scheme 4.24

Scheme 4.25

Although the electronic effect of allylic substituents has been detected in the absence of any steric bias, the dominance of sterics has been demonstrated by Vedejs. A number of substrates with various allylic substituents were designed and studied (Scheme 4.26). E-substrates with bulky X groups (PhSO₂, PhMe₂Si) provided mixtures of diols with syn-isomers as the major products which contradicts
the Kishi model ("anti-Kishi") while Z-substrates afforded anti-isomers as major products.

Scheme 4.26

\[
\text{Me} = \begin{array}{c}
\text{Me} \\
\text{X} \\
\text{Me}
\end{array}
\xrightarrow{\text{OsO}_4/NMO}
\begin{array}{c}
\text{Me} \\
\text{X} \\
\text{Me} \\
\text{OH}
\end{array}
\]

\[
\begin{align*}
X = \text{SiPhMe}_2 &: 66 : 34 \\
X = \text{SiPhMe}_2 &: 78 : 22 \\
X = \text{SPh} &: 67 : 33 \\
X = \text{OAc} &: 38 : 62
\end{align*}
\]

\[
\text{Me} = \begin{array}{c}
\text{Me} \\
\text{X} \\
\text{Me} \\
\text{OH}
\end{array}
\xrightarrow{\text{OsO}_4/NMO}
\begin{array}{c}
\text{Me} \\
\text{X} \\
\text{Me} \\
\text{OH}
\end{array}
\]

\[
\begin{align*}
X = \text{SO}_2\text{Ph}, \text{SiPhMe}_2, \text{MeSO}_2, \text{PhS} &: -4 : 1 \\
X = \text{OAc} &: 7 : 3
\end{align*}
\]

Vedejs proposed that the osmylation transition state will have the allylic hydrogen in the sterically most demanding environment and that this depends on olefin geometry (Scheme 4.27). For E-substrate with large \(X\), the allylic hydrogen is oriented toward developing C-O bonds and the \(X\) group is in the outside position. When \(X\) is small, it is placed in the eclipsed position which results in a reversal of the stereo outcome of this reaction. With Z-substrates the \(A^1,3\) interaction again became the decisive force in the reaction (c.f. Stork’s argument). Thus, regardless of \(X\), osmylation of Z-isomers yielded a mixture with predominant anti-products. It should be stressed that regardless of the electronic properties of allylic substituent \(X\) (e.g. \(\pi\)-donor, \(\sigma\)-acceptor) the direction of stereoselectivity is determined, according to this
model, by steric effects. Note that Vedejs's model is an extension of Stork's proposal (compare Scheme 4.23 and Scheme 4.27).

Scheme 4.27  Vedejs's Model

Osmylation of allylic amines has not been studied as intensively as other allylic substrates. A pronounced electronic effect of the allylic amino group on the \( \pi \)-facial stereoselectivity has been demonstrated.\textsuperscript{169} As shown in Scheme 4.28, the prediction of selectivity is not trivial. However, these results can be explained by "Stork-Vedejs" model. The bulkier Boc protection on the nitrogen results in its expulsion from the electronically preferred eclipsed position and therefore, a reversal
of stereoselectivity. The Z-olefin is even more sensitive to the bulk of nitrogen protecting group which was reflected in better selectivity (1:4 vs 1:2).

Scheme 4.28

Hauser et al

\[
\begin{align*}
\text{Me} & \quad \text{CO}_2\text{Mc} \\
\text{NHCOPh} & \quad \text{OsO}_4\text{NMO} \\
\end{align*}
\]

Anti : Syn

3 : 2

Krysan et al

\[
\begin{align*}
\text{NHBoc} \\
\end{align*}
\]

1 : 4

\[
\begin{align*}
\text{NHBoc} \\
\end{align*}
\]

1 : 2

As was mentioned at the beginning of this section, osmylation has become extremely popular in organic synthesis and therefore, a large number of examples can be found in literature. 170

We explored osmylation conditions introduced originally by Yamamoto. 171 According to this protocol, the reaction is run in a 1:1 mixture of t-BuOH and H$_2$O in the presence of potassium ferricyanide (3 eq.) as an oxidant, and potassium carbonate which accelerates hydrolysis of the osmate ester. Either osmium tetroxide or potassium osmate ($K_2$Os$_2$(OH)$_2$), a non-volatile equivalent of OsO$_4$, were used as catalysts. K$_3$Fe(CN)$_6$ oxidizes reduced metals via an outer sphere electron transfer
mechanism and is inert to diol products. Thus, both major disadvantages of other oxidants (c.f. t-BuOOH, NMO), overoxidation of diol products and inertness toward hindered olefins, were improved. For these reasons we explored the K$_3$Fe(CN)$_6$ protocol with our substrates. Also, the basic character of the aqueous medium was suitable for the acid sensitive imine protection.

Substrate 16a was subjected to the osmylation conditions described above with various amounts of potassium osmate (Scheme 4.29). The smallest amount of osmium catalyst required for the completion of the reaction was 3 mol%. The reaction was also run at 0.15 and 0.06 M concentrations. Dilution had no effect on the reaction time, yet a better yield (60% vs 55%) was obtained. Also, the yield and stereoselectivity was not affected by changes in temperature (-10°C, 2°C, 10°C, rt). A mixture of diastereomers 24a and 24b was obtained. The ratio of 6 : 1 was determined by $^1$H NMR of the peracetylated crude mixture. The major diastereomer 24a possessed the desired stereochemical configuration (galacto) with respect to our synthetic goals. It should be noted that the absolute configuration had to be considered tentative until the total synthesis of N-methyl-fucosamine was completed and comparison with the natural product was made. The O-acetyl derivative 16b provided a 3 : 1 mixture of 24a and 24b upon osmylation.

Optimized conditions for 16a provided only 50% conversion of substrates 18 and 19a (Scheme 4.29). Based on TLC, the reaction did not reach completion after 5 days. Approximately 50% of the starting material was consumed. Also, the dark brown color of the reaction indicated the presence of Os(VI) species. The "active" catalytic system ranged from gold yellow to light brown in color. 10 mol% of osmium tetroxide at 0.15 M concentration was required for a complete consumption of substrates 18 and 19a. Presumably, the combination of the larger steric bulk of
Scheme 4.29

1) \( \text{K}_2\text{OsO}_2(\text{OH})_4 \) 3mol%
MeSO_2NH_2
\( \text{K}_2\text{CO}_3 / \text{K}_3\text{Fe(CN)}_6 \)
tBuOH / H_2O 1:1

2) Ac_2O / Pyridine
60% yield

16a

16b

18, 19b

25a/b \( R = \text{Bn} \) 4 : 1
26a/b \( R = \text{TBS} \) 6 : 1

19b

1) \( \text{K}_2\text{OsO}_2(\text{OH})_4 \) 1.2 mol%
MeSO_2NH_2
50%

2) Ac_2O / Pyridine
60%

27a 9 : 1
27b

14 : 1

(DHQ)_2-PHAL
benzylxy and silyloxy substituents (position 1), combined with the electron with drawing effect of the oxygen resulted in the lower reactivity of the double bond and/or greater stability of the osmate ester. The second major improvement of this methodology came from Sharpless laboratory, where it was found that methanesulfonamide (MeSO₂NH₂) enhances the hydrolysis rate of the osmate ester, and that previously inert tri- and tetrasubstituted hindered alkenes could be dihydroxylated efficiently. Addition of 1 eq. of MeSO₂NH₂ allowed the completion of osmylation of 18 and 19a with 3 mol% of osmium tetroxide in 24 hours. Benzyl-protected compound 18 afforded a 4 : 1 mixture of diastereomers, while t-butyl-dimethylsilyl-protected derivative 19a provided a higher selectivity (6 : 1). Again, the absolute configuration was assigned tentatively and subsequently proven after completion of the L-azafucose synthesis.

Pivalation of 19a with pivaloyl chloride, pyridine and DMAP yielded compound 19b in 89% yield. Osmylation of pivalate 19b with 1.2 % of osmium tetroxide in the presence of 1 eq. of MeSO₂NH₂ yielded 80% consumption (20% of recovered starting material) of the starting material and 50% of the isolated mixture of 27a and 27b. The additional steric bulk improved the ratio to 9:1 in the desired syn sense.

The dimeric ligand (DHQ)₂-PHAL, designed by Sharpless, was added (3 mol%) to the reaction mixture with 19b. Addition of this ligand resulted in an increase in chemical and stereoselective yield. A 14 : 1 mixture of 27a and 28b was isolated in 60% yield. The (DHQ)₂-PHAL ligand was synthesized according to a published procedure, and is commercially available. Originally, we thought that 19b and (DHQ)₂-PHAL constituted the matched pair of a chiral substrate and a chiral catalyst. However, results with substrate 28 revealed an interesting phenomena (Scheme 4.30). Thus, substrate 28 was osmylated in the absence of a chiral auxiliary, which
provided a 6 : 1 mixture of diastereomers 29a and 29b. As expected, in the presence of (DHQ)₂-PHAL ligand stereoselectivity was improved to 10.5 : 1. However, the "mismatch" conditions with pseudo-enantiomeric (DHQD)₂-PHAL did not result in decreased selectivity, but improved the selectivity in the same direction (8 : 1).

Scheme 4.30

We speculate that the interaction between OsO₄ and the Schiff base nitrogen may unfavorably compete with the stereoelectronic effect of the allylic ester moiety (Scheme 4.31). As is well known, sp³ tertiary amines (especially sterically accessible ones such as quinuclidine) possess much higher complexation constants toward osmium tetroxide than sp² amines. Therefore, the presence of such ligands may shift the equilibrium to the right and preclude the participation of the Schiff base nitrogen. Also, Schiff base protected 1,2-amino alcohols exist in both acyclic and cyclic form (section 4.4), and the question which form is the actual reactive species must be considered. Clearly, this complication is eliminated by the protection of the
Scheme 4.31

Figure 12
alcohol group (substrates 16b, 19b). Note that all substrates we studied gave the same anti selectivity. These results can be rationalized by both Kishi and Stork-Vedejs models (Figure 12). It should be stressed that we have no direct evidence for the actual mechanism at present.

In summary, optimal conditions have been found for substrates 16, 18, 19a, 19b and 28. Manipulation of hydroxyl protection and reaction conditions provided significant improvement in stereoselectivity (from 4:1 to 14:1) while keeping the concentration of osmium tetroxide below 3 mol%. It is worth mentioning that such an increase in selectivity of the osmylation step greatly simplified the chromatographic separation of the diastereomeric mixtures.
4.6 Manipulation of the Protecting Groups

As was described in the previous two sections, we have developed a two step method (tandem C-C/C-O bond formation) which allows us to establish four contiguous chiral centers in two steps with excellent stereoselectivity. This section will address the subsequent protecting group manipulation.

Treatment of compound 24a with tetrabutylammonium fluoride (TBAF) in THF resulted in the migration of two acetyl groups (Scheme 4.32). Compound 30 was isolated in 65% yield. Although the migration of acyl groups has ample precedent, especially in the direction of the primary alcohol, the observed migration of two acetates without significant scrambling was remarkable. For this reason, the order of steps in the synthesis of N-methyl-fucosamine was altered. The Schiff base imine was reduced and the secondary amine was reductively methylated to yield N-benzhydryl-N-methyl compound 32 (Scheme 4.32). This substrate was treated with HF in acetonitrile, which provided primary alcohol 33. Thus, the migration was suppressed under acidic conditions. Swern oxidation of 30 and 33 afforded aldehyde 34 and ketone 31, respectively. These carbonyl compounds were valuable for structural confirmation of alcohols 30 and 33.

Aldehyde 34 proved to be very unstable and could not be purified by chromatography on silica gel. Aldehyde 34 decomposed under the mildly basic conditions of the Zemplen procedure (cat. NaOMe/MeOH, pH=9) (Scheme 4.33). An attempt to protect the aldehyde group as an acetal with trimethylorthoformate under acidic conditions failed to provide any identifiable products. Even mild CeCl₃-catalyzed acetalization (Luche conditions) failed. Finally, deprotection of the three acetate groups was achieved with 0.5 eq. of KCN to yield 35 as a mixture of pyranose and furanose anomers in 78% yield.
Scheme 4.32

TBSO

\[ \text{R}_1\text{R}_2\text{N} \]

\[
\begin{array}{c}
\text{A. TBAF / THF} \\
R_1=R_2=\text{CPh}_2 \to 65% \\
\end{array}
\]

\[
\begin{array}{c}
24a: R_1, R_2=\text{CPh}_2 \\
32: R_1=\text{Me}; R_2=\text{CHPh}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{NaBH}_3\text{CN} \\
\text{AcOH, CH}_3\text{CN} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Swern ox.} \to 70% \\
\end{array}
\]

\[
\begin{array}{c}
\text{B. 4% HF, 4% H}_2\text{O} \\
\text{CH}_3\text{CN; 92%} \\
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\end{array}
\]

Scheme 4.33

\[
\begin{array}{c}
\text{34} \\
\text{NaOMe/MeOH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{HC(O)Me}_3 \\
\text{HCl, MeOH} \\
\text{HC(O)Me}_3 \\
\text{C}_{6}\text{H}_5\text{Cl, MeOH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{KCN/MeOH} \to 78% \\
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\text{Ph}_2\text{CH-N} \text{Me} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Me} \\
\text{N-CHPh}_2 \text{Me} \\
\end{array}
\]
While the weak Bronsted base CN⁻ is less likely to attack protons than MeO⁻ due to simple basicity, it may be that rapid cyanohydrin formation can reduce the acidity of the amino aldehyde 31a, permitting deacetylation without β-elimination of HOAc.

Similarly to the previous example, triacetate 26a yielded compound 38, when treated with TBAF, a result of a double migration event (Scheme 4.34). The benzoate and even the robust pivalate underwent the same migration. Acidic treatment with HF in acetonitrile did not suppress the migration (procedure B). Reflux with ammonium fluoride in methanol (procedure C) did not provide reproducible results. In some instances low yields (10%) of migrated products were isolated. Compound 40 with the free hydroxyl group in position 3 was oxidized to ketone 41.

With the idea in mind that greater amounts of water may break up the intramolecular interactions during the HF-catalyzed desilylation, we performed a series of experiments with varying amounts of HF and H₂O. The most successful conditions (6 eq. HF, 20% H₂O/THF or CH₃CN) are depicted in Scheme 4.35. However, this protocol provided compound 42 which resulted from deprotection of the Schiff base and the concomitant O to N benzoate migration. It was observed that with increasing amounts of H₂O the Schiff base was hydrolyzed faster than the silyl ether. Prolonged reaction times led to desilylation and migration. Therefore, a base stable methoxymethyl (MOM) protecting group was used and the subsequent desilylation with TBAF proceeded without problems. The details will be discussed in section 4.9.

NMR data for selected acyclic compounds are summarized in Tables 4-6. Direct comparison of NMR data from related substrates was very helpful, especially for the structural confirmation of the migration products.
Scheme 4.34

A. TBAF / THF
B. 4\% HF, 4\% H_2O
PyCN
C. NH_4F / MeOH

26a R=Ac
36a R=Bz
37a R=Piv

Scheme 4.35

36a

HF (6 eq)
20\% H_2O / THF (CH_3CN)

55\%

42

O

Ph

Ph

Ph

Ph
### Table 4. $^1$H NMR Data for Acyclic Compounds (250 MHz, CDCl$_3$)

![Chemical Structure of Acyclic Compounds]

<table>
<thead>
<tr>
<th></th>
<th>chemical shifts (ppm)</th>
<th>coupling constants (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16a  16b  24a  24b  32 33 34</td>
<td>J$_{1,1}$,1' 16a 16b 24a 24b 32 33 34</td>
</tr>
<tr>
<td>H$_1$</td>
<td>3.62 3.78 3.79 3.75 3.69 3.59 9.46 1.1'</td>
<td>10.6 - - - -</td>
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<tr>
<td>H$_1'$</td>
<td>3.84 3.78 3.79 3.75 3.69 3.59 1.1' 1.2</td>
<td>3.5 - - - -</td>
</tr>
<tr>
<td>H$_2$</td>
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<td>2.2 - - - - 5.8 0.0</td>
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<tr>
<td>H$_3$</td>
<td>4.29 5.38 5.39 5.50 5.32 5.31 5.65 2.3</td>
<td>8.3 - 3.3 3.6 4.7 7.3 5.5</td>
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<tr>
<td>H$_4$</td>
<td>5.27 5.38 5.20 5.20 5.38 5.48 5.45 3.4</td>
<td>6.7 - 7.0 7.0 7.4 3.1 8.5</td>
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<tr>
<td>H$_5$</td>
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<td>15.2 15.2 3.7 3.6 4.5 6.5 2.7</td>
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<tr>
<td>H$_6$</td>
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<td>6.5 6.6 6.4 6.4 6.5 6.6</td>
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<tr>
<td>NMe</td>
<td>2.24 2.27 2.44</td>
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<tr>
<td>Ph$_2$CH</td>
<td>4.83 4.79 5.10</td>
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</table>

### $^{13}$C NMR Shifts for Acyclic Compounds (CDCl$_3$)

|        | 16a  16b  24a  24b  32 33 34 |
|--------|-----------------------|--------------------------|
| C-1    | 59.5 64.1 63.4 63.4 59.8 56.7 200.7 |
| C-2    | 65.1 66.8 63.6 63.7 59.4 58.5 58.9 |
| C-3    | 80.8 75.0 72.9 73.2 73.6 73.1 72.0 |
| C-4    | - - 70.5 70.8 71.6 71.3 68.3 |
| C-5    | - - 68.2 69.2 68.6 68.4 68.0 |
| C-6    | 17.8 17.8 16.6 16.4 16.9 16.8 16.7 |
| N-Me   | 34.9 35.2 36.8 |
| Ph$_2$C | 74.3 74.2 73.6 |
Table 5. $^1$H NMR Data for Acyclic Compounds (250 MHz)

\[
\begin{array}{cccccc}
\text{chemical shifts (ppm)} & \\
18 & 19a & 19b & 52 & 53 & 54 \\
H_1 & 4.00 & 4.13 & 4.17 & 4.46 & 4.44 & 10.14 \\
H'_1 & 4.00 & 4.13 & 4.17 & 4.36 & 4.06 \\
H_2 & 5.87 & 5.79 & 5.84 & 5.72 & 5.98 & 6.01 \\
H_3 & 5.60 & 5.51 & 5.64 & 5.22 & 5.32 & 6.29 \\
H_4 & 3.92 & 3.89 & 5.45 & 5.62 & 5.69 & 6.01 \\
H_5 & 3.00 & 2.95 & 3.57 & 3.56 & 3.62 & 3.62 \\
H_6 & 1.21 & 1.19 & 1.13 & 1.14 & 1.09 & 1.08 \\
\end{array}
\]

\[
\begin{array}{cccccc}
\text{coupling constants (ppm)} & \\
J_{H_1H_1} & 1,1' & - & - & 13.8 & - \\
J_{1,2} & 1,2 & 5.6 & 4.3 & 5.9 & - \\
J_{1,2} & 1,2 & 5.6 & 4.5 & 4.3 & 5.7 & 7.8 \\
J_{2,3} & 2,3 & 15.4 & 15.4 & 15.3 & 11.2 & - \\
J_{3,4} & 3,4 & 7.7 & 7.9 & 6.9 & 8.8 & - & 9.4 \\
J_{4,5} & 4,5 & 4.0 & 7.2 & - & - & - \\
J_{5,6} & 5,6 & 6.3 & 6.3 & 6.5 & - & 6.5 & 6.6 \\
\end{array}
\]

$^{13}$C NMR Shifts for Acyclic Compounds (CDCl$_3$)

\[
\begin{array}{cccccc}
\text{C-1} & 69.9 & 63.0 & 63.0 & 73.9 & 59.6 & 191.5 \\
C-2 & 130.0 & 131.2 & 129.8 & - & 128.5 & 136.4 \\
C-3 & 131.7 & 134.7 & 133.6 & - & 139.3 & 139.3 \\
C-4 & 72.1 & 70.0 & 77.5 & 75.0 & 74.2 & 73.0 \\
C-5 & 59.7 & 59.7 & 60.2 & 60.1 & 58.2 & 59.6 \\
C-6 & 16.0 & 16.0 & 18.3 & 17.9 & 17.5 & 17.7 \\
\end{array}
\]
Table 6. $^1$H NMR Data for Acyclic Compounds (250 MHz, CDCl$_3$)

<table>
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<tr>
<th>H</th>
<th>25a</th>
<th>26a</th>
<th>36a</th>
<th>37a</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
<th>42</th>
<th>27a</th>
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<td>H$_4$</td>
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Table II. $^{13}$C NMR Shirts for Acyclic Compounds (CDCl$_3$)

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4.7 Total Synthesis of N-Methyl-D-Fucosamine

Amino sugars have attracted much attention as an important class of natural products since the early 1950's when 2-deoxy-2-methylamino-L-glucosamine was found to be a component of the antibiotic streptomycin. Aminosaccharides are found in glycoproteins and glycolipids, as well as in various antibiotics. 5-Deoxy-5-amino sugars (azasugars) have been found to be effective glycosidase and glycosyltransferase inhibitors, and have been studied intensively (see Section 2.2).

N-Methyl-fucosamine is the glycosidic portion of the antibiotic Neocarzinostatin (Figure 13). The enediyne class of antibiotics has been of interest for the past decade, and the aminoglycoside structures of the calicheamicins and the esperamycins have been shown to play a crucial role in the recognition and binding to the DNA substrate. It has also been recently demonstrated that the N-methyl-fucosamine moiety participates as an internal base in thiol activation of the neocarzinostatin chromophore.

![Figure 13](image-url)
Amino acids appear to be ideal chiral starting materials for the de novo synthesis of amino sugars and related compounds. The introduction of hydroxyl groups with the desired configuration into amino acid-derived substrates can be more efficient than the manipulation of pre-existing hydroxyl-bearing centers in naturally occurring sugars. In addition, the availability of either enantiomer of many amino acids provides access to rare L-sugars. Although L- and D-amino acids have been widely used in the synthesis of natural products, they have not yet become common starting materials for the synthesis of amino sugars and related compounds. The more reactive α-amino aldehydes have been used, but there are some problems associated with this approach, since α-amino aldehydes undergo racemization, and N-Boc protected amino aldehydes do not give, in many cases, very stereoselective results.

Convenient, highly stereoselective reduction-alkylation methods using amino acids as a source of chirality have been developed, and several research groups have now utilized this approach to avoid the isolation of configurationally labile α-amino aldehydes. In our approach, benzophenone imine derivatives of α-amino esters (O'Donnell's Schiff bases) are cooled to -78° in CH2Cl2, treated with iBu5Al2H (1:1 mixture of iBu3Al and iBu2AlH), followed by addition of a carbon nucleophile. We have shown that under these conditions, significant reduction of the ester does not occur until after the carbon nucleophile is added, which presumably generates an aluminum "ate" species which is a stronger reducing agent than iBu2AlH itself. Thus, a one-pot reaction provides access to β-amino alcohols which can be elaborated in a number of ways to yield numerous nitrogen-containing structures (i.e. sphingosines, aminosugars, azasugars, alkaloids). We have chosen N-methyl-2-amino-2,6-dideoxy-D-galactose (N-methylfucosamine, Figure 12), the aminoglycoside
fragment of the antibiotic neocarzinostatin\textsuperscript{189} as a model system which is also of biological interest.\textsuperscript{190}

The synthesis of N-methylfucosamine began with the crystalline L-serine Schiff base 1. (Scheme 4.36) Addition of iBu$_5$Al$_2$H to the Schiff base ester, followed by the addition of the alkenyllithium provided the protected syn-amino alcohol 16a with excellent stereoselectivity (>20:1); the principal by-product consisting of the over-reduced primary alcohol 17. While long-chain trans-alkenyllithiums could be easily generated with tBuLi from the corresponding iodides in hexane, the shorter propenyllithium analog precipitated from hexane. The solid trans-propenyllithium was isolated and then redissolved in toluene (see Section 4.3). Chromatographic isolation of pure (S,S)-16 in 70% yield was facilitated by the equilibrium between the cyclic oxazolidine structure and the more polar hydroxy-imine tautomer which is capable of chelation. The undesired erythro-amino alcohol favors the open chain structure, is much more polar, and is thus retained on SiO$_2$.

Catalytic osmylation using the Sharpless procedure \textit{in the absence of a chiral auxillary} gave a mixture of triol diastereomers which was acetylated \textit{in situ}, and easily separated by flash chromatography on SiO$_2$ to provide the protected amino tetros 24a and 24b (70% combined, 6:1 ratio). The major diastereomer 24a had the desired stereochemical configuration. That is— all four contiguous chiral centers were introduced stereoselectively in high yield in two steps.

Orthogonal manipulation of the protecting groups, N-methylation, and oxidation of the primary alcohol yielded the desired target structure in 30% overall yield (Scheme 4.36). Reduction of the Schiff base tri-acetate 24a with NaBH$_3$CN under anhydrous acidic conditions to generate benzhydryl 2° amine,\textsuperscript{191} and subsequent reductive methylation was accomplished in a one-pot reaction sequence to provide
compound 32. Desilylation was accomplished with HF in aqueous acetonitrile, and yielded the primary alcohol 33. Desilylation with basic fluoride (nBu₄N⁺F⁻ or

Scheme 4.33

1. DIBAL: TRIBAL hexane, -78°C

2 eq. tBuLi, 0°C

hexane toluene

16a 70% >20 : 1

1) K₂OsO₂(OH)₄ 3mol%

K₂CO₃ / K₃Fe(CN)₆

2) Ac₂O / Pyridine 60 % yield

60

16a 70% >20 : 1

24a 6 : 1

24b

1. HF/CH₃CN

92%

2. Swern ox.

70%

32, R=TBS

33, R=H

34
HF•pyridine) was accompanied by migration (see section 4.6). The α-amino aldehyde 34 was obtained by standard Swern oxidation of 33. This key aldehyde intermediate proved to be very labile under basic conditions. The compound decomposed upon attempts at column chromatography, and Zemplén deacylation (cat. NaOMe/MeOH) led to decomposition of the amino aldehyde. Several attempts to protect the aldehyde as an acetal under acidic conditions also failed (See section 4.6). Deacetylation was finally accomplished with KCN in anhydrous MeOH. In any case, the protected N-methyl-N-diphenylmethylfucosamine 35 was produced in good yield as a mixture of pyranose and furanose anomers.

Conversion of 35 to N-methylfucosamine 43 was accomplished by hydrogenolysis of the benzhydryl group (H₂/Pd-C/MeOH) (Scheme 4.37). Purification of the amino sugar was done on acidic ion-exchange resin, and the desired product was recovered after lyophilization as the free base. In order to confirm the stereochemical configuration of the product, compound 35 was also acetylated (pyridine, Ac₂O) to give the protected pyranose structure 44. Vicinal coupling constants observed in the 250 MHz ¹H-nmr spectrum of 44 in CDCl₃ clearly indicate that the product has the desired galacto configuration. Similar coupling constants are observed with N-methylfucosamine 43 itself in D₂O, although the presence of anomeric species complicates the analysis of this sugar in solution. NMR data for N-methyl-D-fucosamine compounds 35,43,44 is summarized in Table 7.
Scheme 4.37

Scheme 4.37

Table 7 $^1$H NMR and $^{13}$C NMR Data for Cyclic Compounds 35,43,44

<table>
<thead>
<tr>
<th></th>
<th>$^1$H NMR (CDCl$_3$) $\delta$ (J,Hz)</th>
<th>$^1$C NMR (CDCl$_3$) $\delta$</th>
<th>$^1$H NMR (D$_2$O) $\delta$ (J,Hz)</th>
<th>$^1$C NMR (D$_2$O) $\delta$</th>
<th>$^1$HNMR (CDCl$_3$) $\delta$ (J,Hz)</th>
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<td>5.50 (3.5) 87.8</td>
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<td>5.79 (8.8) 3.30 (8.8,11.2)</td>
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<td>2</td>
<td>2.91 (8.3,9,9) 59.6</td>
<td></td>
<td>3.21 (3.25, 11.2) 60.4</td>
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<td>5.16 (11.2, 3.1) 5.08 (3.0)</td>
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<tr>
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*a a mixture of pyranose and furanose anomers is observed in the solution; resonances of major anomers are listed in the table.

b NMR data is in a good agreement with published data$^{189}$

* these resonances differ from published data by Edo et al.$^{189a}$ (1.65, 3.23 ppm)
4.8 The Total Synthesis of L-Azafucose

We applied our synthetic methodology toward the synthesis of L-azafucose (1-deoxyfuconojirimicin) 51 (Scheme 4.5), a known fucosidase inhibitor. The synthesis of began with the crystalline L-alanine Schiff base ester 2 (Scheme 4.38). Addition of iBu₅Al₂H to the Schiff base ester in dichloromethane at -78°C, followed by the addition of the alkenyllithium, generated in hexane from the TBS-protected iodoallylic alcohols 13a, and pivalation of the free hydroxyl group provided the syn-amino alcohol 19b with excellent stereoselectivity (>20:1) and in very good yield (70%). Optimized catalytic osmylation in the presence of (DHQ)₂-PHAL chiral auxiliary (see section 4.5) gave a mixture of triol diastereomers 27a and 27b (14:1) which was separated by flash chromatography on SiO₂ to provide the pure amino tetrol 27a (Scheme 4.38).

Scheme 4.38

```
1. DIBAL : TRIBAL / hexane - 78°C
2. 1 \[\text{OTBS} \]
   \[\text{OTBS} \]
   2 eq. iBuLi / hexane, 0°C; 70%
3. PivCl, Py, DMAP; 89%

\[\text{K}_2\text{OsO}_4\text{(OH)}_2, 1.2\text{mol}\%} \]
\[(\text{DHQ})_2\text{-PHAL, MeSO}_2\text{NII}_2] \]
\[\text{K}_2\text{CO}_3 / \text{K}_3\text{Fe(CN)}_6 \]
\[\text{tBuOH} / \text{H}_2\text{O} 1:1 \]

14 : 1
```

27a
27b
In the presence of acyl protecting groups, both acid-catalyzed and fluoride catalyzed deprotection of the silyl ether was complicated by acyl migration (Section 4.6). To circumvent the migration problem, we chose the base stable MOM group, and examined acylation of the allylic alcohol prior to the osmium catalyzed oxygenation reaction. Thus, pivalate 27a was treated with MOM-Cl\textsuperscript{193} to provide 45 in good yield after purification. Desilylation with TBAF was accomplished without any problems. Oxidation of the 1° alcohol provided the aldehyde 46, which cyclized to the piperidine 47 when treated with NaCNBH\textsubscript{3} in the presence of acetic acid. This "MOM route" (Scheme 4.39) was not completely satisfactory, not only because the MOM-protection/deprotection adds two steps to the synthesis, but also because a considerable amount of inefficiency was introduced due to the requisite chromatography and non-quantitative yields. Also, the high toxicity of MOMCl made this strategy less attractive.

Scheme 4.39
Thus, the protection step was eliminated entirely and selective oxidation of the primary alcohol in the presence of the two secondary alcohols was attempted using TEMPO/NaOCl.\textsuperscript{194} Diol 27a was desilylated with nBu\textsubscript{4}NF in THF to provide the partially protected amino tetrol 48 in 90% yield (Scheme 4.40). TEMPO-mediated oxidation proceeded in excellent yield to provide aldehyde 49 which was cyclized without purification to the protected piperidine diol 50 in 75—80% yield for the two steps.

Scheme 4.40

\[
\begin{align*}
\text{27a} & \xrightarrow{\text{TBAF/THF}} \text{48} \\
\text{48} & \xrightarrow{(\text{TEMPO}) \text{, NaClO, NaHCO}_3, \text{CH}_2\text{Cl}_2} \text{49} \\
\text{49} & \xrightarrow{\text{NaBH}_3\text{CN, AcOH, CH}_3\text{CN}} \text{50}
\end{align*}
\]

This cyclization step deserves a few comments. As shown in Scheme 4.41, the Schiff base imine, probably protonated at pH=5, was reduced selectively in the presence of a very reactive aldehyde to provide the N-benzhydryl secondary amine
which is in equilibrium with the cyclic iminium form. The second hydride addition to the iminium moiety yielded the cyclic tertiary amine.

Scheme 4.41

Deprotection of the piperidine was straightforward (Scheme 4.42). Removal of the pivaloyl group from 50 was accomplished in 80% yield by saponification with nBu₄NOH in aqueous dioxane,¹⁹⁵ and hydrogenolysis of the benzhydryl group was accomplished in MeOH in the presence of HCl and 10% Pd-C with H₂ at atmospheric pressure to provide the potent and specific fucosidase inhibitor, deoxyfuconojiromycin hydrochloride 51 in 95% yield. L-Azafucose was prepared in 8 steps from protected L-alanine in 20% overall yield, which represents the most efficient synthesis at present.
Scheme 4.42

1) nBu4NOH
   Dioxane / H2O
   80%

2) H2 / Pd·C / MeOH
   95%

L - Azafucose
(1 - deoxy - fuconojirimicin)
4.9 The Total Synthesis of L-Azagulose

The application of the same methodology with a reversal of the dihydroxylation and cyclization steps provided L-azagulose, the 3-epimer of azafucose, in 8 steps. Protected L-alanine 2 was subjected to the reduction-alkenylation methodology, this time with Z-iodoalkene 13b. Pure Z-isomer 52 was obtained by subsequent pivalation and flash chromatography (Scheme 4.43). Desilylation with TBAF provided allylic alcohol 53 which was converted to aldehyde 54 in 70% yield. The same cyclization conditions were applied to obtain the unsaturated cyclic amine 55 in 85% yield. Thus, the cyclization was accomplished prior to the double bond dihydroxylation. Osmylation in the presence of 1.2 mol% of K2OsO2(OH)4 and 1 eq. of MeSO2NH2 gave only one diastereomer 56. Stereoselectivity in the cyclic case proved to be superior to that with the corresponding acyclic substrates. Again, the same deprotection conditions as for azafucose provided L-azagulose 57 in 76% yield. The overall yield from methyl L-alaninate 2 was 18%. L-Azagulose is a new compound and investigations of its inhibitory activity against various glycosidases are underway.
Scheme 4.43

1. DIBAL: TRIBAL / hexane - 78 °C
2. 2 eq. tBuLi / hexane, 0 °C
3. PivCl, Py, DMAP

1) nBu₄NOH
Dioxan / H₂O; 80%

2) H₂ / Pd-C / MeOH
95%

L-Azagulose
4.10 Entry to 1-Cyano-1-deoxy-azasugars (Aza-C-Glycosides)

Aldehyde 49 can be viewed as a protected acyclic form of fuconojirimycin. Therefore, we decided to maintain the same oxidation state at the carbon 1 in the cyclic form. We treated aldehyde 49 with HCl in methanol which provided the crude hydrochloride salt of L-fuconojirimycin 58 after removing benzophenone by extraction (Scheme 4.44). The crude material was dissolved in dioxane and water and KCN was added. After 12 hours 1α-cyano-L-azafucose derivative 59a was isolated. The crystalline 59a was obtained in 40% yield over three steps (TEMPO oxidation, deprotection and cyanide treatment). Only a trace of β-isomer 59b was detected by $^1$H NMR.

Alternatively, the Schiff base benzophenone was deprotected with SO$_2$-saturated H$_2$O. Crude sulfite 60 was then treated with an excess of KCN in dioxane and water to yield 40% of α-isomer 59a only. Reprotection with benzyl bromide provided perbenzyl compound 61 (Scheme 4.45). Thus, a straightforward method was developed for the synthesis of α-cyano-azafucose with excellent stereoselectivity. Such compounds are of great use for further manipulations of the cyano group and preparation of fucosidase inhibitors of our design. NMR data for azasugars are summarized in Table 8 and 9.
Scheme 4.44

\[
\begin{align*}
&\text{49} \xrightarrow{\text{HCl/MeOH}} \text{58} \xrightarrow{\text{KCN, dioxane/H}_2\text{O}} \text{59a} \quad 45\% \\
&\quad \quad \quad \text{over three steps}
\end{align*}
\]

Scheme 4.45

\[
\begin{align*}
&\text{59a} \xrightarrow{\text{BnBr xs, NaH, DMF}} \text{61} \quad 60\%
\end{align*}
\]
Table 8

$^{1}H$ NMR and $^{13}C$ NMR Data for Cyclic Compounds

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J$_{1H,1H}$

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Table 9

$^1$H NMR and $^{13}$C NMR Data for Cyclic Compounds

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Ph2CH

J_{1,1}
CHAPTER FIVE

EXPERIMENTAL
General Methods. All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks using modified Schlenk methods. All solvents were dried over the standard drying agents and freshly distilled prior to use. For flash chromatography, 400-230 mesh silica gel 60 (E. Merck No. 9385) was employed. All compounds described were characterized by IR as well as $^1$H- and $^{13}$C-NMR spectroscopy. The $^1$H- and $^{13}$C-nmr spectra were obtained on a Bruker WM-250 spectrometer at 250 and 62.9 MHz, or on a Varian Gemini instrument at 300 MHz. COSY spectra were obtained on a Bruker AM-500 spectrometer at 500 MHz. Chemical shifts are reported in $\delta$ vs Me$_4$Si in $^1$H spectra and vs CDCl$_3$ in $^{13}$C spectra. Infrared spectra were obtained on a Perkin Elmer 1600 Series FTIR. Optical rotations were measured on a Randolph Research, AutoPol III polarimeter using the Na-D line. Mass spectra were obtained on a JEOL JMS-01SG-2 or a Finnegan MAT-90.
5.1 Preparation of Haloalkenes.

**Methyl O-(tert-butylidimethylsilyl)-N-(diphenylmethylene)-L-serinate (1):**

Compound 1 was prepared following the published procedure.¹³⁷

**Methyl N-(diphenylmethylene)-L-alaninate (2):**

Compound 1 was prepared following the published procedure.¹³⁷

**1-Bromo-1E-Propene (3):**

The commercially available mixture of 1-bromo-1-propene (50.0 g, 0.41 mol, 3:7 E/Z) was treated with NaOH (17.0 g, 0.30 mol) in 200 mL of n-butanol. The mixture was refluxed for 5 days. N-Butanol and 1-bromo-1-propene were separated by fractional distillation with a distillation column. The liquid was filtered through anhydrous Al₂O₃ (grade I) to yield 10.0 g of anhydrous 1-bromo-1-propene (30%, b.p. 57°C, E only).

3: 

³H NMR (250 MHz, CDCl₃) δ 6.12 (dq, J = 13.2, 6.6 Hz, 1H), 5.99 (dq, J = 13.3, 1.5 Hz, 1H), 1.68 (dd, J = 6.6, 1.5 Hz).

**3-Bromo-2E-propene-1-ol (4a):**¹⁴⁰

CAUTION: 1-halo-propynes are potentially explosive. Do not heat, and perform these operations behind a blast shield. Bromine (48.00 g, 300 mmol) was added to vigorously stirring solution of 45 g KOH in 120 mL of water at -5°C. The yellow solution was kept at 0°C and added dropwise to propargyl alcohol (17.83 g, 1.06 eq.; freshly distilled) in 39 mL of water at -7 - 0°C. The addition took approximately 3
hours. The mixture was warmed to 10°C and then extracted four times with ether. The ether layer was washed with Na₂S₂O₃, dried over K₂CO₃ and the solvent was removed to provide 28.50 g of the crude 1-bromoopropyne-3-ol. DO NOT DISTILL!

A 2L flask was charged with LiAlH₄ (20.30 g, 2 eq.) and AlCl₃ (53.50g, 1 eq.). Anhydrous ether (270 mL, distilled from K/Na/benzophenone) was added carefully at -5°C. The crude product, prepared in the first step, was added dropwise and the mixture was refluxed for four hours. The reaction was quenched with 200mL of wet ether (H₂O saturated), followed by 20 mL of water and 20 mL of 5% NaOH at -10°C. Additional 60 mL of water was added to break up the solid mass. The liquid was decanted and extracted three times with ether. The ether layer was dried over K₂CO₃ and the solvent was removed. The crude material was vacuum distilled (67°C, 110 mbar) to yield pure bromoalkene 4a (27.7 g, 70% for two steps).

4a: ¹H NMR (250 MHz, CDCl₃) δ 6.36 (m, 2H), 4.13 (apparent d, 2H), 2.51 (1H, OH).

1-Tert-butyldimethylsilyloxy-3-iodo-2E-propene (13a):

TBS-propynol (20 g, 117.4 mmol), tributyltin hydride (63.2 mL, 152.6 mmol, 1.3 eq.) and AIBN (150 mg) were stirred and heated at 110 - 130°C for 2 hours. Then, the mixture was distilled in vacuo. An excess of tributyltin hydride can be separated as the first fraction (65 - 70°C, 0.4 mm). The second fraction (100 - 130°C) yielded 53.68 g (99%) of vinylstannanes (E:Z:Terminal 69% : 16% : 15%). 53.68 g (116 mmol) of vinylstannanes was dissolved in 700mL of CH₂Cl₂ and the solution of I₂ (32.41 g, 1.1 eq.) was added dropwise at rt until the solution in the reaction vessel remained brown. The mixture was washed with saturated Na₂S₂O₃, water and dried over K₂CO₃. The solvent was removed and the crude product was purified by vacuum
distillation. The first fraction (58 - 61°C, 0.4 mm) yielded 21.03g of vinyliodides (E:Z:Terminal 3:1:1) and the second fraction (61 - 65°C, 0.4mm) yielded E-enriched vinyliodides (E:Z: Terminal 8:1:1). A total yield of 84% was accomplished. The product was stored in a brown reagent bottle at low temperature (-20°C). A piece of copper wire was added to slow decomposition. With the proper storage, as described above, vinyliodides can be stored for several months without significant decomposition.

13a: E-isomer: $^1$H NMR (250MHz, CDCl$_3$) δ 6.58 (dt, J= 14.3, 4.4 Hz, 1H), 6.27 (dt, J= 14.5, 1.8 Hz, 1H), 4.09 (dd, J= 4.7, 1.8 Hz, 2H), 0.88 (s, 9H), 0.05 (s, 6H); Z isomer: 4.22 (dd, J= 5.3, 1.8 Hz, 2H); Terminal isomer: 4.15 (t, J= 1.8 Hz); $^{13}$C NMR (250MHz, CDCl$_3$) δ 136.69 (C3), 106.06 (C2), 63.24 (C1), 25.80 (tBu), -5.40 (SiMe).

1-Benzylxoo-3-iodo-2E-propene (12a):

Compound 12a was prepared following the same procedure as for TBS-protected vinyliodide 13a. The product (105°C, 0.5mm) was obtained in 80% yield as a mixture of E and Z (E:Z 8:1).

12a: $^1$H NMR (250MHz, CDCl$_3$) δ E isomer: 7.39 - 7.25 (m, 5H), 6.66 (dt, J= 14.6, 5.7 Hz, 1H), 6.40 (dt, J= 14.6, 1.5 Hz, 1H), 4.51 (s, 2H), 3.95 (dd, J= 5.6, 1.5 Hz, 2H); Z isomer: 4.22 (dd, J= 5.3, 1.8 Hz, 2H); Terminal isomer: 4.15 (t, J= 1.8 Hz, 2H).
5.2 Preparation of Alkenyllithiums and Protected Amino Alcohols.

Preparation of E-1-Lithio-1-propene:
Distilled E-BrCH=CHCH₃ (4.0 mmol, 484 mg) and 8 ml hexane were cooled to 0°C and tBuLi (4.8 ml, 2.2 eq) added dropwise with vigorous stirring. The mixture was stirred 30 min. at 0°C and then warmed to RT and stirred for another hour to form a white precipitate (LiBr and propenyllithium). The mixture was allowed to stand at RT for 30 min., and the upper hexane layer was syringed out and 12 ml toluene added. The mixture was stirred vigorously for 15 min before use. Gilman titration showed that the solubility of propenyllithium is four times greater in toluene than in hexane.

Preparation of E-1-Lithio-3-benzyloxy-1-propene and E-1-Lithio-3-tert-butyldimethylsilyloxy-1-propene:
Distilled E-BnO-CH₂CH=CH-I (4.0 mmol, 1.096 g) or E-TBDMSO-CH₂CH=CH-I (4 mmol, 1.197 g) and 8 ml of hexane were cooled to 0°C and tBuLi (4.8 ml, 2.2 eq) added dropwise with vigorous stirring. The mixture was stirred for 30 min. at 0°C to form a white precipitate (LiBr). It is strongly recommended to monitor temperature inside of the reaction vessel, especially when run on a large scale.

(2S,3S)-2-[N-(Diphenylmethylene)amino]-4E-hexen-3-ol (14):
Schiff base ester 2 (1.0 mmol, 267 mg) and 10 ml of CH₂Cl₂ were cooled to -78°C and 1.1 eq iBu₅Al₂H (2.2 ml of 0.5M solution in hexane) was added via syringe pump over 15—20 min. After addition was complete, 4.0 eq. E-1-Lithio-1-propene (12 ml of 1.6 M solution in toluene) was slowly cannulated into the reaction flask. The reaction was stirred for 1 hr at -78°C, then warmed to RT. After stiring 1 hr at RT, the reaction was quenched with 1% NaHCO₃ at -10°C. The reaction mixture was extracted
with Et₂O, dried over K₂CO₃, filtered through Celite® and the solvent removed in vacuo. The product was purified via flash chromatography to provide pure 14 (196 mg, 70%).

\(^1\)H NMR (250 MHz, CDCl₃) δ 7.69 - 7.16 (m, 10H), 5.73 (dq, J = 15.3, 6.5, 0.6 Hz, 1H), 5.33 (dd, J = 15.3, 8.4 Hz, 1H), 3.82 (dd, J observed = 8.4 Hz; J₂,₃ = 8.5 Hz, determined via decoupling at position 1; 1H); 2.95 (m, 1H), 1.68 (dd, J = 6.5, 1.7 Hz, 3H), 1.17 (d, J = 6.4, 3H); \(^1\)C NMR (APT, CDCl₃) δ 145.84, 145.36 (quart. aromatic), 130.00, 129.90, 128.23, 127.95, 127.42, 127.06, 126.27, 125.42 (aromatic), 99.30 (C=N), 87.31 (CH-O), 59.52 (CH-N), 17.83, 15.90 (Me); IR (neat) \(\nu_{\text{max}}\) 3295.9 (NH), 2961.9, 2916.8 (C-H), 1597.9, 1449.8 (C-O), 967.2; \([\alpha]_D^\circ +5.3°.

(2S,3S,4E)-2-[N-(Diphenylmethylene)amino]-1-O-(tert-butyl-dimethylsilyl)-4E-hexen-1,3-diol (16a):

Schiff base ester 1 (1.0 mmol, 398 mg) and 10ml of CH₂Cl₂ were cooled to -78° and 1.1 eq iBu₅Al₂H (2.2ml of 0.5M solution in hexane) was added via syringe pump over 15—20 min. After addition was complete, 4.0 eq. E-1-Lithio-1-propene (12 ml of 1.6 M solution in toluene) was slowly cannulated into the reaction flask. The reaction was stirred for 1hr at -78° C, then warmed to RT. After stirring 1hr at RT, the reaction was quenched with 1% NaHCO₃ at low temperature. The reaction mixture was extracted with Et₂O, dried over K₂CO₃, filtered through Celite® and the solvent removed in vacuo. The product was purified via flash chromatography to provide pure 16a (287 mg, 70%).

\(^1\)H NMR (250MHz, CDCl₃) δ 0.01 (s, 3H, SiCH₃), 0.03(s, 3H, SiCH₃), 0.82 (s, 9H, tBu), 1.67 (dd, 3H, J=1.6, 6.5 Hz, CH₃), 2.92 (ddd, unresolved, 1H, CH-N),
3.62 (1/2 AB dd, 1H, J= 10.6, 2.2 Hz, TBDMS-OCH₃), 3.84 (1/2 AB dd, 1H, J= 10.6, 3.45 Hz, TBDMS-OCH₃), 4.29 (t, 1H, J= 8.3 Hz), 5.27 (ddd, 1H, J= 15.2, 6.7, 1.6 Hz, CH=CH-CH₃), 5.69 (dq, J= 15.2, 6.5, CH=CH-CH₃), 7.15 - 7.81 (m, 10H, aromatic); $^{13}$C NMR (APT,CDCl₃) δ -5.47 (SiCH₃), 17.8 (CH₃), 25.77 (tBu), 59.45 (CH₂O), 65.08 (CH-N), 80.84 (CH-O), 99.48 (CH=N), 125.71, 126.66, 127.13, 127.43, 127.92, 128.097, 128.28, 129.69, 130.66 (aromatic CH), 145.55 (q aromatic); IR (neat) $\nu_{\text{max}}$ 3309.9, 3059.1, 2928.3, 2856.6, 1664.1, 1598.8, 1449.9 cm$^{-1}$; MS (Cl) 411(MH$^+$, bp), 410 (M$^+$), 394 (MH - OH), 392 (M - H₂O), 368 (M - CH₃CH=CH₂), 352 (M - (CH₃)$_3$CH ), 339 (M - CH₃CH=CH₂,CHO), 338 (M - CH₃CHCH₂ - CH₂O); [α]D $^{22}$° (c=0.1, CHCl₃)

(2S,3S,4E)-3-Acetoxy-2-[N-(diphenylmethylene)amino]-1-O-(tert-butyldimethylsilyl)-4-hexen-1-ol (16b):

Compound 16a was dissolved in pyridine (1 mL), a catalytic amount of DMAP was added and the mixture was chilled to 0°C. Acetic anhydride was added dropwise. The reaction mixture was stirred for 24 hours at room temperature. Toluene was added and the solvent was removed in vacuo. The crude product was purified by flash chromatography (5% EtOAc/Hexane) to yield 220 mg (95%) of pure 16b.

$^1$H NMR (CDCl₃) δ -0.05 (s, 3H, SiCH₃), 0.00 (s, 3H, SiCH₃), 0.83 (s, 9H, tBu), 1.66 (dd, 3M, J= 1.1, 6.6 Hz, CH₃), 2.00 (s, 3H, Acetate CH₃), 3.67 - 3.81 (m, 3H, CH-N, CH₂-O, unresolved), 5.26-5.43 (m, 2H, CH-OAc, CH=CH-CH₃), 5.73 (dq, 1H, J = 6.5, 15.2 Hz, CH=CH-CH₃), 7.20 - 7.60 (m, 10 H, unresolved, aromatic); $^{13}$C NMR (CDCl₃) δ -5.5 (SiCH₃), 17.8 (CH₃), 21.3 (CH₃COO), 25.9 (tBu), 64.1 (CH₂O), 66.8 (CH-N), 75.0 (CH-OAc), 127.2, 127.9, 128.1, 128.2, 128.5, 128.6, 129.8, 130.6 (CH, aromatic), 136.8 (q aromatic), 139.4 (q
aromatic), 170.0 (CH$_3$COO); IR (neat) ν 3060.3, 2929.0, 1740.2, 1662.1, 1595.2, 1371.0, 1221.0 cm$^{-1}$; [α]$_D$ +2.7° (C= 1.1, CHCl$_3$).

(4S,5S,2E)-5-[N-(Di phenyl methyl ene) amino]-1-O-benzyl-2E-hexen-1,4-diol (18):

Schiff base ester 2 (1.0 mmol, 267 mg) and 10 ml of CH$_2$Cl$_2$ were cooled to -78° and 1.1 eq iBu$_5$Al$_2$H (2.2ml of 0.5M solution in hexane) was added via syringe pump over 15—20 min. After addition was complete, 3.0 eq. E-1-lithio-3-benzyloxy-1-propene was slowly cannulated into the reaction flask. The reaction was stirred for 1 hr at -78° C, then warmed to RT. After stirring 1hr at RT, the reaction was cooled and quenched by adding wet ether and 10 ml of 1% NaHCO$_3$. The reaction mixture was extracted with Et$_2$O, dried over K$_2$CO$_3$, filtered through Celite and the solvent removed in vacuo. The product was purified via flash chromatography to provide pure 18 (259 mg, 60%).

$^1$H NMR (250 MHz, CDC$_3$) δ 7.82 - 7.18 (m, 15H), 5.87 (dt, J=5.6, 15.4 Hz), 5.60 (dd, J= 7.7, 15.4 Hz, 1H), 4.49 (s, 2H), 4.00 (d, J=5.5 Hz, 2H), 3.92 (t, J=4.0 Hz, 1H), 3.00 (broad quintet, 1H), 1.21 (d, J=6.3 Hz, 3H); $^{13}$C NMR (CDCl$_3$) δ 145.6, 145.1 (quaternary aromatic), 131.7, 130.0 (CH=CH), 128.5, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.1, 126.3, 125.4 (aromatic), 99.7 (OCN), 86.3 (CH$_2$Ph), 72.1 (CH-O), 69.9 (OCH$_2$), 59.7 (CHN), 16.0 (CH$_3$); IR (neat) ν$_{max}$ 3152.3 (C-H), 2930.2 (C-H), 1645.5 (C=C), 1326.7 (C-O). [α]$_D$ + 3.1° (0.1, CHCl$_3$).
(4S,5S,2E)-5-[N-(Diphenylmethylene)amino]-1-O-(tert-butyldimethylsilyl)-2E-hexen-1,4-diol (19a).

Schiff base ester 2 (1.0 mmol, 267 mg) and 10 ml of CH₂Cl₂ were cooled to -78° and 1.1 eq iBu₅Al₂H (2.2 ml of 0.5M solution in hexane) was added via syringe pump over 15—20 min. After addition was complete, 3.0 eq. E-1-Lithio-3-tert-butyldimethylsilyloxy-1-propene was slowly cannulated into the reaction flask. The reaction was stirred for 1 hr at -78° C, then warmed to RT. After stirring 1 hr at RT, the reaction was cooled and quenched by adding wet ether and 1-mL of 1% NaHCO₃. The reaction mixture was extracted with Et₂O, dried over K₂CO₃, filtered through Celite and the solvent removed in vacuo. The product was purified via flash chromatography to provide pure 19a (290 mg, 70%).

¹H NMR (250MHz, CDCl₃) δ 7.81-7.15 (m, 10H), 5.79 (ddt, J=0.6, 4.4, 15.4 Hz), 5.51 (ddt, J=1.65, 7.85, 15.4 Hz, 1H), 4.13 (dd, J=1.6, 4.5 Hz, 2H), 3.89 (t, J=8.1 Hz, 1H), 2.95 (broad m, 1H), 2.39 (broad s, 1H), 1.19 (d, J=6.3 Hz, 3H), 0.85 (s, 9H), 0.01 (s, 3H), -0.02 (s, 3H); ¹³C NMR (APT,CDCl₃) δ 145.60, 145.20 (CH=CH), 128.31, 128.12, 126.78 (aromatic), 70.03 (CH-O), 63.05 (CH₂-O), 59.73 (CH-N), 15.96 (CH₃); IR (neat) νmax 3315.1, 3048.3, 2937.3 (C-H), 1662.9 (C=O), 1584.3, 1350.0 (C-O); [α]D 4.4° (c=0.1, CHCl₃)

(4S,5S)-5-[N-(Diphenylmethylene)amino]-4-pivaloyloxy-1-O-(tert-butyldimethylsilyl)-2E-hexen-1-ol (19b):

The alcohol 19a (820 mg, 2mmol) and DMAP (48.8mg, 0.4mmol) was dissolved in 8 mL of pyridine. Pivaloyl chloride (1.48 mL, 12 mmol) was added dropwise via syringe while stirring the mixture at rt. Stirring was continued until completion (2 days). The mixture was poured into water and ice and the aqueous solution was
extracted three times with CH$_2$Cl$_2$. The organic solution was dried with MgSO$_4$, filtered through celite and the solvent was removed. The crude sample was flash chromatography on silica gel (5% to 10% EtOAc/ hexanes) to yield 880 mg (89%) of pure 19b.

19b: colorless oil; $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 7.58 - 7.17 (m, 10H), 5.84 (dt, $J$=4.3, 15.3 Hz), 5.64 (tdd, $J$=1.6, 6.9, 15.3), 5.45 (pseudo t, $J$=7.2 Hz), 4.17 (d, $J$=3.7 Hz), 3.57 (pseudo q, $J$=6.5 Hz), 1.14 (s, 9H), 1.13 (d, 3H), 0.88 (s, 9H), 0.04 (s, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 139.87, 133.55, 129.84, 128.49, 128.37, 128.29, 127.76, 125.52 (aromatic), 77.53, 62.97, 60.18, 27.22 (tBu), 25.86 (tBu), 18.30 (CH$_3$), -5.25 (CH$_3$-Si); IR (neat) $\nu_{max}$ 3250.1, 2945.3 (C-H), 1729.5 (C=O), 1345.1, 1215.3 (C-O); $[\alpha]_D$ 5.6 (c=0.5, CHCl$_3$); elemental anal: C$_{30}$H$_{43}$N$_2$O$_3$Si theoretical C 72.98%, H 8.78%, found C 73.10%, H 8.72%.

(4S,5S,2E)-5-[N-(Diphenylmethylene)amino]-4-pivaloyloxy-1-O-(tert-butyldimethyl-silyl)-2Z-hexen-1-ol (52):

The compound 52 was prepared in the same way as 19b. (30% EtOAc/ hexane, 90% yield) oil.

$^1$H NMR (CDCl$_3$) $\delta$ 7.60 - 7.15 (m, 10H), 5.72 (pseudo q, 1H), 5.61 (pseudo t, $J$=8.8Hz, 1H), 4.46 (ddd, $J$=1.7, 5.9, 13.8Hz, 1/2AB, 1H), 4.36 (ddd, $J$=1.8, 5.7, 13.8 Hz, 1/2AB), 3.56 (pseudo q, 1H), 1.14 (d, CH$_3$), 1.11 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 177.16 (C=O), 136.67, 135.70, 129.90, 128.49, 128.40, 127.96, 127.72, 125.34 (aromatic), 74.97 (CH-O), 73.94 (CH$_2$-O), 60.06 (CH-N), 27.19 (tBu), 25.90 (tBu), 17.93 (CH$_3$), -5.19
(CH₃); IR (neat) νmax 2956.3 (C-H), 1731.7 (C=O), 1631.3 (C=C), 1352.1 (C-O); [α]D 7.3 (c=0.07, CHCl₃).

(4S,5S)-4-Methyl-5-[-(E)-prop-1-en-1-yl]-2-oxazolidinone (21):
Amino alcohol 20 (45 mg, 0.39 mmol) was dissolved in dry THF and carbonyldimidazole (CDI, 91 mg, 0.56 mmol) was added. The mixture was stirred for two hours at RT (completion detected by TLC). The solvent was removed and the crude material was purified via flash chromatography (50% EtOAc/ hexanes) to yield pure 21 (47 mg, 85%).

21: colorless oil; ¹H NMR (250 MHz, CDCl₃) δ 5.85 (ddq, J = 15.2, 6.6, 0.7 Hz), 5.51 (m, 1H), 4.41 (pseudo t, J = 7.7 Hz; J₂,3 = 7.5 Hz determined via decoupling of Me group); 3.61 (m, 1H), 1.72 (dd, J = 6.6, 1.4 Hz, 3H); 1.24 (d, J = 6.2 Hz, 3H); ¹³C NMR (APT, CDCl₃) δ 132.65, 126.67 (CH=CH), 85.08 (CH-O), 54.14 (CH-N), 19.26, 17.76 (Me).

(4S,5S)-2,2-Diphenyl-4-methyl-5-[-(E)-prop-1-en-1-yl]-N-(trichloroacetyl-carbamoyl)-oxazolidine (22):
Compound 22 was prepared by addition of one drop of trichloroacetyl-isocyanate (TAI) to the NMR sample of alcohol 14 (5 mg of alcohol 14 in 0.5 mL of CDCl₃). Cyclic product 22 was formed instantaneously and quantitatively as judged by NMR.

22: ¹H NMR (250 MHz, CDCl₃) δ 7.81 - 7.21 (m, 10H), 5.81 (dq, J = 15.2, 6.4 Hz), 5.61 (ddd, J = 15.2, 7.8, 1.4 Hz, 1H), 4.15 (dq, J = 8.6, 6.1 Hz, 1H), 3.82 (dd, J = 8.2 Hz; J₂,3 = 8.7 Hz, determined via decoupling of Me group, 1H), 1.73 (dd, J = 6.4, 1.4 Hz, 3H), 1.36 (d, J = 6.1 Hz); ¹³C NMR (APT, CDCl₃) δ 139.56,
(4S,5S)-2,2-Diphenyl-4-[(tert-butyldimethylsiloxy)-methyl]-5-[(E)-prop-1-en-1-yl]-N-(trichloroacetyl-carbamoyl)-oxazolidine (23):

23: $^1$H NMR (250 MHz, CDCl$_3$) δ 7.54-7.17 (m, 10H), 5.82 (dq, $J = 15.4$, 6.5 Hz, 1H), 5.64 (ddd, $J = 15.3$, 7.4, 1.5 Hz, 1H), 4.35 (apparent t, $J = 7.7$ Hz, 1H), 4.16 (ddd, $J = 8.2$, 5.9, 3.5 Hz, 1H), 3.94 (m, 1H, 1/2 AB), 3.82 (dd, $J = 10.3$, 5.9 Hz, 1H), 1.72 (dd, $J = 6.3$, 1.3 Hz, 3H), 0.75 (s, 9H), -0.05 (s, 6H); $^{13}$C NMR (APT, CDCl$_3$) δ 139.83, 138.67 (quart. aromatic), 132.15, 130.32, 130.04, 129.22, 128.83, 128.69, 128.24, 128.15, 127.77, 127.72, 127.56 (aromatic), 79.25 (CH-O), 64.66 (CH$_2$-O), 60.96 (CH-N), 25.76 (tBu), 17.85 (Me), -5.39 (SiMe), -5.54 (SiMe).
5.3 Catalytic Dihydroxylation.

**Procedure A (in the absence of a chiral auxiliary):**

A flask was charged with 5 mL of water, 5 mL of tert-butanol, 0.98 g of K$_3$Fe(CN)$_6$ (3 mmol), 0.42 g K$_2$CO$_3$ (3 mmol), 10.8 mg of K$_2$OsO$_2$(OH)$_4$ (3 mol %) and 95 mg of MeSO$_2$NH$_2$. The mixture was stirred at room temperature until both phases were clear. The olefin (1 mmol) was added at once, and the heterogeneous mixture was vigorously stirred at room temperature until the starting material was consumed (TLC). Na$_2$SO$_3$ (2.3 g) was added and the mixture was stirred for 20 min. The phases were separated and the aqueous layer was extracted with three portions of chloroform. The combined organic phase was dried over MgSO$_4$, filtered through celite and dried in vacuo to give a crude mixture of diastereomeric triols.

**Procedure B (in the presence of a (DHQ)$_2$PHAL chiral auxiliary):**

A container was charged with 5 mL of water, 5 mL of tert-butanol, K$_3$Fe(CN)$_6$ (0.98 g, 3 mmol), K$_2$CO$_3$ (0.42 g, 3 mmol), K$_2$OsO$_2$(OH)$_4$ (4.3 mg, 1.2 mol %), and MeSO$_2$NH$_2$ (95 mg, 1 mmol) and (DHQ)$_2$PHAL (23.8 mg, 3 mol %). The mixture is stirred at room temperature until both phases were clear, and then added to a flask with the olefin (1 mmol) at once, and the heterogeneous mixture was vigorously stirred at room temperature until the starting material was consumed (followed by TLC). Na$_2$SO$_3$ (2.3 g) was added and the mixture was stirred for 20 min. Two phases were separated and the aqueous layer was extracted with three portions of chloroform. Combined organic phase was dried over MgSO$_4$, filtered through Celite and dried in vacuo to give a crude mixture of diastereomeric triols.
(2S,3S,4S,5R)-3,4,5-Triacetoxy-2-[N-(diphenylmethylene)amino]-1-O-(tert-butyldimethylsilyl)hexan-1-ol (24a):

A mixture of K₃Fe(CN)₆ (0.49 g, 1.5 mmol, 3 eq), K₂CO₃ (0.21 g, 1.5 mmol, 3 eq), and K₂O₂SO₂(OH)₄ (5.6 mg, 3 mol%) in 6.7 ml H₂O was prepared. Compound 16a was dissolved in 6.7 ml of tBuOH and added to the aqueous solution. The mixture was stirred vigorously at RT. After 24 hrs the reaction was complete (TLC). Na₂SO₃ (0.375 g) was added and the mixture was stirred for 20 min. Two phases were separated and the aqueous layer was extracted with three portions of chloroform. The combined organic phase was dried over MgSO₄, filtered through Celite and dried in vacuo. Without purification the mixture was dissolved in 1 ml pyridine, catalytic amount of DMAP was added and the mixture was chilled to 0°C. One ml of Ac₂O was added dropwise. The reaction was complete in 3 hrs. Solvent was removed and the mixture of 24a and 24b was separated by flash chromatography (5% EtOAc in hexane increased to 25% EtOAc in hexane during chromatography) to yield pure 24a (170 mg, 60%) and pure 24b (28 mg, 10%).

24a: colorless oil; ¹H NMR (250 MHz, CDCl₃) δ -0.07 (s, 3H, SiCH₃), 0.00 (s, 3H, SiCH₃), 0.84 (s, 9H, tBu), 1.09 (d, 3H, J= 6.4 Hz, CH₃), 1.85 (s, 3H, Ac), 1.97 (s, 3H, Ac), 2.06 (s, 3H, Ac), 3.65 - 3.69 (m, 1H, unresolved, CH-N), 3.72 - 3.85 (m, 2H, unresolved, CH₂O), 5.13 (dq, 1H, J= 3.65, 6.5 Hz, C(5)H-OAc), 5.20 (dd, 1H, J= 3.6, 7.0 Hz, C(4)H-OAc), 5.39 (dd, 1H, J= 3.3, 7.0 Hz, C(3)H-OAc), 7.23 - 7.58 (m, 10H, unresolved); ¹³C NMR (CDCl₃) δ -5.48 (CH₃Si), 16.6 (CH₃), 20.84 (acetate CH₃), 20.97 (acetate CH₃), 21.06 (acetate CH₃), 25.85 (tBu), 63.43 (CH₂O), 63.62 (CH₃-N), 68.22 (CH-OAc), 70.54 (CH-OAc), 72.93 (CH-OAc), 127.94, 128.22, 128.51, 128.62, 128.71, 130.04 (aromatic CH), 135.92 (q aromatic), 140.20 (q aromatic), 169.85 (acetate C=O), 170.00 (acetate
C=O), 170.25 (acetate C=O); IR (neat) νₘₐₓ 3058.0, 3023.9, 2928.1, 2856.9, 1742.2, 1698.9, 1625.0, 1578.1, 1370.2, 1251.6, 1220.8 cm⁻¹; MS (CI) 570 (MH⁺), 512 (MH - (CH₃)₃CH), 424 (MH - TBDMS-OMe), 364 (MH - (TBDMS-OMe) - CH₃COOH), 338 (TBDMS-OMeCH₂=N=CPh₂), 280 (338 - (CH₃)₃CH); [α]²⁵d +2.15° (c= 0.04, CHCl₃).

24b: ¹H NMR (CDCl₃) δ -0.05 (s, 3H, SiCH₃), 0.01 (s, 3H, SiCH₃), 0.86 (s, 9H, tBu), 1.23 (d, 3H, J= 6.4 Hz, CH₃), 3.63 - 3.70 (m, 1H, unresolved, CH-N), 3.74 - 3.77 (m, 2H, unresolved, CH₂-O), 4.89 (quintet, 1H, J= 6.6 Hz, C(5)H-OAc), 5.25 (dd, 1H, J= 3.6, 6.9 Hz, CH-OAc), 5.50 (dd, 1H, J= 3.6, 7.0 Hz), 7.20 - 7.62 (m, 10H, unresolved, aromatic); ¹³C NMR (CDCl₃) δ -5.5 (CH₃Si), 16.42 (CH₃), 20.41 (CH₃COO), 20.92 (2 CH₃COO), 25.80 (tBu), 63.35 (CH₂O), 63.65 (CH-N), 69.15 (CH-OAc), 70.76 (CH-OAc), 73.15 (CH-OAc), 127.99, 128.17, 128.29, 128.40, 128.62 (aromatic CH), 130.14 (q aromatic), 167.75 (CH₃COO), 169.95 (2 CH₃COO).

(2R,3R,4R,5S)-2,3,4-Triacetoxy-5-[N-(Diphenylmethylene)amino]-1-O-benzylhexan-1-ol (25a):

Compound 18 was prepared following procedure A. Without purification the crude mixture was dissolved in 2 ml pyridine, a catalytic amount of DMAP was added and the mixture was cooled to 0° C. Two ml of Ac₂O were added dropwise. The reaction was complete in 3 hrs. Solvent was removed and the mixture of 25a and 25b (4 : 1) was separated by gradient flash chromatography (5 - 20% EtOAc in hexanes) to yield pure 25a (110 mg, 52%) and pure 25b (28 mg, 10%).

25a: colorless oil; ¹H NMR (250 MHz, CDCl₃) δ 7.58 - 7.12 (m, 15H), 5.43 (dd, J = 2.8, 7.6 Hz, 1H), 5.35 (m, J= 2.8 Hz, 1H), 5.25 (dd, J = 4.5, 7.5 Hz, 1H), 4.38
(dd, J = 10.1, 11.75 Hz, 2H), 3.64 (m, 1H), 3.47 (1/2 AB dd, J = 5.2, 10.3 Hz, 1H), 3.39 (1/2 AB dd, J = 6.1, 10.3, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 1.87 (s, 3H), 1.19 (d, J = 6.5 Hz, 3H); ¹³C NMR (CDCl₃) δ 170.3, 169.8 (Ac), 137.7 (quartenary aromatic), 130.1, 128.6, 128.4, 128.3, 128.1, 127.9, 127.7, 127.6 (aromatic), 73.4, 73.1, 70.1 (CHOAc), 69.4 (CH₂Ph), 68.6 (CH₂O), 56.9 (CH-N), 21.1, 20.9, 18.5 (CH₃); IR (neat) νmax 2983.1 (C-C), 1745.1 (C=O), 1665.8 (aromatic), 1358.7, 1261.3 (C-O).

(2S, 3S, 4S, 5R)-3, 4, 5-Triacetoxy-2-[N-methyl, N-(diphenylmethyl)-amino]-1-O-(tert-butyldimethylsilyl)hexan-1-ol (32):
A reaction flask was charged with NaBH₃CN (26.4 mg, 1.6 eq) under an argon atmosphere. Compound 24a (150 mg, 0.26 mmol) in 1.2 ml of anhydrous acetonitrile was added to the flask via syringe. Glacial acetic acid was added dropwise to keep the pH near neutrality. The reduction was complete in 10 min. (TLC). Aqueous formaldehyde solution (37%, 420 μl, 20 eq) and additional NaBH₃CN (102 mg, 6 eq) were added. An exothermic reaction ensued. The reaction was allowed to cool to RT while stirring. Glacial acetic acid was added dropwise until the solution tested neutral on wet pH paper. After 2 hrs the reaction was diluted with ether and washed with conc. NaHCO₃ solution, then with water. The organic phase was dried over K₂CO₃, filtered through Celite and dried in vacuo. The crude product was purified by flash chromatography (5% EtOAc in hexane) to yield pure 32 (140 mg, 89%).

32: colorless oil; ¹H NMR (CDCl₃) δ 0.03 (s, 3H, SiCH₃), 0.04 (s, 3H, SiCH₃), 0.89 (s, 9H, tBu), 1.07 (d, 3H, J= 6.5 Hz), 1.96 (s, 3H, acetate CH₃), 2.03 (s, 3H, acetate CH₃), 2.18 (s, 3H, acetate CH₃), 2.24 (s, 3H, N-CH₃), 3.00 - 3.10 (m, 1H, unresolved, CH-N), 3.65 - 3.73 (m, 2H, unresolved, CH₂-O), 4.83 (s, 1H, N-
(2R,3R,4R,5S)-2,3,4-Triacetoxy-5-[N-(diphenylmethylene)amino]-1-O-(tert-butylidimethylsilyl)-hexan-1-ol (26a):

$^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 7.54 - 7.22 (m, 10H), 5.40 (dd, $J = 7.5$, 4.4 Hz, 1H), 5.13 (m, 1H), 3.64 (m, 1H), 3.56 (dd, $J = 10.4$, 5.3, 1H, 1/2AB), 3.43 (dd, $J = 10.4$, 5.8 Hz, 1H, 1/2AB), 2.07 (s, 3H), 2.01 (s, 3H), 1.92 (s, 3H), 0.86 (s, 9H), 0.91 (d, 1H), -0.05 (s, 3H), -0.07 (s, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 170.51, 170.30, 169.71 (Ac), 140.21 (quart. arom.), 131.31, 127.51, 127.30, 127.12, 127.00 (arom.), 73.10, 72.63, 70.23 (CH-OAc), 67.31 (CH2-O), 55.93 (CH-N), 22.32, 21.61, 20.68 (CH$_3$), 17.91 (CH$_3$); IR (neat) $\nu_{max}$ 2953.1 (C-H), 1740.1 (C=O), 1365.3, 1262.1, 1250.1, 1212.3 (C-O). $[\alpha]_D +5.4^\circ$ (c=0.4 CHCl$_3$); elemental anal. calculated for C$_{31}$H$_{43}$NO$_7$Si C 65.35%, H 7.61%, found C 65.39%, H 7.65%.
(2R,3R,4R,5S)-2,3,4-Tribenzoyloxy-5-[N-(diphenyl-methylene)-amino]-1-O-(tert-butyldimethylsilyl)hexan-1-ol (36a/36b):

Compound 19a (900 mg, 2.2 mmol) was dihydroxylated (c.f. A) and benzoyl protected following the standard protocol (BzCl/Py) to yield a mixture of 36a/37b (915 mg, 65%), which could not be separated by flash chromatography.

$^1$H NMR (250 MHz, CDCl$_3$) δ 7.92 - 6.83 (m, 25H), 5.84 (dd, J= 5.0, 3.35 Hz, 1H), 5.71 (pseudo t, 1H), 5.46 (m, 1H), 3.86 (m, 1H), 3.36 (m, 2H), 1.20 (d, J= 6.4 Hz, 3H), 0.66 (s, 9H), -0.24 (s, Si-CH$_3$), 0.21 (s, Si-CH$_3$); $^{13}$C NMR (CDCl$_3$) δ 165.28 (C=O), 133.05, 133.00, 132.78, 132.73, 129.86, 129.78, 129.67, 128.55, 128.40, 128.29, 128.13, 128.08, 127.87, 127.87, 127.52 (aromatic), 75.71 (CH-O), 72.24 (CH-O), 70.35 (CH-O), 61.30 (CH$_2$-O), 57.56 (CH-N), 25.65 (tBu), 18.45 (CH$_3$), -5.64 (SiMe); IR ν max 2928.3 (C-H), 1724 (C=O), 1109.2, 1068.7, 708.0.

(2R,3R,4R,5S)-2,3,4-Tripivaloyloxy-5-[N-(diphenylmethylen)-amino]-1-O-(tert-butylidimethylsilyl)hexan-1-ol (37a):

37a: colorless oil; $^1$H NMR (CDCl$_3$) δ 7.58 - 7.12 (m, 10H), 5.42 (pseudo t, 1H), 5.33 (pseudo t, 1H), 5.14 (pseudo q, 1H), 3.67 (m, 1H), 3.56 (m, 2H), 1.18 (s, 9H), 1.11 (s, 9H), 1.07 (s, 9H), -0.02 (s, 3H), -0.06 (s, 3H); $^{13}$C NMR (CDCl$_3$) δ 177.25 (C=O), 128.49, 127.82 (aromatic), 74.59 (CH-O), 72.20 (CH-O), 70.48 (CH-O), 61.56 (CH$_2$-O), 56.83 (CH-N), 27.25 (tBu), 27.04 (tBu), 25.75 (tBu-Si), 18.92 (Me), -5.53 (SiMe$_3$); IR ν max 2981.3 (C-H), 1725.3 (C=O), 1275.3, 1145.1 (C-O).
(2R,3R,4R,5S)-4-Pivaloyloxy-5-[N-(diphenylmethylene)-amino]-1-O-(tert-butyldimethylsilyl)hexan-1,2,3-triol (27a):

27a: colorless oil; $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 7.49 - 7.20 (m, 10H), 4.94 (dd, J= 3.7, 9.6 Hz), 4.17 (d, J= 9.4 Hz), 3.92 (m, 1H), 3.69 (pseudo d, 2H), 3.48 (m, 1H), 1.14 (s, 9H), 1.13 (d, 3H), 0.87 (s, 9H), 0.05 (d, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 177.16 (C=O), 130.72, 128.72, 128.86, 128.55, 128.22, 127.7 (aromatic), 71.44 (CH-O), 70.04 (CH-O), 68.59 (CH-O), 63.77 (CH2-O), 58.25 (CH-N), 27.05 (tBu), 25.83 (tBu), 15.16 (CH3), -5.39 (SiCH3); IR $\nu$ max 3500.0 (OH), 2957.3, 2930.2, 2856.9 (C-H), 1732.3 (C=O), 1151.7 (C-O); $[\alpha]_D$ +37.6°; elemental anal.: calculated for C$_{30}$H$_{45}$N$_5$Si C 71.53%, H 9.00%; found C 71.58%, H 9.06%.
5.4 TBAF Deprotection of tButyldimethylsilyl protecting group:

tButyldimethylsilyl group was deprotected under standard conditions. Substrate (1 mmol) was dissolved in THF (4 mL), TBAF (1.1 mmol) was added and the mixture stirred at RT to completion (typically 2-3 hrs). The solvent was removed in vacuo and the residue purified via flash chromatography on silica gel.

\[(2S,3S,4S,5R)-1,3,5-Triacetoxy-2-[N-(diphenylmethylene)-amino]-hexan-4-ol\] (30):

30: colorless oil, 65%; \(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 7.57 - 7.24 (m, 10H), 5.08 (dq, J = 6.6, 1.7 Hz, 1H), 4.93 (dd, J = 10.2, 3.9 Hz, 1H), 4.44 (dd, J = 10.8, 3.0 Hz, 1H), 4.27 (dd, J = 10.8, 8.5 Hz, 1H), 4.13 (m, 1H), 4.07 (dd, J = 10.3, 1.7 Hz), 2.03 (s, 3H), 2.00 (s, 3H), 1.91 (s, 3H), 1.40 (d, J = 6.6 Hz, 3H); IR \(v_{\text{max}}\) 3432.3 (OH), 3014.1 (C-H), 1745.3 (C=O), 1235.8 (C-O).

\[(2R,3R,4R,5S)-1,2,4-Triacetoxy-5-[N-(diphenylmethylene)amino]-hexan-3-ol\] (38):

38: 50% yield colorless oil; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.60 - 7.21 (m, 10H), 5.30 (ddd, J = 1.7, 4.3, 8.3 Hz, 1H), 4.87 (dd, J = 3.9, 9.9 Hz, 1H), 4.51 (dd, J = 4.35, 11.6 Hz, 1H), 4.39 (dd, J = 1.7, 9.9 Hz, 1H), 4.27 (dd, J = 8.3, 11.6 Hz, 1H), 3.90 (dq, J = 3.9, 6.5 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.13 (d, J = 6.5 Hz); \(^{13}\)C NMR APT (CDCl\(_3\)) \(\delta\) 170.81 (C=O), 170.69 (C=O), 169.58 (C=O), 138.49 (quart. arom.), 134.75, 130.90, 129.02, 128.63, 128.28, 127.59 (aromatic), 70.35 (CH-O), 69.74 (CH-O), 68.88 (CH-O), 63.39 (CH\(_2\)-O), 58.53 (CH-N), 20.84
(2R,3R,4R,5S)-1,2,4-Tribenzoxyloxy-5-[N-(Diphenylmethylene)-amino]-hexan-3-ol (39):

Compound 39 was obtained from 36a with TBAF as described above. The crude residue was purified via gradient flash chromatography (6 - 15% EtOAc/hexane).

Pure 39 was obtained in 60% yield as a colorless oil.

39: $^1$H NMR (CDCl$_3$) $\delta$ 7.93 - 7.15 (m, 25H), 6.55 (s, 1H, OH), 5.71 (m, 1H), 5.17 (dd, J= 3.9, 9.6 Hz, 1H), 4.79 (dd, J= 4.1 11.6 Hz, 1/2 AB), 4.68 (m, 1H, 1/2AB), 4.00 (m, 1H), 1.18 (d, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 169.75, 166.30, 165.84 (C=O), 134.76, 133.13, 133.05, 132.87, 123.94, 129.90, 129.79, 129.67, 129.58, 128.99, 128.64, 128.34, 128.26, 128.20, 127.58, 125.79, 125.23 (aromatic), 71.18 (CH-O), 70.77 (CH-O), 69.56 (CH-O), 64.17 (CH$_2$-O), 58.63 (CH-N), 15.03 (Me); IR $\nu_{\text{max}}$ 3250.1 (OH), 3063.4 (C-H), 1724.58 (C=O), 1269.32 (C-O); $[^{\alpha}]_{D}$ +25.6° (CHCl$_3$).

(2R,3R,4R,5S)-1,2,4-Tripivaloxyloxy-5-[N-(Diphenylmethylene)-amino]-hexan-3-ol (40):

Compound 40 was obtained from 37a with TBAF as described above. The crude residue was purified via gradient flash chromatography (6 - 15% EtOAc/hexane).

Pure 40 was obtained in 85% yield as a colorless oil.

40: $^1$H NMR (CDCl$_3$) $\delta$ 7.54 - 7.20 (m, 10H), 5.22 (m, 1H), 4.79 (dd, J= 3.65, 9.65 Hz, 1H), 4.50 (dd, J= 4.3, 11.7 Hz, 1/2AB), 4.36 (dm, 1H), 4.25 (dd, 1H), 3.85 (m, 1H), 1.25 (d, 3H), 1.21 (s, 9H), 1.20 (s, 9H), 1.17 (s, 9H); $^{13}$C NMR
(CDCl₃) δ 168.30 (C=O), 133.56, 130.50, 129.13, 127.41 (aromatic), 71.31, 70.97, 69.93 (C-O), 63.56 (CH₂-O), 58.6 (CH-N), 15.34 (CH₃); IR vmax 3321.3 (OH), 2960.3 (C-H), 1721.91 (C=O), 1320.3, 1157.3 (C-O).

(2R,3R,4R,5S)-4-Pivaloyloxy-5-[N-(diphenylmethylene)-amino]-hexan-1,2,3-triol (48):
48: white crystals, m.p. 92-94°C (hexane/EtOAc); ¹H NMR (CDCl₃) δ 7.48 - 7.20 (m, 10H), 4.94 (dd, J= 3.8, 9.6 Hz, 1H), 4.15 (d, J= 9.8 Hz, 1H), 3.93 (m, 1H), 3.86 (dd, J= 5.7, 11.3 Hz, 1H, 1/2AB), 3.71 (dd, J= 4.4, 11.3 Hz, 1H, 1/2AB), 3.52 (t, pseudo t, 1H), 1.13 (s, 9H), 1.12 (d, 3H); ¹³C NMR (CDCl₃) δ 177.13 (C=O), 138.63 (quart. arom.), 134.69 (quart. arom.), 130.90, 128.60, 128.56, 128.31, 127.66 (aromatic), 71.59 (CH-O), 71.09 (CH-O), 69.60 (CH-O), 65.32 (CH₂-O), 58.38 (CH-N), 38.80 (quart. Bu), 27.04 (tBu), 14.95 (Me); IR vmax 3213.4 - 3425.1 (broad, OH), 1732.2 (C=O), 1274.2 (C-O); [α]D +51.3°.

(4S,5S)-5-[N-(Diphenylmethylene)amino]-4-pivaloyloxy-2Z-hexen-1-ol (53).
53: ¹H NMR (CDCl₃) δ 7.56 - 7.13 (m, 10H), 5.98 (m, 1H), 5.69 (pseudo t), 5.32 (pseudo t, 1H), 4.44 (m, 1H, 1/2AB), 4.06 (m, 1H, 1/2AB), 3.62 (pseudo q, 1H), 3.10 (s, 1H), 1.12 (s, 9H), 1.09 (d, J=6.5 Hz, 3H); ¹³C NMR (CDCl₃) δ 133.97, 130.17, 128.49, 127.90, 127.73 (aromatic), 74.17 (CH-O), 59.63 (CH₂-O), 58.15 (CH-N), 27.1 (tBu), 17.54 (CH₃); ¹³C NMR (CDCl₃) δ 133.97, 130.17, 128.49, 127.90, 127.73 (aromatic), 74.17 (CH-O), 59.63 (CH₂-O), 58.15 (CH-N), 27.1 (tBu), 17.54 (CH₃); IR vmax 3445.3 (OH), 2974.6 (C-H), 1726.5 (C=O), 1626.2 (C=C), 1159.37, 696.39; [α]D +29°; m.p. 114-115°C.
5.5 HF Deprotection of t-Butylidimethylsilyl Group.

(2S,3S,4S,5R)-3,4,5-Triacetoxy-2-[(N-Methyl,N-(Diphenylmethyl)-amino)hexan-1-ol (33):

2.8 mL of a solution, prepared by mixing 0.4 mL of 49% aqueous HF and 9.6 mL of acetonitrile, was added to the compound 32 (140 mg, 0.24 mmol) and the mixture was stirred at RT for 2 hrs. It was quenched by adding enough conc. NaHCO3 solution to make the mixture basic then extracted three times with chloroform, dried over K2CO3, filtered through Celite and dried in vacuo. Pure alcohol 5 (100 mg, 92%) was obtained by flash chromatography (35% EtOAc in hexane).

5: colorless oil; 1H NMR (CDCl3) δ 1.14 (d, 3H, J= 6.5 Hz), 1.96 (s, 3H, acetate CH3), 2.06 (s, 3H, acetate CH3), 2.17 (s, 3H, acetate CH3), 2.27 (s, 3H, N-CH3), 3.06 - 3.12 (m, 1H, unresolved, CH-N), 3.51 - 3.66 (m, 2H, unresolved, CH2-O), 4.79 (s, 1H, CHPh2), 5.17 (dq, 1H, J= 3.1, 6.5 Hz, C(5)H-OAc), 5.31 (dd, 1H, J= 5.8, 7.3 Hz, C(3)H-OAc), 5.48 (dd, 1H, J= 3.1, 7.3 Hz, C(4)H-OAc), 7.17 - 7.41 (m, 10H, unresolved, aromatic); 13C NMR (CDCl3) δ 16.79 (CH3), 21.03 (acetate CH3), 21.11 (acetate CH3), 21.20 (acetate CH3), 35.17 (CH3-N), 56.70 (CH2-O), 58.48 (CH-N), 68.44 (CH-OAc), 71.34 (CH-OAc), 73.14 (CH-OAc), 74.16 (CHPh2), 127.07, 127.24, 127.75, 128.00, 128.10, 128.36, 128.49, 128.62, 128.94 (aromatic CH), 142.56 (q aromatic), 170.2 (COO), 170.6 (COO), 171.1 (COO); IR (neat) νmax 3519.0, 2968.9, 1740.0, 1371.4, 1226.8, 1028.7, 753.9, 707.9; MS (Cl) 472 (MH+), 412 (MH - AcOH), 394 (M - AcOH - H2O), 334 (M - 2AcOH - H2O), 282 (AcOCH2CH=N(CH3)CHPh2), 240 (HOCH2CH=N(CH3)CHPh2), 167 (PhCH, bp); [α]D 25 +4.4° (c= 5.1, CHCl3).
(2R,3R,4R,5S)-2,3-Dibenzoyloxy-5-N-benzoylamino-1-O-(tert-butyldimethylsilyl)hexan-4-ol (42):

Compound 36a (40 mg, 0.053 mmol) was dissolved in 0.8 mL of THF/H₂O (4:1) and 266 µL of 4% aqueous HF was added. The mixture was stirred at RT for 3.5 hours. The reaction mixture was quenched with concentrated NaHCO₃ and aqueous layer was extracted with CH₂Cl₂. The organic phase was washed with H₂O, dried over MgSO₄, filtered and dried in vacuo. The crude product was purified by flash chromatography to yield pure 42 (20 mg, 64%).

42: ¹H NMR (CDCl₃) δ 8.06 - 7.24 (m, 15H), 6.59 (d, J= 8.8 Hz, 1H, N-H), 5.59 (pseudo dt, J= 2.4, 6.4 Hz, 1H), 5.43 (J= 2.3, 9.4 Hz, 1H), 4.22 (m, 1H), 3.86 (m, 3H), 3.33 (d, J= 6.8 Hz, 3H), 0.74 (s, 9H), -0.05 (s, 6H); ¹³C NMR (CDCl₃) δ 167.52, 167.43, 165.43 (C=O), 134.89, 133.58, 133.23, 133.11, 131.49, 131.29, 130.00, 129.85, 129.70, 129.60, 129.25, 128.49, 128.44, 128.35, 127.00, 126.94 (aromatic), 74.35 (CH-O), 71.83 CH-O), 71.63 (CH-O), 62.00 (CH₂-O), 45.71 (CH-N), 25.57 (tBu), 18.18 (Me), -5.57 (Si-Me); IR νmax 3200.0 - 3450.1 (broad, OH), 2930.2 (C-H), 1726.5 (C=O), 1261.6 (C-O);
5.6 Oxidation of Partially Protected Tetrols.

(2S,3S,4S,5R)-3,4,5-Triacetoxy-2-[N-methyl,N-(diphenylmethyl)-amino]-hexan-1-al (34): Swern Oxidation:
Oxalychloride (22μl) and 0.55ml of dry CH2Cl2 were mixed in a flask under an argon atmosphere. The mixture was cooled to -65°C (CHCl3,CO2) and 37 μl of DMSO in 110 μl of dichloromethane was added. The reaction was stirred for 2 min. at -65°C and then 100 mg of alcohol 33 in 0.22 ml CH2Cl2 was added. The reaction was stirred at -65°C for 15 min., and then 154 μl of triethylamine was added. After stirring for additional 10 min. the mixture was allowed to warm to RT and then was quenched with 6 ml of water. The mixture was extracted twice with chloroform. Combined organic phase was subsequently washed with 1% HCl, H2O, 3% NaHCO3, H2O. The solution was dried over MgSO4 and the solvent was removed to provide crude aldehyde 34 (70 mg, 70%). Aldehyde 34 decomposed on the silica gel column very rapidly precluding further purification. The aldehyde should be used for the subsequent step without storing it longer than 12 hrs.

34: colorless oil; 1H NMR (CDCl3) δ 1.12 (d, J= 6.6 Hz, CH3), 1.91 (s,3H, acetate CH3), 2.01 (s,3H, acetate CH3), 2.02 (s,3H, acetate CH3), 2.44 (s,3H, N-CH3), 3.45 (d, 1H, J= 5.4 Hz, CH-N), 5.07 (dq, 1H, J= 6.6, 2.7 Hz, C(5)H- OAc), 5.45 (dd, 1H, J= 2.8, 8.5 Hz, C(4)H-OAc), 5.65 (dd, 1H, J= 8.5, 5.5 Hz, C(3)H- OAc), 7.10 - 7.40 (m, 10H, unresolved, aromatic), 9.46 (s, 1H, CH=O); 13C NMR (CDCl3) δ 16.7 (CH3), 21.03 (acetate CH3), 21.09 (acetate CH3), 21.17 (acetate CH3), 36.78 (CH3-N), 58.87 (CH-N), 67.99 (CH-OAc), 68.25 (CH-OAc), 72.02 (CH-OAc), 73.60 (CHPh2), 127.1, 127.3, 127.4, 127.6, 127.9, 128.6, 128.7, 128.8 (aromatic CH), 141.6 (q aromatic), 142.8 (q aromatic), 169.6 (COO), 169.8
(COO), 170.3 (COO), 200.7 (CHO); IR (neat) $v_{\text{max}}$ 2931.0, 2731.3, 1746.6, 1371.2, 1215.2, 1024.0, 708.0 cm$^{-1}$.

$(2S,3S,5R)-1,3,5$-Triacetoxy-2-[N-(diphenylmethylene)amino]-hexan-4-on (31): Swern oxidation. Compound 31 was prepared from alcohol 30 as described for 34.

$^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 7.61 - 7.18 (m, 10H), 5.53 (m, 2H), 4.34 (dd, $J$= 10.6, 6.0 Hz, 1H), 4.25 (m, 1H), 4.00 (dd, $J$= 10.6, 6.7 Hz, 1H), 2.18 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.40 (d, $J$= 7.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 203.08 (ketone C=O), 171.99 (C=O), 169.91 (C=O), 146.35 (CH$_2$-O), 139.01, 135.60 (quart. aromatic), 130.71, 128.83, 128.55, 128.31, 128.14, 127.75, 126.01 (aromatic), 75.55 (CH-O), 73.02 (CH-O), 62.84 (CH$_2$-O), 61.20 (CH-N), 20.79 (CH$_3$CO), 20.61 (CH$_3$CO), 15.76 (CH$_3$); $[\alpha]_D$ 12.2°.

$(2R,4R,5S)-1,2,4$-Tri-pivaloyloxy-5-[N-(diphenylmethylene)amino]-hexan-3-on (41):

Oxalylchloride (22 µL) and 0.55 mL of CH$_2$Cl$_2$ (anhydrous) were placed in a dry flask and cooled to -60°C under argon. DMSO (37µL) was mixed with 110 µL of CH$_2$Cl$_2$ in the syringe and the solution was added to the flask and stirred for 2 minutes at -60°C. Alcohol 40, dissolved in 0.22mL of CH$_2$Cl$_2$ was added dropwise within 1 min. The mixture was stirred for 15 min at -60C. Subsequently, triethylamine (154 µL) was added, the mixture was stirred for 10 min and then warmed up to RT. The reaction was quenched with H$_2$O and the aqueous layer was washed three times with CH$_2$Cl$_2$. The organic layer was extracted with 2% NaHCO$_3$, H$_2$O and then dried over MgSO$_4$. After evaporation the residue was chromatographed on silica gel to yield pure 41 (70%).
41: colorless oil; $^1$H NMR (CDCl$_3$) $\delta$ 7.62 - 7.16 (m, 10H), 5.81 (dd, J = 2.7, 6.6 Hz, 1H), 5.81 (dd, J = 2.7, 6.6 Hz, 1H), 5.45 (d, J = 5.0 Hz, 1H), 4.91 (dd, J = 2.8, 12.3 Hz, 1H), 4.30 (dd, J = 6.6, 12.3 Hz, 1H), 3.97 (m, 1H), 1.25 (s, 18H), 1.11 (s, 9H), 1.11 (d, J = 6.4 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 199.75 (C=O, ketone), 177.10 (C=O), 137.30 (quart. aromatic), 130.32, 128.32, 128.61, 128.55, 128.31, 128.11, 127.47 (aromatic), 78.68 (CH-O), 76.23 (CH-O), 61.84 (CH$_2$-O), 58.38 (CH-N), 29.69 (QMe$_3$), 27.08 (tBu), 16.87 (Me); IR $\nu_{\text{max}}$ 2993.4 (C-H), 1741.3 (C=O), 1725.3 (C=O), 1259.3, 1150.1 (C=O).

$(4S,5S)-5$-[N-(Diphenylmethylen)amino]-4-pivaloyloxy-2Z-hexen-1-al (54): Swern oxidation. Aldehyde 54 was prepared as described for 41.

54: 70% yield; oil; $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 10.14 (d, J = 7.8 Hz, 1H), 7.51 - 7.08 (m, 10H), 6.29 (dd, J = 9.4, 11.4 Hz, 1H), 6.01 (m, 2H), 3.62 (pseudo q, J = 6.6 Hz), 1.09 (s, 9H), 1.08 (d, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 191.48 (CH=O), 177.48 (C=O), 139.34, 136.40 (CH=CH), 132.46, 130.28, 128.61, 128.49, 128.68, 127.50 (aromatic), 72.95 (CH-O), 59.59 (CH-N), 27.08 (tBu), 17.74 (CH$_3$).

$(2S,3R,4R,5S)-2,3$-bis(Methoxymethoxy)-4-pivaloyloxy-5-[N-(diphenylmethylen)amino]-1-al (46): Swern Oxidation. Compound 46 was prepared as described for 41.

46: 75%; colorless oil; $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 9.63 (d, J = 1.0 Hz, 1H), 7.61 - 7.14 (m, 10H), 5.41 (dd, J = 5.8 Hz, 1H), 4.71 (d, J = 6.9 Hz, 1H), 4.64 (d, J = 7.0 Hz, 1H), 4.57 (d, J = 6.9 Hz, 1H), 4.52 (d, J = 6.9 Hz, 1H), 4.16 (dd, J = 6.0, 2.7 Hz, 1H), 3.94 (pseudo quint. 1H), 3.87 (dd, J = 2.6, 0.9 Hz, 1H), 3.34
(s, 3H), 3.30 (s, 3H), 1.21 (s, 9H), 1.10 (d, 6.5 Hz, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \( \delta \)
201.68 (CH=O), 177.63 C=O), 139.52 (quart. arom.), 130.00, 128.37, 127.93, 127.58 (arom.), 97.84 (O-CH\textsubscript{2}-O), 96.82 (O-CH\textsubscript{2}-O), 82.29 (CH-O), 76.15 (CH-O), 74.50 (CH-O), 56.45 (CH-N), 45.68 (CH\textsubscript{3}O), 27.20 (tBu).
(2S,3R,4R,5S)-5-[N-(Diphenylmethylene)amino]-4-pivaloyloxy-2,3-dihydroxy-hexan-1-al (49): TEMPO OXIDATION:

Triol 48 (0.53 mmol, 220 mg; Rf = 0.25) was dissolved in 1.4 mL of dichloromethane, TEMPO (2,2,6,6-tetramethylpiperidine-N-oxide; 1 mol%, 0.8 mg; 320 µL of the solution prepared from 5 mg of TEMPO and 2 mL CH2Cl2 was added) and 0.87 mL of saturated NaHCO3 containing 5.3 mg of KBr and 7.3 mg of tetrabutylammonium chloride hydrate were added. The mixture was cooled to -5°C. While stirring vigorously a mixture of 1.56 mL of NaClO (4% solution, Aldrich), 0.76 mL of saturated NaHCO3 and 1.49 mL of brine was added dropwise to a cooled flask. The addition time was 12 - 15 minutes. The addition time was very important. The extension of the addition time resulted in deactivation of the catalyst. The reaction is monitored by TLC. At the end of the reaction the mixture was diluted with an excess of dichloromethane, dried with MgSO4, filtered and the solvent was removed in the dark and at low temperature (0-10°C). The crude aldehyde 49 (210 mg, 96%, Rf = 0.77) was used immediately for the next reaction. The low stability of aldehyde 49 prevented its spectral characterization.
5.7 Preparation of Cyclic Amino Sugar Analogs.

2-[N-Methyl,N-(diphenylmethyl)amino]-2,6-dideoxy-D-galactose (35):
Aldehyde 34 (70 mg, 0.15 mmol) was dissolved in freshly distilled MeOH and KCN (5 mg, 0.5 eq) was added (KCN was dried in vacuo over P2O5 prior to use). After 12 hrs the reaction was diluted with MeOH, filtered through a pad of silica gel and dried. The crude mixture was chromatographed (2.5% MeOH in CHCl3) to yield 40 mg (78%) of 35 (a mixture of α,β-pyranose and α,β-furanose).

35: colorless oil; 1H NMR (CDCl3) δ 1.2 - 1.29 (four doublets, 3H, J= 6.5, CH3), 2.21, 2.26, 2.38, 2.44 (four singlets, 3H, ratio = 1:4:1:1, N-CH3), 2.91 (dd, J= 8.3, 9.9 Hz, β-pyranose form, position 2), 3.15 - 3.34 (m, undetermined, CH-N), 3.60 (q, J= 6.6, C(5)H-OH), 3.76 (s, undetermined), 3.79 - 3.84 (m, undetermined, CH-OH), 4.06 (m, undetermined), 4.10 - 4.20 (m, undetermined), 4.83 (d, J= 8.2 Hz, β-pyranose form, position 1), 4.93 (d, J= 10.8 Hz, furanose form, position 1), 5.20 (s, furanose form, position 1), 5.50 (d, J= 2.7 Hz, α-pyranose, position 1), 7.14 - 7.45 (m, 10H, aromatic); 13C NMR δ 16.4 (CH3), 33.98 (CH3-N), 59.6 (CH-N), 65.21, 65.97, 67.61, 69.38, 70.32, 70.73, 71.23, 72.74, 73.97 (CH-O, CHPh2, undetermined), 87.6 (α-pyranose C1), 92.91 (β-pyranose C1), 95.47 (furanose C1), 97.6 (furanose C1), 126.99, 127.08, 127.17, 127.26, 127.31, 127.52, 127.76, 127.93, 128.23, 128.39, 128.49, 128.64, 128.72, 128.93 (aromatic CH), 142.19 (q aromatic), 143.34 (q aromatic); IR (neat) v_max 3565.2, 2974.2, 1598.3, 1033.2; MS (El) 343 (M+), 314 (M - CHO), 252 (M - CH3CH(OH)-O-CHOH), 239 (HO-CHCH=N(CH3)CHPh2), 210
(CH$_2$=N(CH$_3$)CHPh$_2$), 167 (CHPh$_2$, bp), 42 (CH$_2$=N=CH$_2$); [α]$_d^{25}$ +37.1° (c= 2.7, CHCl$_3$, 24 hrs).

2-N-Methyl-D(+) 2,6-dideoxy-galactose (N-Methylfucosamine) (43):  
20 mg (0.05 mmol) of 35 was dissolved in 15 ml of MeOH and the solution was added to the flask with 5 mg Pd/C (5%) under argon. The mixture was vigorously stirred under H$_2$ atmosphere (1 atm) for 5 hrs. The flask was then flushed with argon several times, the palladium catalyst was filtered off, and the solvent was removed in vacuo. The crude product was dissolved in water, the mixture was acidified to pH= 2 with 2M HCl and extracted with toluene. The aqueous phase was lyophilyzed and the crude material was dissolved in 1M NaOH and purified by ion exchange chromatography (Dowex 50 H$^+$form), MeOH : 3M NH$_4$OH : H$_2$O, 2:5:3) to yield crystalline product 43 (6 mg, 68 %).  

8: colorless crystals; $^1$H NMR (D$_2$O) δ 1.19 - 1.32 (four doublets, 3H, J= 6.7 Hz, CH$_3$), 2.67 (s, CH$_3$-N), 2.73 (s, CH$_3$-N), 3.21 (dd, J= 3.5, 11.2 Hz, CH-N), 3.55 (s, undetermined), 3.80 (m, C(4)H$_2$-OH), 4.03 (dd, J= 3.2, 11.2 Hz, C(3)H$_2$-OH), 4.22 (q, J= 6.3 Hz, C(5)H$_2$-OH), 5.50 (d, 3.5 Hz, α-pyranose, position 1); $^{13}$C NMR (D$_2$O) δ 16.66 (CH$_3$), 33.9 (CH$_3$-N), 58.2 (CH-N), 60.4 (CH-N), 65.2, 66.7, 67.4, 68.1, 69.2, 71.0, 71.7, 72.9 (CH-O, undetermined), 87.8 (α-pyranose C$_1$, major isomer), 92.6 (β-pyranose C$_1$), 95.1 (furanose C$_1$), 98.0 (furanose C$_1$); MS (FAB) 178 (MH$^+$), 176 (MH - H$_2$), 160 (MH - H$_2$O), 132 (MH - CH$_2$=CH$_2$ - H$_2$O), 130 (MH - H$_2$ - HC00H), 103 (MH - CH$_2$=CH$_2$ - CH$_2$=NH); m.p. 158.5 - 162 °C (158 - 161°C, ref X); [α]$_d^{25}$ +2.6° (c= 0.3, H$_2$O, 24 hrs).
1,3,4-O-Triacetyl-2-[N-methyl,N-(diphenyl)methyl]-2,6-dideoxy-β-D(+)galactopyranose (44):

Protected amino sugar 35 (15 mg) was dissolved in 0.5 ml of dry pyridine. The solution was cooled to 0°C and 0.5 ml of acetic anhydride was added. The reaction mixture was stirred at 0°C for 3 hrs. The solvent was removed in vacuo. The crude material was purified by preparative TLC (Kieselgel 60,F254, 1mm; CHCl3, developed twice) to yield pure 44 (4 mg, 58%).

44: Colorless oil; Rf = 0.11 (CHCl3); 1H NMR (CDCl3) δ 1.06 (d, 3H, J = 6.5 Hz), 1.83 (s, 3H, acetate CH3), 2.12 (s, 3H, acetate CH3), 2.16 (s, 3H, acetate CH3), 2.23 (s, 3H, CH3-N), 3.30 (dd, 1H, J = 8.8, 11.2 Hz, CH-N), 3.75 (q, 1H, J = 6.6 Hz, C(5)H-0), 4.75 (s, 1H, C(3)H-Ph2), 5.08 (d, 1H, J = 3.0 Hz, C(4)H-OAc), 5.16 (dd, 1H, J = 3.1, 11.2 Hz, C(3)H-OAc), 5.79 (d, 1H, J = 8.8 Hz, C(1)H-OAc), 7.10 - 7.38 (m, 10H, unresolved); MS (El) 469 (M+), 409 (M - AcOH), 392 (M - C6H5), 349 (M - 2 AcOH), 322 (CH3CH=CHCH(OAc)CH=N(CH3)CHPh2), 281 (AcOCH=CH=N(CH3)CHPh2), 237 (Ph2CH(CH3)N=CH-0), 222 (281 - AcO), 167 (Ph2CH, bp); IR (neat) Vmax 2934.1, 1747.7, 1493.1, 1454.5, 1371.6, 1224.9, 1045.6 cm⁻¹.

N-Diphenylmethyl-1-deoxy-2,3-di-(methoxymethyl)-4-pivaloyl-L-azafucose (47):

Aldehyde 46 (90 mg, 0.18 mmol) in 0.52 mL of anhydrous CH3CN was acidified with glacial acetic acid (8-10 eq.) to keep the pH = 5-6. NaBH3CN (12.4 mg, 0.2 mmol) was added to the mixture at once. The reaction was complete in 10 min (TLC). The mixture was diluted with H2O and extracted with EtOAc. The organic layer was dried
and evaporated to provide the crude 47 (48 mg), which was purified via flash chromatography (50% EtOAc/ hexanes) to yield pure 47 (34mg, 88%).

47: colorless oil; $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 7.45 - 7.19 (m, 10H), 5.26 (pseudo t, $J$=2.4, 3.5 Hz), 5.22 (s, 1H), 4.69 (d, $J$=6.7 Hz, 2H), 4.56 (dd, $J$=6.8, 8.9 Hz), 3.93 (pseudo dt, $J$=4.5, 9.0 Hz), 3.50 (dd, $J$=3.3, 8.8 Hz), 3.33 (s,3H), 3.25 (s, 3H), 3.05 (dd, $J$=4.3, 8.8 Hz), 2.71 (pseudo q, $J$=6.8 Hz), 1.93 (m,1H), 1.29 (s, 9H), 1.24 (d, $J$= 6.5 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 142.52, 129.75, 128.14, 128.05, 127.84, 127.31, 126.69(aromatic), 96.03 (OCH$_2$O), 95.14 (OCH$_2$O), 73.42 (CH-O), 72.26 (Ph$_2$CH), 65.15 (CH-O), 55.42, 55.19, 54.61, 49.64 (CH-N), 39.16 (CH-N), 27.35 (tBu), 15.19 (CH3); IR $\nu$max 2972.7, 2891.7 (C-H), 1730.4 (C=O), 1479.6, 1165.5, 1033.9; $\left[\alpha\right]_D$ -55.3° (CHCl$_3$).

N-Diphenylmethyl-1-deoxy-4-pivaloyl-L-(−)-azafucose (50):

Crude aldehyde 49 was converted to azafucose 50 (80% yield) following the same protocol as for 47.

50: colorless oil; $^1$H NMR (CDCl$_3$) $\delta$ 7.41 - 7.18 (m, 10H), 5.16 (s, 1H), 5.12 (pseudo t, $J$= 3.0 Hz), 3.86 (ddd, 1H), 3.49 (dd, $J$=3.3, 7.7 Hz), 3.03 (dd, $J$= 4.0, 11.6 Hz), 2.99 (dq, 1H), 1.96 (dd, $J$=8.6, 11.6 Hz), 1.28 (s, 9H), 1.23 (d, $J$= 6.5 Hz); $^{13}$C NMR (CDCl$_3$) $\delta$ 142.30, 129.55, 128.28, 128.19, 127.84, 127.39, 126.76 (aromatic), 74.81 (Ph$_2$CH), 74.33 (CH-O), 69.09 (CH-O), 65.42 (CH-O), 54.37 (CH-N), 49.77 (CH$_2$-N), 27.31(tBu), 14.38 (CH3); IR $\nu$max 3443.4 (OH), 2976.5 (C-H), 1728 (C=O), 1165.15 (C-O); $\left[\alpha\right]_D$ -41°.
N-Diphenylmethyl-2S-methyl-3S-pivaloyloxy-Δ4,5-azidine (55):
Crude aldehyde 54 yielded 55 (84%) as described for 47.

\[ \text{1H NMR (CDCl}_3\text{) } \delta 7.46 - 7.12 (m, 10H), 5.71 (m, 1H, 1/2AB), 5.49 (m, 2H), 4.65 (s, 1H), 3.42 (pseudo q, 1H), 2.99 (pseudo d, 1H, 1/2AB), 2.75 (pseudo d, 1H, 1/2AB), 1.14 (s, 9H), 0.86 (d, J= 6.6 Hz, 3H); \text{13C NMR (CDCl}_3\text{) } \delta 128.76, 128.55, 127.93, 127.46, 127.08, 127.03, 124.2 \text{ (aromatic), 71.83 (PhCH), 70.15 (CH-O), 49.30 (CH-N), 45.5 (CH}_2\text{-N), 27.1 (tBu), 4.2 (CH}_3\text{); IR } \nu_{\text{max}} 2974.6 (C-H), 1728.4 (C=O), \text{[α]D } +58.6°. \]

N-Diphenylmethyl-1-deoxy-4-pivaloyl-L(-)-azagulose (56):
Cyclic alkene 55 was converted to azagulose 56 (80% yield) following the osmylation protocol A. Only the guloisomer 56 was detected (1H NMR).

56: colorless oil; 80%; \text{1H NMR (CDCl}_3\text{) } \delta 7.40 - 7.11 (m, 10H), 5.09 (dd, J= 5.5, 10.0 Hz, 1H), 4.62 (s, 1H), 3.73 (m, 1H), 3.58 (dt, 3.3, 9.8 Hz), 3.34 (pseudo q, J= 6.8 Hz), 2.73 (dd, J=2.7, 12.8 Hz), 2.50 (dd, J= 9.5, 12.8 Hz), 1.12 (s, 9H), 0.88 (d, J=6.8 Hz, 3H); \text{13C NMR (CDCl}_3\text{) } \delta 128.93, 128.80, 127.49, 127.30 \text{ (aromatic), 72.12 (PhCH), 70.83 (CH-O), 70.12 (CH-O), 69.10 (CH-O), 51.85 (CH-N), 47.15 (CH}_2\text{-N), 27.10 (tBu), 3.70 (tBu); IR } \nu_{\text{max}} 3462.2 (OH), 2992.2 (C-H), 1743.2 (C=O), 1143.3 (C-O); \text{[α]D } -85.4 (CHCl}_3\text{); calcd. for C}_{27}\text{H}_{31}\text{NO}_4: C 74.80 \%, H 7.21\%. \text{ Found C 74.89 \%, H 7.30 \%.}
Deprotection of pivalate and benzhydral group:
N-Diphenylmethyl-4-pivaloyl-L-azasugar (313.4 mg, 1 mmol) was dissolved in 10 mL of dioxane and 6.5 mL of H₂O. Aqueous solution of nBu₄NOH (720 µL, 1.1 mmol) was added at 0° C. The mixture was stirred to completion (TLC, 5% MeOH/CH₂Cl₂). Upon completion the mixture was diluted with an excess of CH₂Cl₂ and the two phases separated. The aqueous layer was extracted three times with CH₂Cl₂. The combined organic phase was washed with brine (water will generate an emulsion), dried over MgSO₄ and evaporated. When necessary, the crude material was purified via gradient flash chromatography (2 - 5% MeOH/CH₂Cl₂) to yield a pure triol (typically 85%), which was submitted to hydrogenolysis in MeOH. One mmol of a substrate was dissolved in an excess of methanol (250mL) and the solution was added to an argon-purged flask with 5% Pd/C catalyst. Hydrogen was applied via rubber balloon. The mixture was vigorously stirred at RT for 3 hours, then it was filtered and evaporated to dryness. The residue was dissolved in H₂O, acidified with HCl (2 mmol) and washed with hexane twice. The aqueous solution was lyophilized and the crude sample was purified via ion exchange column (Dowex H⁺form, MeOH : 3M NH₃ : H₂O 2:5:3) to yield a pure azasugar (typically 95% for the second step).

1-Deoxy-L-(−)-azafucose (1-deoxy-L-fuconojirimycin) (51):
51: ¹H NMR (250 MHz, D₂O) 3.58 (dd, J= 1.4, 3.2 Hz, 1H), 3.48 (ddd, J= 5.3, 9.8, 10.8 Hz, 1H), 3.26 (dd, J= 3.2, 9.8 Hz, 1H), 2.85 (dd, J= 5.3, 13.0 Hz, 1H), 2.56 (dq, J=1.4, 6.8 Hz, 1H), 2.13 (dd, 10.8, 13.0 Hz, 1H), 0.87 (d, J= 6.8 Hz, 3H); ¹³C NMR (D₂O) δ 75.58 (CH-O), 73.16 (CH-O), 68.31 (CH-O), 53.63 (CH-N), 49.28 (CH₂-N), 16.7 (CH₃); [α]D -50⁰; calcd for: C₆H₁₂NO₃: C 48.96%, H 8.22%. Found C 49.01, H 8.25%.
1-Deoxy-L-(-)-azagulose (1-deoxy-L-gulonojirimycin) (57):

57: $^1$H NMR (250 MHz, D$_2$O) $\delta$ 3.95 (m, 1H), 3.80 (pseudo t, J= 3.0, 4.4 Hz), 3.71 (dd, J= 1.5, 3.2 Hz), 3.35 (pseudo q, J= 6.9 Hz), 2.99 (dd, J= 5.1, 12.0 Hz), 2.86 (dd, J= 11.3, 11.7 Hz, 1H), 1.07 (d, J=6.9 Hz, 1H); $^{13}$C NMR (250 MHz, D$_2$O) $\delta$ 71.31 (CH-O), 70.05 (CH=O), 63.91 (CH-O), 51.71 (CH=N), 43.91 (CH$_2$-N), 15.22 (CH$_3$); $[\alpha]_D^\circ$ +13.5° (H$_2$O, MeOH); calcd for C$_6$H$_{13}$N$_3$O$_3$Cl: C 39.46%, H 7.17%. Found C 39.46%, H 7.22%.
1α-Cyano-1-deoxy-4-pivalyl-L-(−)-azafucose (59a):

Procedure A: Crude aldehyde 49 (720 mg, 1.71 mmol, assuming 100% purity) was dissolved in 23 mL of methanol. The pH was adjusted to 3 with 3% HCl (about 3.5 mL). The mixture was stirred at RT. The reaction was complete in two hours as judged by TLC. The solvent was removed in vacuo, the residue dissolved in water and the aqueous layer washed twice with hexane to remove benzophenone. The solution was lyophilized to yield 482 mg of the crude aminal. The crude material was dissolved in 8.2 mL of dioxane and 6.6 mL of water. KCN (120 mg, 1 eq.) was added and the mixture was stirred at RT overnight. The mixture was dried in vacuo and the crude aminonitrile was purified by flash chromatography on silica gel (3→4→5→7% MeOH/CH₂Cl₂; gradient chromatography) to yield pure α isomer 59a (209 mg, 46% over 3 steps).

59a: crystalline, m.p. 126-128°C; ¹H NMR (250 MHz, CDCl₃) δ 5.16 (dd, J= 1.8, 2.5 Hz, 1H), 4.34 (d, J= 4.8 Hz, 1H, aX), 3.91 (m, 2H), 3.37 (dq, J= 1.5, 6.5 Hz, 1H), 1.25 (s, 9H), 1.09 (d, J= 6.5 Hz, 3H); ¹³C NMR (250 MHz, D₂O) δ 179.30 (C=O), 117.83 (CN), 72.53 (CH-O), 72.17 (CH-O), 67.58 (C-O), 51.52 (CH-CN), 49.48 (CH-N), 29.69 (Me3), 27.24 (tBu), 16.84 (Me); [α]D -91.7° (CHCl₃); calcd for C₁₂H₂₀N₂O₄: C 56.24%, H 7.87%. Found C 56.28%, H 7.95%; β-isomer 59b was detected: ¹H NMR 8₁β = 4.11 (dd, J = 10.2, 2.9 Hz).

Procedure B: Crude aldehyde 49 (120 mg, 0.285 mmol, assuming 100% purity) was covered with distilled water and SO₂ gas was bubbled through it for 1 hour while the mixture was agitated. The mixture was tightly capped and stirred for 12 hours at RT. The insoluble starting material gradually disappeared and a milky solution was formed. The mixture was diluted with more water and washed twice with hexane to remove benzophenone. The mixture was lyophilized to yield crude 60 (86 mg, 97%). Crude
60 (86 mg) was dissolved in 0.5 mL of dioxane and 0.2 mL of H2O and KCN (18.2 mg, 1 eq. assuming 100% purity of crude 60) in 0.2 mL of H2O was added. As judged by TLC, only a small amount of desired product was formed after 24 hours and therefore another portion of KCN (9.1 mg, 0.5 eq.) was added. After three days the solvent was removed and the crude material was purified via flash chromatography as described in procedure A to yield pure 59a (30 mg, 41% over three steps, α only).
APPENDIX

SELECTED $^1$H AND $^{13}$C NMR SPECTRA
25a/b  R = Bn  4:1
25a/b  R = Bn  4:1
L - Azafucose

(1 - deoxy - fuconojirimicin)
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