A SERINE-DERIVED BUTENOLIDE AS A VERSATILE CHIRAL BUILDING BLOCK: APPLICATIONS IN THE SYNTHESIS OF NATURAL AND NATURE-LIKE COMPOUNDS OF BIOLOGICAL SIGNIFICANCE

By

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Dedication

In the name of Lord Shiva ..

To my parents and wife

for their unwavering love, support and encouragement

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I am indebted to many individuals for their support and assistance in accomplishing this major milestone in my life. I would first like to sincerely thank my major advisor Dr. Apurba Dutta for his motivation, guidance, training, and unwavering support during my graduate studies. The word "Advisor" in Sanskrit stands for "Guru". To me, my Guru Dr. Dutta is equally "*Gurur Brahma Gurur Vishnuhu Guru Devo Maheswaraha Guru Saakshaat Parabhrahma Tasmai Sri Gurave Namaha"* (meaning: the Guru is creator, preserver, and destroyer. To that very Guru I bow, for He is the Supreme Being, right before my eyes).

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Abstract

Part I. HIV Protease Targeted Synthesis of Conformationally‑Rigid Hydroxyethylene Dipeptide Isosteres: A Combinatorial approach

With the success of Highly Active Anti-Retro Viral therapy (HAART), the life expectancy of HIV patients has dramatically increased. However, emergence of drug resistance, toxicity, pharmacokinetic issues, and side effects etc. are some of the concerns still associated with the currently available drugs. To address these issues, there is a continuous need for the development of novel and improved anti-HIV agents.

Drugs acting via potent inhibition of the critical enzyme HIV protease constitute a major class of drugs in AIDS therapy. Currently available HIV protease inhibitors utilize peptide hydrolysis transition-state isosteres as the key element for their anti-HIV activity. Hydroxyethylene, dihydroxyethylene, hydroxyethyl amine, and methyleneamine moieties are some of the strategic nonhydrolyzable transition state isosteres that have been employed in the construction of various HIV protease inhibitors.

As part of our research towards medicinal chemical studies of bioactive compounds, the present research investigates the design and synthesis of conformationally rigid hydroxyethylene dipeptide mimics. Among the various structural motifs useful in imparting conformational rigidity, cyclopropane rings remain a popular choice in drug discovery endeavors. Accordingly, in an as yet unreported investigation, synthesis of cyclopropane ring-constrained dipeptide isosteres containing a hydroxyethylene ‘warhead’ has been initiated. Utilizing a multifunctional enantiopure aminobutenolide (developed in our research group) as a ‘second-generation’ chiral building block, the synthesis involved an initial diastereoselective cyclopropanation to construct a strategic [3,5]-bicyclic lactone. Utilizing two diversification sites in the target analogs, and employing select sets of amines and amino acids as the diversity elements, a combinatorial

parallel synthetic approach has been followed towards rapid construction of a demonstration library of cyclopropane ring containing hydroxyethylene dipeptide isosteres. The synthetic route designed also offers additional flexibility in terms of further diversification in future studies.

Part II. Total Synthesis and Structure–Activity Relationship Studies of the Cytotoxic Anhydrophytosphingosine Jaspine B

Jaspine B, also known as Pachastrissamine, is a cytotoxic marine natural product isolated from the sponges *Pachastrissa sp*. and *Jaspis sp*. In biological assays, Jaspine B exhibited sub-micromolar cytotoxicity ($IC_{50} \leq 0.5$ μM) against several different cancer cell lines (murine B16, human Sk-Mel28 melanoma etc.). Exposure of these cells to Jaspine B triggered cell death by typical apoptosis, as indicated by phosphatidylserine externalization, the release of cytochrome C, and caspase processing. Recent studies have indicated that interference with ceramide metabolism via inhibition of *sphingomyelin synthase* is most probably responsible for the apoptotic effects of this natural product. Despite its impressive biological activity, detailed structure-activity relationship (SAR) investigations of Jaspine B are relatively limited.

The present research describes an efficient and practical total synthetic route to Jaspine B, and application of the method thereof in the structure-activity relationship (SAR) studies of this bioactive natural product. Starting from the previously mentioned chiral aminobutenolide as an advanced building block, a key reaction in our synthetic route involved a stereocontrolled synthesis of the *cis*-fused [5,5]-bicyclic lactone. Subsequent functional group transformations of this lactone led to a short-step total synthesis of enantiopure Jaspine B. Easy amenability of various advanced chiral intermediates from the total synthesis route has also allowed us to perform strategic structural modifications to access a number of unique Jaspine B analogs for biological investigations. Subsequent *in vitro* cytotoxicity assays of the analogs thus obtained

have provided important SAR information, which is expected to provide useful direction in the potential development of new anti-cancer agents, based on the Jaspine B structural lead.

Simultaneously to the above studies, a previously reported synthetic route to Jaspine B from our group has also been reinvestigated in order to help clarify some unanswered questions. Gratifyingly, the results from the above study have also confirmed the utility of the previous route in stereoselective synthesis of enantiopure Jaspine B.

Part III. A Rapid Approach Towards Diastereoselective Synthesis of Azacarbohydrate Scaffolds of Biological Significance

A wide range of monocyclic and bicyclic polyhydroxylated alkaloids (azacarbohydrates/iminosugars) have been isolated from various plants, insects and microorganisms. Many of these compounds have been found to be potent inhibitors of glycosidase and glycosyl transferase enzymes of clinical importance. Advancements involving stereoselective synthesis, structural modification, and biological evaluation of these compounds resulted in therapeutic agents Glyset® and Zavesca® for use in patients with type II diabetes and type I Gaucher's disease respectively.

In further application of the L-serine-derived chiral aminobutenolide as an advanced building block, a rapid stereoselective synthesis of azacarbohydrate structural scaffolds have been investigated in the present study. The key reaction steps involved, utilization of an enone in the starting material towards stereoselective installation of key functional groups, and an intramolecular cyclization of an appropriately located amine into the lactone carbonyl resulting in the formation of the desired azacarbohydrate structural scaffolds. Subsequent reduction and deprotection provided an efficient and short-step route to variously functionalized azacarbohydrates.

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List of Compounds

List of Abbreviations

Ac – acetyl

br – broad

Bn – benzyl

Boc – *tert*-butoxycarbonyl

n-BuLi – butyl lithium

^{t}Bu – *tert*-butyl

Bz – benzoyl

Cbz – carbobenzyloxy

CSA – camphorsulfonic acid

d – doublet

dd – doublet of doublets

DBU – 1,8-diazabicyclo[5.4.0]undec-7-ene

DCE – 1,2-dichloroethane

DIAD – diisopropylazodicarboxylate

DIBAL-H – diisobutylaluminum hydride

DIPEA – diisopropylethylamine

DMAP – 4-*N*,*N*-dimethylaminopryidine

DMF – *N*,*N*-dimethylformamide

DMP – 2,2-dimethoxypropane

DMSO – dimethylsulfoxide

EDCI – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

ee – enantiomeric excess

Et – ethyl

EtOAc – ethyl acetate

HMBC – hetero multiple bond correlation

HRMS (ESI-TOF) *m/z*: – high resolution mass spectroscopy

HWE – Horner-Wodsworth-Emmons

Hz – hertz

IC_{50} – concentration required to inhibit 50% growth

Imd – imidazole

IR – infrared

K_i – inhibitory constant

m – multiplet

m-CPBA – m-chloroperoxybenzoic acid

Me – methyl

MeOH – methanol

MHz – megahertz

MOM – methoxymethyl

NBS – *N*-bromosuccinimide

NMM – *N*-methylmorpholine

NMO – *N*-methylmorpholine oxide

NMR – nuclear magnetic resonance

NOE – nuclear Overhouser effect

Pd-C – Palladium on Carbon

PI – protease inhibitor

Piv – pivaloyl

PMB – *para*-methoxybenzyl

PTSA – *para*-toluene sulfonic acid

Py – pyridine

RCM – ring-closing metathesis

rt – room temperature

s – singlet

SAR – structure-activity relationship

t – triplet

TBAF – tetrabutylammonium fluoride

TBDMSCl (or TBSCl) – *tert*-butyldimethylsilyl chloride

TBDPS – *tert*-butyldiphenylsilyl

TEA – triethylamine

Tf – triflate

TFA- trifluoroacetic acid

THF – tetrahydrofuran

TLC – thin layer chromatography

TMS – trimethylsilyl

TMSOTf – trimethylsilyl triflate

TPP – triphenylphosphine

μM– micromolar

Chapter 1

Amino Acids: Versatile Chiral Synthons in Organic Synthesis

1.1 Background

α-Amino acids are among the most widely used single class of chiral pool materials in contemporary organic synthesis. In addition to their utility as enantiopure starting materials for various synthetic targets, amino acids are also often used as chiral catalysts in a number of asymmetric synthetic methodologies.[1,2] Some of the advantages as associated with the use of α-amino acids in asymmetric synthesis include,

- easy availability in both enantiomeric forms
- possible incorporation as a chiral fragment into target products
- utilization of the inherent chirality to induce asymmetry in the formation of new stereocenters
- presence of both nucleophilic ($-NH_2$) and electrophilic ($-CO_2H$) functional groups in the molecule allowing for a variety of regioselective synthetic transformations
- utility of the side chain as an additional site for further chemical transformations

Accordingly, amino acids continue to be an important source of enantiopure starting materials, chiral auxiliaries, and chiral catalysts in the stereoselective synthesis of a variety of organic molecules of structural, biological, and pharmaceutical importance.

In previous research from our group, amino acids have been extensively utilized as versatile chiral starting materials in the stereoselective syntheses of a variety of natural and non-natural products of medicinal chemical significance.[3-28] Representative examples of some of the compounds thus synthesized are shown below (Figure 1).[7,9-14,18-20,23,24]

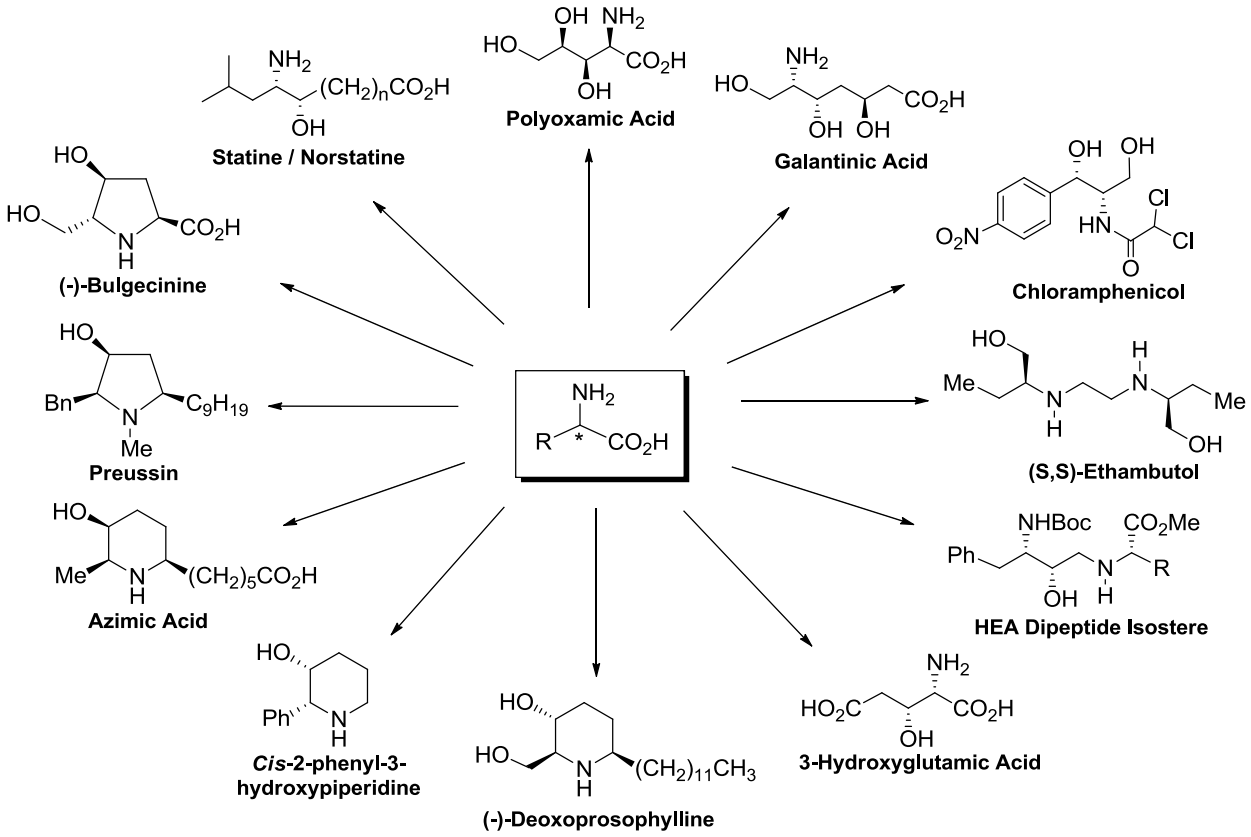


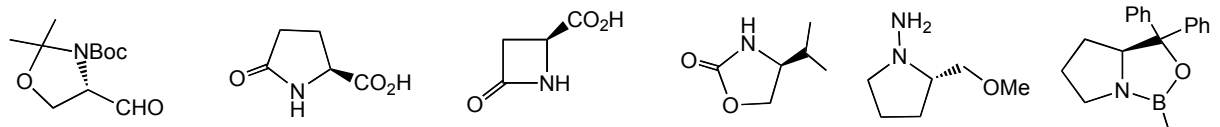
Figure 1. From amino acids to bioactive compounds: A chiral pool approach

1.2. Amino acid-derived advanced chiral intermediates in organic synthesis

In addition to their direct use as chiral materials in organic synthesis, chemically modified 'second generation' products obtained from amino acids are also a valued reservoir of chiral molecules with wide-ranging applications as enantiopure building blocks (chirons), chiral auxiliaries, and catalysts in contemporary asymmetric/stereoselective synthetic protocols.

Representative examples of some of the amino acid-derived chiral molecules, with varied applications as mentioned above, are shown in the figure below (Figure 2). Thus, Garner's aldehyde (**I**),[29] easily available from serine, has proven to be a versatile intermediate in numerous synthetic endeavors, and is an especially valuable chiral precursor for the rapid synthesis of a variety of amino acids, amino alcohols, amino sugars, azacarbohydrates, and many other biologically important compounds.[30] Along similar lines, pyroglutamic acid (**II**) and azetidinone

carboxylic acid (**III**) as shown below (Figure 2) have been gainfully employed as advanced chiral building blocks for the stereoselective syntheses of a number of natural and non-natural products of medicinal chemical significance.[1]



I. Garner's aldehyde (From L-serine) **II.** Pyroglutamic acid (from L-glutamic acid) **III.** Azetidin-2-one carboxylic acid (from L-aspartic acid) **IV.** Evans oxazolidinone (from L-valine) **V.** SAMP (Enders) (from L-proline) **VI.** CBS catalyst (from L-proline)

Figure 2. Amino acid-derived '2nd generation' chiral building blocks, auxiliaries, and catalysts

On the other hand, the valine-derived oxazolidinone chiral auxiliary **IV** is a critical component in Evans aldol and alkylation protocols, providing the respective products in a highly stereoselective manner.[31] Similarly, employing the proline-derived RAMP or SAMP (**V**) as chiral auxiliaries, Enders has developed a versatile method for the stereoselective α-alkylation of aldehydes and ketones.[32,33] In further illustration of the utility of chemically modified amino acids in asymmetric synthesis, the proline-derived CBS-oxazaborolidine **VI**, as developed by Corey's group, has been shown to be a highly efficient catalyst for the rapid and enantioselective reduction of achiral ketones in the presence of borane as the reducing agent.[34]

Syntheses of amino acid-derived strategically functionalized advanced chiral building blocks, and their further utilization in various synthetic endeavors have also been the focus of investigations in our own research group. Towards this end, starting from L-, or D-serine and following a concise and efficient synthetic protocol we have developed synthetic routes to the enantiopure 5- and 6-member amino lactones (**1)** as shown below (Figure 3).[26] In subsequent research, these chiral lactones have proven to be versatile structural platforms for the stereoselective synthesis of a variety of more complex molecules. For example, the amine functionality in the side chain is a potentially useful site for nucleophilic modifications, whereas,

the protected terminal primary hydroxy group is a masked precursor for a carbonyl group and can also be easily manipulated towards nucleophilic displacement reactions. The ring olefinic linkage provides a convenient handle for carrying out both nucleophilic and electrophilic modifications at this site. Similarly, the lactone carbonyl can be utilized for introduction of various nucleophiles, especially carbon- and nitrogen-based, as desired. Importantly, the inherent chirality of the lactones can be easily utilized to control and introduce asymmetry in subsequent reactions, thereby providing access to various stereoisomeric products. In subsequent studies demonstrating the utility of the above chirons, our group has also achieved the syntheses of several natural and non-natural compounds of biological importance (Figure 3).[21,26,35]

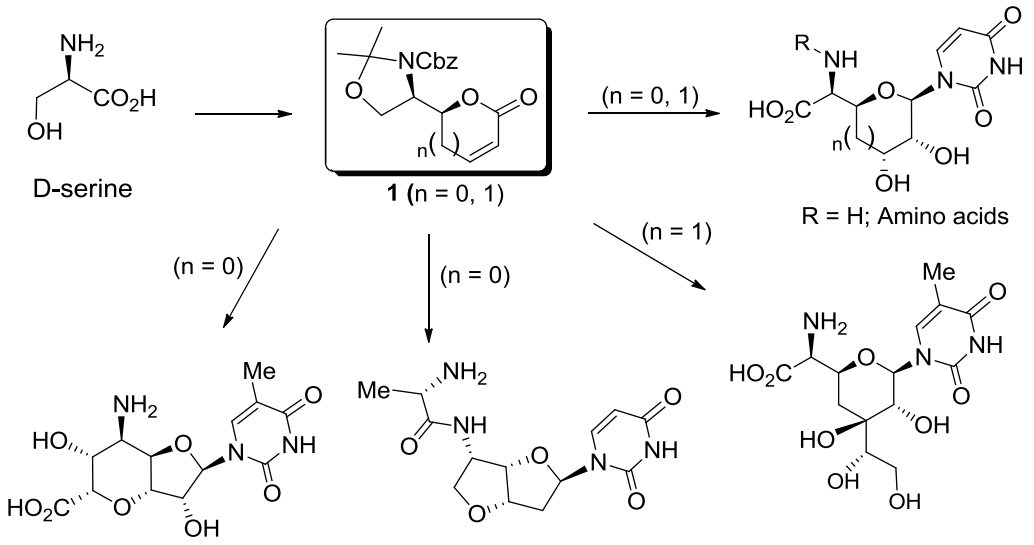


Figure 3. Serine-derived chiral lactones (1) and selected synthetic applications thereof

In continuation of the above studies, in my doctoral research, I have initiated further exploration of the chiral aminobutenolide **1** (n=0) in the syntheses of selected targets of medicinal chemical significance. The details of the studies are described in the following chapters.

Chapter 2

HIV Protease Targeted Synthesis of Conformationally–Rigid Hydroxyethylene Dipeptide Isosteres: A Combinatorial approach

2.1 Background

Acquired immunodeficiency syndrome (AIDS) was first reported in 1981 in a small cohort of individuals, but soon thereafter developed into a major health concern of epidemic proportions.[36-38] AIDS is caused by infection with the retrovirus, Human Immunodeficiency Virus (HIV), and the infected patients can exhibit a variety of symptoms such as fever, lymphadenopathy, sore throat, rash, myalgia/arthralgia, and headache. Although these are not specific for acute HIV infection only, prolonged duration of the above symptoms with mucocutaneous ulcers recommends for immediate diagnosis and initiation of appropriate treatment.[39-41] The HIV retrovirus can be divided into two major subtypes, HIV-1 and HIV-2, with HIV-1 being the more common and pathogenic strain. Latest statistical analysis by World Health Organization (WHO) indicates that more than 34 million people are currently living with HIV, the highest rate being observed in Sub-Saharan Africa (69%), with 1.8 million deaths worldwide in 2010 alone.[42] Thus, HIV infection and AIDS continue to be a global health concern with huge impacts on social, economic, and ethical issues.

Since the first clinical detection of HIV in 1981, there have been substantial advancements in the understanding of the HIV life cycle, infection prevention, and its treatment. Currently, there are 37 FDA approved drugs/drug regimens for the treatment of AIDS.[43] The available AIDS drugs target different stages of the HIV life cycle, and are useful in preventing the increase in viral load. At the beginning of the AIDS epidemic, early mortality was very significant, however, subsequent advancements in the development of new drugs and the

introduction of combination therapeutic strategies, also known as Highly Active Antiretroviral Therapy (HAART), have resulted in profound reductions in morbidity and mortality.[44-46]

Zidovudine (also known as azothymidine or AZT), clinically introduced in 1987, is the first FDA-approved drug for HIV treatment.[47] A synthetically modified nucleoside analog, the prodrug AZT is an inhibitor of HIV-reverse transcriptase, an essential enzyme in the viral life cycle. Since then, extensive drug discovery efforts have continued to add new drugs towards the HIV drugs armamentarium. Depending on their binding targets and function, the currently available AIDS therapeutic agents can be categorized into seven different categories (Table 1). The first column in the table lists the various drug classes and their mode of action, while the second column provides representative examples of drugs belonging to each class.[43]

Table 1. Anti-HIV drug classes and representative agents in current clinical use

Drug Classes	Representative Drugs
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Zidovudine; Emtricitabine
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Delaviridine; Nevirapine
Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)	Tenofovir disoproxil fumarate
Protease Inhibitors (PIs)	Darunavir; Ritonavir
Fusion Inhibitors	Enfuvirtide
Entry Inhibitors	Maraviroc
HIV Integrase Strand Transfer Inhibitors	Raltegravir; Dolutegravir

2.1.1 HIV protease structure, function and mechanism

Among the various anti-HIV drugs, the HIV protease inhibitors (PI) comprise the single largest class of drugs in current clinical use.[43,48] HIV protease is a homodimeric aspartyl protease, wherein each of the identical monomeric units consists of 99 amino acid residues. The catalytic active site of this C2-symmetric enzyme is located at the dimeric interface, with each

protein subunit contributing the tripeptide sequence, Asp-Thr-Gly. The HIV protease is closely related to the other retroviral proteases, with similar amino acid sequences and molecular structures.[49,50] The enzyme has broad substrate specificity, however, unlike mammalian proteases, the HIV protease can also hydrolyze peptidic bonds formed between proline and aromatic amino acid residues (Figure 4). Additionally, the symmetrical nature of this viral enzyme and its active site also represents structural features that are absent in mammalian proteases. The above structural and functional differences between the HIV protease and its mammalian counterparts thus provide an opportunity for the design of drugs selective against the viral enzyme.

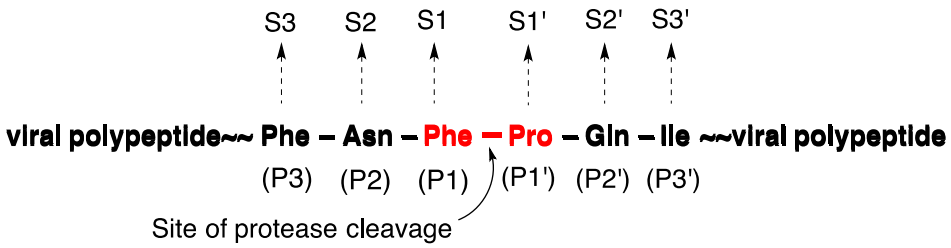
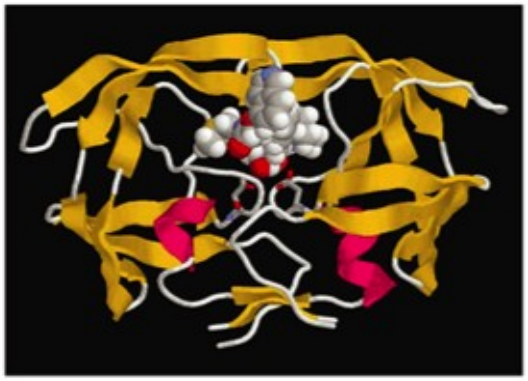
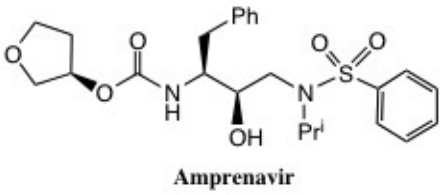


Figure 4. The polypeptide substrate of HIV protease and the cleavage site [showing 6 of the binding subsites (S1 etc.), and the corresponding amino acid residues (P1 etc.) of the substrate]

In further illustration of the HIV protease structure and function, a ribbon diagram of HIV protease (PR), bound to the peptidomimetic protease inhibitor Amprenavir, is shown below (Figure 5).[51] Apart from the catalytic tripeptide sequence Asp-Thr-Gly, the retroviral PR also has a conserved Gly-Arg-Asn/Asp near the C-terminus and a Gly-rich sequence in the middle of the protein structure. The main function of this enzyme involves the proteolytic cleavage of precursor proteins $Pr55^{Gag}$ and $Pr160^{Gag\text{-}Pol}$ using two aspartates located at D25 and D25'.[52,53]





Amprenavir

Figure 5. HIV protease bound to the protease inhibitor Amprenavir (adapted from Ref. 51)

The ceiling of the active site of the enzyme is formed by two beta-hairpin structures or flaps that are highly flexible, and can undergo large localized conformational changes during the binding and release of substrates. The body of the enzyme contains β-sheet and α-helical segments defining an extended hydrophobic core with a curved tubular shape. Embedded in the convex region of this core lies the catalytic Asp25 and Asp25' residues.

Mechanistically, the above-mentioned aspartate residues and a bridging water molecule at the active site of the HIV protease are responsible for hydrolysis of the substrate peptide bond (Figure 6: Panel A). Subsequent to activation of the water molecule, the reaction proceeds via formation of a tetrahedral *gem*-diol as a transition-state (TS) intermediate (Figure 6: Panel B), followed by cleavage of the amide linkage and regeneration of the catalytic enzyme (Figure 6: Panel C).[53-57] Breaking of the tetrahedral intermediate is reversible at a low level as established by ^{18}O exchange into reformed substrates.[58] Enzyme kinetic studies of the reaction mechanism provided evidence that the dissociation of tetrahedral intermediate is the rate-determining step (RDS).[58-61] A non-chemical slow step was also observed for the substrate with P'2 Gln instead of Glu.[60,62] An alternate RDS was proposed to be the conformational change of the two flaps during the entry of the substrate.

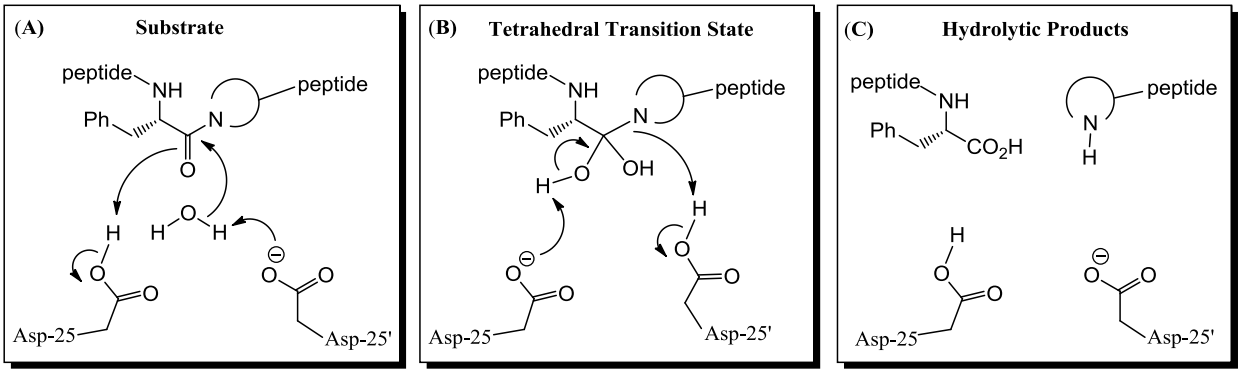


Figure 6. HIV protease catalyzed hydrolysis of the viral polypeptide substrate

2.1.2. Transition State Mimicry in the Design of HIV Protease Inhibitors

In 1989, Wlodawer *et al* reported the first X-ray co-crystal structure of HIV protease bound to a non-hydrolysable peptide analog.[63,64] Structural and binding interaction data gleaned from this and several subsequent studies provided crucial information towards rational design and development of various HIV protease inhibitors. The first generation HIV protease inhibitors were developed based on the TS peptidomimetic principles in which the hydrolyzable P1-P1' amide bond was replaced by non-hydrolysable TS isosteres.[52] As illustrated in the HIV protease catalyzed peptide hydrolysis mechanism (Figure 6), the transition state involves the formation of a gem-diol tetrahedral intermediate. However, the inherent chemical instability of such a tetrahedral intermediate necessitates the design of inhibitors incorporating transition state mimicry, while being stable to hydrolysis. Several such isosteres meeting the above structural guidelines have been investigated in the design and development of inhibitors of renin, a mammalian aspartyl protease. Representative examples of peptide hydrolysis transition state isosteres thus explored include methyleneamine, hydroxyethylene, dihydroxyethylene, hydroxyethylamine, ketomethylene, and phosphonamide etc. (Figure 7). As non-hydrolysable structural mimics of the transition state *gem*-diol, the strategic secondary hydroxy (or a related)

group in these isosteres (colored blue) are expected to participate in hydrogen-bonding interactions with the two aspartates in the HIV protease active site, leading to enzyme inhibition.

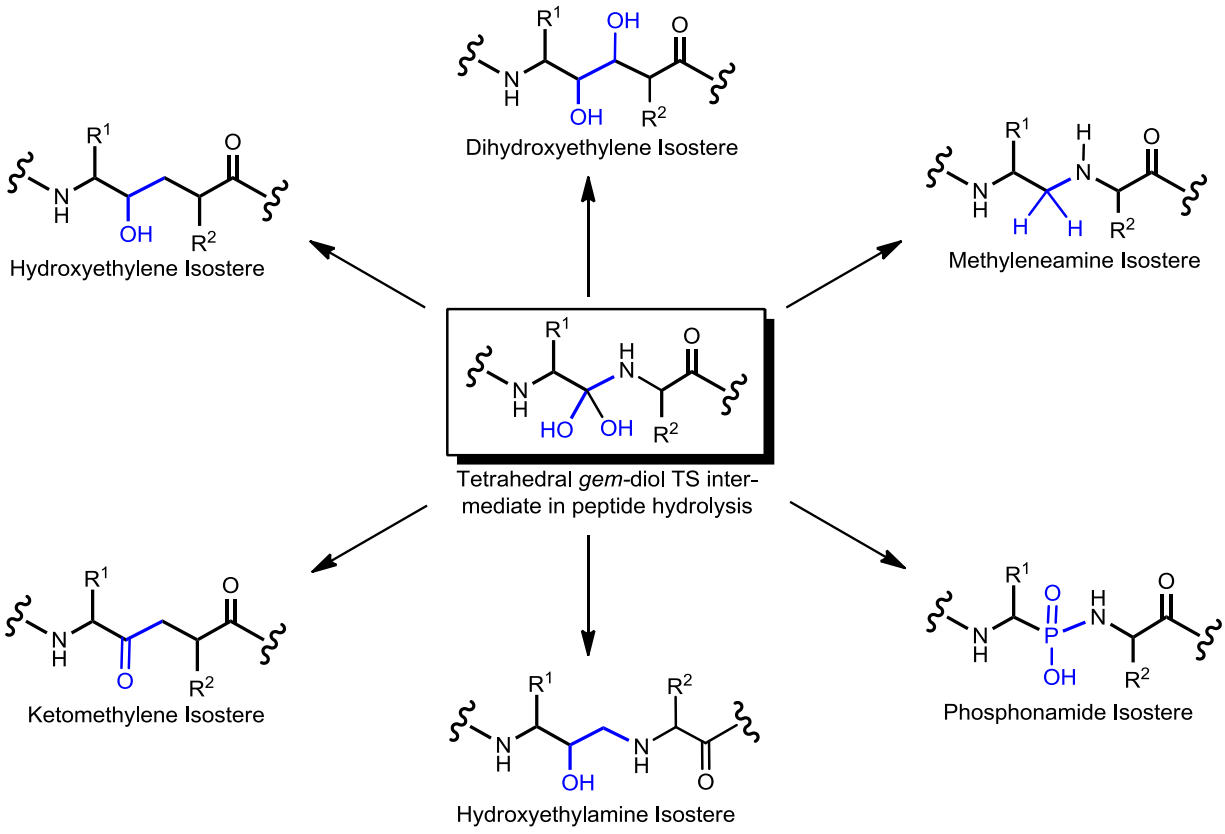


Figure 7. Transition-state isosteres of peptide bond hydrolysis

Structures of protease inhibitor anti-HIV drugs in clinical use, incorporating some of the above transition-state mimic structural components (color coded in red), are shown below (Figure 8). FDA-approved in 1995, Saquinavir as the first protease inhibitor drug in AIDS treatment, and served as a benchmark for all the subsequently introduced PIs. Several other PIs, such as Indinavir, Ritonavir, Nelfinavir and Amprenavir, and their utility in various combination therapy regimens have significantly contributed towards the success of the Highly Active Anti-Retroviral Therapy (HAART).[65-69]

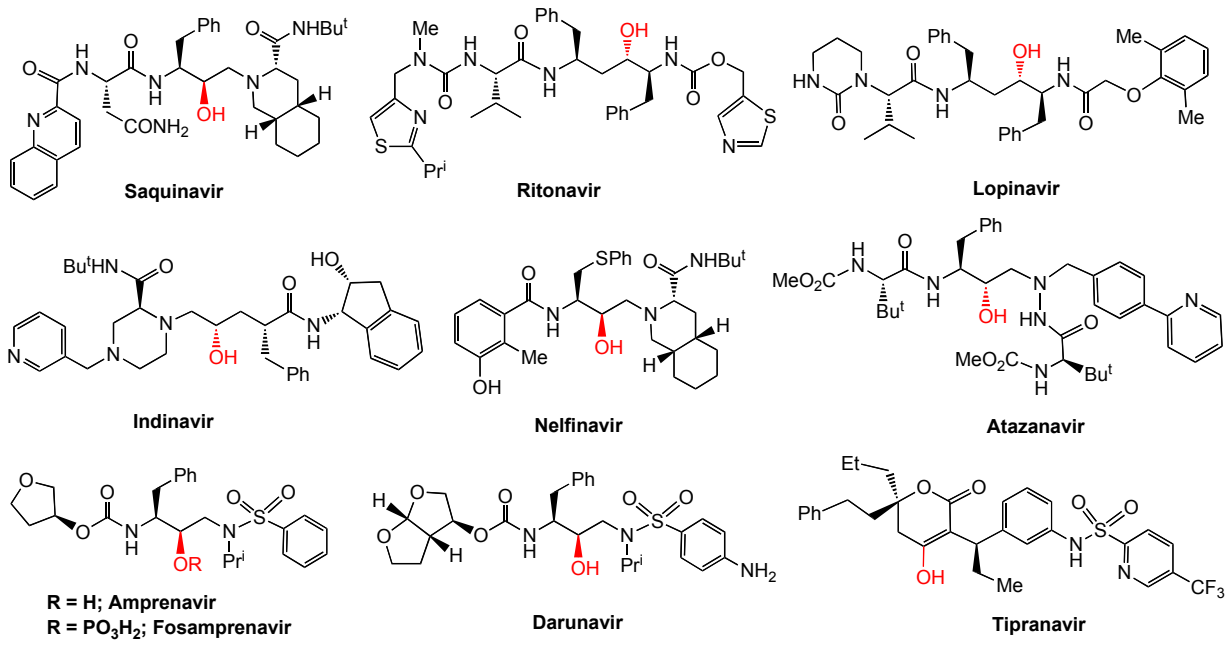


Figure 8. FDA-approved HIV protease inhibitors in AIDS therapy

2.1.3. HIV Protease inhibitors: Design, development and concerns

Having identified appropriate transition-state isosteres, the design of the first generation PI's were based on the natural peptidic substrates of the enzyme, targeting binding interactions spanning the S1/S1', S2/S2', and S3/S3' subsites (Figure 4). Thus, Saquinavir, a hydroxyethylamine transition-state isostere analog, flanked by amino acid units phenylalanine and aspargine at P1 and P2 respectively (Figure 8), is the first clinically approved HIV protease inhibitor drug, displaying strong enzyme inhibitory activity (IC_{50} < 0.4 nm). However, the resulting peptide-like character and relatively high molecular weight of this and similar drugs results in poor pharmacokinetic properties, while also contributing to some of the deleterious side effects.[70,71] Addressing the above limitations, the more recent generation of PI's were developed with an emphasis on the reduction of peptidic features, lowering of molecular weight, and the introduction of novel structural entities capable of strong binding interactions with the enzyme. The second generation PI's thus developed include Lopinavir,[72] Atazanavir,[73]

Amprenavir,[74] Fosamprenavir (a prodrug),[75] Tipranavir,[76] and the most recently approved drug Darunavir[77] (Figure 8). All of these agents demonstrate superior antiviral activities, including activity against resistant viral strains, and also have better oral bioavailability. For example, in the design of Darunavir (Figure 8), the decahydroisoquinoline carboxamide group of Saquinavir has been replaced by an *N*-isopropyl-*N*'-p-aminosulfonamide group, thereby improving water solubility (enhanced oral bioavailability). Similarly, replacement of the P2 and P3 groups of Saquinavir by the bistetrahyrdofuryl ring-fused carbamate group in Darunavir reduced the peptidic character in this molecule, while also providing a better space-filling group at the hydrophobic S2 binding pocket. It was also found that the oxygen atoms of the bicyclic furan substituent of Darunavir participate in potent hydrogen bonding interactions with the peptide backbone of the target enzyme. Such binding interaction with the peptidic backbone of the enzyme, rather than with an amino acid residue, reduces the likelihood of mutation-influenced development of drug resistance.

Although each successive generation of drugs for anti-retroviral therapy (ART) has been associated with an overall improved activity profile, toxic side effects, and development of drug resistance however continues to be of concern. For example, Tenofovir, an NRTI class of first-line drug, commonly used in various combination regimens, has small but measureable adverse effects on kidney function.[78-80] A rapidly growing and consistent evidence base suggests that many markers of inflammation are higher in ART adults than in age-matched uninfected individuals.[81,82] Liver toxicity has also been observed in HIV patients receiving novel second generation non-nucleoside reverse transcriptase inhibitors etravirine and rilpivirine.[83,84]

Apart from the toxicity issues, development of viral resistance against the HIV drugs is also a growing concern. According to a recent report, usage of HIV-1 reverse transcriptase (RT)

inhibitors have been found to contribute towards the development of resistance by introducing mutations into the viral genome.[85,86] Gratifyingly, the current treatment regimens of HAART, in which multiple classes of anti-HIV-1 drugs are employed, have been shown to significantly increase the life expectancy of HIV-1 infected patients. Additionally, first-line HAART with boosted protease inhibitor (PI)-based regimens resulted in less resistance within and across drug classes.[87-90] However, the ability to provide effective long-term anti-HIV therapy is complicated, as 40-50% of those patients, who initially achieved favorable viral suppression to undetectable levels, subsequently experienced treatment failure. Between 10-40% of antiviral therapy-naïve individuals with HIV-1 infection have shown persistent viral replication (plasma HIV RNA > 500 copies/mL) under HAART regimens.[91] Possibly due to the transmission of drug-resistant HIV-1 regimens, only partial immunologic reconstitution is attained in patients with advanced HIV-1 infections or AIDS.[51]

With the above concerns in HIV therapy, there is a continuing need for further development of more active / less toxic anti-HIV drugs, identification of novel targets, and new treatment strategies / regimens to combat resistance.[92,93]

2.2. Present work

In addition to identification of important binding elements required for biological activity, the pharmacophore of a drug molecule is also defined by its bioactive conformation at the receptor-binding site. Elucidation of the bioactive conformation, the conformation adopted by an otherwise structurally flexible compound upon binding to its biomacromolecular target, is a critical element in successful drug discovery efforts. An important strategy in such investigations include strategic introduction of conformational restraints in the structural framework of the compound of interest, resulting in the locking of the molecule into a more rigid

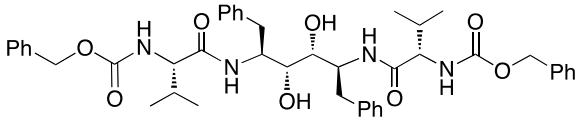
conformation. As a conformationally more flexible bioactive compound has an increased likelihood of interacting with multiple biological receptors, locking the compound to a more rigid active conformation can lead to selective binding to the desired receptor while avoiding off-target interactions. From an entropic viewpoint too, compared to a flexible molecule, a rigidified analog in its bioactive conformation is expected to have better overall binding affinity.

Molecules incorporating structural features capable of causing decreased freedom of intramolecular motion, particularly of rotation around single bonds, exhibit conformational restriction.[94] Some of the common strategies towards introduction of conformational restriction in an otherwise flexible structural scaffold include, incorporation of olefinic linkages or cyclic moieties, extended networks of intramolecular hydrogen bonding, and bulky substituents preventing free rotations across single bonds. However, in designing conformationally rigid analogs, it is an important criterion that, in terms of size, shape, and molecular weight, the designed analogs retain similarity to the parent (lead) compound to a certain extent, so as not to compromise critical structural features associated with desirable activity. Following the above strategy and approach, rational design of agents for various therapeutic areas has been investigated.[62-66]

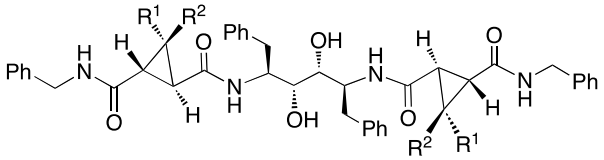
Among the various structural motifs useful in imparting conformational rigidity, cyclopropane rings remain a popular choice. The characteristically rigid and small structure of a cyclopropane ring is highly effective in restricting the conformation of a molecule while retaining the chemical and physical properties of the parent compound. Cyclopropane ring-rigidified peptidomimetics have been found to be very useful in the strategic design of conformationally pre-organized analogs imitating turns or helices of the peptide secondary

structure. In another study, it has been shown that by controlling the cyclopropane ring substitution pattern (*cis/trans*) in a peptide backbone, in addition to attaining conformational rigidity, it is also possible to simultaneously project the amino acid side chains in defined orientations. Cyclopropane-containing conformationally rigid analogs have thus been variously investigated in designing inhibitors of renin,[95,96] HIV-1 protease,[97] Ras farnesyltransferase,[98] enkephalin analogues,[99] SH2 antagonists,[100] and H_3 and/or H_4 receptor ligands.[101] Development of selective α4β2-nicotinic acetylcholine receptor partial agonists exhibiting an antidepressant profile in which cyclopropane-induced rigidity improved metabolic stability and provided directionality towards H-bonding have also been reported.[102]

In a pioneering example, Martin and co-workers have investigated cyclopropane-containing peptidomimetic analogs (**B**) designed along the lines of known C2-symmetric 1,2-dihydroxyethylene dipeptide isosteric HIV protease inhibitors (**A**) (Figure 9).[97]



(**A**) Dihydroxyethylene dipeptide isostere (lead compound)



(**B**) Cyclopropane substituted dihydroxyethylene dipeptide isostere (backbone rigidified analog): R^1= H, Me; R^2 = H, Me

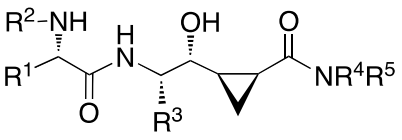
Figure 9. An HIV Protease inhibitor C2-symmetric dihydroxyethylene peptide isostere (**A**), and peptide backbone rigidified cyclopropane analogs (**B**) thereof (Ref. 97)

Some of the noteworthy inhibitor design features (**B**) in the above study include: installation of two cyclopropane rings spanning the P2 and P2' subsites, resulting in local rigidification of the peptidic backbone; peptide hydrolysis transition-state mimicry via the incorporation of a 1,2-dihydroxyethylene structural motif; and retention of the overall C2 symmetry as in the parent compound (**A**). The *trans*-1,2 substitution pattern across the two cyclopropane rings are meant to orient the resulting peptidic substrate in an extended

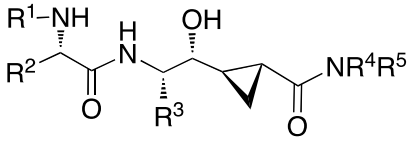
conformation. Several of the rigid analogs (**B**) thus synthesized were found to be competitive inhibitors (K_is in sub-nM range) of the wild-type-HIV-1 protease, and were approximately equipotent to the known lead compound (**A**).[97]

The above study demonstrates the promise of conformationally rigid peptide isosteres in probing the bioactive conformation of HIV-protease inhibitor peptidomimetics, and potential application thereof in the design and development of a new class of agents for AIDS therapy.

Intrigued by the above observations, we decided to initiate a study aimed at the design, synthesis and evaluation of a hitherto unexplored class of analogs combining conformational rigidity and peptide hydrolysis transition state mimicry in a common structural framework. Based on the above design elements, the general structural scaffolds of two hypothetical analogs we envisioned are shown below (Figure 10).



Cis-1,2-disubstituted cyclopropane



Trans-1,2-disubstituted cyclopropane

Figure 10. Proposed cyclopropane ring-constrained hydroxyethylene dipeptide isosteres

Mimicking relevant structural features of some of the known HIV protease inhibitors (e.g. Ritonavir and Lopinavir: Figure 8), we conceptualized the above analogs combining the functionally critical hydroxyethylene transition-state isostere moiety, and the introduction of a strategically placed cyclopropane ring to impart conformational rigidity in the peptide-like backbone. It is noteworthy that, depending on the 1,2-substitution pattern (*cis*/*trans*) across the cyclopropane ring, the above analog design offers the additional possibility of spatial placement of the two substituents either in a folded (*cis*), or an extended (*trans*) conformation. Side chain

orientations of peptides/pseudopeptides are often critical determinants of enzymatic binding interactions and specificity, therefore, the ability to control the orientations of the above appendages is a highly desirable feature in the strategic design of the above analogs. With the above aims, the details of the synthetic studies undertaken by me are described below.

2.2.1. Retrosynthetic strategy and combinatorial synthetic approach towards a new class of *cis*-1,2-disubstituted cyclopropane ring-containing hydroxyethylene dipeptide isosteres

Initiating the desired study, we began our pursuit by targeting the synthesis of conformationally rigid hydroxyethylene dipeptide isosteres containing a *cis*-1,2-cyclopropane ring in the structural backbone. Accordingly, utilizing a versatile chiral aminobutenolide **1** [reported previously from our laboratory (*vide infra*)] as an advanced building block, we envisioned the following combinatorial synthetic approach towards rapid and efficient synthesis of a select library of the desired dipeptide isostere analogs **4** (Figure 11).

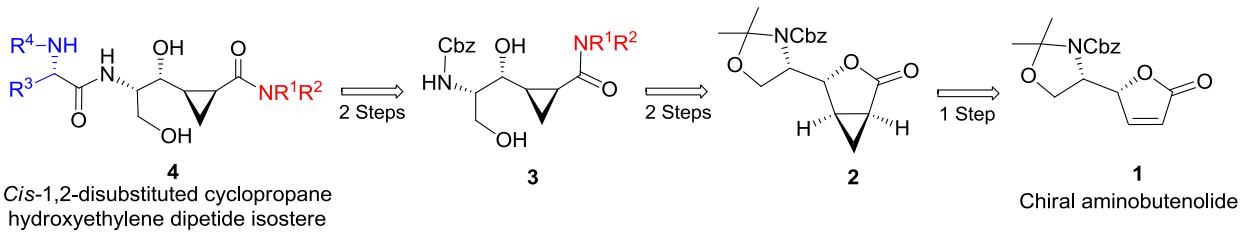
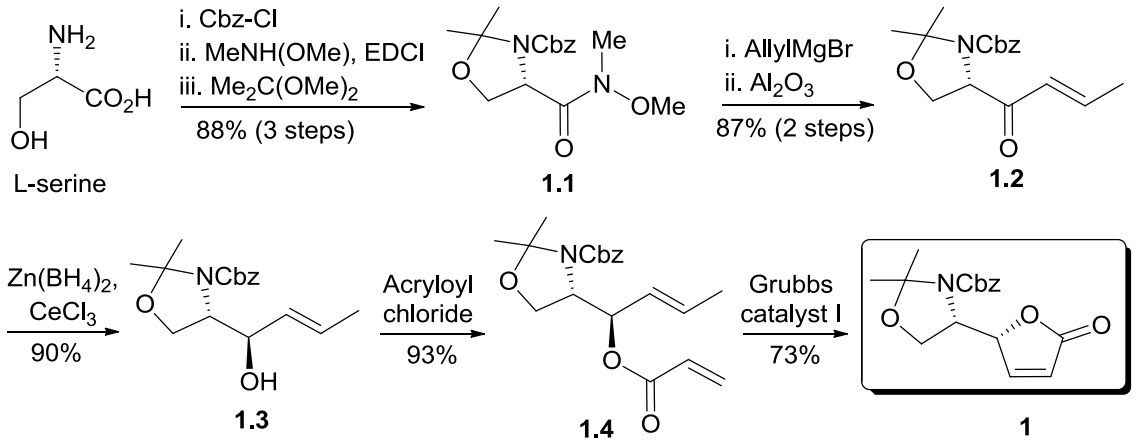


Figure 11. Retrosynthetic strategy and approach towards conformationally-restricted hydroxy-ethylene dipeptide isosteres: A combinatorial approach

As evident, the strategically functionalized aminobutenolide **1** already incorporates two of the required stereogenic centers of the final product. Through asymmetric induction, the existing chiral center of the butenolide ring is expected to allow stereoselective installation of the cyclopropane ring in compound **2** (Figure 11) as shown. Exploiting the inherent ring-strain of the [3, 5]-bicyclic scaffold **2**, the subsequent lactone ring-opening with amines will easily furnish the

corresponding *cis*-1,2-disubstituted cyclopropane carboxamide derivatives **3**, with simultaneous unmasking of the desired hydroxyethylene isostere structural motif. Employing a chosen set of amines in the above lactone aminolysis step, this reaction will also provide the first diversification point (color coded red) towards the intended synthesis of the combinatorial library of target analogs. Finally, *N*-Boc deprotection and standard coupling of the resulting amine with various amino acids (second diversification step in the library synthesis; colored blue) will culminate in the target cyclopropane ring-containing hydroxyethylene dipeptide isosteres (**4**). Although not included in the present study, appropriate functionalization and nucleophilic substitution involving the primary hydroxyl group of **3** could easily provide a third possible diversification site in further combinatorial library construction efforts.

Accordingly, acquisition of the aminobutenolide (**1**) was initiated following a previously developed synthetic route from our laboratory as shown below (Scheme 1).[26]



Scheme 1

Starting from L-serine, the key steps in the synthesis involved, conversion of the Weinreb amide **1.1** to the strategic enone **1.2**, followed by a chelation-controlled reduction of the ketone to the corresponding *anti*-aminoalcohol **1.3** in a highly stereoselective manner.[103] Subsequent

derivatization of **1.3** to form the diene **1.4**, and subjecting it to Grubbs ring-closing metathesis provided the desired enantiopure lactone **1** (Scheme 1).[104] Some of the salient features of the above synthesis involve:

- A practical, efficient, and reproducible reaction sequence
- Good overall yield, and easy scalability to obtain multi-gram quantities (15–20g) of the product lactone in each reaction batch
- The product lactone obtained as a stable, crystalline solid, and can be stored indefinitely without any apparent decomposition
- Starting from D-serine, the above route also provides an equally efficient protocol towards obtaining *ent*-**1**

It is worth mentioning that, in several other previous, and ongoing research projects in our group, the above lactone has proven to be a highly valuable chiral building block in the synthesis of a variety of natural and non-natural products of biological significance.

Having synthesized sufficient quantities of the lactone **1** for the proposed study, introduction of the cyclopropane moiety to form the desired bicyclic lactone **2** was next investigated. Cyclopropanation of olefins with diazoalkanes in the presence of transition metal catalysts is a widely used synthetic protocol.[105-111] The simplest diazoalkane used in such cyclopropanation reactions is diazomethane (CH_2N_2). Although several metal catalysts (e.g. Ni, Pd, Cu, Fe, Co, Rh etc.)[112] have been found to be useful in performing the above cyclopropanation reaction, palladium catalysts are generally among the most effective.[113-116] A probable mechanistic pathway explaining the palladium-catalyzed cyclopropanation of olefins in the presence of diazomethane is shown below (Figure 12).

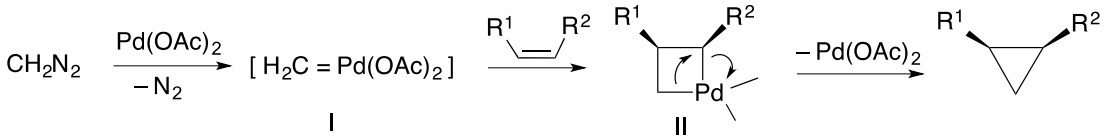
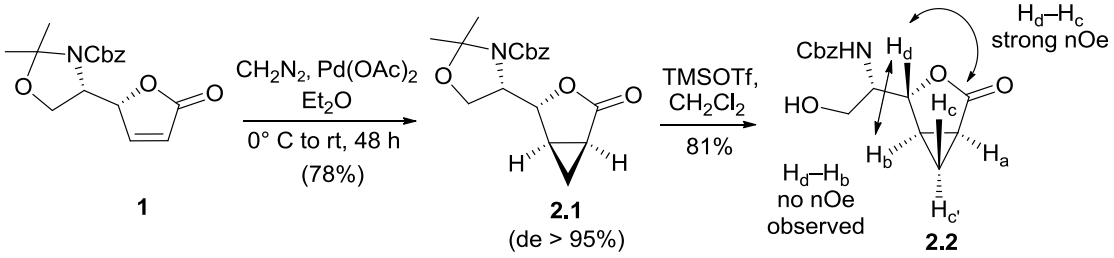


Figure 12. Cyclopropanation of olefins in the presence of diazomethane and palladium

The overall reaction involves initial coordination of diazomethane to the palladium catalyst to form the metal-carbenoid species **I**, with concomitant extrusion of nitrogen. Subsequent concerted addition of the metal-carbenoid to the olefin results in the formation of the palladocyclobutane **II**. This unstable intermediate on further reductive elimination affords the *cis*-cyclopropane derivative in a stereospecific manner (Figure 12).[94-117]

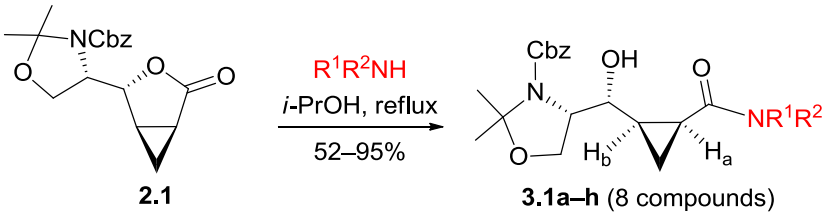
Accordingly, following the above method, the aminobutenolide **1** was reacted with freshly prepared diazomethane ethereal reagent[118] in the presence of a catalytic amount (10 mol%) of $Pd(OAc)_2$. Subsequent work-up and chromatographic purification of the reaction mixture afforded the cyclopropane ring-fused bicyclic lactone **2.1** as the only product (Scheme 2), along with the recovery of minor quantities of unreacted starting material. During efforts to optimize the above reaction, it was found that addition of the diazomethane in two batches (0 h and after 24 h) afforded the best product yield. The structure of the product **2.1** was confirmed on the basis of high-resolution NMR and mass-spectrometric studies. To further confirm the assigned stereochemistry of the newly formed cyclopropane stereocenters, the *N,O*-acetonide linkage of **2.1** was subjected to cleavage so as to simplify the ^{1}H NMR spectrum (to eliminate peak-broadening caused by the presence of rotamers in **2.1**). Accordingly, employing a mild and efficient reaction protocol previously developed in our laboratory,[119] selective removal of the *N,O*-acetonide protecting group cleanly afforded the aminoalcohol **2.2** (Scheme 2) in high yield. In subsequent high-resolution NMR experiments, significant nOe enhancement was observed

between the H_d and H_c protons, while there was no such effect involving the H_d and H_b protons (Scheme 2), thereby confirming the assigned stereochemistry at the cyclopropane stereocenters. True to expectation, the observed stereoselectivity in the above reaction is influenced by the chiral center adjacent to the olefin directing the approach of the incoming reagent from the less hindered β-face (propagation of asymmetry).[120,121]



Scheme 2

Continuing with our studies, the next step in the synthesis involved aminolysis of the bicyclic lactone to form the *cis*-1,2-disubstituted cyclopropane containing hydroxyethylene isostere segment of the target compound. Accordingly, refluxing a solution of the lactone **2.1** with an excess of n-butylamine in the presence of i-propanol as solvent cleanly afforded the corresponding ring-opened cyclopropane carboxamide **3.1a** (Scheme 3).



R^1R^2NH = (**a**) n-Butylamine; (**b**) Cyclopropyl amine; (**c**) Cyclohexyl amine; (**d**) Dimethylamine; (**e**) Pyrrolidine; (**f**) Morpholine; (**g**) Tetrahydroisoquinoline; (**h**) (1*R*,2*S*)-1-aminoindan-2-ol

Scheme 3

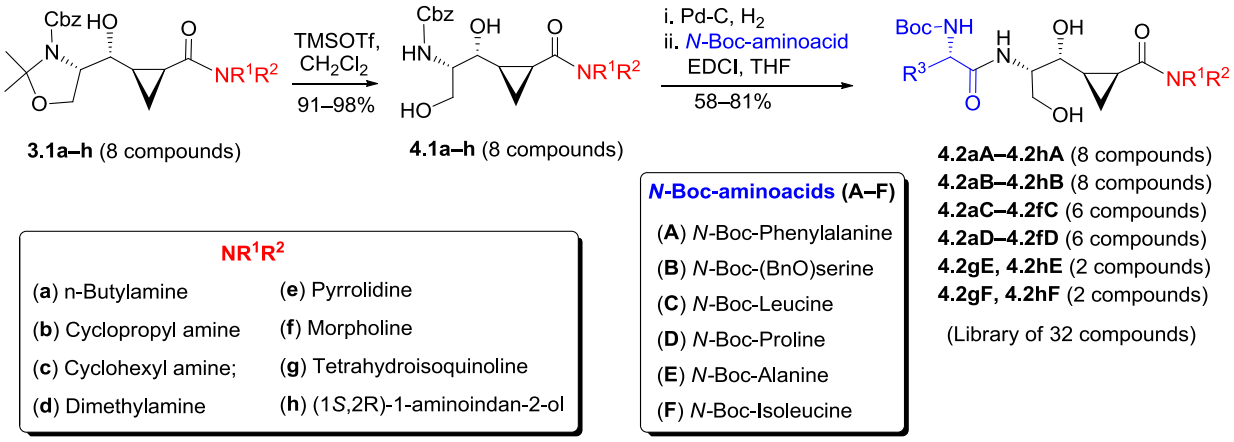
In addition to formation of the right-hand side portion of the carboxamide functional group, the above reaction also simultaneously unveiled the critical 'warhead' hydroxyethylene isostere component as designed in the target conformationally rigid dipeptide analogs.

A potential concern in this reaction has been the possibility of stereochemical inversion at the chiral center adjacent to the carboxamide group, so as to orient the two bulky substituents at the 1,2-position of the cyclopropane ring in a more favorable *trans*-relationship. Gratifyingly, ^{1}H NMR spectrum of the above product (**3.1a**) indicated the coupling constant between the respective methine protons ($J_{Ha,Hb}$=8.1Hz) of the cyclopropane ring to be in the desired *cis*-relationship, thereby confirming the assigned product structure and stereochemical integrity.

Subsequently, in a parallel combinatorial synthetic approach, a select set of representative amines with different steric, electronic, and lipophilic properties were also employed in the above aminolysis reaction to obtain a total of eight different carboxamide analogs **3.1a-h** as shown (Scheme 3). In addition to belonging to either to a primary or secondary amine class, the chosen amines can also be broadly classified into aliphatic, cyclic and heterocyclic categories. Moreover, the tetrahydroisoquinoline, and the aminoindanol-derived carboxamide moieties in two of the above analogs (**3.1g–h**) have also been deliberately designed so as to mimic similar structural components as present in some of the known HIV protease inhibitors (Figure 8).

In continuation of the studies, incorporation of the remaining peptidic core in the above structural scaffold was next undertaken. Towards this goal, selective removal of the *N,O*-acetonide linkage of **3.1** was performed using a mild and efficient protocol developed previously in our laboratory.[119] Accordingly, treatment of the oxazolidines **3.1a-h** with TMSOTf at ambient temperature resulted in rapid formation of the corresponding aminodiol derivatives **4.1a-h** in uniformly high yields (Scheme 4). Subsequent hydrogenolytic removal of the *N*-Cbz-protecting group, followed by reaction of the resulting free amines with a chosen set of six *N*-Boc-aminoacids (**A**–**F**) under standard peptide coupling conditions[122] led to the uneventful formation

of a combinatorial library of the desired dipeptide isosteres **4.2aA–4.2hf** (Scheme 4, and Figure 13). Thus, following a concise and efficient combinatorial synthetic route, a 32-member library of a novel class of conformationally rigid hydroxyethylene dipeptide isosteres was successfully constructed.



Scheme 4

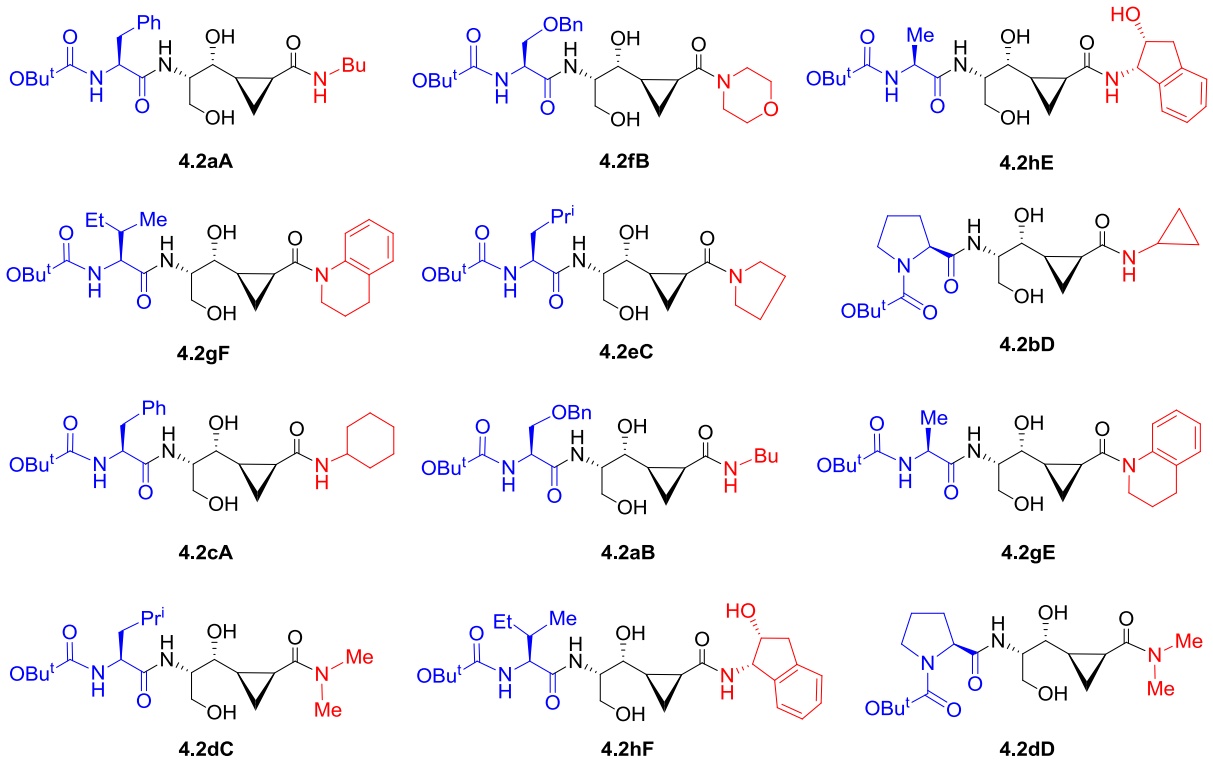
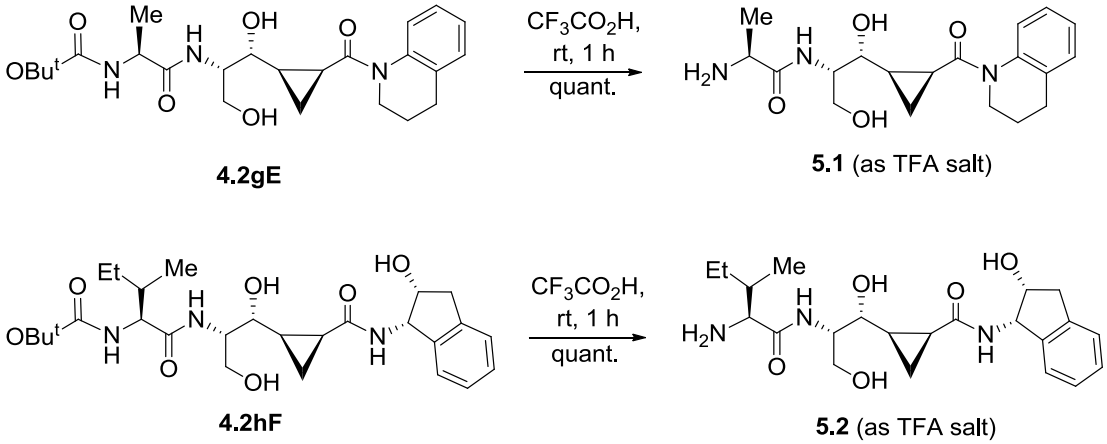


Figure 13. Representative examples of the conformationally-rigid hydroxyethylene dipeptide isostere combinatorial library of compounds

As the Boc-protected amine functional groups in this library of compounds represent a potential diversification site in future library design, it was considered worthwhile to investigate the possibility of removal of this group without compromising the structural or stereochemical integrity of the above dipeptide isosteres.

Accordingly, two representative analogs from the library, **4.2gE** and **4.2hF** (Figure 13), were selected for Boc-deprotection study. Thus, treatment of the above compounds with trifluoroacetic acid (neat) at ambient temperature, followed by removal of the excess acid under high vacuum cleanly afforded the respective amines **5.1** and **5.2** (Scheme 5) as the corresponding TFA-salts. Spectral and analytical data confirmed the assigned structure and purity of the products formed.



Scheme 5

2.3. Concluding remarks

Structural inspection of the various HIV protease inhibitor dipeptide isosteres (Figure 8) indicates the presence of a linear peptide isosteric backbone to be a common motif, with individual compounds differing mostly with respect to their side chain functionalities. Surprisingly, modifications involving the conformational features of the peptidic backbone of the HIV protease inhibitors have remained relatively scarce. Contemporary studies have shown that

preorganization of otherwise flexible peptidic molecules in a structurally rigid conformation can result in higher target binding affinity and improved selectivity. In such studies, strategies for introduction of conformational restrain on peptidic backbones generally involved incorporation of olefin motifs, or the formation of cyclic framework.

In view of the above observations, our present study was initiated to investigate the design and synthesis of a new class of conformationally-restricted hydroxyethylene dipeptide isosteres as potential HIV-protease inhibitors. Accordingly, utilizing an L-serine-derived polyfunctional chiral lactone as a strategic building block, we have developed an efficient combinatorial synthetic route to construct a unique library of the above dipeptide isosteres. In the structural design of these analogs, local conformational rigidity on the dipeptidic backbone has been achieved by stereoselective incorporation of a strategic 1,2-disubstituted cyclopropane structural motif. Utilizing two diversification sites in the target analogs, and employing select sets of amines and amino acids as the diversity elements, a parallel synthetic approach has been followed towards rapid construction of a demonstration combinatorial library of 32 analogs.

The synthetic route designed also offers additional flexibility in terms of further diversification. For example, the primary hydroxy and the amine functional groups in the molecule (Figure 14) can be easily manipulated to incorporate appropriate functionalities to optimize interactions at the S1–S3 subsites of the enzyme binding pocket.

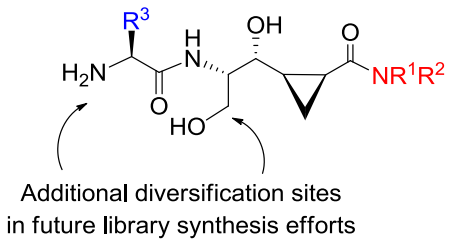


Figure 14. Further diversification sites towards future combinatorial libraries

Continued synthetic studies along the above lines, and biological evaluation of the analogs will be the target of future investigations.

Chapter 3

Total Synthesis and Structure-Activity Relationship Studies of the Cytotoxic Anhydrophytosphingosine Jaspine B

3.1. Introduction

Cancer, a broad group of diseases characterized by uncontrolled cell growth, is a leading cause of death in the United States, accounting for more than 580,000 deaths in 2013 alone.[123,124] According to Weinberg *et al,* the major hallmarks of cancer include: sustained proliferative signaling, evasion of growth suppressors, cell death resistance, enabled replicative immortality, induction of angiogenesis, activation of invasion, metastasis, reprogrammed energy metabolism, and evasion of immune-destruction[125].

Despite major breakthroughs in many areas of medicine over the past century, successful treatment of cancer remains a daunting challenge. Thus, advances in the elucidation of molecular mechanisms that promote cancer, and identification of small molecules that modulate these mechanisms are providing new insights into potential therapeutic targets for cancer chemotherapy.

Natural products have a long history of use in the treatment of cancer and many other diseases. Discovery of several effective anti-cancer agents from natural products, or leads thereof, may be attributed directly or indirectly, to a history of use of the relevant plants in traditional medicine. Representative examples of some of the natural product-inspired drugs in contemporary cancer chemotherapeutic use include the taxanes, camptothecins, and vinca alkaloids etc. Thus, natural products continue to be a rich resource for the discovery and development of new chemotherapeutic agents.[126,127]

3.2. Background

3.2.1. Isolation of marine natural product Jaspine B and biological significance

Jaspine B (Figure 15), also known as Pachastrissamine, is a marine natural product initially isolated from the Okinawan marine sponge *Pachastrissa sp*. (family Calthropellidae) in 2002.[128] It is the first example of a cyclic anhydrophytosphingosine structural feature in a natural product. In cell cytotoxicity studies, Jaspine B exhibited an IC_{50} value of 0.01 μg/mL against P388, A549, HT29 and SK-Mel28 cancer cell lines.[128]

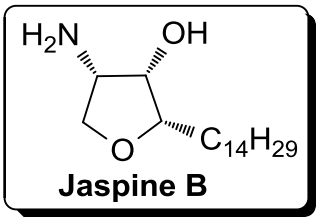


Figure 15. Structure of the cytotoxic marine natural product Jaspine B

Shortly thereafter, Debitus *et al* have independently reported the isolation of the same bioactive natural product from a different marine sponge *Jaspis* sp. in Vanuatu, New Zealand. [129] In their biochemical studies, Jaspine B exhibited toxicity towards brine shrimp, *Artemia salina,* at a concentration as low as 0.1 μg/mL. In another cell-based cytotoxicity study, Jaspine B exhibited an IC_{50} value of 0.24 μM against A549 human lung carcinoma cell line. [129]

Since its isolation, there has been a keen interest in identifying the molecular mechanisms by which Jaspine B exhibits cytotoxicity in cancer cell lines. One such study by Salma *et al* have reported that time and dose-dependent cell growth inhibition was exhibited by Jaspine B in murine B16 and human SK-MEL28 melanoma cells.[130] In their studies, exposure of these melanoma cells to Jaspine B triggered cell death by typical apoptosis involving phosphatidyl serine externalization, release of cytochrome C, and caspase processing.[130] These biochemical events were associated with increased intracellular ceramide levels, owing to perturbed ceramide

metabolism caused by strong inhibition of *sphingomyelin synthase* (SMS). SMS belongs to a *transferase* enzyme family, which catalyzes the transfer of phosphatidyl choline to the primary hydroxyl of ceramide to form sphingomyelin (Figure 16).

In another study by Delgado *et al*, exposure of lung cancer cells to Jaspine B suggested that dihydroceramide-mediated autophagy might be involved in its observed cytotoxicity.[131] Autophagy (self-eating) is a survival pathway responsible for the breakdown of damaged organelles, protein aggregates, and long-lived proteins towards recycling during periods of starvation in an effort to maintain cellular homeostasis.[132] During tumorigenesis, cancer cells need a continuous supply of nutrients. However, solid tumor cancer cells are deprived of nutrients due to poor vascularization. Under these metabolic stressful circumstances cells initiate autophagy as a survival mechanism.[133]

Recently, Kim *et al* reported that Jaspine B induces apoptosis in melanoma cells and inhibits cell cycle progression by activating Forkhead box O3 (FOXO3) via suppression of the Extracellular Regulatory Kinase (ERK) pathway and inhibiting Cyclin-Dependent Kinase (CDK) production.[134] FOXO3 is a tumor suppressor transcription factor that negatively regulates melanoma tumorigenesis.[135] It has been identified as a component of phytosphingosine (PS)-induced cell death machinery.[136] FOXO3 induces apoptosis by upregulation of genes necessary for cell death, such as *BIM* (BCL2 Family member that promotes apoptosis); *PUMA (*p53 Upregulated Modulator of Apoptosis)[137] or down regulation of anti-apoptotic proteins such as *FLIP* (FLICE Like Inhibitory Protein).[138]

Though the exact mechanism of action of Jaspine B is not yet fully understood, preliminary biochemical studies supporting its involvement in ceramide metabolism pathways and anti-cancer potential offers an interesting lead for further investigation.[139]

3.2.2. Sphingolipids biosynthesis, metabolism and biological significance

Sphingolipids (SLs) constitute a heterogeneous class of lipids derived from aliphatic amino alcohol sphingosine (Figure 16). In mammalian cells, SLs play key role both as ubiquitous component of cellular membrane, circulating lipoproteins, and as bioactive lipids involving in a variety of fundamental processes including cell growth, cell death, cell differentiation, cell senescence, migration, angiogenesis, inflammation and immune response.[140-151] Their functional diversity is related to their structural complexity and is reflected by their implication in numerous human pathological processes.

Among the SLs, ceramide (Figure 16) is the central precursor of sphingolipid metabolism and has been studied extensively for its pro-apoptotic and anti-proliferative effects. Pharmacological approaches targeting ceramide metabolism in apoptosis-related diseases such as cancer have been proposed.[140-142,148-154] The three major biosynthetic pathways leading to ceramide formation (Figure 16) include: (1) *de novo* synthesis from serine and palmitoyl CoA; (2) *SMase* pathway: *sphingomyelinase* assisted catabolism of Sphingomyelin, and (3) Salvage pathway: where more complex sphingolipids are metabolized to sphingosine followed by re-acylation to form ceramide (Figure 16). Ceramide thus formed is subject to various metabolic pathways such as, C-1-*O*-functionalization into several metabolites such as: sphingomyelin (catalyzed by *sphingomyelin synthase,* SMS); glucosyl ceramide (glycosylated by *glucosyl ceramide synthase,* GlcCer); ceramide-1-phosphate (C1P) (phosphorylated by *ceramide kinase*, CERK); and 1-*O*-acyl ceramide (acylated by *1-acyl ceramide synthase)*. In another important metabolic pathway, Sphingosine-1-phosphate (S1P) is derived from ceramide by the actions of the enzymes ceramidase followed by sphingosine kinase. In contrast to ceramide, S1P is a key facilitator of pro-cancerous cellular events such as proliferation, migration, angiogenesis, as well

as resistance to apoptosis. [140-142,148-151 155-158] In view of the opposing actions of ceramide and S1P, it has been suggested that the cellular balance between these two sphingolipids could be an important determining factor in cell survival or death. [141-143,149-152,159,160]

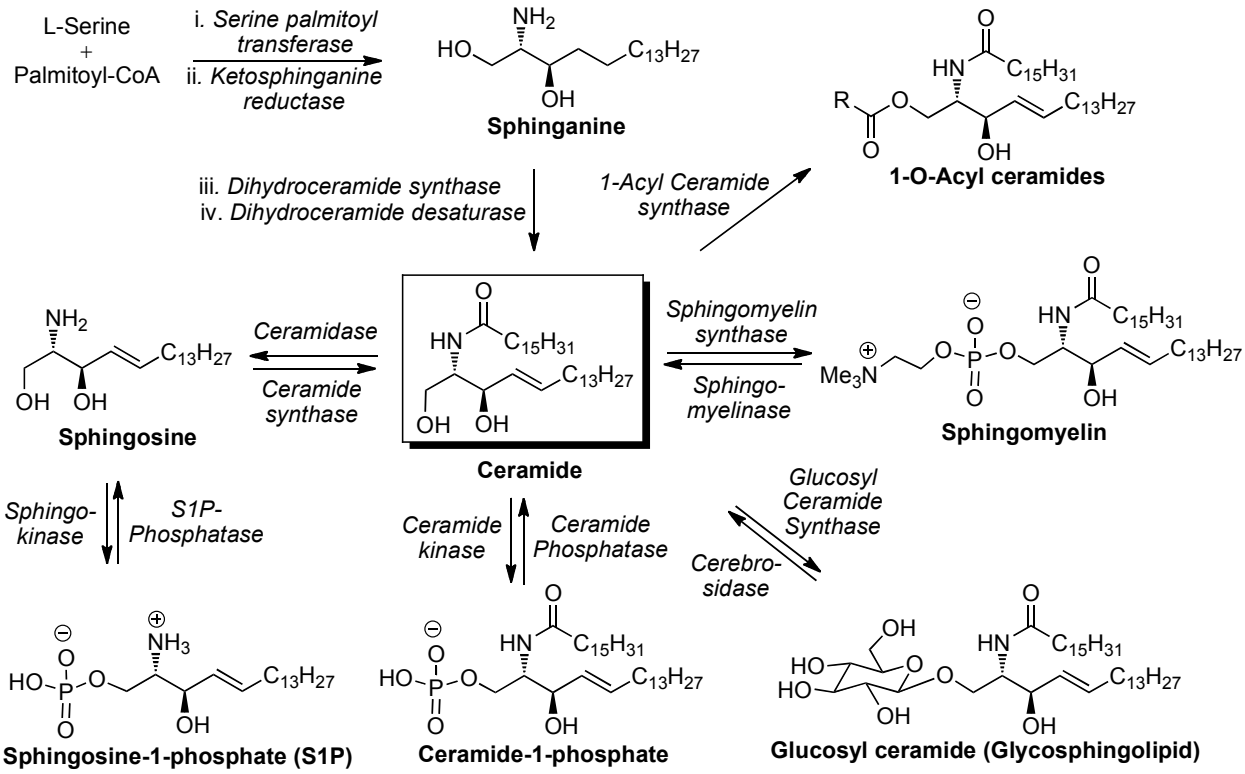


Figure 16. Ceramide biosynthesis and metabolism: An overview

As evident, ceramide is ultimately at the crux of sphingolipid metabolic pathways, and modulating these metabolic pathways will significantly contribute towards controlling intracellular ceramide concentration.

Among the various biological functions of ceramide, of particular relevance is its apoptotic potency and specificity.[140-142,148-153,161-165] Administration of exogenous ceramides to cells *in vitro* has been shown to cause apoptosis, whereas, reducing cellular ceramide levels lead to drug resistance. Modulation of ceramide-induced apoptosis is therefore a viable strategy for sensitizing neoplastic cells to anticancer agents.[140-142,148-151,166] The table shown below (Table 2)

lists several sphingolipid pathway-associated enzymes as potential targets for ceramide-based therapeutics.[142,166,167]

Table 2. Sphingolipid biosynthetic pathways-associated enzymes as potential anticancer targets

Enzyme	Known Modulators	Action
Serine palmitoyl transferase (SPT)	4-HPR; PSC 833	Activation
Ceramide synthase (CS)	4-HPR; Daunorubicin	Activation
Ceramidase	B13; D-MAPP	Inhibition
Ceramide kinase (CERK)	K1; NVP-231	Inhibition
Sphingomyelinase (SMase)	Many cancer drugs	Activation
Sphingomyelin synthase (SMS)	D609 and prodrugs; Jaspine B	Inhibition
Sphingosine kinase (SK)	Safingol; DMS	Inhibition
Sphingosine-1-phosphatase (S-1-P)	-	-
Glucosyl ceramide synthase (GCS)	P-drugs; NBDJ	Inhibition
1-O-Acylceramide synthase (ACS)	PPMP	Inhibition

As shown in the table, manipulation of the above biosynthetic pathways can provide a means for enhancing drug action, overcoming drug resistance, protecting normal cells from cytotoxic actions, and even creation of new chemoprevention possibilities.

In view of the anti-tumor role of ceramide, enhancement of endogenous ceramide levels is considered an effective way to regulate cell growth. Several anticancer drugs, such as Daunorubicin, Camptothecin, Etoposide, Fludarabine, and Gemcitabine promote *de novo* synthesis of ceramide, whereas, Ara-C increases the cellular ceramide level through a SMase activation pathway.[146 168-170] An alternative strategy to prolong ceramide half-life is the inhibition of the enzymes responsible for its metabolism or activation of enzymes involved in the

biosynthesis of ceramide (Table 2). For example, when tested in a murine model of metastatic colon cancer, as well as a prostate cancer xenograft model, B13 (a potent ceramidase inhibitor) was found to prevent tumor growth via accumulation of ceramide without affecting the normal ceramide level or survival of normal liver cells.[171,172] Inhibition of *Sphingomyelin synthase* (SMS) has been shown to increase ceramide accumulation in cancer cells.[173,174] In one particular study, a synergistic combination of exogenous ceramide and paclitaxel was found to enhance apoptotic death of Tu 138 head and neck cancer cells.[142]

Strategies leading to agonism/antagonism of these sphingolipids, or modulation of their levels could provide attractive cancer therapeutic approaches. Though the study of the bioactive sphingolipids is of relatively recent origin, an increased understanding of the specific functions of these bioeffector molecules, and rational modulations of these functions could provide novel avenues for cancer therapy. The growing body of ongoing investigation suggests that ceramide-related pathways are a fertile area for the identification and development of novel cancer chemotherapeutic agents.

Encouraged by the above observations, and the promising activity of Jaspine B in enhancing cellular ceramide level (via inhibition of sphingomyelin synthase), I initiated a study aimed at the synthesis and structure-activity relationship investigation of this novel natural product. Details of the studies thus undertaken are reported in the following sections.

3.3 Previous syntheses of Jaspine B

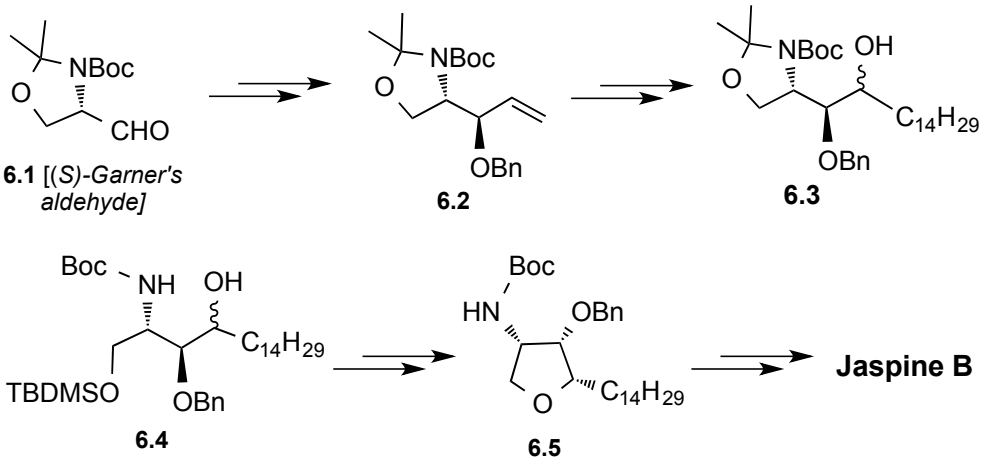
For a relatively small molecule (mol. wt. 299), the structural features of Jaspine B are somewhat challenging. Aside from three contiguous asymmetric centers, the sterically unfavorable all-*syn* substitution pattern as present in this molecule is also a potential concern in any synthetic efforts. The appealing structural / stereochemical challenges and the biological

significance of Jaspine B have resulted in more than 20 total syntheses since its isolation (2002).[175-198] The various approaches employed in these synthetic endeavors include chiral pool approach, asymmetric synthesis, and organocatalysis. Among these approaches, the chiral pool strategy utilizing naturally available enantiopure starting materials such as L-serine and various carbohydrates have been the most popular. Among the asymmetric synthetic methods, Sharpless asymmetric dihydroxylation of appropriate achiral precursors were the most commonly employed approaches.[193,194,197] The following section briefly reviews a representative cross-section of the total synthetic endeavors towards Jaspine B.

L-Serine-based syntheses:

B.V. Rao's approach

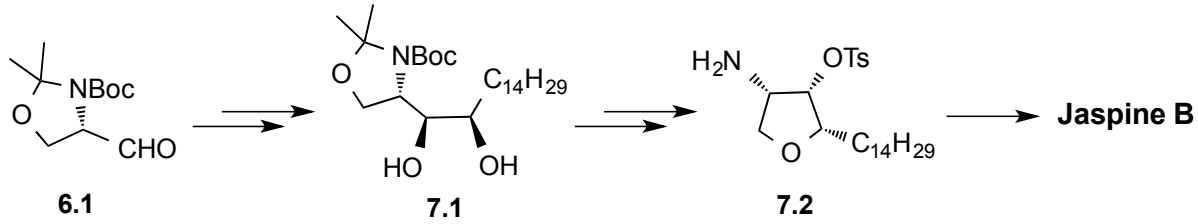
The first total synthesis of Jaspine B as reported by Rao in 2005, utilizes L-serine derived Garner's aldehyde **6.1** as the source of chirality (Scheme 6).[179] Thus, Garner's aldehyde **6.1** was subjected to Grignard addition using vinyl magnesium bromide to afford a mixture of separable diastereomeric alcohols (*anti:syn* = 86:14). The enantiopure major diastereomeric alcohol was protected as its benzyl ether derivative **6.2.** Ozonolysis of olefin in **6.2** to the corresponding aldehyde, followed by its reaction with tetradecylmagnesium bromide afforded an inseparable (70:30) mixture of diastereomeric alcohols **6.3**. Acetonide deprotection of **6.3** followed by selective protection of primary alcohol as TBS ether gave a mixture of the corresponding diastereomeric D-*ribo*- and D-*lyxo*-phytosphingosines **6.4**. Mesylation of secondary alcohol in **6.4** followed by TBAF promoted TBS deprotection and concomitant cyclization afforded a separable mixture (70:30) of the desired tetrahydrofuran **6.5**. Subsequent debenzylation followed by Boc-deprotection afforded Jaspine B as its TFA salt.



Scheme 6

Ohno's approach

In the synthesis by Ohno *et al,*[184] starting from (*S*)-Garner's aldehyde **6.1**, an initial step in the synthesis involved formation of the intermediate diol **7.1** (Scheme 7) by a sequence of Wittig olefination[199] using pentadecyl triphenylphosphoniumbromide to form the corresponding olefin (as a mixture of *Z:E* = 13:1), followed by its osmium tetroxide mediated dihydroxylation providing the product diol **7.1** as a diastereomeric mixture (77:23).

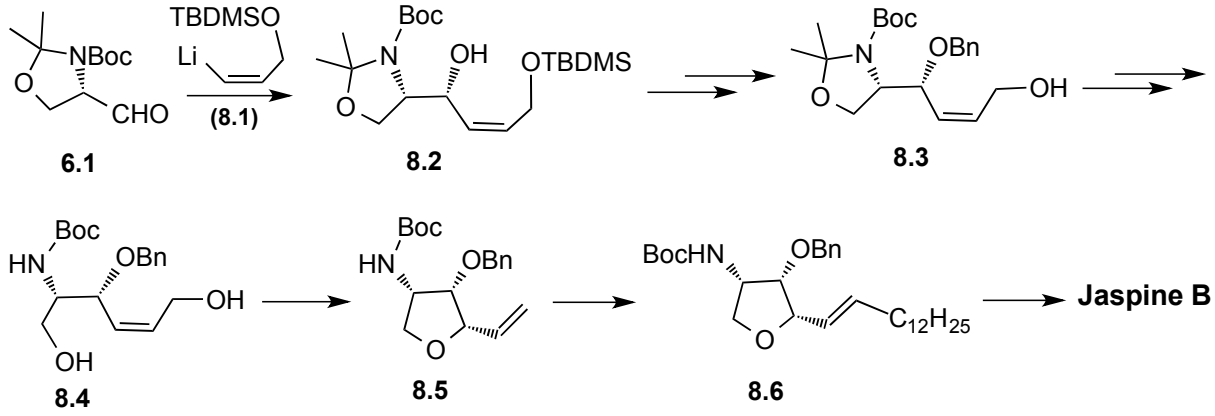


Scheme 7

Separation of the enantiopure major diol **7.1** and its derivatization to a ditosylate, followed by subsequent removal of the acetonide and Boc-protecting groups and resulting cyclization led to the required tetrahydrofuran intermediate **7.2**. Finally, cleavage of the tosyl-protection completed the synthesis of Jaspine B.

Passiniemi's approach

In Passiniemi and Koskinen's synthesis of Jaspine B, addition of the lithiated vinylic species **8.1** to Garner's aldehyde (**6.1**) afforded a separable diastereomeric mixture (92:8) of secondary alcohol intermediate **8.2** (Scheme 8)[180] . Benzyl protection of the secondary hydroxy group of **8.2**, and silyl-deprotection formed the allylic alcohol intermediate **8.3**. Acetonide deprotection forming the diol **8.3** and subjecting it to a Pd (0)-mediated intramolecular 1, 5-cyclization afforded a separable diastereomeric mixture (2:1) of tetrahydrofuran derivative **8.5**. Grubbs catalysis assisted cross-metathesis of **8.5** with 1-tetradecene afforded the C2-alkenyl adduct **8.6**. Tandem hydrogenation and hydrogenolysis of **8.6** with Pd $(OH)_2$/C under H_2, followed by treatment with dry HCl afforded Jaspine B.

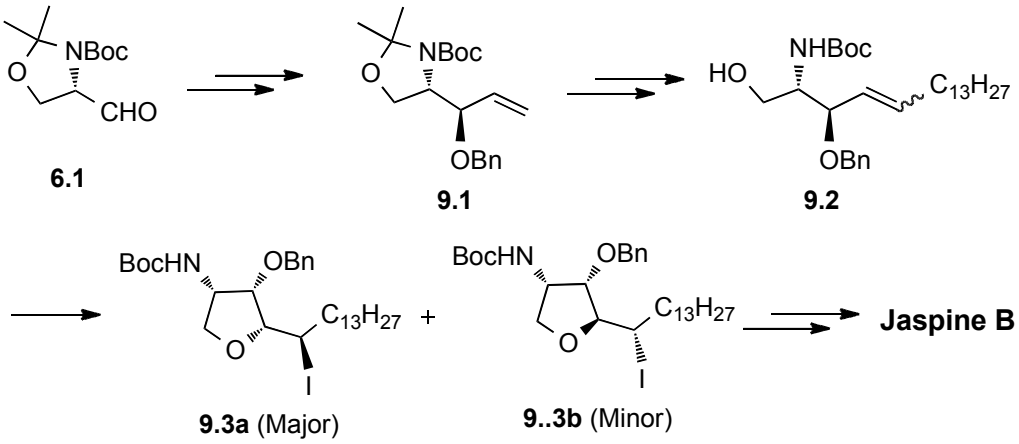


Scheme 8

Shaw's approach

Shaw *et al* have reported a stereoselective total synthesis of Jaspine B utilizing iodocyclization as a key reaction key step.[185] Thus, the allylic alcohol derivative **9.1** (Scheme 9) was obtained by a sequence of reactions involving: addition of vinyl magnesium bromide to (*S*)-Garner's aldehyde (**6.1**) to afford a mixture of the corresponding *anti* and *syn* allylic alcohols (6:1), followed by separation of the desired *anti*-isomer and *O*-benzyl protection of the hydroxy

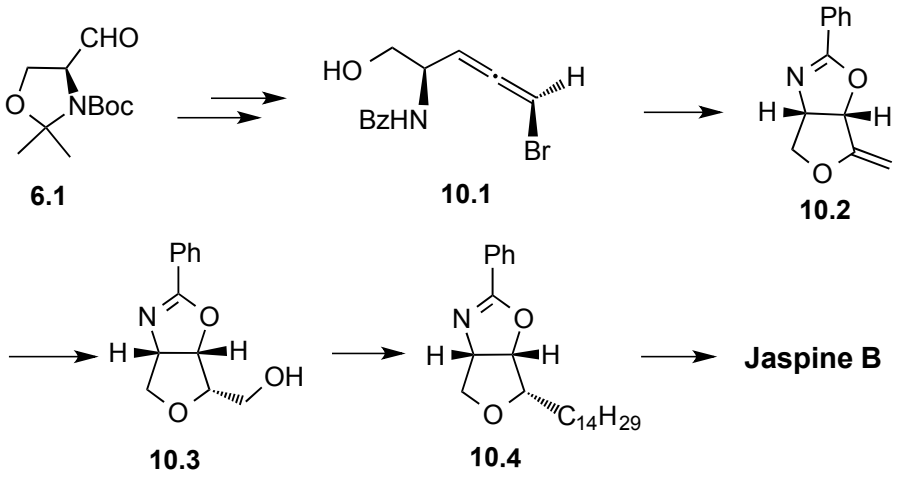
group. Subjecting **9.1** to olefin cross-metathesis with 1-pentadecene in the presence of Grubbs' second generation catalyst, and subsequent acetonide deprotection provided a mixture of the olefins **9.2** (*E:Z*=94:6). The 1, 2-amino alcohol **9.2** on iodocyclization with I_2 and *N*-iodosuccin-imide (NIS), furnished a diastereomeric mixture (10:1) of the tetrahydrofuran intermediates **9.3a** (major) and **9.3b** (minor). Separation of the major diastereomer **9.3a**, its reductive de-iodination using *n*-Bu_3SnH/AIBN,[200] followed by subsequent debenzylation and *N*-Boc-deprotection afforded Jaspine B as a TFA salt.



Scheme 9

Fujii and Ohno's approach

In Fujii and Ohno's total synthesis of Jaspine B, a palladium-catalyzed bis-cyclization of bromoallene **10.1** (Scheme 10) was utilized as a key reaction step.[201] The bromoallene **10.1**, derived from (*S*)-Garner's aldehyde,[29,202] when subjected to palladium-catalyzed reaction using $Pd(PPh_3)_4$ and Cs_2CO_3, resulted in a cascade cyclization to form the bicyclic product **10.2.** Hydroboration-oxidation of the exocyclic olefin in **10.2** afforded primary alcohol **10.3** with the desired stereochemistry. Triflation of the alcohol **10.3** followed by copper-catalyzed alkylation with a Grignard reagent gave tetrahydrofuran intermediate **10.4** bearing all the requisite functionalities. Finally, acidic hydrolysis of **10.4** afforded Jaspine B.



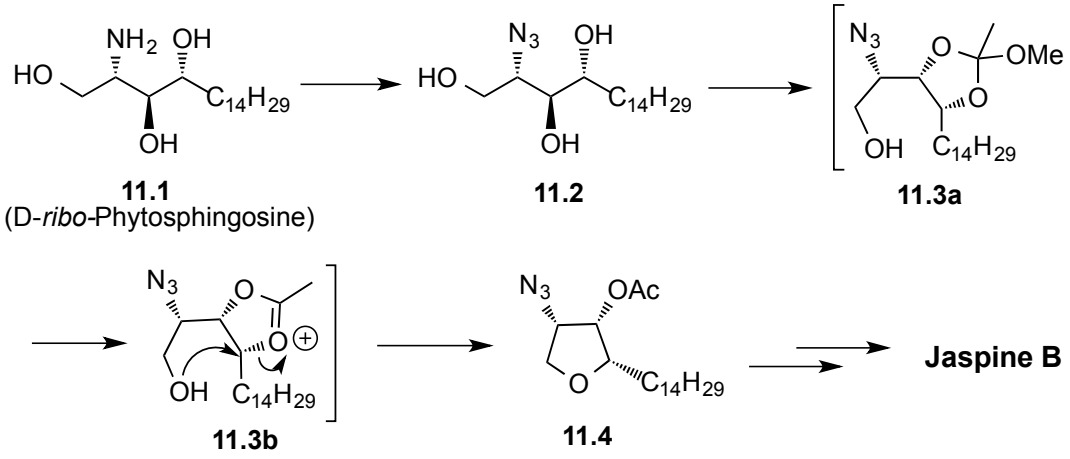
6.1 10.1 10.2 10.3 10.4

Scheme 10

Phytosphingosine-based approaches:

Overkleeft's approach

Overkleeft *et al* have reported the synthesis of Jaspine B, utilizing D-*ribo*-phytosphingosine (**11.1**) as a chiral precursor (Scheme 11).[177] Standard functional group transformation of the amine **11.1** resulted in the corresponding azide **11.2**.



11.1 (D-*ribo*-Phytosphingosine) **11.2** **11.3a** **11.3b** **11.4**

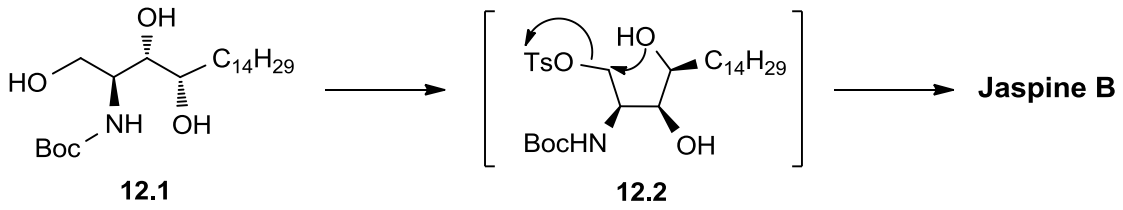
Scheme 11

Treatment of **11.2** with trimethylorthoacetate in the presence of a catalytic amount of Lewis acid afforded the desired tetrahydrofuran intermediate **11.4**. The process is believed to proceed through a 5-*exo*-tet cyclisation involving the ortho ester-derived oxonium intermediate

11.3b. Base catalyzed acetate hydrolysis followed by Staudinger reduction of azide in **11.4** gave Jaspine B.

Delgado's approach

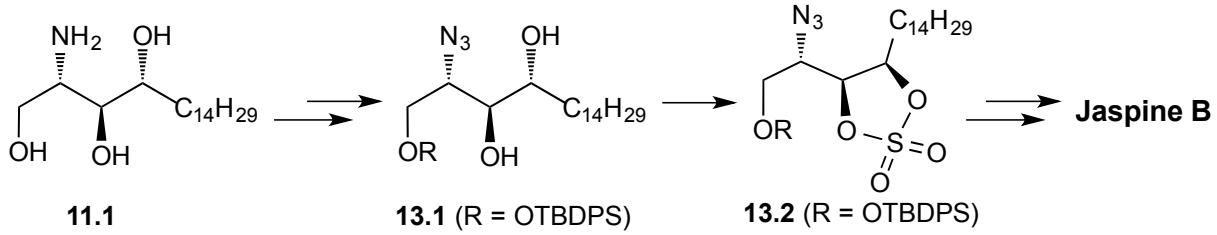
Delgado *et al* have reported the total synthesis of Jaspine B involving a key intramolecular cyclization of protected *N*-Boc-phytosphingosine **12.1** (Scheme 12).[131] The primary hydroxy group of *N*-Boc-phytosphingosine **12.1** was tosylated to afford **12.2**, which upon intramolecular S_N2 displacement afforded the required tetrahydrofuran intermediate. Deprotection of *N*-Boc using TFA afforded Jaspine B as its TFA salt.



Scheme 12

Kim's approach

Kim *et al* have reported a stereodivergent synthesis of Jaspine B utilizing a D-*ribo*-phyto-sphingosine-derived key cyclic sulfate intermediate **13.3** (Scheme 13).[203]



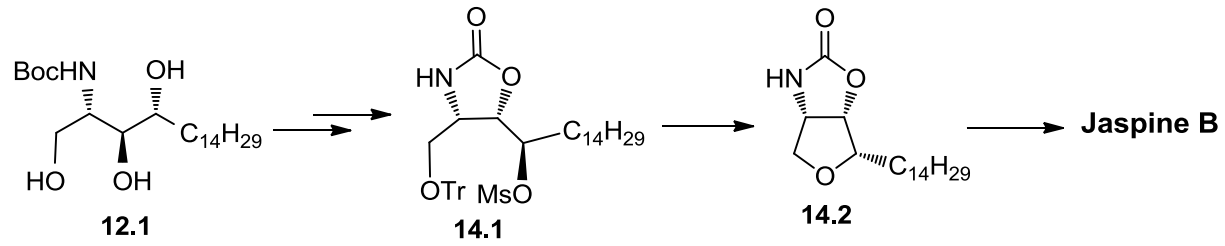
Scheme 13

Thus, starting from D-*ribo*-phytosphingosine, its conversion to the corresponding TBS-protected azido alcohol **13.1** was achieved following standard reaction procedures. Subsequent treatment of **13.1** with $SOCl_2$ provided the cyclic sulfite **13.2**. Desilylative cyclisation of **13.2**

with TBAF forming the desired tetrahydrofuran core, and subsequent reduction of azide afforded Jaspine B.

Sanghee Kim's approach

In Sanghee Kim's total synthesis of Jaspine B, *N*-Boc-phytosphingosine **14.1** was initially converted to the corresponding orthogonally protected oxazolidinone derivative **14.1** (Scheme 14).[186] Subsequent deprotection of *O*-trityl ether and concomitant spontaneous intramolecular cyclization provided the required tetrahydrofuran oxazolidinone intermediate **14.2**. Treatment of **14.2** under alkaline conditions afforded Jaspine B.



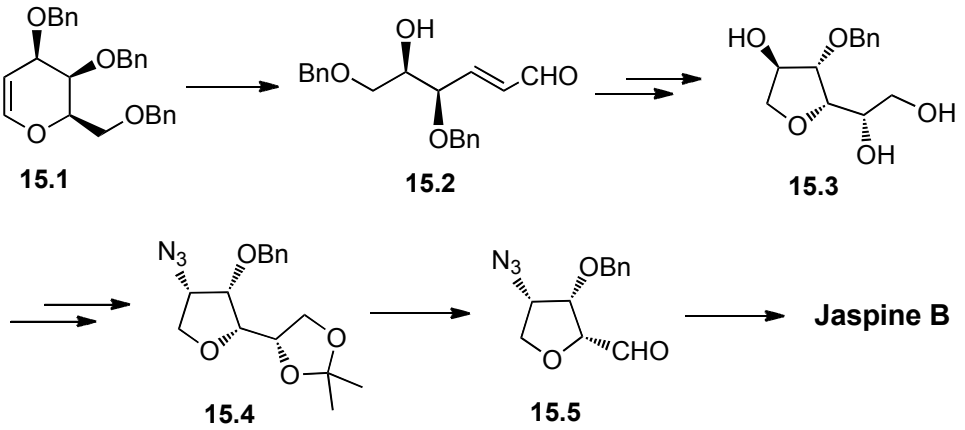
Scheme 14

Carbohydrates as starting materials:

Shaw's approach

Several carbohydrate starting materials have also been utilized as chiral starting materials for the enantiospecific syntheses of Jaspine B. Thus, in Shaw's total synthesis of Jaspine B,[181] the D-galactose-derived 3,4,6,-tri-*O*-benzyl-galactal (**15.1**) was initially converted to *α,β*-unsaturated aldehyde **15.2** (Scheme 15). Luche reduction of the *α,β*-unsaturated aldehyde **15.2** to the corresponding allylic alcohol, subsequent Sharpless asymmetric epoxidation, and intramolecular ring opening of the resulting epoxide resulted in the tetrahydrofuran diol intermediate **15.3**. Acetonide protection of the side chain diol of **15.3** and mesylation of the secondary hydroxy group in the tetrahydrofuran ring, followed by its S_N2 displacement with azide provided **15**.**4**. Deprotection of the acetonide linkage in **15.4** and oxidative cleavage of the

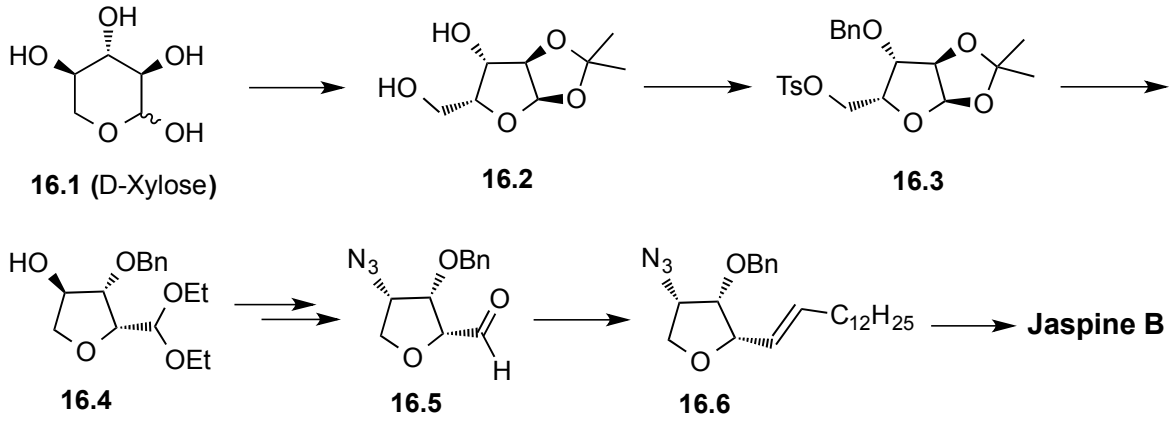
diol formed the C2 formyl tetrahydrofuran intermediate **15.5**. Wittig olefination of **15.5** using tridecyltriphenylphosphoniumbromide to form the corresponding olefin, and global reduction via transfer hydrogenation afforded Jaspine B.



Scheme 15

Du and Linhardt's approach

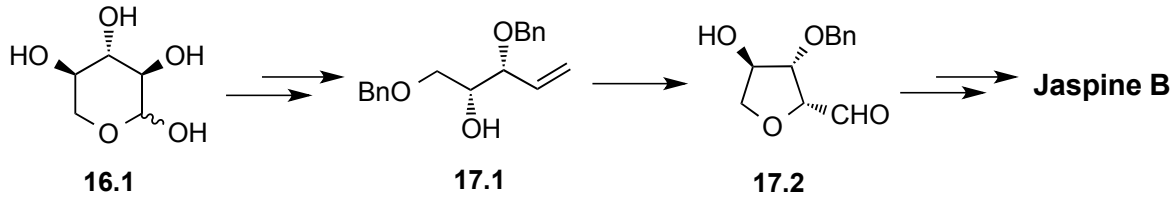
Starting from D-xylose, Du and Linhardt have reported a total synthesis of Jaspine B (Scheme 16).[182] The synthetic sequence started with structural rearrangement of D-xylose (**16.1**) into the corresponding pentose, followed by acetonide protection to afford the acetal **16.2** (Scheme 16)**.** Subsequent regioselective tosylation of the primary hydroxyl group and benzylation of the secondary alcohol afforded **16.3**. Acid catalyzed furan ring reconstruction then afforded the 2,5-anhydro intermediate **16.4**. The secondary hydroxyl of **16.4** was then converted to the corresponding azide with inversion of stereochemistry followed by acetal deprotection to form the 4-azido-aldehyde intermediate **16.5**. Wittig olefination of **16.5** with a C-13 alkyl donor provided an inseparable mixture of *E*- and *Z*- isomers of the corresponding C-14 olefinic side chain derivative **16.6**. Finally, in a single step, conversion of azide to amine, *O*-benzyl deprotection, and hydrogenation of the olefinic side chain furnished Jaspine B.



Scheme 16

Du's approach

Soon after the above synthetic endeavor, Du *et al* have described a further refined synthesis of Jaspine B.[204] The key intermediate in this route involved the development of a closely related formylfuran intermediate **17.2** via an iodine-induced diastereoselective cyclization of an appropriately functionalized olefinic intermediate **17.1** (Scheme 17).

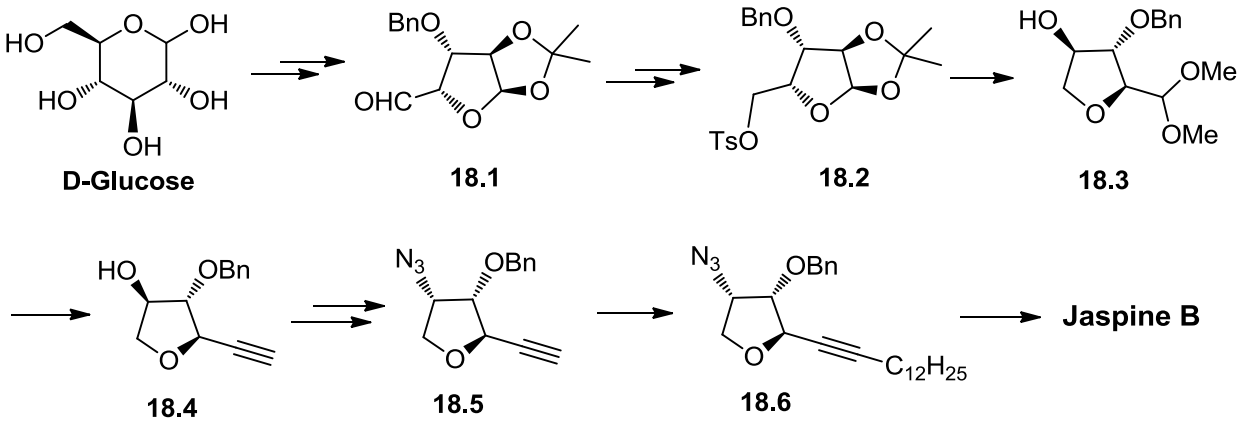


Scheme 17

The olefinic triol **17.1** was obtained by oxidative degradation and subsequent Wittig olefination of a D-xylose-derived precursor. Subsequently, an iodine promoted olefin activation/ debenzylation/cyclization cascade involving **17.1**, and further functional group transformation led to the 2-formyl tetrahydrofuran derivative **17.2**. Employing a similar sequence of reaction as in the previous route (Scheme 16) a total synthesis of Jaspine B was achieved.

Ramana's approach

Ramana *et al* have reported the synthesis of Jaspine B starting from D-Glucose.[188] A key intermediate in this route involved the formation of a strategic C-2 alkyne substituted tetrahydrofuran **18.4** (Scheme 18). Following a literature procedure,[205,206] conversion of D-Glucose to the aldehyde **18.1** and its conversion to the corresponding tosylate **18.2** was followed by acidic hydrolysis of the acetonide linkage with concomitant cyclization to the tetrahydrofuran acetal **18.3**. Unmasking of the aldehyde, and an Ohira-Bestmann alkynation[207,208] afforded the key intermediate **18.4**. Conversion of the secondary hydroxy group to azide with inversion of stereochemistry to form **18.5**, and base promoted alkylation of the terminal alkyne with dodecyl bromide provided the penultimate alkynyl tetyrahydrofuran **18.6**. Global hydrogenolysis completed the synthesis of Jaspine B.



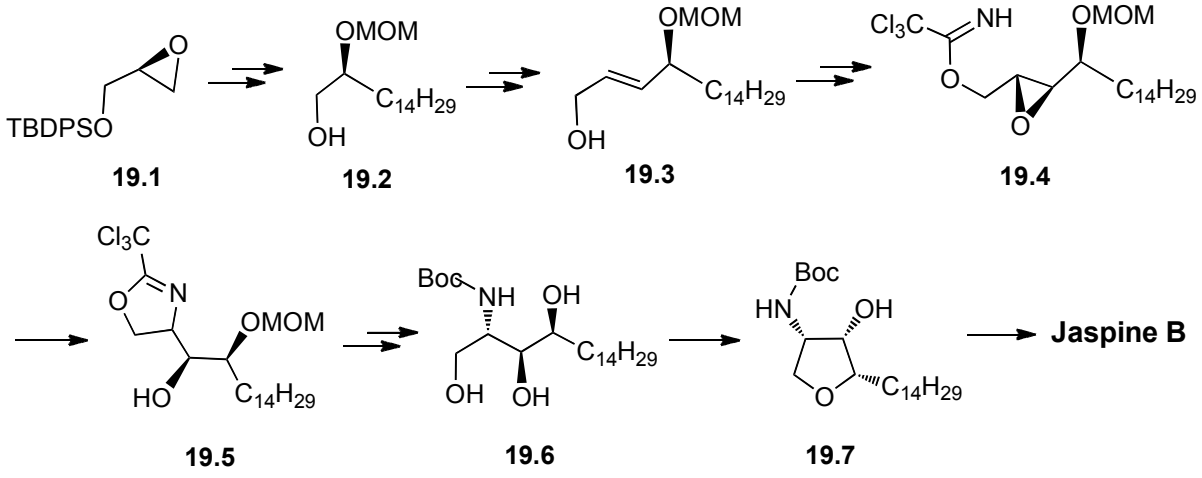
Scheme 18

Miscellaneous chiral starting materials:

Marco's approach

Marco *et al* have reported an enantiospecific synthesis of Jaspine B starting from commercially available (*R*)-glycidol (Scheme 19).[189] Thus, *O*-TBDPS protected (*R*)-glycidol **19.1** was treated with tridecylmagnesium bromide to afford the corresponding alcohol, which

was then protected as its *O*-methoxymethyl (*O*-MOM) derivative followed by *O*-desilylation to afford the mono-protected diol **19.2**. Swern oxidation of the primary alcohol in **19.2** followed by HWE olefination and DIBAL reduction of the resulting *α,β*-unsaturated ester afforded the allylic alcohol intermediate **19.3**. Sharpless asymmetric epoxidation of allylic alcohol **19.3** using (-)-DET afforded the epoxy alcohol which on further treatment with trichloroacetonitrile formed the acetimidate **19.4**. Structural rearrangement leading to the oxazoline **19.5** and its subsequent hydrolysis followed by *N*-Boc protection afforded the phytosphingosine derivative **19.6**. Tosylation of the primary alcohol in **19.6** followed by base induced cyclization afforded the tetrahydrofuran **19.7** Boc-deprotection of **19.7** completed the synthesis of Jaspine B.

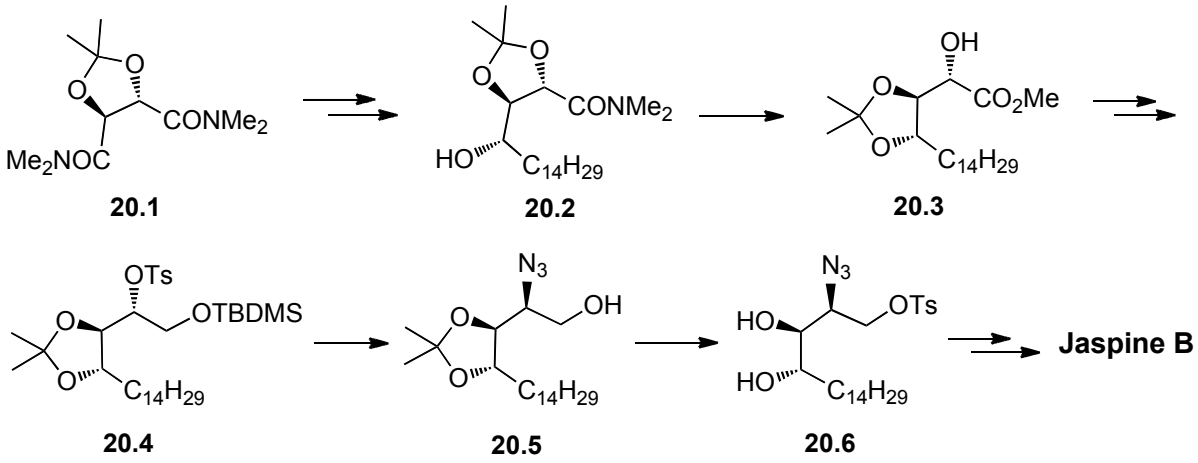


Scheme 19

Chandrakumar's approach

Chandrakumar *et al* have reported synthesis of Jaspine B utilizing D-tartaric acid (Scheme 20).[190] Starting with addition of tetradecylmagnesium bromide to tartaric acid bisdimethylamide (**20.1)** provided the corresponding ketoamide, which was subjected to stereoselective reduction furnishing a mixture of diastereomeric alcohols (*dr* = 95:5) with **20.2** as the major diastereomer. Treatment of **20.2** with excess 2,2-dimethoxyropane and *p*-TSA resulted in the acetonide rearranged methyl ester derivative **20.3**. Subsequent reduction of the methyl

ester to primary alcohol and selective *O*-TBDMS and *O*-tosyl protections afforded the fully protected tetraol derivative **20.4**. Displacement of the tosylate with azide with concomitant inversion of stereochemistry, and deprotection of the primary hydroxyl group yielded the azidohydrin **20.5**. Tosylation of hydroxyl group of **20.5** and hydrolysis of the acetonide linkage unveiled the diol **20.6**. A base promoted cyclization to form the trisubstituted tetrahydrofuran core and subsequent azide reduction led to Jaspine B.

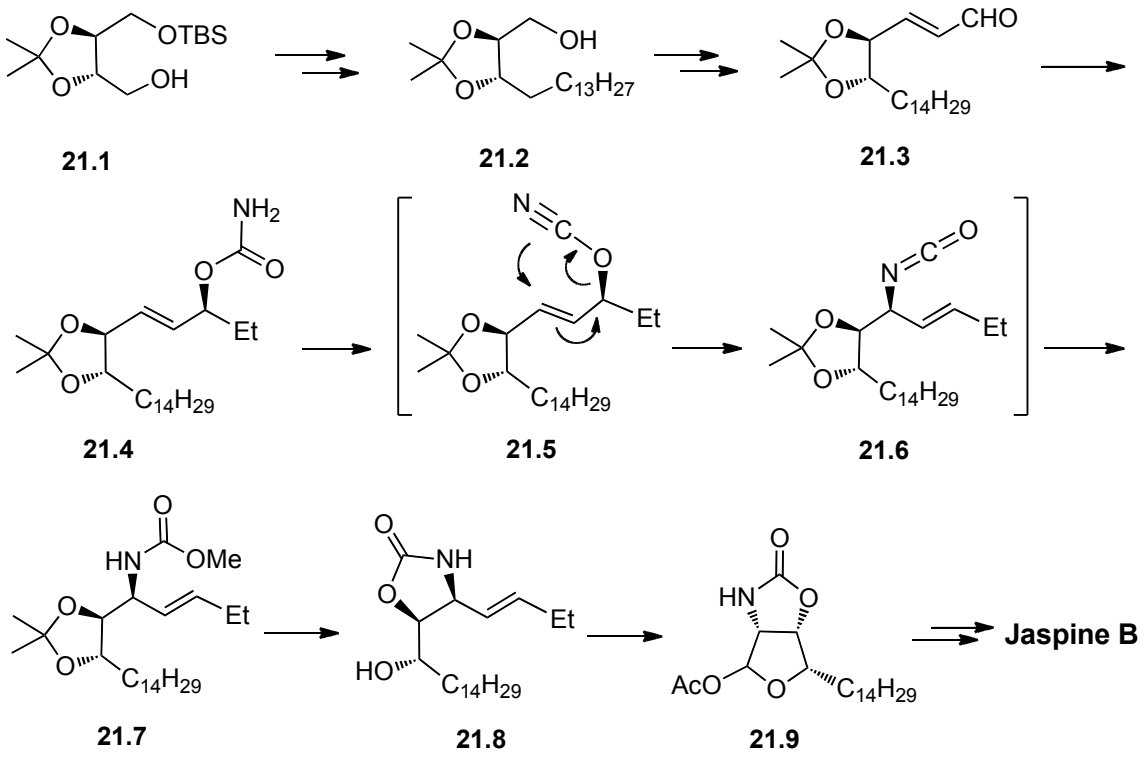


Scheme 20

Ichikawa's approach

Ichikawa *et al* have reported the synthesis of Jaspine B employing a highly stereocontrolled [3,3] sigmatropic rearrangement of an allyl isocyanate intermediate as a key reaction step (Scheme 21).[191,209] Thus, following a four-step reaction sequence, the L-tartaric acid-derived tetraol **21.1** was converted to the long-chain alkylated triol **21.2**. Subsequent oxidation of the primary hydroxyl group to aldehyde, its HWE olefination, DIBAL-H reduction of the resulting α,β-unsaturated ester, and oxidation of the resulting primary alcohol provided the aldehyde **21.3.** Enantioselective addition of Et_2Zn to the aldehyde **21.3**, in the presence of 3-*exo*-morpholinoisoborneal (MIB) as a chiral ligand, to form the corresponding carbinol adduct, conversion of the resulting hydroxyl group to a trichloroacetimidate, and subsequent hydrolysis

yielded the allyl carbamate **21.4**. Triphenyl phosphine promoted conversion of the allyl-carbamate **21.4** to the allyl cyanate **21.5** as a transient intermediate, followed by its spontaneous [3,3]-sigmatropic rearrangement to the allylisocyanate **21.6**, and quenching of the reaction with methanol afforded the methyl carbamate **21.7**. Acetonide deprotection of **21.7** and base promoted intramolecular cyclization afforded the oxazolidinone **21.8**. Ozonolytic cleavage of the olefin in **21.8** led to the formation of a lactol, which on treatment with acetic anhydride provided the acetylated derivative **21.9**. Reductive cleavage of the acetoxy group, and alkaline hydrolysis of the cyclic carbamate culminated in Jaspine B.



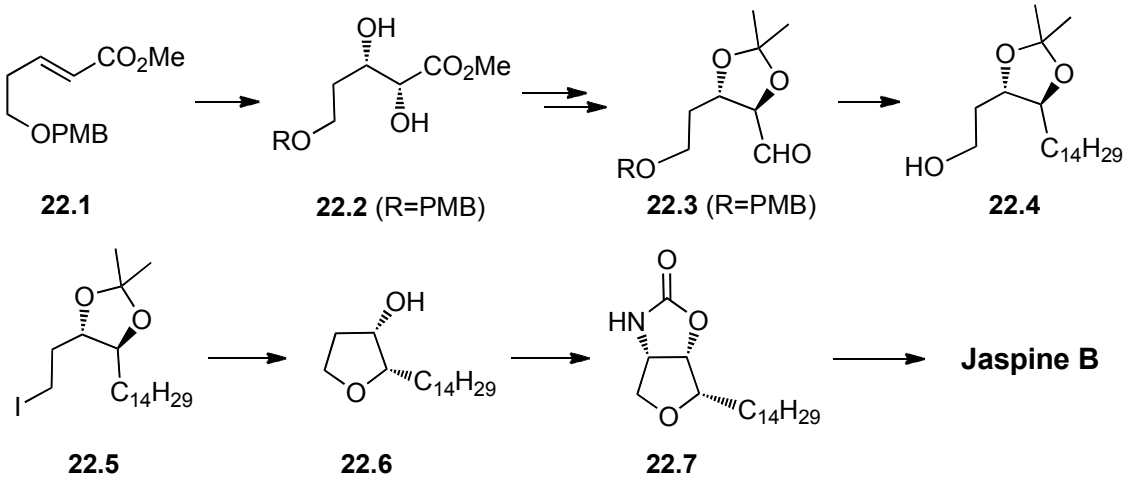
Scheme 21

Asymmetric synthetic approaches:

Yakura's synthesis

The first asymmetric synthesis of Jaspine B was reported by Yakura *et al* and utilized a Sharpless asymmetric dihydroxylation protocol as a key reaction step.[210] Thus, Sharpless

asymmetric dihydroxylation of *α,β*-unsaturated ester **22.1** provided the diol **22.2** (Scheme 22). Acetonide protection of the diol and partial reduction of the methyl ester with DIBAL afforded the formyl intermediate **22.3**. Subsequent Wittig olefination of the aldehyde **22.3**, followed by hydrogenation of the resulting olefin and PMB-deprotection in a one-pot reaction resulted in the hydroxy alkane **22.4**. Conversion of the primary hydroxy group of **22.4** to the iodoalkane **22.5**, and acid-catalyzed acetonide deprotection followed by intramolecular cyclization formed the tetrahydrofuran intermediate **22.6**. Subsequent reaction with trichloroacetyl isocyanate, and Rh-mediated C-H amination gave a diastereomeric mixture, from which the desired enantiopure bicyclic carbamate **22.7** was isolated. Hydrolysis of the carbamate linkage completed the synthesis of Jaspine B.

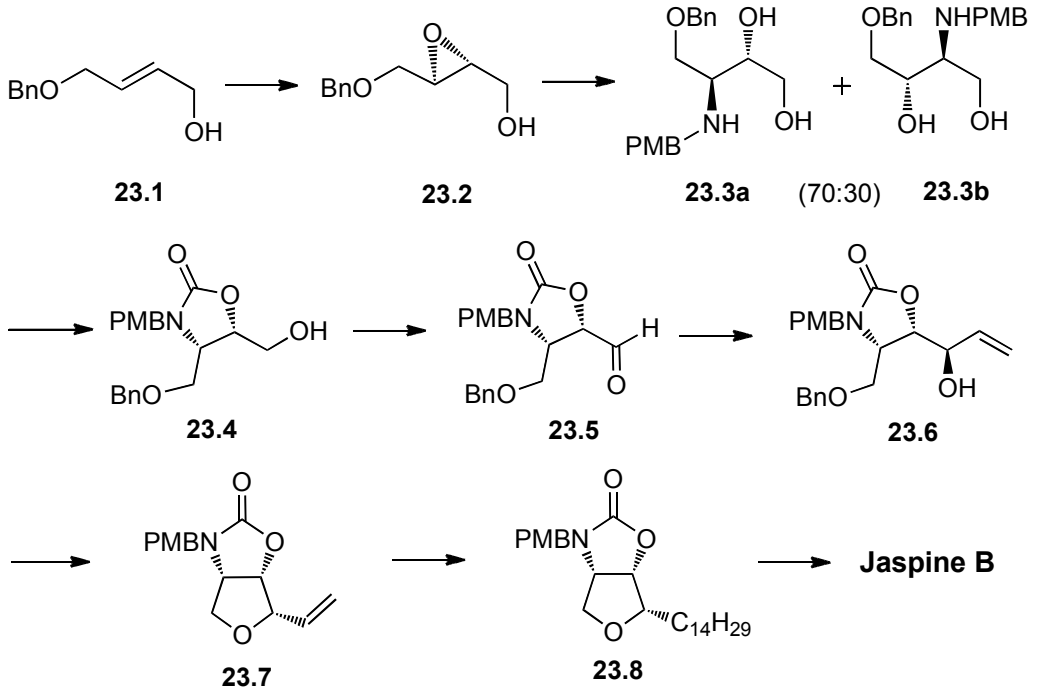


Scheme 22

Génisson's approach

The synthesis of Jaspine B as developed by Génisson *et al* started with the easily available mono-benzylated *trans*-butenediol **23.1** (Scheme 23).[193,194] Thus, Sharpless' asymmetric epoxidation of **23.1** delivered the key epoxide **23.2**, which upon ring opening with *p*-methoxybenzylamine provided the regioisomeric aminoalcohols **23.3a** and **23.3b** as an inseparable mixture of products (70:30). However, treatment of the above product mixture with

methyl chloroformate allowed the separation of the desired oxazolidinone **23.4**. Dess-Martin oxidation of **23.4** to the corresponding aldehyde **23.5**, and its *anti*-selective addition with a vinylcerium reagent afforded the allylic alcohol **23.6.** Subsequent conversion of the secondary hydroxy group to its mesylate resulted in spontaneous cyclization to form the all-*cis*-tetrahydrofuran **23.7**. Cross-metathesis of **23.7** with 1-tetradecene using Grubbs second-generation catalyst, and subsequent hydrogenation of the product olefin resulted in the oxazolidinone derivative **23.8** Subsequent PMB deprotection and hydrolytic cleavage of the cyclic carbamate afforded Jaspine B.



23.1 **23.2** **23.3a** (70:30) **23.3b**

23.4 **23.5** **23.6**

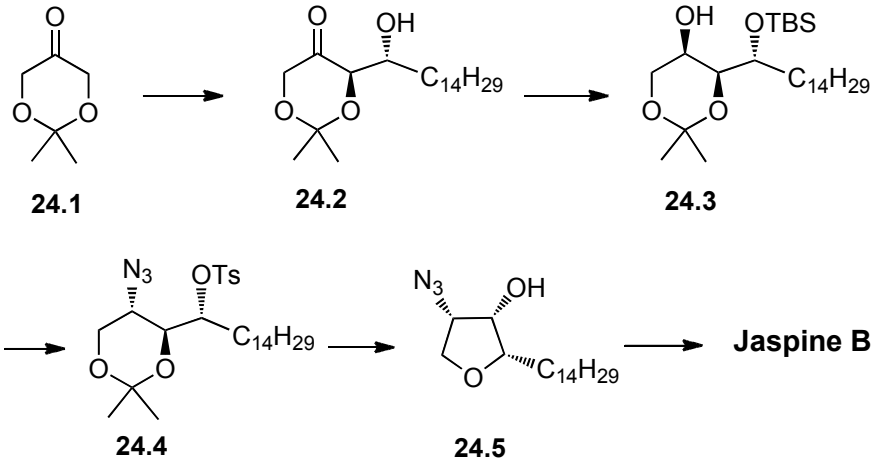
23.7 **23.8**

Scheme 23

Enders' approach

Ender's asymmetric synthesis of Jaspine B involved a (*R*)-proline catalyzed diastereo- and enantioselective aldol condensation of dioxanone **24.1** and 1-pentadecanal as a key reaction step towards the stereoselective formation of the *anti*-diol **24.2** (Scheme 24).[195] Silyl-protection of the hydroxy group, followed by reduction of the ketone with L-selectride provided the

secondary alcohol derivative **24.3** with high selectivity. Conversion of the resulting hydroxy group to its mesylate and its S_N2 displacement with NaN_3 with concomitant inversion of the stereocenter, followed by a two-step conversion of the silyl-protected hydroxy group to the corresponding tosylate afforded the azidotriol **24.4**. Acetonide deprotection under acidic conditions followed by an intramolecular S_N2 displacement of the tosylate afforded the all-*syn*-trisubstituted tetrahydrofuran **24.5**. Finally, hydrogenation of the azide **24.5** afforded Jaspine B.

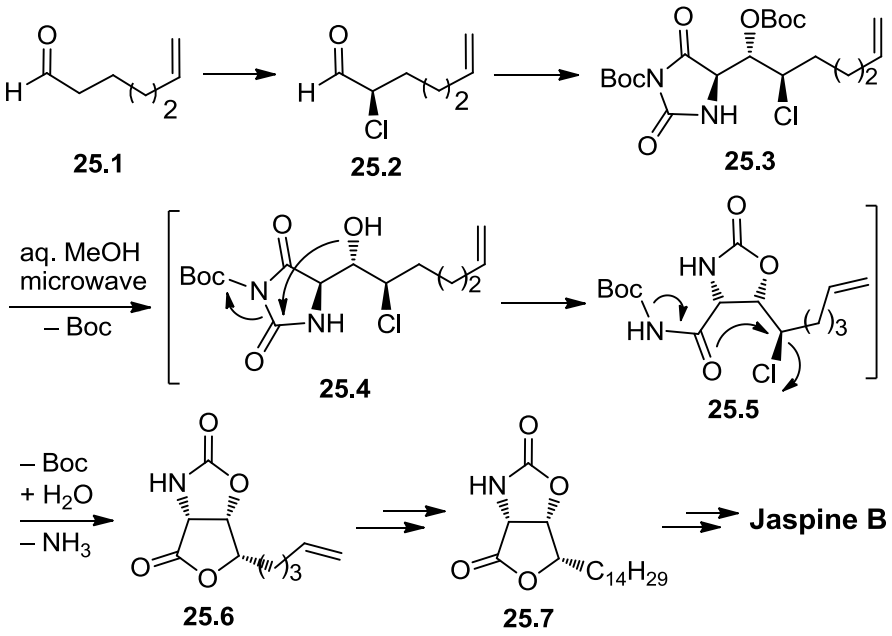


Scheme 24

Britton's approach

In Britton's synthesis of Jaspine B, the first step involved an asymmetric chlorination of 1-heptenal (Scheme 25). Thus, following McMillan's method,[211] 1-heptenal (**25.1**) was converted to *α*-chloro-6-heptenal (**25.2**) with high selectivity (>99% ee).[196] A subsequent aldol reaction of **25.2** with Boc-protected hydantoin afforded a mixture of diastereomeric chlorohydrins (dr= 10:1:1:1), with the desired aldol adduct **25.3** being the major diastereomer. Heating a solution of **25.3** in aq. methanol under microwave conditions resulted in the formation of the bicyclic lactone **25.6** in moderate yield. The formation of the bicyclic lactone (**25.6)** has been explained via a reaction cascade, wherein initial cleavage of the *O*-Boc linkage resulted in the chlorohydrin intermediate **25.4**. Subsequent nucleophilic attack at the highly activated

hydantoin carbonyl by the hydroxy group to generate the ring-opened carbamate **25.5**, followed by *N*-Boc deprotection and intramolecular displacement of the chloride in **25.5** by amide carbonyl oxygen and hydrolysis of the resulting imine ultimately led to the lactone **25.6**. Subjecting olefin **25.6** to cross-metathesis with 1-undecene and hydrogenation of the resulting alkene installed the tetradecyl side chain in compound **25.7**. Subsequent conversion of the lactone carbonyl to methylene and alkaline hydrolysis of the cyclic carbamate completed the synthesis of Jaspine B.



Scheme 25

3.4. Structure-activity relationship (SAR) studies of Jaspine B

As described in the previous section, since its isolation in 2002, several total and formal syntheses of Jaspine B have been reported in the literature. In contrast, despite its known anticancer potential, any systematic or detailed studies aimed at co-relating the structural elements and biological activity of this unique natural product remains relatively scarce. Based on the available information from the reported studies, the major structure-activity relationships (SAR) of Jaspine B are summarized below (Figure 17).[129,131,193,194,203,212,213]

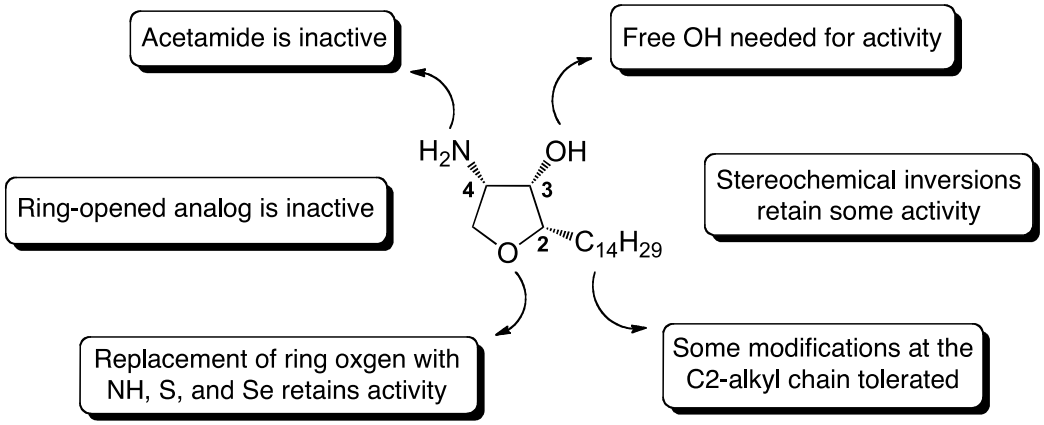


Figure 17. Jaspine B structure-activity relationships: An overview

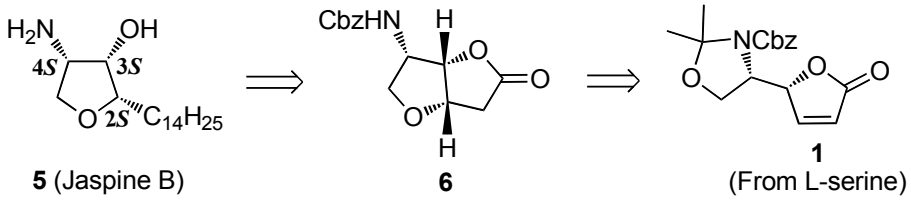
Evidently, the cyclic form of Jaspine B is essential for biological activity as the corresponding open chain analog (D-*ribo*-phytosphingosine) was found to be inactive. It was observed that, the epimeric analogs at various stereocenters were partially active. The alkyl chain at C2 is amenable to minor modifications with the resulting analogs retaining activity. However, any modifications of the C3-hydroxy group were found to be detrimental to activity. Interestingly, bioisosteric replacements of the core tetrahydrofuran ring oxygen atom with amine (NH), sulfur (S), and selenium (Se) groups resulted in activity retention. However, all the Jaspine B analogs reported thus far have been found to be less active than the natural product.

With the aim to carry out a detailed SAR investigation of Jaspine B, we initiated the present study, wherein, development of a flexible synthetic route to the natural product, and further application of the method for systematic structural modification studies were targeted. Results of the studies thus undertaken are described hereunder.

3.5. Towards Jaspine B: Previous studies from our research group

Soon after the isolation and biological activity of Jaspine B (2002–2003) were reported, our research group undertook a preliminary study aimed at the total synthesis of this bioactive natural product. The retrosynthetic strategy for the intended synthesis envisioned utilization of

the serine-derived chiral aminobutenolide **1** (Figure 18) as a strategic chiral platform towards rapid construction of the all *syn*-2,3,4-trisubstituted structural framework of Jaspine B.



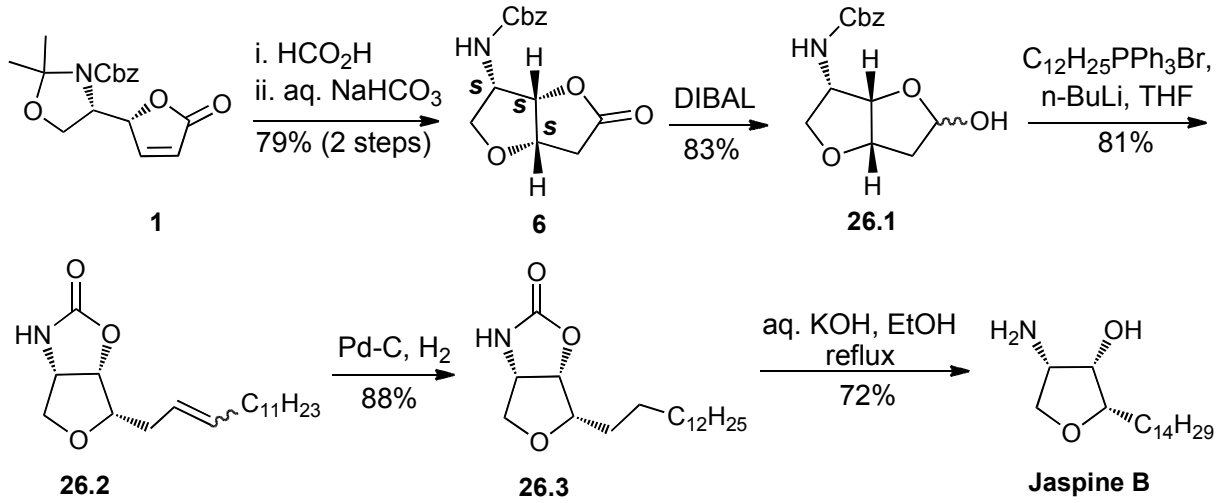
5 (Jaspine B) **6** **1** (From L-serine)

Figure 18. Jaspine B: Retrosynthetic strategy

As mentioned previously, the aminobutenolide **1** has been shown to be a highly versatile and useful chiral building block in our research. In further utilization of this multifunctional lactone, it was proposed that, starting from **1**, selective deprotection of the *N*, *O*-acetonide linkage of **1** and possible Michael addition of the resulting primary hydroxy group into the β-carbon of the butenolide should provide the bicyclic lactone **6** (Figure 18). Stereoselective formation of the *cis*-fused [5,5]-bicyclic scaffold of **6** is expected due to the highly unfavorable ring strain associated with the alternative *trans*-ring junction between the two five-membered rings. The formation of the bicyclic lactone **6** should also install the C2, C3, and C4-stereo-centers as present in Jaspine B. Standard functional group transformations involving the right hand side lactone moiety will complete the synthesis of the desired all *syn*-tetrahydrofuran natural product **5**.

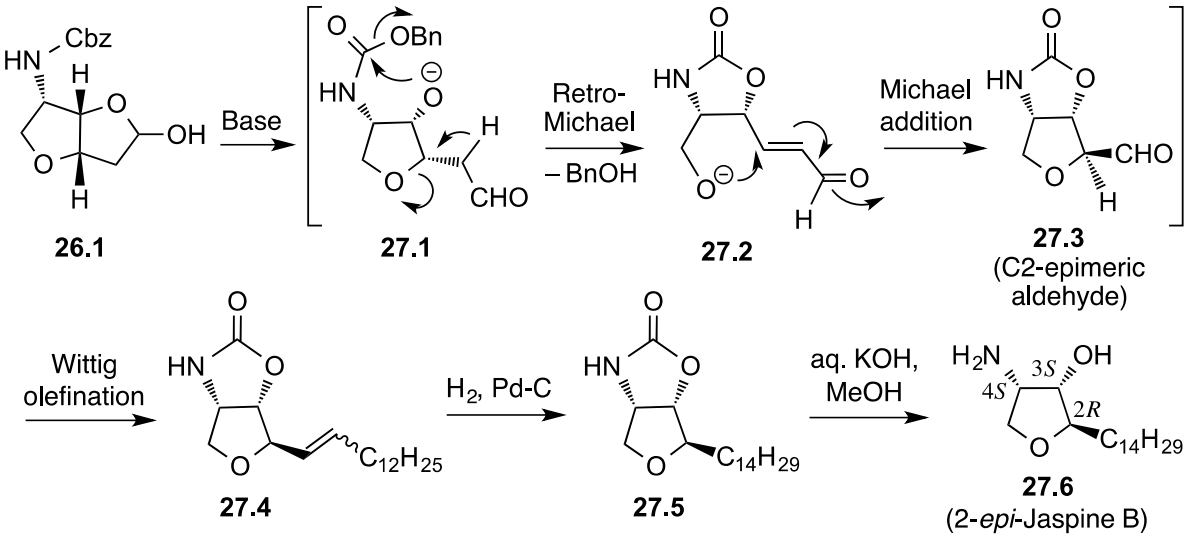
Accordingly, acid catalyzed hydrolysis of the *N,O*-acetonide linkage of **1**, followed by treatment of the crude product with aqueous bicarbonate directly afforded the bicyclic lactone **6** (Scheme 26) as the only product. The assigned stereochemistry and the all *syn*-relationship between the three contiguous asymmetric centers as present in **6** were confirmed on the basis of its X-ray crystal structure. As expected, the favorable energetics involved in the *cis*-fused [5,5] bicyclic ring forming reaction during the above intramolecular Michael addition contributed to

the exclusive formation of product **6** as shown. Towards introduction of the C2-tetradecyl side chain, DIBAL-assisted partial reduction of the lactone carbonyl to the lactol derivative **26.1** and its standard condensation with a Witting reagent derived from dodecylphosphoniumbromide resulted in the corresponding olefins **26.2** as an inseparable mixture of *E*- and *Z*-isomers. Interestingly, the C_3-secondary oxyanion generated during the Wittig reaction mediated ring opening of the lactol underwent intramolecular addition to the favorably disposed *N*-Cbz-carbonyl group, resulting in the concomitant formation of the cyclic carbamate protected tetrahydrofuran derivative **26.2**. Hydrogenation of the side chain olefin of **26.2** to the corresponding saturated alkane **26.3**, followed by alkaline hydrolysis of the cyclic carbamate culminated in the synthesis of Jaspine B (Scheme 26). A manuscript detailing the results of the above study was subsequently published (early 2005),[28] and became only the second reported total synthesis of 'Jaspine B' at that time.



Scheme 26

However, in a subsequent study (2008), Davies and co-workers found that, the product formed in our above reported synthesis is not the natural (2*S*,3*S*,4*S*) Jaspine B, but the corresponding C2- epimeric analog **27.5** instead (Scheme 27).[175]



Scheme 27

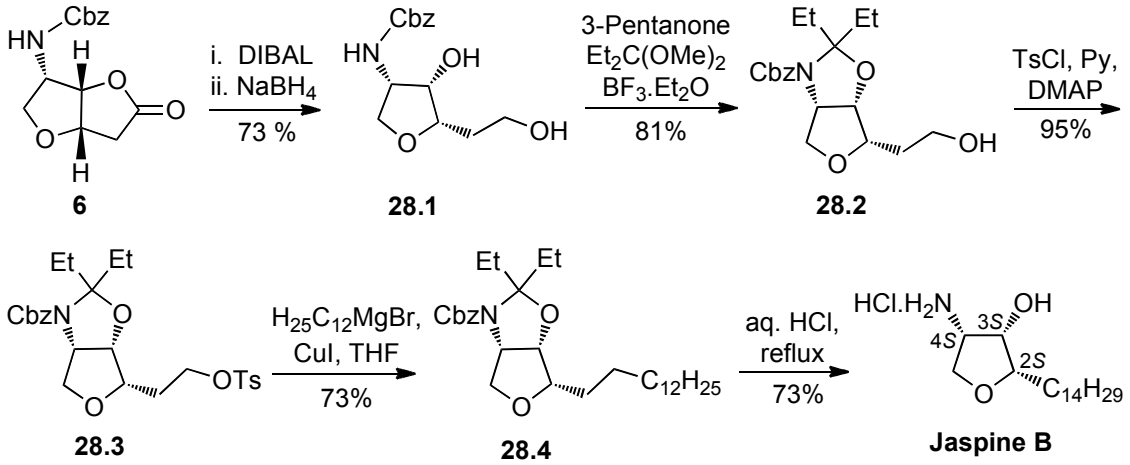
Davies' reassignment of the above stereochemistry was based on comparison of the spectroscopic data of Jaspine B and the C2-epimer **27.5**, as available from several studies published subsequent to our publication. To rationalize the formation of this unexpected product, Davies also suggested an alternative reaction pathway, wherein the *in situ* formed aldehyde intermediate **27.1** (Scheme 27), generated from the lactol **26.1** under the base mediated Wittig olefination reaction condition, undergoes facile C2-epimerization via a retro-Michael–Michael addition sequence (through intermediacy of **27.2**), resulting in the sterically more favorable C2–C3 *trans*-substituted 2-formyl tetrahydrofuran intermediate **27.3**. Subsequent transformations involving the (2*R*)-aldehyde **27.3** ultimately led to the C2-*epi*-Jaspine B as obtained.

3.5.1. Present research: A revised total synthetic route to Jaspine B

In view of the rather surprising and unexpected outcome of the above Jaspine B synthetic endeavor, in my present research, I decided to investigate an alternative reaction strategy to circumvent the undesired C2-epimerization.

Accordingly, starting from the advanced bicyclic lactone **6**, exhaustive reduction of the ester functionality provided the diol **28.1** in good yield (Scheme 28). Although the above

reduction could also be achieved using an excess of DIBAL alone, the recovery of the product diol was found to be poorer compared to the two step sequence involving initial DIBAL (1.2 equiv) reduction of the lactone to the corresponding lactol, followed by further reduction of the crude reaction product with sodium borohydride. Subsequent regioselective protection of the C3-C4 amino alcohol functionalities as the oxazolidine **28.2**, and conversion of the free primary hydroxy group to the corresponding tosylate **28.3** was achieved in good overall yield.



Scheme 28

Towards installation of the natural tetradecyl substituent at C2, facile S_N2 displacement of the tosylate with an *in situ* generated dodecyl organocuprate reagent as shown, uneventfully provided the fully protected Jaspine B derivative **28.4**. Finally, global deprotection of **28.4** under acidic conditions afforded Jaspine B as its HCl Salt.

The specific rotation {$[\alpha]_D$ = 2.5 (c 0.46, MeOH); lit.[175] $[\alpha]_D$ = 2.6 (c 0.4, MeOH)}, and the NMR spectral data of the Jaspine B salt as obtained above, are in good agreement with the literature reported values,[175] thereby unambiguously confirming the product structure.

As reported by the Davies study,[175] a major difference in the ^{1}H-NMR spectra between Jaspine B and its diastereoisomeric 2-*epi*-analog is the chemical shift pattern involving one of the C5-protons. Thus, in the proton NMR spectrum of 2-*epi*-Jaspine B, the above diagnostic peak is

observed at δ 4.14 ppm, whereas the corresponding spectrum of the natural, all *cis* Jaspine B is characterized by the absence of any peaks above δ 4.0 ppm. In accordance with the above observations, comparison of the ^{1}H-NMR spectra of Jaspine B from the present study (in the free amine form, obtained by neutralization of the corresponding salt), and the corresponding 2-*epi*-analog (as obtained by us previously), displayed the characteristic chemical shift differences as expected (Figure 19), confirming the structural identity and stereochemical integrity of the products obtained (full spectra data provided in the Experimental Section).

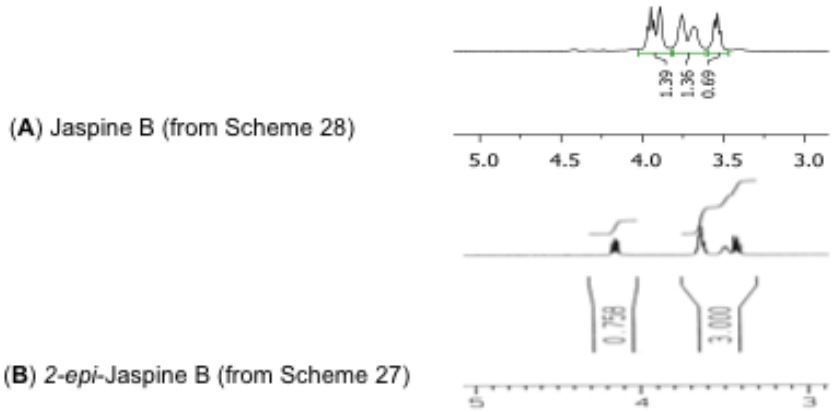


Figure 19. Comparison of the 1HNMR spectra (δ 3–5 ppm) of Jaspine B and 2-*epi*-Jaspine B

In conclusion, starting from the same bicyclic lactone **6** as in the previous effort from our research group, but employing a modified synthetic route, a stereoselective total synthesis of enantiopure Jaspine B has been accomplished.

3.5.2. Wittig olefination approach to Jaspine B: A reinvestigation of our initial synthesis

While pursuing the above synthesis, it was considered worthwhile to also reinvestigate the earlier reported synthetic route (Scheme 26) from our initial studies, as it could provide a complimentary method towards synthesizing 2-*epi*-Jaspine B analogs for possible SAR studies. Accordingly, following the previously developed synthetic route from our group (Scheme 26), the bicyclic lactone **6** was subjected to DIBAL reduction to form the lactol **26.1**. Subsequent

Wittig olefination, followed by employment of the same reaction protocol as earlier completed the purported total synthesis of 2-*epi*-Jaspine B. However, to my utter surprise, spectral and analytical data of the product thus obtained was found to be identical to the natural Jaspine B stereoisomer, and not the expected 2-*epi*-analog. As the C2-epimerization in our previous synthesis of Jaspine B step was suspected to have taken place under the basic reaction conditions of the Wittig olefination (**27.1** $\rightarrow$ **27.3:** Scheme 27), I therefore repeated the sequence once again, and performed the Wittig reaction in the presence of an excess amount of base (5 equiv). However, the product Jaspine B ultimately obtained from this effort too was found to be the natural stereoisomer, and not the C2-epimer thereof. The above experiments point to the fact that, the epimeric Jaspine B, as obtained during our previous synthetic endeavor, did not result from the proposed base mediated epimerization of the transient aldehyde intermediate **27.3**. While we were pondering the implications of the above study, an interesting publication appeared, adding another intriguing element to the overall mystery.[186] In this study, pursuing the total synthesis of Jaspine B and its 2-*epi*-analog, Kim *et al* developed a route, wherein a strategically functionalized common precursor **I** was utilized to selectively obtain either the all-*syn*-substituted bicyclic oxazolidinone **II**, or the corresponding 2-*epi*-analog **III** (Figure 20). Subsequent oxazolidinone ring cleavage led to Jaspine B, and 2-*epi*-Jaspine B respectively.

Surprisingly, while comparing the spectral and analytical data of the above oxazolidinones **I** and **II** with the corresponding intermediate as reported in the earlier synthesis from our group, Kim observed that the oxazolidinone formed during our study is indeed the initially assigned all-*syn* compound **26.3** (Scheme 26), and not the subsequently suggested C2-epimeric analog **27.5** (Scheme 27) thereof.

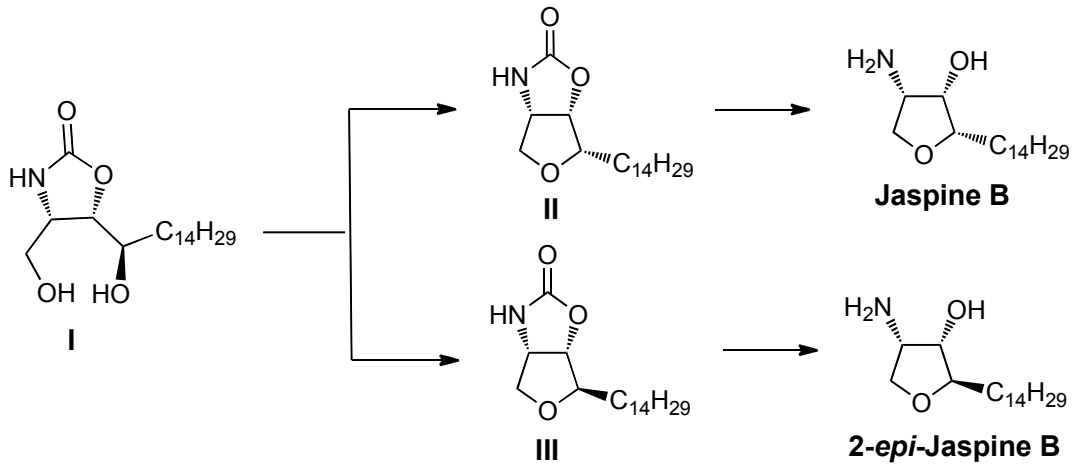


Figure 20. Kim's bicyclic oxazolidinones **I** and **II** *en route* Jaspine B and 2-*epi*-Jaspine B[186]

The above observations from Kim's study, and our own studies repeating the Wittig olefination sequence therefore confirmed that, while the final product obtained from our initial synthesis is indeed 2-*epi*-Jaspine B, the epimerization could not have been caused by base/basic conditions of the Wittig reaction process.

In view of the above conclusion, we herein propose an alternative mechanistic pathway rationalizing the epimeric Jaspine B formation in our previously reported synthesis. It is possible that, an acid catalyzed epimerization occurring during the lactone to lactol reduction step could be responsible for the C-2 epimeric Jaspine B in our previous synthesis. In a probable scenario, during the material build-up process of the synthesis, use of an older/improperly stored lot of DIBAL-H, contaminated with partially decomposed reagent as impurities, could have provided the Lewis acidic metal ion responsible for catalyzing the epimerization of the lactol intermediate (Figure 21). Thus, it can be postulated that, in the presence of a Lewis acidic contaminant present in the DIBAL reagent used, the hydroxyaldehyde **A** in equilibrium with the lactol **26.1**, undergoes a tetrahydrofuran ring-opening reaction via a β-elimination process (Figure 21). Recyclization involving an intramolecular Michael addition of the alkoxide **B** onto the α,β-

unsaturated aldehyde then results in the sterically more favorable 2,3-trans-substituted C2-epimeric aldehyde (Figure 21), leading to the subsequently obtained 2-*epi*-Jaspine B.

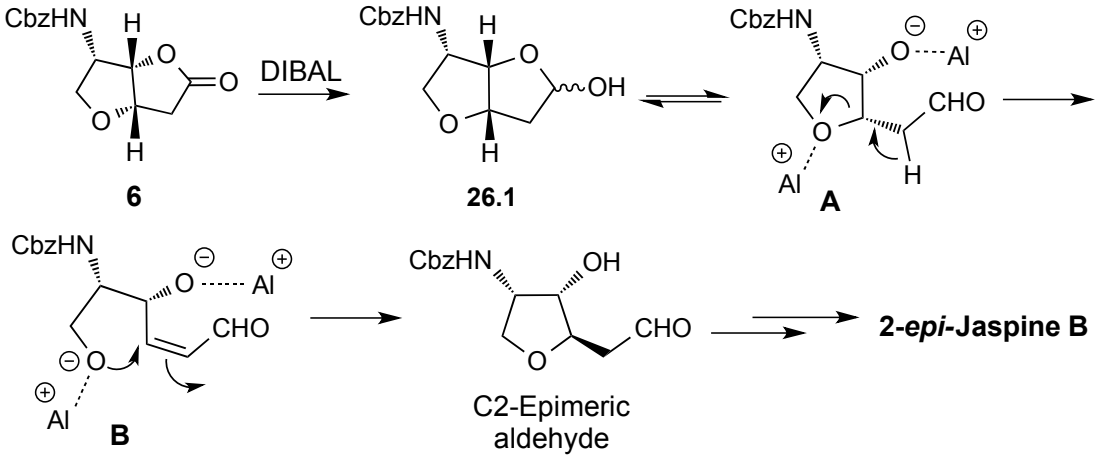


Figure 21. Formation of 2-*epi*-Jaspine B: An alternative mechanistic rationale

Accordingly, it is possible that, during the previously reported synthesis from our group, the compound characterization data as reported for the bicyclic oxazolidinone **26.3** was obtained from an initial batch of reaction product (standardization step), wherein non-contaminated DIBAL was used as the reducing agent. However, in moving forward with the total synthesis endeavor, in the subsequent scaled-up version of the reaction, possible use of a contaminated reagent bottle could have led to the formation of the epimeric aldehyde, ultimately leading to 2-*epi*-Jaspine B.

However, future studies, involving carefully designed experiments mimicking the postulated acid catalyzed reaction conditions, will have to be performed to confirm (or disprove) the above-suggested alternative mechanistic pathway (Figure 21).

3.5.3. Structure-activity relationship studies of Jaspine B

In devising the total synthetic route to Jaspine B during my present studies, we also intended to utilize the method developed to investigate structure-activity relationships of this bioactive natural product. Thus, following the above synthetic approach, and utilizing various

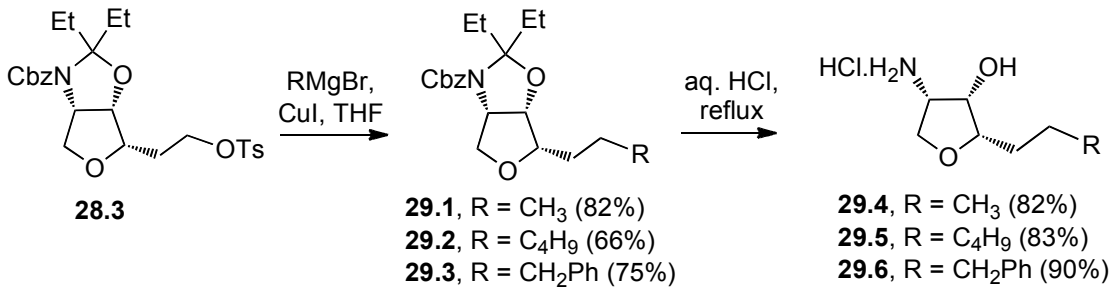
advanced intermediates from the total synthesis endeavor, I embarked on the following SAR-targeted modifications of Jaspine B. Unfortunately, there are no X-ray crystal structures of *Sphingomyelin synthase*, limiting any structural information of the target-binding site of the enzyme. Therefore, the present structural modification studies have been devised basing on classical medicinal chemistry approaches.

3.5.3.1. C2-Side chain modified analogs of Jaspine B

C2-Alkyl analogs:

Jaspine B has a long lipophilic alkyl chain at the C2-position. Although some of the previous studies have targeted modification of this tetradecyl side chain, there has been no systematic study co-relating optimum chain length and bioactivity. Accordingly, our first SAR aim has been the identification of optimum chain length required for activity. Thus, replacement of the tetradecyl side chain of Jaspine B with a short chain (propyl); medium chain (hexyl) and a benzylic functionality was planned for the present study. In addition to its lipophilicity, the benzyl functional group containing analog was also intended to probe possible π-π interactions with any suitably disposed aromatic amino acid residues at the enzyme-binding pocket.

Syntheses of the designed analogs (Scheme 29) were initiated from an advanced chiral intermediate **28.3** from our total synthetic route. Thus, following the same reaction protocol as in the synthesis of Jaspine B, displacement of the tosylate **28.3** with *in situ* generated alkyl magnesiocuprates as shown, afforded the corresponding C2 alkylated analogs **29.1-29.3** (Scheme 29) in good yields. One-pot removal of both the acetonide, and the carbamate protecting groups under acidic conditions provided the three desired analogs **29.4-29.6** as the corresponding hydrochloride salts.

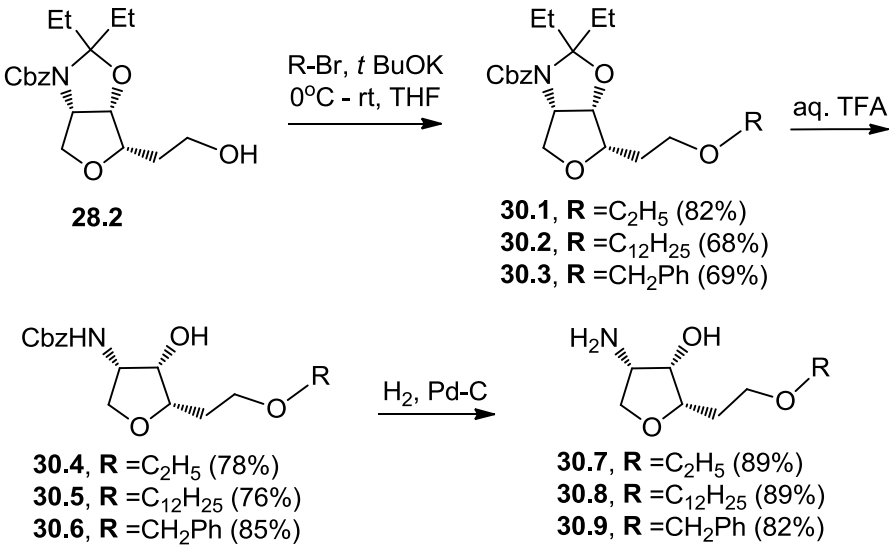


Scheme 29

C2-Ether analogs:

The natural tetradecyl side chain of Jaspine B renders the molecule highly lipophilic (CLogP = 6.21). Aiming to modulate the lipophilicity of the above substituent, we decided to investigate analogs wherein a methylene group in the C2-alkyl chain will be subjected to bioisosteric replacement with a more polar oxygen functionality. It was reasoned that, in addition to increased polarity of the intended analogs, the oxygen atom in the side chain could also serve as a potential H-bond acceptor at the target-binding site.

Once again, utilizing an advanced intermediate **28.2** from our total synthesis, the desired analogs could be accessed in a rapid and efficient manner (Scheme 30).



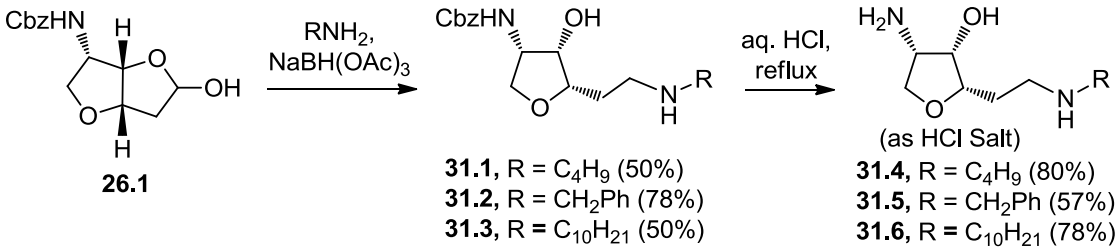
Scheme 30

Thus, starting from the bicyclic tetrahydrofuran intermediate **28.2**, deprotonation of the primary hydroxyl group and subsequent *O*-alkylation with appropriate alkyl halides led to the formation of the corresponding alkoxy analogs **30.1-30.3** (Scheme 30) in good yields. Following a two-step deprotection sequence, initial hydrolytic cleavage of the oxazolidine ring forming the *N*-Cbz-protected amino alcohols **30.4-30.6**, followed by hydrogenolytic removal of the amine protecting group yielded the target C2-alkyloxyalkyl analogs **30.7-30.9** in good overall yields.

C2-Alkylamine analogs:

In continued studies aimed at bioisosteric modification of the C2- alkyl chain of Jaspine B, bioisosteric replacement of one of the side chain methylene groups with an amine (NH) group (Scheme 31) was undertaken. In addition to increasing the hydrophilicity of the molecule, it was also envisioned that, the amine moiety thus introduced in the side chain could provide a favorable tool towards probing potential H-bond interactions (both as donor and acceptor) with appropriate amino acid residues at the enzyme-binding pocket.

The syntheses of the intended analogs were initiated with the bicyclic lactol intermediate **26.1** from our total synthesis route. Thus, subjecting the lactol **26.1** to standard reductive amination with selected primary amines in the presence of sodium acetoxyborohydride provided the corresponding C2-alkylamino tetrahydrofuran derivatives **31.1-31.3** (Scheme 31) in good yields. Subsequent hydrolytic removal of the Cbz-protecting group uneventfully resulted in the desired C2-alkylamine substituted Jaspine B analogs **31.4-31.6** as the corresponding HCl salts.



26.1

31.1, R = C_4H_9 (50%)
31.2, R = CH_2Ph (78%)
31.3, R = $C_{10}H_{21}$ (50%)

31.4, R = C_4H_9 (80%)
31.5, R = CH_2Ph (57%)
31.6, R = $C_{10}H_{21}$ (78%)

Scheme 31

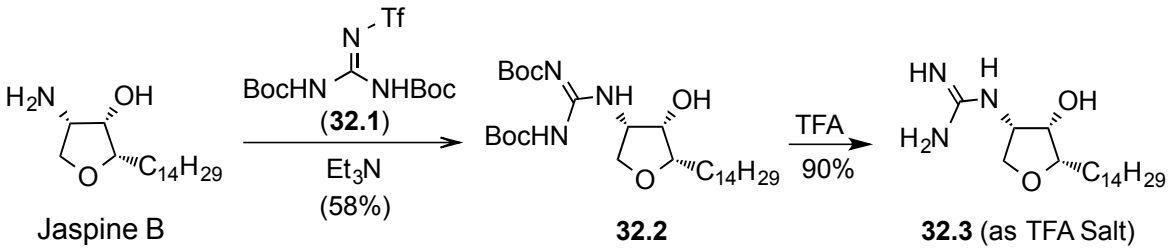
3.5.3.2. C4-Amine modified analogs of Jaspine B

Except for a surprisingly few reports involving functionalization of the C4-amine group of Jaspine B, there have been no SAR studies to determine the role, or effect of the above amine in the biological activity of this cytotoxic natural product. We therefore decided to carry out strategic modification of the C4-amine and evaluate the effect on biological activity.

C4-Guanidine analog:

In addition to strong H-bond forming capabilities, the relatively higher basicity and increased affinity for ionic interactions with appropriate counter anions at enzyme binding pockets render the guanidine moiety a preferred replacement for amine groups in investigational bioactive molecules. The potent neuraminidase inhibitor anti-influenza drug Zanamavir is an example of such an approach, wherein, an amine to guanidine modification of a previous generation lead compound resulted in several fold increase in binding affinity of the drug.[214]

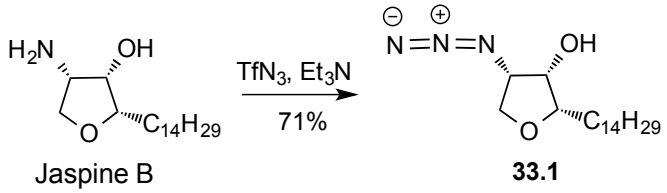
Accordingly, with an aim to probe/modulate the possible biological role of the C4-amine of Jaspine B in ionic, or salt-bridge forming interactions, we decided to investigate the synthesis of the corresponding C4-guanidine analog of Jaspine B. Thus, staring from Jaspine B, as obtained from our total synthesis, its reaction with the known guanidine transfer reagent **32.1** (Scheme 32) under standard conditions cleanly provided the corresponding di-Boc-protected guanidine derivative **32.2**. Removal of the Boc-protecting groups with trifluoroacetic acid resulted in the desired C4-guanidino Jaspine B analog **32.3**.



Scheme 32

C4-Azide analog:

Replacement of the C4-amine of Jaspine B with a polar and linear azide moiety was next investigated. Thus, in a one-step process, reaction of Jaspine B with freshly prepared triflic azide (Scheme 33) directly provided the corresponding C4-4-azido-Jaspine B (**33.1**) in good yield.

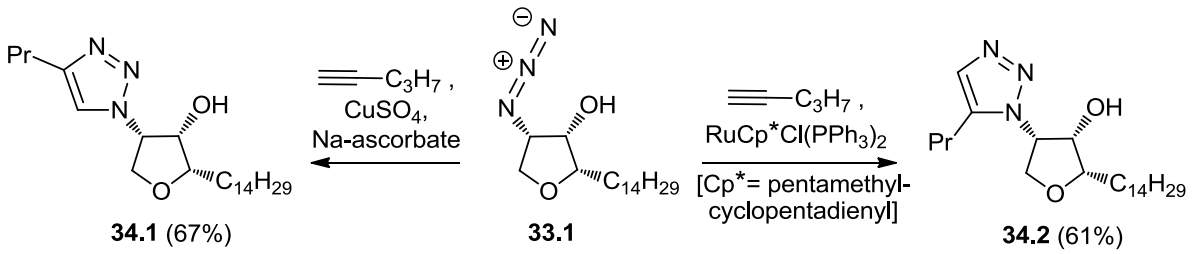


Scheme 33

C4-Triazolo analogs:

Having easy access to the above C4-azide analog (**33.1**) also created an opportunity to investigate replacement of the amine group of Jaspine B with a biologically relevant triazole motif. Structurally, the triazole moiety possesses a large dipole, is capable of H-bond interactions, and is a mimic of amide bond planarity. In drug design strategies, the triazole moiety is often used as a bioisosteric replacement for amides, and also to modulate polarity and bioavailability via H-bond interactions of the analog.[215]

Installation of the target triazole group at the C4-position of Jaspine B was accomplished following a Sharpless click reaction.[216] Thus, subjecting the azido analog **33.1** to a Cu (I) catalyzed 1,3 dipolar cycloaddition with 1-pentyne led to the regioselective formation of the corresponding 4-(1,4-disubstituted-triazolo) Jaspine B analog **34.1** (Scheme 34) in good yield.



Scheme 34

Similarly, employing the Fokin protocol,[217] 1,3-dipolar cycloaddition of the azide **33.1** with 1-butyne in the presence of catalytic RuCp*Cl$(PPh_3)_2$ afforded the regioisomeric 4-(1,5-disubstituted-triazolo)Jaspine B analog **34.2** (Scheme 34).

3.5.3.3. C2, C4 Modified Analogs: Synthesis of 'Jaspine B–Ceramide' hybrids

A free amine or amide thereof is an integral structural component of the bioactive sphingolipids (Figure 16). For example, in ceramide, the amine group is derivatized to a fatty acid amide. Considering the ceramide and Jaspine B structural similarities (highlighted red in Figure 22 below), it is considered worthwhile to investigate whether the attachment of appropriate acyl/alkyl groups onto the C4-amine of Jaspine B, matching the lipophilic/steric/electronic features of ceramide even more closely, would result in derivatives with improved interactions at the various ceramide processing enzyme binding site(s). Accordingly, we performed a molecular modeling study to probe the possible structural overlaps among: (**i**) ceramide, (**ii**) Jaspine B, and two hypothetical analogs, (**iii**) 4-*N*-palmitoyl Jaspine B (mimicking the corresponding amide functionality of ceramide), and (**iv**) a C2, C4 doubly modified 'ceramide-Jaspine B hybrid' (Figure 22).

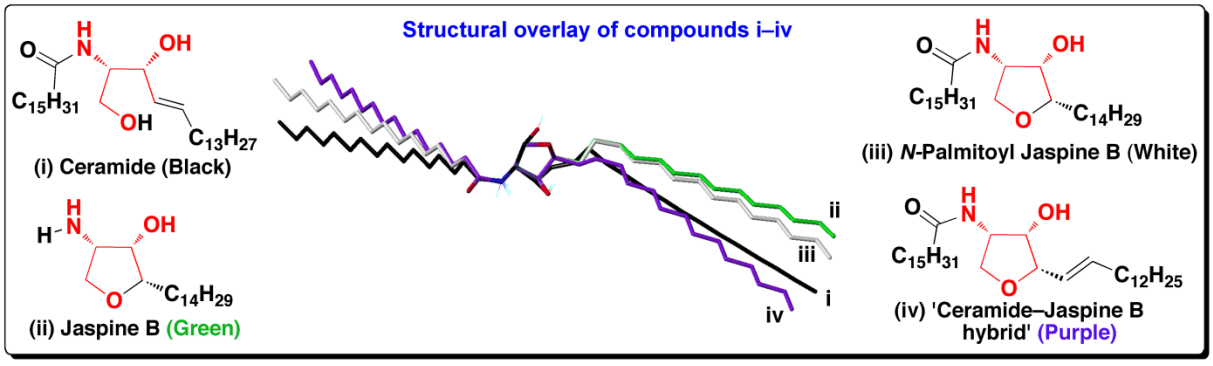


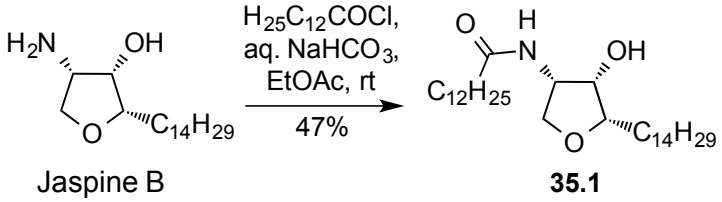
Figure 22. Ceramide, Jaspine B, and hypothetical analogs **iii** and **iv**. Similarities and overlap

The structures were optimized in SYBYL according to default optimization thresholds and were then overlaid according to positions of functionally conserved heteroatoms. As evident,

in addition to the excellent overlap among the central structural cores of all the above four molecules, the close spatial orientation of the lipophilic C2/C4 functionalities of these compounds provide a strong incentive to investigate the proposed 'ceramide-mimic' Jaspine B analog as a potential chemical tool towards beneficial modulation of ceramide action.

C4-Fatty acid amide analog:

Based on the above premise, synthesis of a C4-caboxamide analog was undertaken. Accordingly, Jaspine B was reacted with tridecanoyl chloride in the presence of sodium bicarbonate in a biphasic medium, providing the corresponding Jaspine B carboxamide analog **35.1** (Scheme 35).

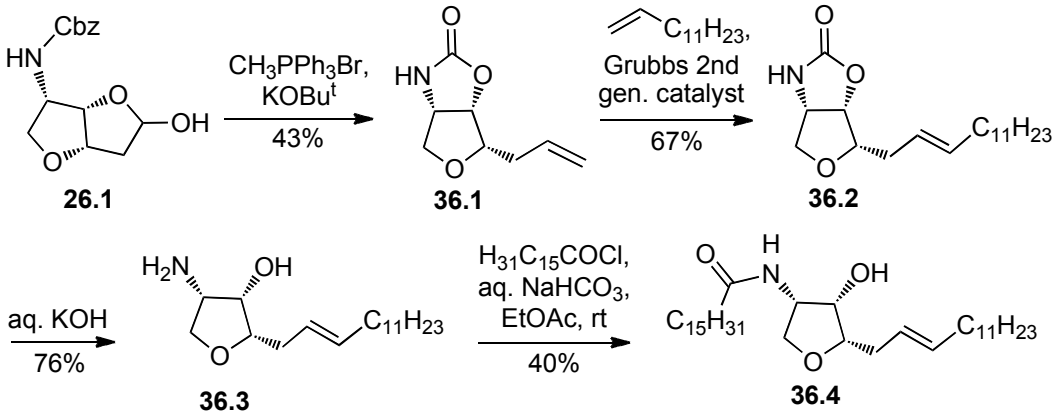


Jaspine B **35.1**

Scheme 35

Jaspine B – Ceramide hybrid:

The synthesis of the desired Jaspine B–Ceramide hybrid was initiated from the same lactol intermediate **26.1** as obtained from our total synthetic route. Accordingly, Wittig olefination of the masked aldehyde **26.1** with a methyl triphenylphosphonium bromide derived reagent, formed the corresponding C2-allyl-substituted bicyclic oxazolidinone **36.1** (Scheme 36). Installation of the desired tetradecenyl side chain at C2 was accomplished by subjecting **36.1** to an olefin cross-metathesis reaction in the presence of Grubbs second- generation catalyst, selectively affording the corresponding *E*-alkene derivative **36.2**. Alkaline hydrolytic cleavage of the oxazolidinone ring afforded the free amine **36.3**. Finally, treatment of the amine **36.3** with palmitoyl chloride culminated in the novel Jaspine B–Ceramide hybrid **36.4**.



Scheme 36

3.5.4. Cytotoxic activity assays of the Jaspine B analogs against B16 melanoma cells

To determine the effects of C2-side-chain modifications on cytotoxic activity, the C2-alkyl chain modified analogs (**29.4-29.6; 30.7-30.9; 31.4-31.6**) were evaluated in murine B16 melanoma cells by resazurin assay (the details of the assay protocol are described in the experimental section). Jaspine B and the anticancer drug paclitaxel (Taxol®) were also included as references in this assay. As can be seen from the figure below (Figure 23), the synthetic Jaspine B exhibited a dose-dependent cytotoxicity comparable to the natural product (IC_{50} = 0.56 μM), and is 20 times more potent than Taxol® (IC_{50} = 10.2 μM). In an interesting trend, it was observed that, shortening of the tetradecyl side chain of the natural product to a hexyl analog (**29.5**) retained substantial activity (IC_{50} = 3.3 μM), whereas further shortening to a propyl side chain (**29.4**), or incorporation of a benzylic moiety (**29.6**) resulted in significant activity loss (IC_{50}> 25 μM). It is worth noting that, the CLogP value (1.978) of the C2 hexyl analog **29.5** indicates a substantial improvement in its hydrophilicity compared to Jaspine B (CLogP 6.21). In the C2-alkoxy series, the bioisosteric replacement of an internal methylene group with oxygen, the long chain alkyloxy analog **30.8** retained substantial activity (IC_{50} 2.04 μM), while the corresponding shorter chain (**30.7**), or the benzyloxy analog (**30.9**) are devoid of activity (IC_{50}>

25 μM). In C2-alkylamine series, the long chain alkyl amine derivative **31.6** (IC_{50} 1.7 μM) is the most active compound among all the analogs synthesized. The other congeners in this series are inactive (IC_{50}> 25 μM).

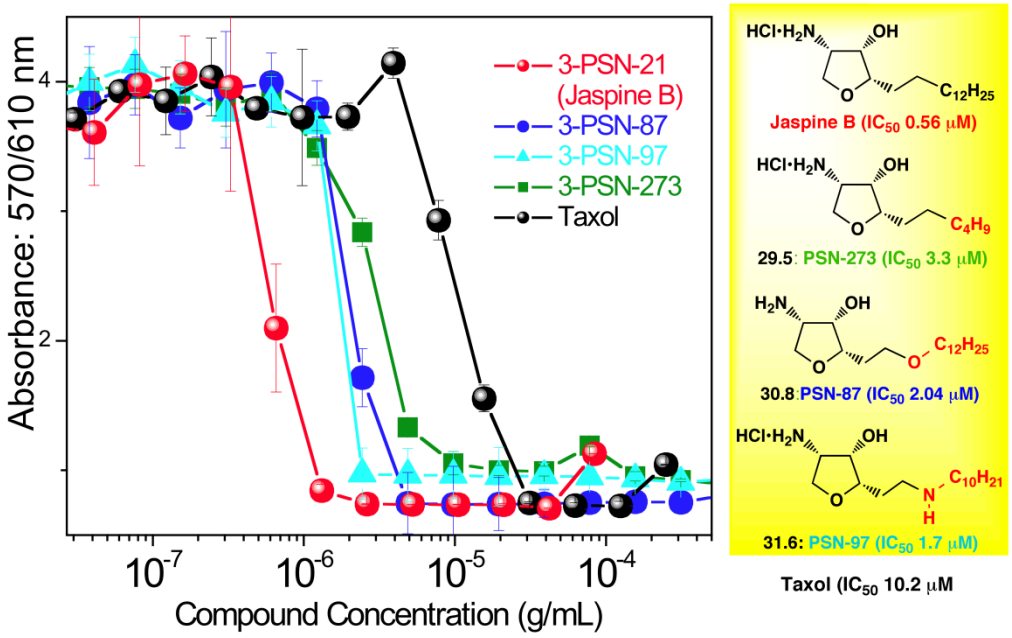


Figure 23. Murine B16 melanoma cell cytotoxic activities of Jaspine B, Taxol, and the C2-side chain modified Jaspine B analogs in Resazurin assay (active analogs shown)

The results from the above preliminary studies clearly indicate that the C2-alkyl substituent of Jaspine B could be a potentially beneficial site for a more detailed and systematic SAR investigation towards improvement of its anticancer activity and efficacy. The encouraging activity of the alkylamino analog **31.6** also points towards a possible approach towards improving the hydrophilicity of these compounds. While the cytotoxic activities of the remaining C4-, and C2, C4-modified analogs (**33.1**, **34.1**, **34.2**, **35.1**, and **36.4**) are yet to be determined, it is expected that, once available, the results from these assays too will provide important directions towards future design of analogs with improved activity.

3.5.5. Synergistic cytotoxic effect of Jaspine B-Paclitaxel combination against B16 melanoma cells

As discussed earlier, various anticancer drugs (including Taxol®) are known to cause cellular accumulation of ceramide and, malignant cells appear to be more sensitive to the effects of ceramide perturbation and/or generate ceramide more selectively in response to drugs. Thus, in a potential synergistic scenario, increased ceramide generation with concomitant inhibition of its metabolism could provide an even more efficient and cancer cell selective approach to chemotherapy with reduced collateral toxicity to normal tissues. As a proposed inhibitor of *sphingomyelin synthase*, Jaspine B action is expected to prolong the half-life of cellular ceramide via prevention of its metabolic conversion to sphingomyelin. It was therefore considered worthwhile to study the effect of a systematic combination of Jaspine B and Taxol® and investigate any potential synergism. The single-agent IC_{50} value of Jaspine B and Taxol® were determined to be approximately, 0.6 μg/mL and 10 μg/mL, respectively, against murine B16 melanoma cells in conventional cytotoxicity assays (using resazurin readouts as in figure 23 above). In experiments designed to test whether the presence of both compounds would result in additive or synergistic effects, a 'checkerboard titration' experiment was performed where both Jaspine B and Taxol® concentrations were simultaneously varied using an automated liquid handling program implemented on a Bio-Tek Precision 2000 XS instrument, wherein one compound is diluted 'down' the plate, while the other compound is diluted 'across' the plate. As shown below (Figure 24), gradual increase in Taxol® concentration results in progressive increase in the cytotoxicity of Jaspine B; and, sub-inhibitory concentrations of Taxol® (0.892 μg/mL) drastically reduces the viability of the melanoma cells when incubated with even <10 ng/

mL of Jaspine B (Fig. 24, left panel). A Schild-type plot of IC_{50} attributable to Jaspine B as a function of concomitant Taxol® concentration (Fig. 24, right panel) also indicated a potential synergistic effect of the two compounds.

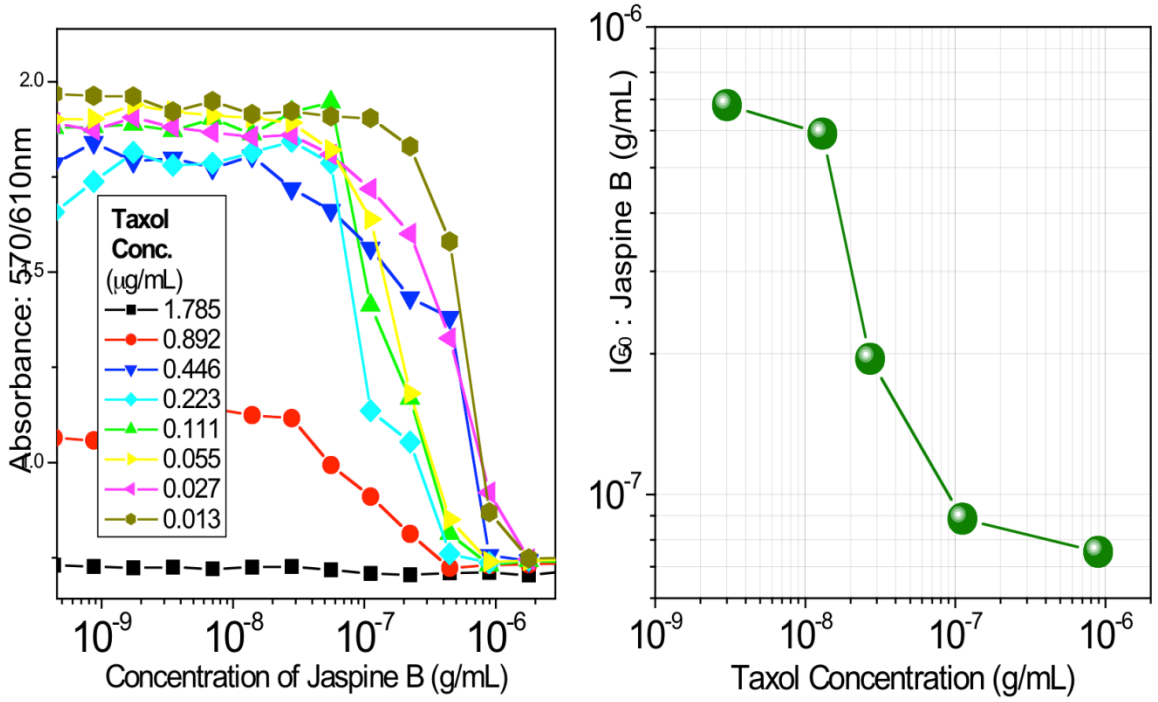


Figure 24. Inhibition of murine B16 melanoma cells as a function of simultaneous variation of Taxol® and Jaspine B concentrations (left panel), and, plot of Jaspine B-induced melanoma cell growth inhibition as a function of Taxol® concentration (right panel).

The above experiments point towards a potentially beneficial chemotherapeutic approach, wherein, combination of Jaspine B with cellular ceramide level enhancing known anti-cancer agents, could provide a synergistic pathway in triggering ceramide-induced apoptotic events in cancer cells. Such a strategy could also offer potential means to avoid/overcome development of drug resistance otherwise caused by evasion of apoptosis.

3.6. Concluding remarks

An efficient and practical total synthetic route to Jaspine B has been developed during the present research. Easy amenability of the above method towards strategic structural

modifications has also allowed easy access to a number of unique Jaspine B analogs for hitherto unknown SAR investigations. Simultaneously to the above studies, a previously reported synthetic route to Jaspine B from our group has also been reinvestigated in order to help clarify some unanswered questions. Gratifyingly, the results from the above study have also confirmed the utility of the previous route in stereoselective synthesis of enantiopure Jaspine B.

Results of the SAR investigations from the present study have contributed useful structural information towards future design and development of further improved analogs based on the Jaspine B structural lead. Results from the studies investigating potential synergism involving Jaspine B and Taxol® combination have been very promising. In future studies, this approach will be further investigated to study possible synergistic combinations of other ceramide level enhancing anticancer drugs (Paclitaxel, Camptothecin, Vincristine, Doxorubicin, Etoposide, Tamoxifen etc), with Jaspine B/active analogs thereof. These studies will be performed in cell lines relevant for Taxol (Breast cancer cell lines) and Taxol-resistant cell lines. The effect of combination (both Taxol and Jaspine B) on normal growing cell lines and in vivo xenograft model studies will also be pursued.

Chapter 4

A Rapid Approach Towards Diastereoselective Synthesis of Azacarbohydrate Scaffolds of Biological Significance

4.1 Introduction

A wide range of monocyclic and bicyclic polyhydroxylated alkaloids (Figure 25), commonly termed as iminosugars/azacarbohydrates, has been isolated from various plants, insects and microorganisms. Acting as carbohydrate mimics, many of these compounds have been found to be potent inhibitors of glycosidase and glycosyl transferase enzymes.[218-220] Since the initial discovery of the glycosidase inhibitory activities of the azacarbohydrates in the 1970's, the promising potential of these compounds as a new generation of therapeutics for the treatment of diseases such as diabetes,[221] viral infections,[222,223] cancer,[224] and metabolic disorders[225] has opened up exciting new opportunities in various drug discovery endeavors.

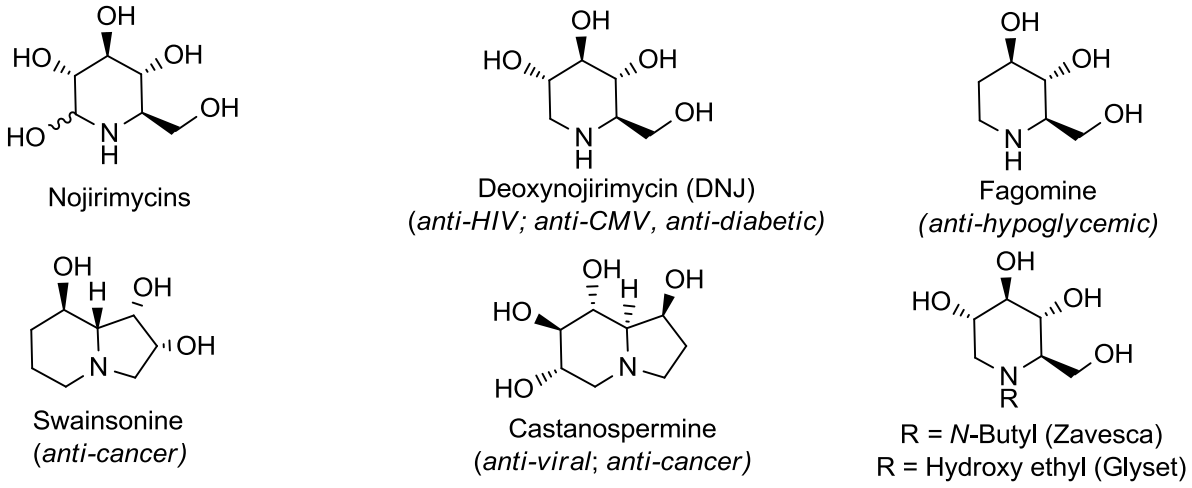
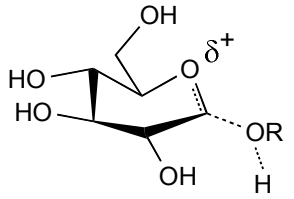


Figure 25. Representative examples of natural azacarbohydrates and analogs thereof

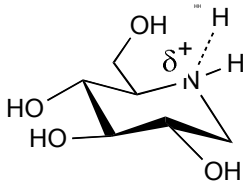
During the last few decades, rapid advancements in the fields of iminosugar chemistry and biology have also extended the scope of this class of compounds as inhibitors of a number of other enzymes such as phosphorylases, metalloproteinases, and glycosyltransferases. In recent years, the azacarbohydrate analogs, Miglitol/Glyset® (*N*-hydroxyethyl-1-deoxynojirimycin), and

Miglustat/Zavesca® (N-butyl-1-deoxynojirimycin) (Figure 25) have received FDA-approval for use in patients with type II diabetes,[226] and type I Gaucher's disease,[227] respectively.

The interest in iminosugars stems from the fact that, both conformationally[228] and electronically[228] they resemble the proposed transition state structure of the enzyme-catalyzed hydrolysis of glycoconjugates (Figure 26). During glycoside hydrolysis, protonation of the anomeric ether linkage is followed by formation of an oxocarbenium ion intermediate that adopts a half-chair conformation and possesses a positively charged endocyclic oxygen atom (**A**: Figure 26). Similarly, iminosugars bear a structural resemblance to the enzyme's natural substrate and mimic this oxocarbenium ion transition state, as the amino group exists as protonated form under physiological conditions. (**B**: Figure 26).[228-230]



(**A**) Oxonium ion transition state



(**B**) Protonated 1-deoxynojirimycin

Figure 26. Structural mimicry between oxonium ion transition-state of glycoside hydrolysis (**A**), and protonated deoxynojirimycin (**B**)

Thus, as transition-state mimetics, iminosugars are often capable of binding more tightly in the enzyme active site than the natural substrate. For example, 1-deoxynojirimycin (Figure 25) has 5 fold greater binding affinity to *α-glucosidase* than the natural substrate glucose.[228] The anomeric carbon in iminosugar is still sp^3 hybridized and they do not adopt a half-chair conformation as expected for oxocarbenium transition-state analogs of glycosidase cleavage processes.[229] Importantly, the replacement of the endocyclic oxygen atom of carbohydrates with a nitrogen atom in iminosugars renders the latter compounds metabolically inert, but does not affect their recognition by glycosidases.[228] Structural resemblance of the iminosugars to

carbohydrates also endows them with unique properties such as, transportation by carbohydrate transporters, and the ability to cross the blood-brain barrier etc, while the polar nature of the molecules helps retain their water-solubility and normal, unchanged excretion from the body.[231]

Broad range of biological activities, and challenging structural features of the azacarbohydrates have thus attracted growing interest in the synthesis, structural modifications, and biological evaluations of this class of compounds. In the following section, I will briefly discuss the recent literature reports on the synthesis of representative azacarbohydrates.

4.2. Overview of azacarbohydrate synthesis

Azacarbohydrates, with multiple chiral centers, several polar hydroxy functionalities, and lack of a chromophore in the structural core, present considerable challenges in any synthetic endeavor. Accordingly, application of chiral pool materials, orthogonal protection-deprotection protocols, and use of specialized techniques for analysis and separation have been some of the useful strategies/tools employed in the synthesis of the various azacarbohydrates.[232-237]

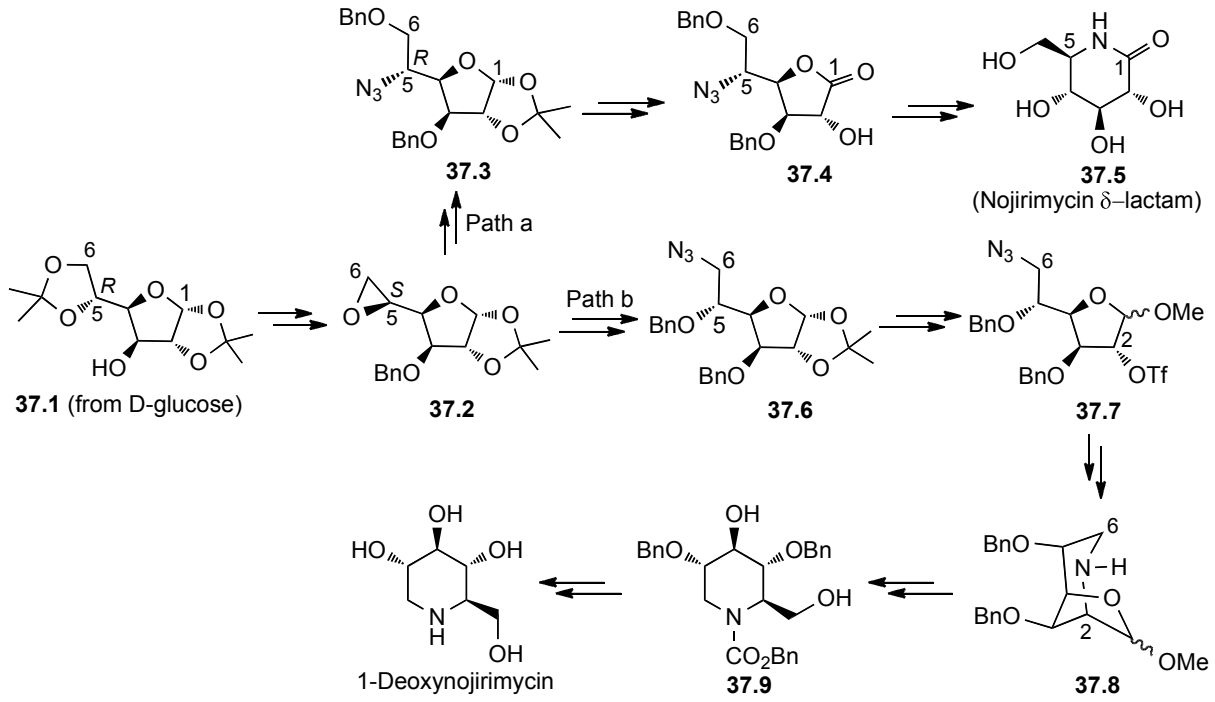
A common synthetic approach for this class of compounds has been the utilization of readily available carbohydrate starting materials, wherein, initial introduction of an amine functional group in the sugar core is followed by aminocyclization towards formation of the piperidine/pyrrolidine ring structure of the target iminosugar.[232-237] Some of the strategies employed for the performance of the above aminocyclization reaction include intramolecular versions of: reductive amination of aldehydes/ketones, S_N2 displacement of suitably located leaving groups by amines, or, addition of amines onto activated olefins. Alternatively, simultaneous amination and cyclization have also been achieved employing a one-pot protocol, via reaction of ammonia (or a primary amine) with a di-functionalized sugar.[232-237]

In addition to carbohydrates, other chiral pool starting materials such as amino acids,[238-241] and tartaric acid[242] etc. have also been utilized in a number of other synthetic approaches to iminosugars. Some of the other reported approaches leading to the azacarbohydrates include, construction of the nitrogen containing cycle via Diels-Alder reactions of appropriate substrates, Grubbs olefin metathesis, asymmetric synthetic methodologies towards stereoselective installation of hydroxy and amine bearing functionalities etc.[237,243-251] Representative synthetic routes to various azacarbohydrates are briefly reviewed in the following section.

Fleet's approach

In extensive studies, Fleet and co-workers have reported the syntheses of azacarbohydrate scaffolds utilizing monosaccharides as starting material.[235] 1-Deoxynojirimycin, an analog of D-glucose in which the ring oxygen has been replaced by nitrogen, is the archetypal azasugar (Scheme 37). In the described synthesis, the formation of the piperidine ring of the azacarbohydrate core has been achieved by connection of nitrogen either through, (a) connection of C-1 and C-5 via an amine bridge, with inversion of configuration at C-5, or (b) installation of the amine between C-2 and C-6, with inversion of configuration at C-2. Accordingly, starting from the D-glucose-derived di-acetonide **37.1**, a common epoxide intermediate **37.2** has been utilized in both of the above ring-forming strategies. Thus, subjecting **37.1** to regioselective acetonide deprotection followed by activation of primary alcohol towards S_N2 displacement by the secondary alcohol at C-5 afforded the 5,6-epoxide intermediate **37.2**. Ring opening of the epoxide **37.2** with benzyloxy anion (path a) and subsequent S_N2 displacement of the resulting C-5 secondary alcohol with an azide nucleophile afforded the glucoazide derivative **37.3**, with concomitant inversion of the C5-stereocenter. Hydrolysis of the acetonide to form the lactol, and its selective oxidation formed the corresponding hydroxy lactone **37.4**. Reduction of the azide to

amine and its subsequent intramolecular cyclization to the lactam, followed by removal of the benzyl protecting groups resulted in the nojirimycin δ-lactam **37.5**.

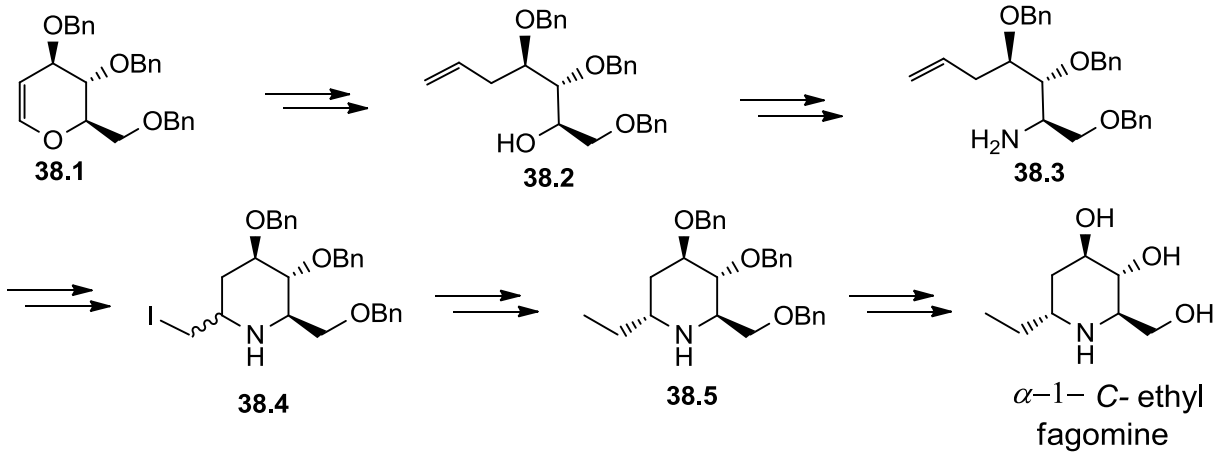


Scheme 37

Alternatively, opening of the epoxide **37.2** with an azide nucleophile (path b), and benzyl protection of the resulting secondary hydroxy group yielded the azide **37.6** (Scheme 37). Opening of the acetonide with methanolic HCl to form the corresponding methyl furanoside, and reaction of the resulting secondary hydroxy group with triflic anhydride led to the triflate derivative **37.7**. Reductive conversion of the azide to amine led to its spontaneous cyclization to the bicyclic amine **37.8**, with inversion of the stereocenter at C2. Protection of the amine as its carbamate, subsequent acid hydrolysis of the methyl furanoside to the corresponding lactol, and its reduction with sodium borohydride formed the tri-protected 1-Deoxynojirimycin derivative **37.9**. Global removal of the protecting groups completed the synthesis of 1-Deoxynojirimycin.

Martin's approach

Starting from the D-glucose-derived 3,4,6-Tri-*O*-benzyl-D-glucal (**38.1**), Martin's synthesis of α-1-*C*-substituted fagomine derivatives involved initial conversion of the glucal **38.1** to the ring-opened terminal olefin **38.2** (Scheme 38).[236] Thus, treatment of **38.1** with NIS, followed by removal of the iodide with sodium dithionate, and subjecting the resulting 2-deoxysugar to Wittig methylenation afforded **38.2**. Mitsunobu inversion of the free hydroxy group of **38.2** to form the corresponding epimer and subjecting the resulting hydroxy group to a second Mitsunobu inversion with phthalimide, followed by cleavage of the phthalimido protection generated the amine **38.3**. NIS mediated activation of terminal alkene in **38.3** followed by an intramolecular cyclisation afforded iminosugar intermediate **38.4** as a mixture of products, with the α-anomer being the major product. Treatment of the iodo intermediate **38.4** with Me_2CuLi and chromatographic separation of the desired stereoisomeric product, followed by removal of the benzyl protecting groups afforded α-1-*C*-ethyl fagomine **38.5**.

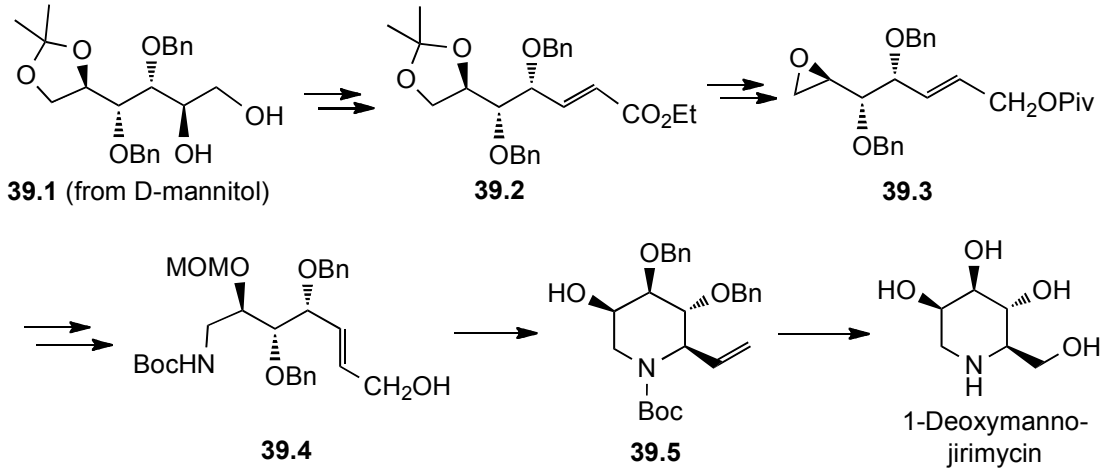


Scheme 38

Hirai's approach

Hirai has reported an original synthesis of 1-deoxymannojirimycin starting from D-mannitol.[252] A key reaction in this route involved a Pd-catalyzed intramolecular cyclization.

Thus, following a known procedure, D-mannitol was initially converted to the tetra-protected polyol **39.1** (Scheme 39). Oxidative cleavage of the diol and HWE reaction of the resulting aldehyde provided the α,β-unsaturated ester **39.2**. Reduction of the ester to alcohol and its protection as a pivalate, followed by acetonide deprotection and conversion of the diol moiety to the epoxide **39.3** was achieved via standard functional group transformations. Regioselective opening of the epoxide with azide anion, MOM-protection of the resulting secondary hydroxyl, conversion of the azide to amine and its Boc-protection, followed by selective pivaloyl deprotection provided the allylic alcohol **39.4**. The intramolecular cyclization of **39.4** was achieved via a Pd(II) catalyzed reaction, forming the piperidine **39.5** with high selectivity. Ozonolysis of the terminal olefin to aldehyde, its reduction with sodium borohydride, and removal of the protecting group completed the synthesis of 1-deoxymannojirimycin.

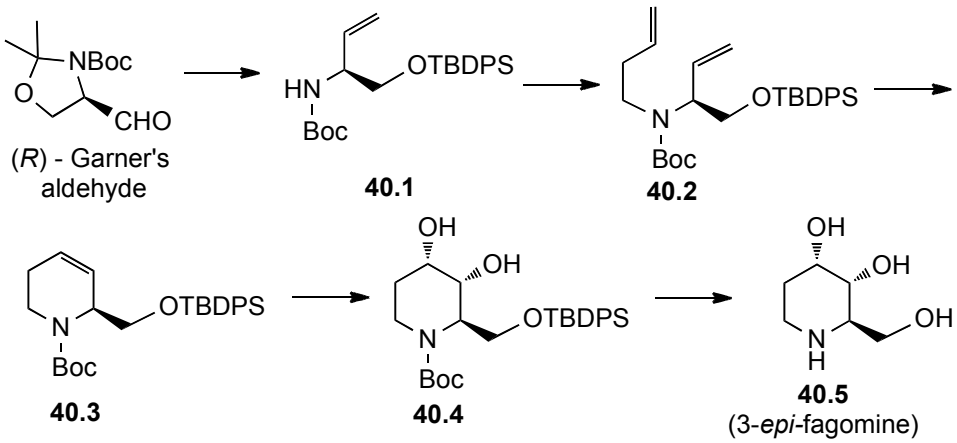


Scheme 39

Takahata's approach

Takahata and co-workers have reported the synthesis of iminosugar analogs using D-serine-derived Garner's aldehyde as a chiral building block. Thus, Wittig olefination of Garner's aldehyde, acetonide deprotection, and re-protection of the primary hydroxy group as its TBS ether formed the allylamine intermediate **40.1** (Scheme 40).[238] Conversion of **40.1** to the *N,N'*-

dialkylated diene **40.2,** and subjecting it to a ring-closing metathesis with Grubbs' second generation catalyst formed the piperidine derivative **40.3**. Dihydroxylation of **40.3** employing modified Upjohn conditions resulted in the stereoselective formation of the diol **40.4**. Subsequent removal of the protecting groups afforded 3-*epi*-fagomine (**40.5**).

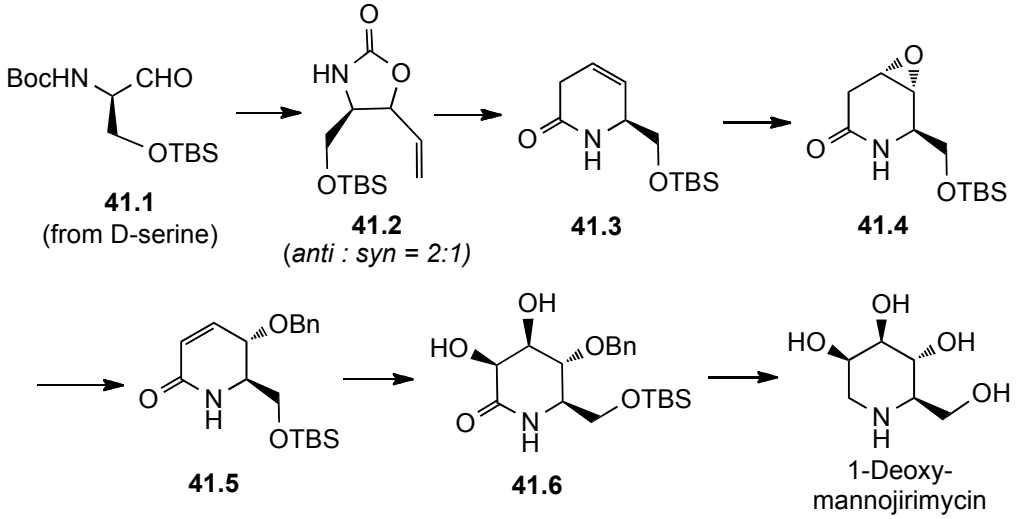


Scheme 40

Knight's approach

Knight and co-workers have reported a total synthesis of 1-deoxymannojirimycin starting from the amino acid D-serine. A key reaction in the synthesis involved palladium-catalyzed decarboxylative carbonylation of 5-vinyloxazolidine-2-one intermediate **41.2** to afford δ-lactam intermediate **41.3** (Scheme 42).[253] Thus, the D-serine-derived aldehyde intermediate **41.1** on reaction with vinyl Grignard reagent, followed by treatment with potassium tert-butoxide furnished a mixture of the *anti*- and *syn*-5-vinyloxazolidin-2-ones **41.2** (2:1 *dr*). This mixture was heated under CO pressure in the presence of a Pd catalyst to form, via a π-allylpalladium intermediate, the unsaturated δ-lactam **41.3**. Subsequent treatment of **41.3** with Oxone® resulted in a 4:1 mixture of the corresponding *anti*-and *syn*-epoxides, from which the major epoxide **41.4** was isolated. A base-mediated ring opening of the epoxide **41.4** and benzyl protection of the resulting hydroxyl led to the corresponding allylic alcohol **41.5**. Dihydroxylation of the olefin

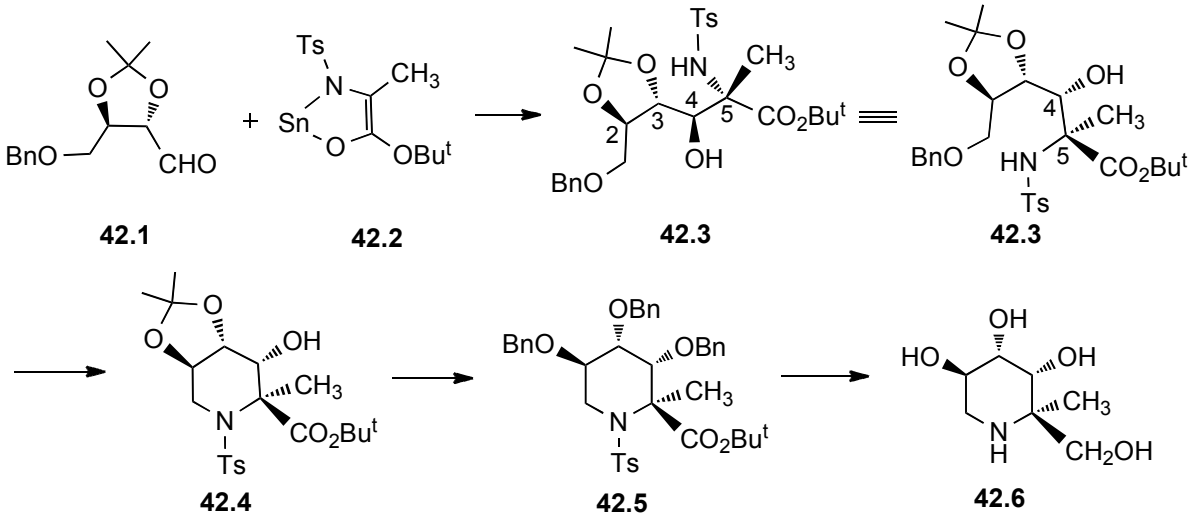
resulted in the diol **41.6** with high selectivity. Reduction of the lactam carbonyl to form the piperidine ring, and removal of the protecting groups afforded 1-deoxymannojirimycin.



Scheme 41

Kazmaier's approach

In Kazmaier's synthesis of an unnatural iminosugar, aldol reaction between a protected threose derivative **42.1** and the metal-chelated *N*-protected alanine *tert*-butyl ester **42.2** (Scheme 43) constituted a key reaction step.[242]

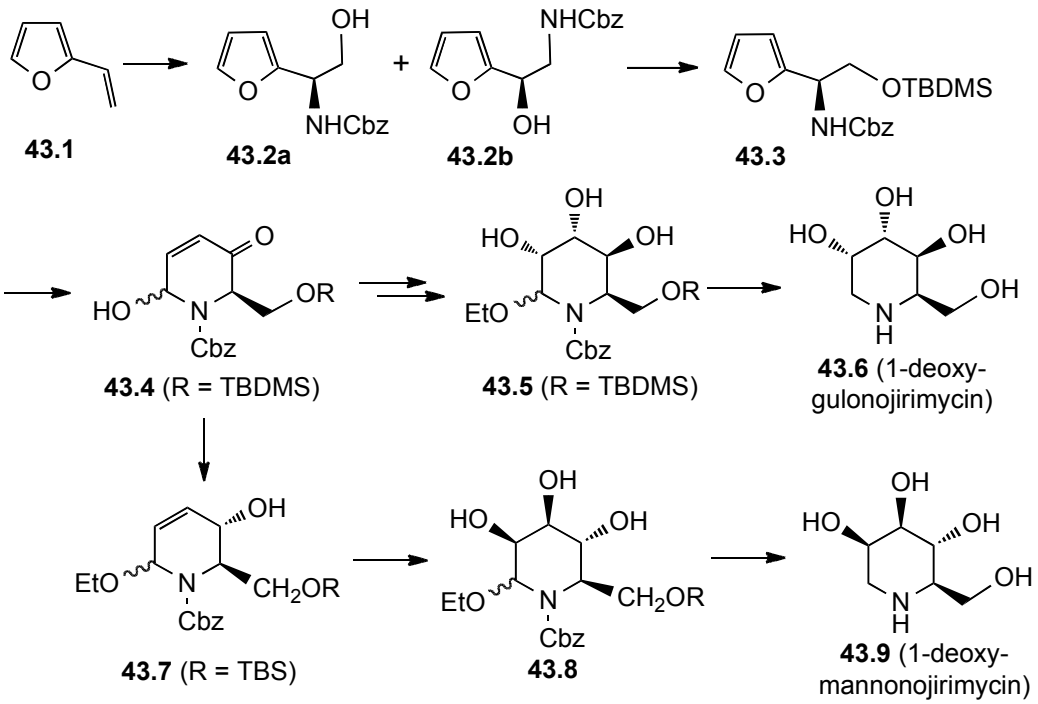


Scheme 42

The above aldol condensation provided a mixture of three diastereomers, with the desired 2,3-*anti*-3,4-*anti* isomer **42.3** being the major isomer. Removal of benzyl group in **42.3** followed by cyclization under Mitsunobu conditions formed the pipecolic acid derivate **42.4**. Subsequent cleavage of the acetonide linkage and benzylic protection of the free hydroxy groups afforded the tribenzyl- protected derivative **42.5**. Subsequent reduction of ester to primary alcohol, and removal of the protecting groups completed the synthesis of the iminosugar **42.6**.

O'Doherty's approach

In an asymmetric synthetic approach, O'Doherty *et al* have employed the Sharpless Asymmetric Aminohydroxylation (SAA) as a key reaction in their synthesis of chiral azasugars. Thus, vinyl furan **43.1** (Scheme 43), obtained from furfural, was subjected to SAA to provide a mixture of the regioisomeric amino alcohols **43.2a** and **43.2b** (1:2 *dr*).[243]



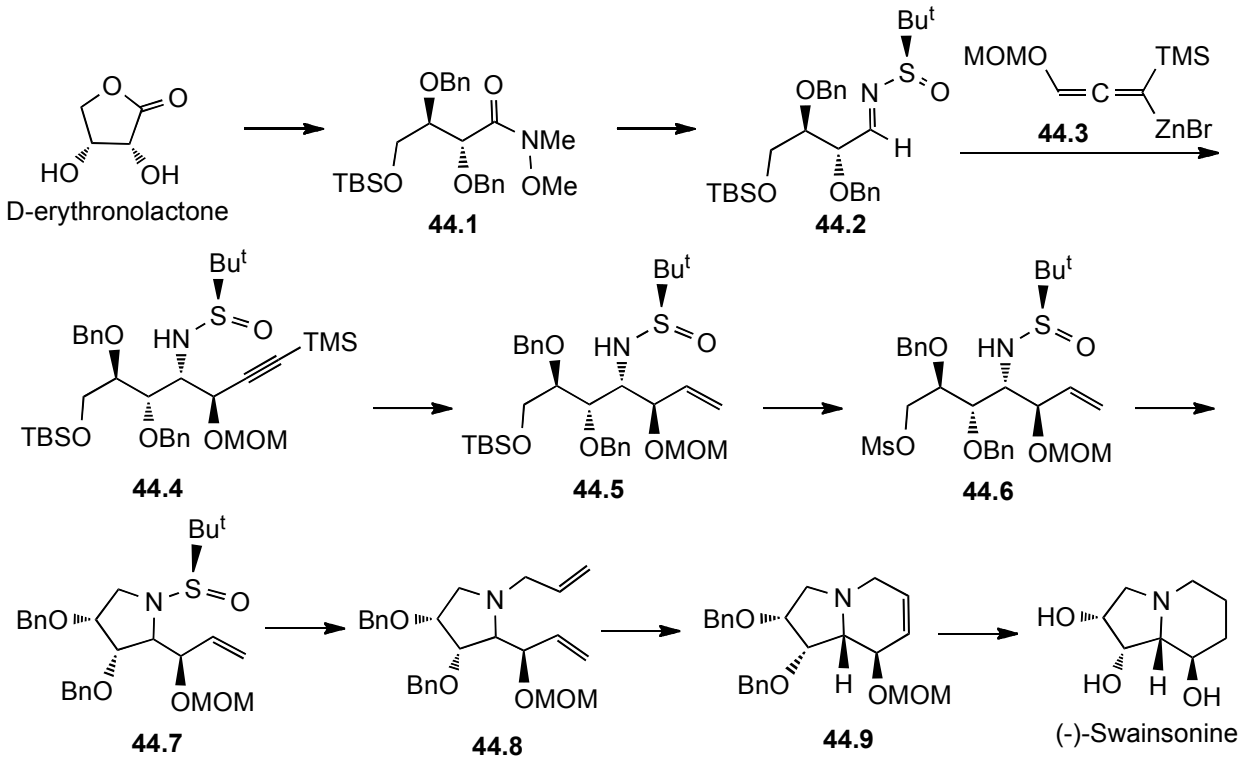
Scheme 43

Selective silyl-protection of the primary hydroxy group of **43.2a** allowed efficient separation of the desired aminoalcohol derivative **43.3**. Subsequent aza-Achmatowicz

rearrangement of **43.3** in the presence of m-CPBA afforded the hemiaminal **43.4**. Acid catalyzed conversion of **43.4** to the corresponding ethylaminal, followed by stereoselective Luche reduction of the ketone, and diastereoselective OsO_4 dihydroxylation yielded the triol **43.5**. Hydrogenolysis of **43.5** in the presence of TsOH completed the synthesis of 1-deoxygulo-nojirimycin **43.6**. Following a similar reaction sequence, the intermediate **43.4** was converted to the epimeric allylic alcohol **43.7** (via a Mitsunobu inversion of the Luche reduction product), followed by its eventual transformation to 1-deox*y*mannojirimycin **43.9** as shown.

Chemla's approach

Chemla and co-workers have reported a stereoselective synthesis of (–)-swainsonine by employing the addition of allenylmetals to chiral α,β-dialkoxy *N*-Boc-sulfinylimines as a key reaction step. Thus, commercially available D-erythronolactone was initially converted to the fully protected Weinreb amide **44.1** (Scheme 44).[254] Reduction of the amide to aldehyde, followed by its condensation with enantiopure *N*-*tert*-butanesulfinamide led to the formation of the sulfinylimine adduct **44.2**. Reaction of imine **44.2** with the *in situ* generated allenylzinc **44.3** provided the corresponding adduct **44.4** as the major diastereoisomer (major/minor = 4:1). Mild, base-catalyzed cleavage of the C-Si bond of **44.4**, and partial reduction of the alkyne afforded the olefin **44.5**. A two-step conversion of the TBS-ether to the mesylate **44.6**, and its subsequent treatment with base resulted in an intramolecular cyclization to form the pyrrolidine **44.7**. Selective removal of the sulfinyl chiral auxiliary, and alkylation of the resulting free amine with allyl bromide led to the formation of the diene **44.8**. Ring-closing metathesis of **44.8** with Grubbs' catalyst (2^{nd} generation) yielded the bicyclic amine **44.9**. Removal of the protecting groups, and hydrogenation of the olefin provided (–)-swainsonine.

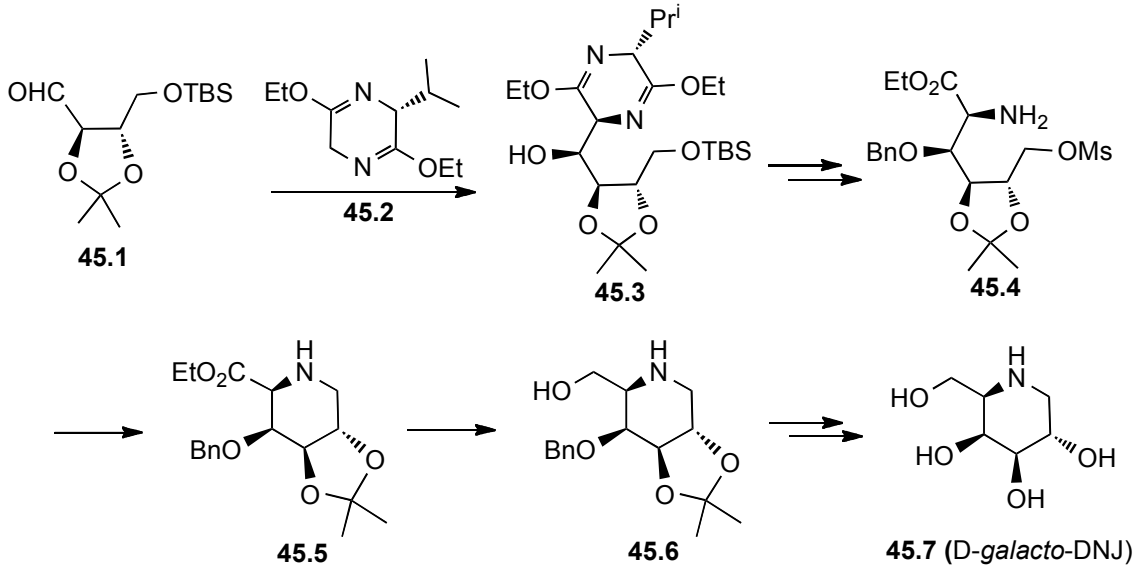


Scheme 44

Ojea's approach

Ojea *et al* have utilized a diastereoselective aldol addition of Schöllkopf's bislactim ether **45.2** to acetonide protected L-threose **45.1** (Scheme 45) in constructing a key precursor in their synthesis of various piperidine imino sugars.[250] The group has reported seven types of piperidine imino sugars by selecting different reaction partners. A representative aldol reaction used towards synthesis of D-*galacto*-deoxynojirimycin (**45.7**) is described below (Scheme 45).[250] 2,3-*O*-isopropylidene derivative of L-threose **45.1** on stereoselective aldol reaction with a metalated azaenolate of bislactim ether **45.2** afforded the adduct **45.3** as a major isomer, along with formation of two other minor diastereoisomers. Benzyl-protection of the free hydroxy group of **45.3** followed by a two-step conversion of the TBS-ether to the corresponding mesylate, and selective hydrolysis of the pyrazine moiety unmasked the amine **45.4**. Treatment of the amino mesylate **45.4** with triethylamine led to an intramolecular cyclization to yield the pipecolate **45.5**.

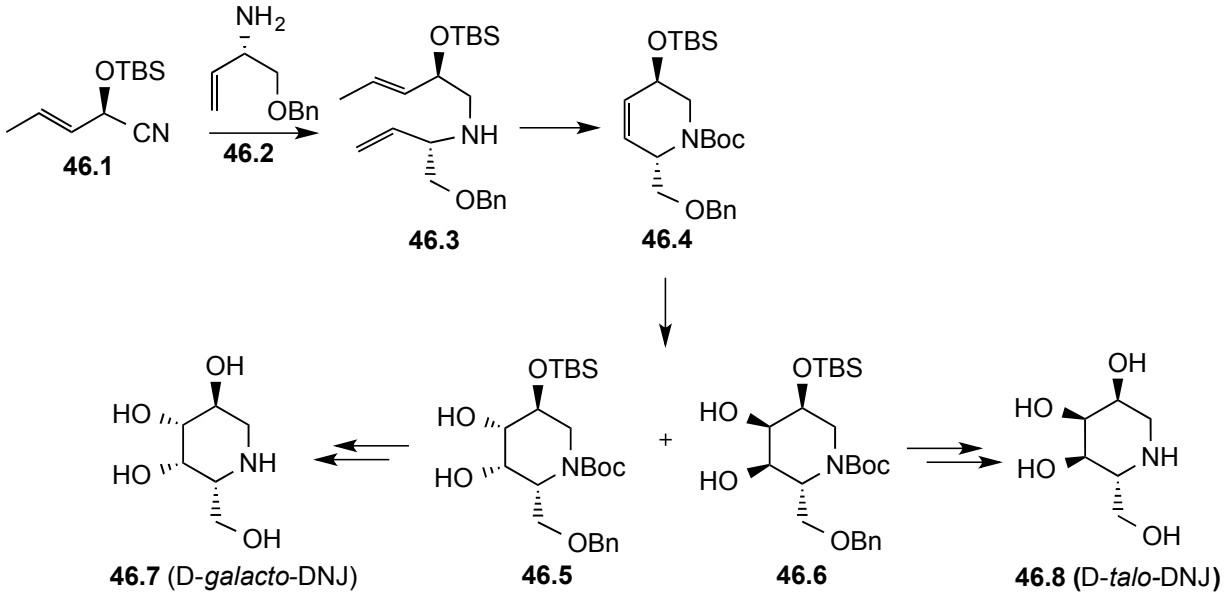
Subsequent ethyl ester reduction forming the primary alcohol intermediate **45.6**, followed by debenzylation and acetonide deprotection afforded D-*galacto*-deoxynojirimycin **45.7**.



Scheme 45

Overkleeft's approach

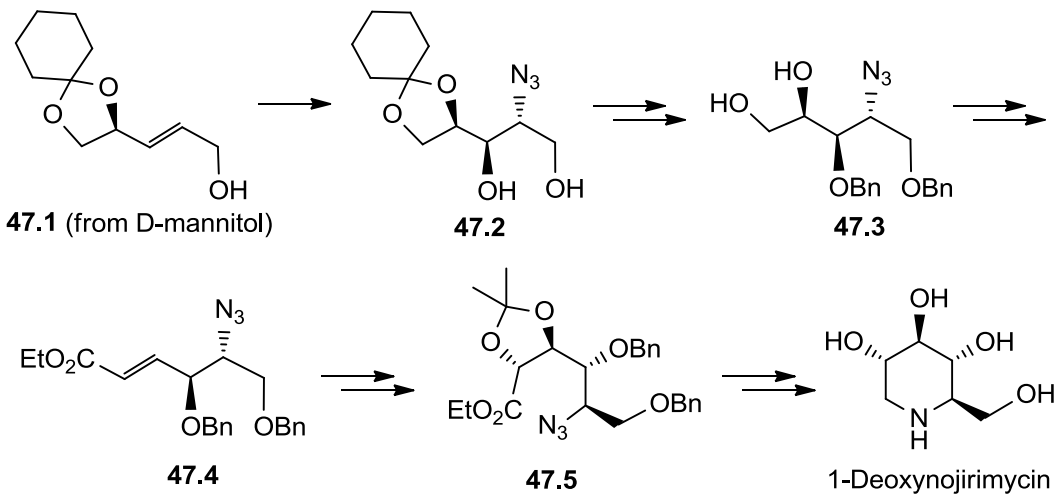
Overkleeft *et al* have synthesized eight diastereomeric 1-deoxynojirimycins starting from a single chiral cyanohydrin **46.1** (Scheme 46).[255] Cyanohydrin derivate **46.1** was synthesized by the reaction between crotonaldeyde and HCN in the presence of purified enzyme *pa*HNL,[256] a hydroxynitrile lyase derived from almonds, affording the enantiopure (99% ee) secondary alcohol which was subsequently protected as its TBS ether to afford **46.1**. Cyanohydrin **46.1** was converted to the secondary amine adduct **46.3** via a one-pot, three step reaction sequence involving DIBAL reduction of **46.1,** transamination with **46.2** and $NaBH_4$ reduction of the adduct imine.[257] Subsequent *N*-Boc protection and ring-closing metathesis yielded **46.4**. Upjohn dihydroxylation of **46.4** afforded a separable mixture of the diols **46.5** and **46.6** (1:1 ratio). Finally, deprotection of the purified products **46.5** and **46.6** led to a straightforward synthesis of the corresponding iminosugars D-galactodeoxynojirimycin and (**46.7**) and D-talo-deoxynojirimycin (**46.8**) respectively.



Scheme 46

Jayathirtha Rao's approach

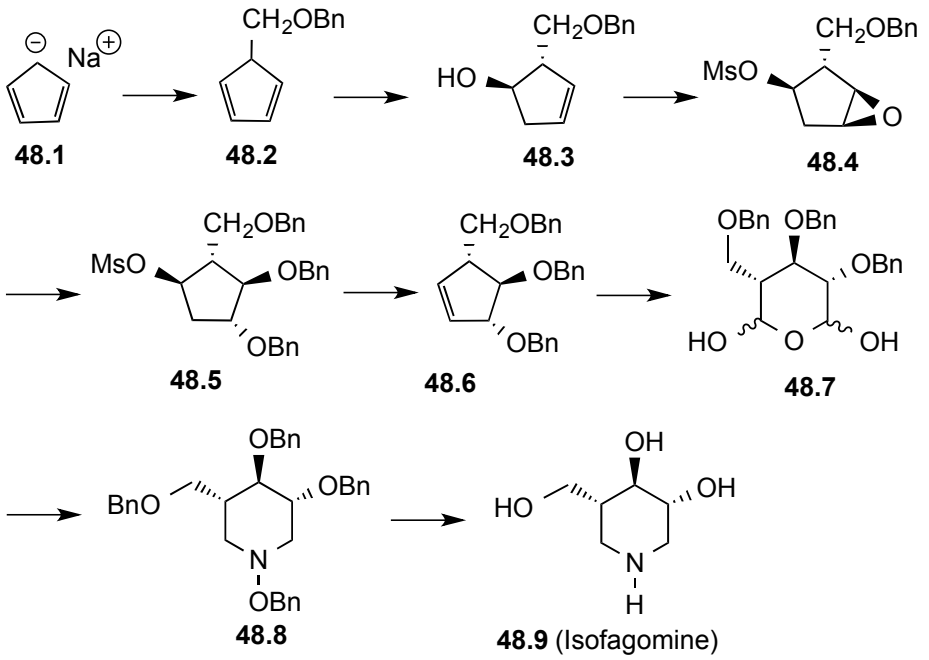
Starting from a D-mannitol-derived advanced chiral intermediate, Jayathirtha Rao *et al* have reported a synthetic route to 1-deoxynojirimycin.[237] Thus, the (*E*)-allylic alcohol **47.1**, obtained from D-mannitol in 4-steps, on Sharpless asymmetric epoxidation, followed by C-2 selective *endo*-mode epoxide opening with an azide afforded the azido alcohol intermediate **47.2** (Scheme 47). Benzylic ether protection of the free hydroxy groups of **47.2** followed by cleavage of the cyclohexylidene protection yielded the 1,2-diol **47.3**. Oxidative cleavage of the diol with $NaIO_4$ followed by HWE olefination of the resulting aldehyde selectively formed the α,β-unsaturated ester **47.4**. Upjohn dihydroxylation of electron deficient olefin **47.4**, forming the corresponding diol with high selectivity (*dr* 99:1), followed by acetonide protection afforded the fully functionalized azido ester derivative **47.5**. Reduction of ethyl ester **47.5** to the primary alcohol, its conversion to a mesylate, reductive cleavage of the azide to an amine, base catalyzed intramolecular cyclization forming the piperidine ring, and removal of the protecting groups completed the synthesis of 1-deoxynojirimycin.



Scheme 47

Crich's approach

Crich *et al* have reported an asymmetric synthetic route to polyhydroxylated piperidines, as exemplified by the synthesis of isofagomine shown below (Scheme 48).[251] Thus, starting with cyclopentadiene, generation of the corresponding sodium cyclopentadienylide and its reaction with benzyloxymethyl chloride formed the corresponding alkylated analog **48.2**. Subjecting **48.2** to a desymmetrizing enantioselective hydroboration with (+)-Ipc_2BH, followed by standard oxidative workup formed the corresponding 1,2-*trans*-substituted cyclopentenol **48.3** with high stereoselectivity (99% ee). Hydroxyl-directed m-CPBA oxidation of the olefin **48.3** and subsequent conversion of the hydroxy group to mesylate provided the epoxide **48.4** as the major product. Lewis acid catalyzed regioselective opening of the epoxide ring with benzyl alcohol yielded the tetra-substituted cyclopropane derivative **48.5**. Base-assisted elimination of the mesylate resulted in the olefin **48.6** as the major regioisomeric product. Subjecting the olefin **48.6** to oxidative cleavage with OsO_4/NMO/PIDA resulted in the hydrated dialdehyde **48.7**. Ring-closing double reductive amination of **48.7** with *O*-benzylhydroxylamine in the presence of sodium cyanoborohydride yielded the *N*-benzyloxypiperidine **48.8** in high yield. Finally, treatment of **48.8** with BBr_3 afforded isofagomine (**48.9**).



Scheme 48

To date, the most commonly used approach for the synthesis of various iminosugars have involved modification of readily available carbohydrate precursors. Close structural similarities between the above two compound classes, and resident chirality of the starting sugars are the major advantages of the above approach. However, when the target azacarbohydrate contains unusual / non-natural residues or stereochemistry, the above synthetic strategy often suffers from lengthy reaction sequence and necessitates extensive protection-deprotection of functional groups. In our present studies, we therefore propose to explore and develop an alternative reaction strategy involving stereoselective *de novo* construction of the azacarbohydrate structural scaffold, starting from a more simple non-carbohydrate synthon. Besides circumventing the disadvantages inherent in the carbohydrate-based approach, our alternative strategy will also make the method more flexible towards potential structural modifications towards detailed SAR investigations of this biologically important class of compounds. The details of my studies

involving the development of a rapid and flexible synthetic route to representative azacarbohydrate structural scaffolds are described in the following section.

4.3 Present Research

In continuation of our research interest in bioactive natural products, I initiated a study aimed at the development of a flexible synthetic route to variously functionalized azacarbohydrates. In this endeavor, the previously described aminobutenolide **1** from our research appeared to be an ideal building block for the proposed studies. Accordingly, starting from the chiral aminobutenolide **1** as a common building block, the retrosynthetic plan (Figure 27) envisioned utilization of the enone moiety of the butenolide towards stereoselective incorporation of appropriate functional groups as present in the target azacarbohydrates. Similarly, the conveniently located amine group in **1** should allow its intramolecular nucleophilic addition to the lactone carbonyl, leading to a rapid construction of the desired piperidine structural core.

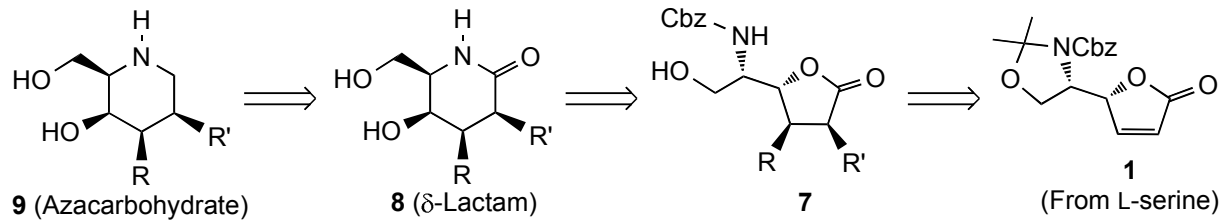
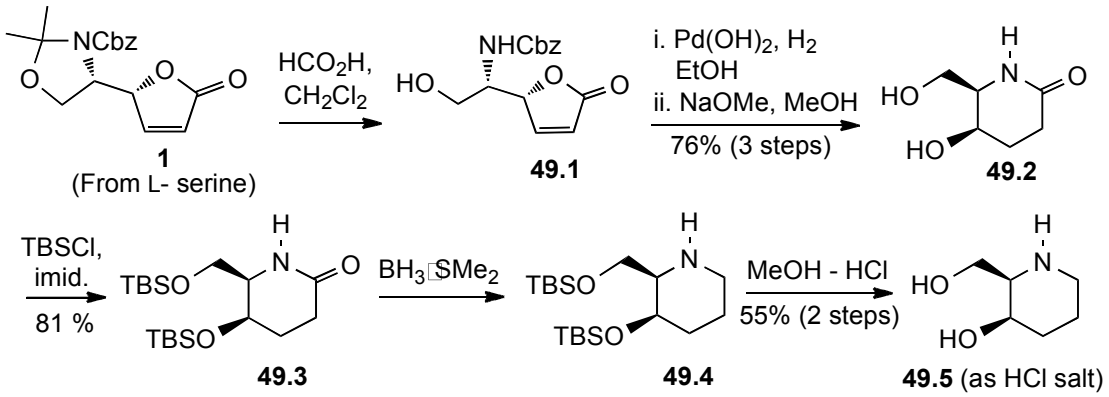


Figure 27. Aminobutenolide (**1**) to azacarbohydrates (**9**): Retrosynthetic strategy and approach

4.3.1. Stereoselective synthesis of a 2,3,4-trideoxyazacarbohydrate

Based on the retrosynthetic strategy described above, the synthesis of a dihydroxylated iminosugar was investigated. Accordingly, the L-serine-derived aminobutenolide **1** was treated with formic acid in methylene chloride to form the acetonide deprotected primary alcohol derivative **49.1** (Scheme 49). After removal of solvent and excess reagent, the crude product was

directly subjected to a further two-step reaction sequence, wherein, hydrogenation of **49.1** resulted in a one-pot *N*-Cbz-deprotection and saturation of the olefin, forming the corresponding free amine derivative. Removal of the hydrogenation catalyst by filtration and solvent removal, followed by subjecting the resulting amino lactone to a base-catalyzed intramolecular cyclization provided the desired piperidinone **49.2** in good overall yield. Borane-mediated standard reduction of the lactam **49.3** to the corresponding piperidine derivative **49.4**, followed by standard removal of the protecting groups provided the target 2,3,4-trideoxyazacarbohydrate analog **49.5**.



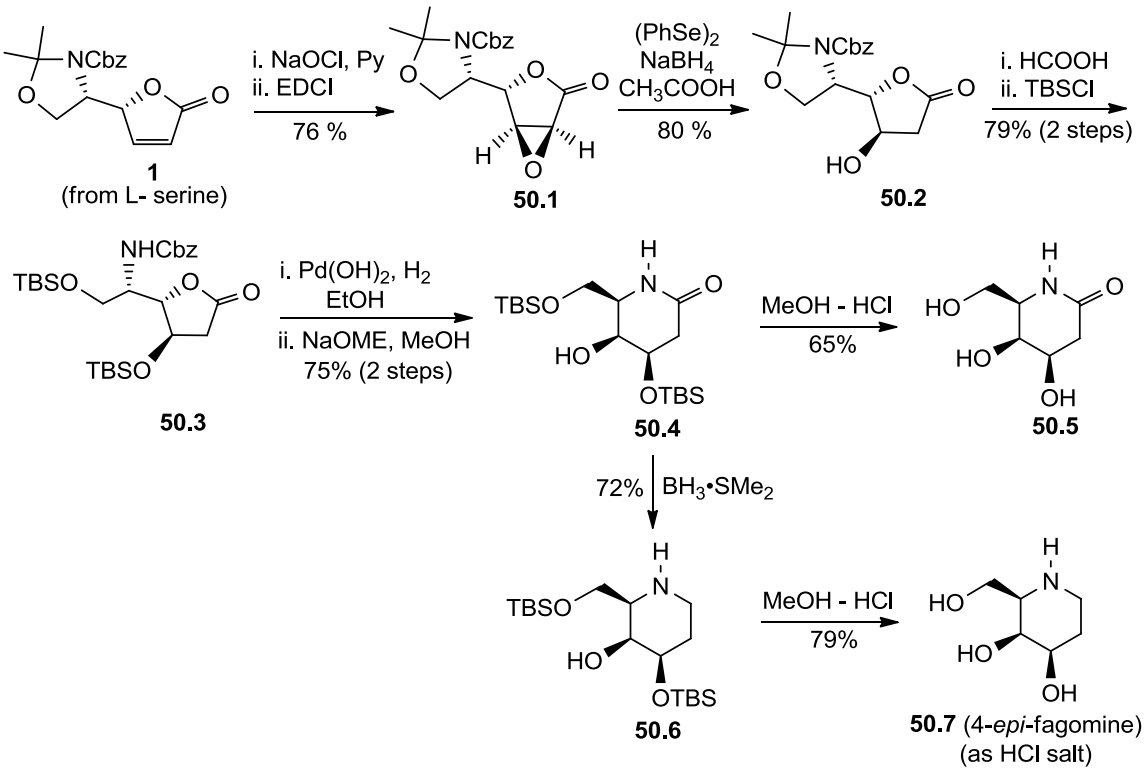
Scheme 49

Successful attainment of our initial synthetic goal towards rapid and efficient conversion of the aminobutenolide **1** to a functionalized azacarbohydrate scaffold encouraged us to further explore the above approach in the synthesis of tri-, and tetra-substituted azacarbohydrates.

4.3.2. Stereoselective synthesis of 4-*epi*-fagomine

Aiming to synthesize a 3,4,5-trihydroxylated azacarbohydrate, the aminobutenolide **1** was subjected to epoxidation at the enone moiety. Accordingly, following a known procedure,[258] reaction of the butenolide **1** with NaOCl led to the stereoselective formation of the epoxide **50.1** (Scheme 50) in moderate yield. Analysis of the crude reaction mixture showed the presence of a polar product in addition to the epoxide **50.1**. Concomitant to epoxide ring formation, the above

epoxidation condition is also known to cause lactone ring opening of the bicyclic epoxylactone to form the corresponding hydroxy carboxylic acid derivative.[258] Accordingly, suspecting the polar product in our reaction mixture to be the lactone ring-opened epoxide, in a subsequent reaction, the crude reaction mixture, obtained after epoxidation of **50.1**, was subjected to re-cyclization of the lactone ring by treatment with EDCI (1.2 equiv), and stirring at room temperature for 1 hr. TLC monitoring of the reaction mixture showed clean and complete disappearance of the polar spot, with increased intensity of the product spot corresponding to the epoxide. Gratifyingly, standard work-up of the reaction mixture, and purification of the crude product afforded the desired epoxide (**50.1**) with an improved yield (76%).



Scheme 50

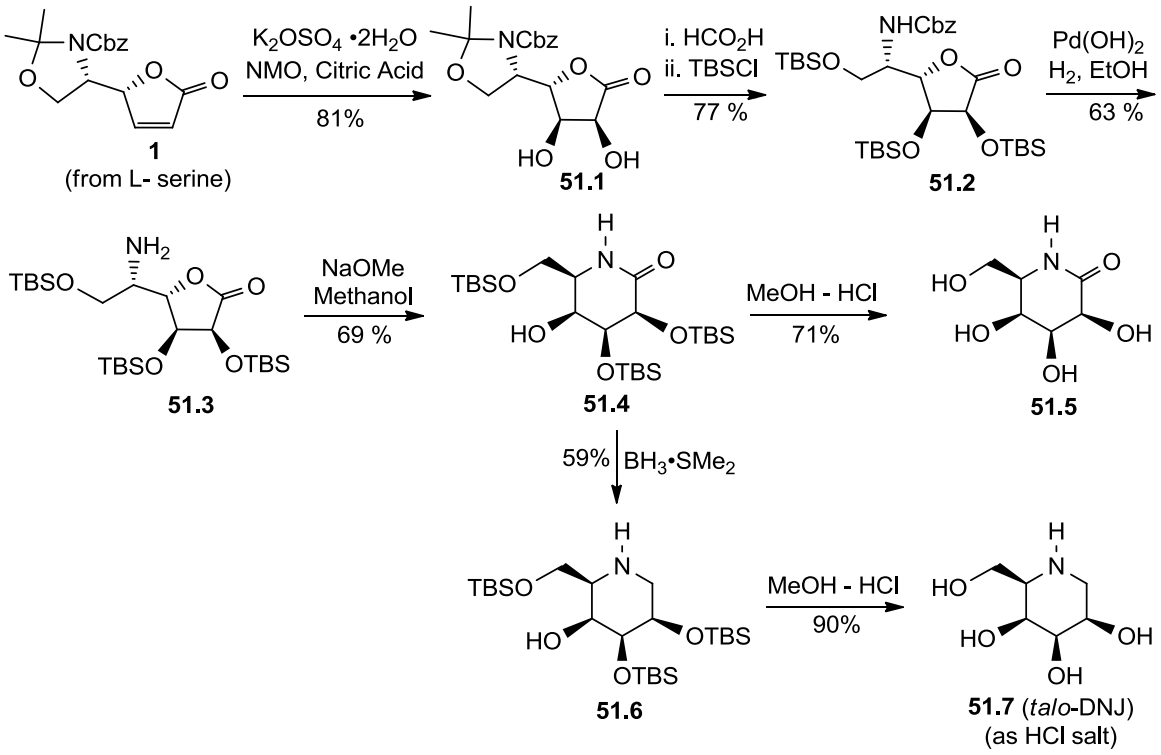
The assigned stereochemistry of epoxide **50.1** was based on literature precedence,[258] as well as our analogous observation involving cyclopropanation of the butenolide **1** (Chapter 2), wherein approach of the incoming nucleophile from the sterically less-hindered face of the olefin

leads to the stereoselective formation of the product. Proceeding with the synthesis, regio-selective ring-opening of the epoxide with an *in situ* generated phenylselenium hydride (PhSeH) formed the *β*-hydroxy lactone **50.2** in high yield. The structure and purity of the product was confirmed on the basis of its spectral and analytical data. The observed regioselectivity in the above epoxide ring-opening reaction can be attributed to the attack of the hydride at the more reactive (electron deficient), and less sterically hindered *α*-carbon adjacent to the carbonyl group.[259-263] Removal of the acetonide protection of the *β*-hydroxy lactone **50.2**, followed by silyl-protection of the free hydroxy groups afforded the di-silyl lactone **50.3**. Hydrogenolysis of the Cbz-protecting group, and exposure of the resulting amine to base catalyzed intramolecular cyclization uneventfully afforded the desired piperidinone **50.4** in good overall yield. Subsequent acidic cleavage of the silyl-protecting groups afforded the trihydroxy- substituted δ-lactam **50.5**. On the other hand, borane reduction of the lactam carbonyl affording the corresponding trisubstituted piperidine **50.6**, followed by removal of the silyl-protecting groups resulted in 4-*epi*-fagomine (**50.7**).

4.3.3. Stereoselective synthesis of *talo*-1-deoxynojirimycin

The next target in our studies involved the synthesis of the tetra-substituted structural framework of the 1-deoxynojirimycins. Thus, following a previously reported synthesis from our group, [26] the aminobutenolide **1** was stereoselectively converted to the corresponding diol **51.1** in good yield (Scheme 51). The high stereoselectivity observed in this reaction is once again attributable to the approach of the oxidizing reagent from the less hindered β-face of the olefin. Subsequent removal of the acetonide protection, and silyl-protection of all the free hydroxy groups provided the tri-silyloxylactone **51.2**. Unmasking of the amine **51.3**, via hydrogenolytic removal of the Cbz-protecting group, followed by treatment of the resulting amino lactone with

catalytic sodium methoxide yielded the expected piperidinone **51.4** in good overall yield. Cleavage of the silylether linkages led to the tetrasubstituted *δ*-lactam **51.5**. Whereas, initial borane-mediated conversion of the lactam **51.4** to the corresponding pyrrolidine derivative **51.6**, and global deprotection completed a straightforward synthesis of *talo*-1-deoxynojirimycin (**51.7**).



Scheme 51

5.2.Conclusions

Continuing with the utilization of a highly functionalized aminobutenolide as a versatile chiral building block, the present research describes a general and highly efficient protocol for the rapid construction of variously substituted enantiopure azacarbohydrate structural scaffolds of biological significance. In terms of brevity, ease of reactions, and overall yields, the present method compares well with the literature reported syntheses of the above class of compounds. From a future structure-activity relationship investigation perspective, the versatility of the key lactone intermediate **1** towards easy structural modifications and consequent possible access to a

variety of natural and non-natural azacarbohydrate analogs is also expected to be an added advantage of the present route.

Chapter 5

Experimental Procedures and Data

5.1 Materials and Methods

Proton and carbon nuclear magnetic resonance spectra were recorded using Bruker DRX 400 MHz, or Bruker DRX 500 MHz, or Avance AV-III 500 MHz spectrometers. All chemical shifts were recorded as parts per million (ppm), and all samples were dissolved in $CDCl_3$ using residual solvent peak as internal standard unless otherwise noted. Mass spectra were obtained from a ZAB HS mass spectrometer (VG Analytical Ltd., Manchester, U.K.) equipped with a 11/250 data system. Fast-atom bombardment mass spectrometry (FAB-MS) experiments were performed with a Xenon gun operated at 8 Kev energy and 0.8 mA emission at the MS laboratory at the University of Kansas. Fast-atom bombardment high-resolution mass spectra (FAB-HRMS (ESI-TOF) *m/z*:) were recorded at 1:10,000 resolution analyzer mode (MCA). HPLC analyses were carried out using a Shimadzu LC-6AL liquid chromatograph coupled to a SPD 20A prominence UV/VIS detector. All columns were purchased from Agilent. A Shimadzu FTIR 8400S spectrophotometer was used to record infrared spectra. Optical rotations were obtained using an Autopol IV Automatic Polarimeter at rt. Melting points were obtained using a Thomas Hoover Uni-melt capillary melting point apparatus and are uncorrected. All moisture-sensitive reactions were performed using either oven or flame dried glassware under a positive pressure of argon unless otherwise noted. Solvents and reagents that are commercially available were used without further purification unless otherwise noted. Tetrahydrofuran and diethyl ether were freshly distilled from sodium benzophenone ketyl under argon. Methylene chloride was distilled freshly from calcium hydride under argon. Starting from L-serine the aminobutenolide **1** was prepared following an earlier reported procedure from our laboratory.[26] The silica gel (230-

400 mesh) used for column chromatography was purchased from Sorbent Technologies, while thin layer chromatography was performed using Silica Gel HLF Uniplates™, purchased from Analtech, Inc. All the compounds were concentrated using standard rotavap and high-vacuum techniques.

5.2. Biological Procedures

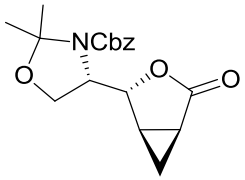
Cytotoxicity assays were performed in the laboratory of our collaborator Dr. Sunil David, in the Medicinal Chemistry Department at the University of Kansas. Jaspine B analogs were evaluated in murine B16 melanoma cells by using a homogeneous resazurin (Alamar Blue) assay,[264] which has been shown to be more sensitive.[265] Resazurin detects cell viability by converting from a nonfluorescent dye to the highly red fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. Continued cell growth maintains a reduced environment while inhibition of growth maintains an oxidized environment. Reduction related to growth causes the REDOX indicator to change from the oxidized (nonfluorescent, purple color) form to the reduced (fluorescent, red color) form.[264,266,267]

Stock solutions (1mM) of compounds are first serially diluted in cell-culture medium (RPMI-1640 supplemented with 10 mM L-glutamine, 10% fetal bovine serum, and antibiotics) across a 384-well plate, resulting in 40 μL in each well. 40 μL aliquots of cells suspended in cell-culture medium at a density of 10^6 cells/well are added to the plate and incubated for 48 h in a humidified atmosphere (37 °C, 5% CO_2). At the end of the incubation period, 40 μL of the supernatant is removed and an equal volume of 2X resazurin (200 μg/mL) was added and incubated for an additional 4 h. Conversion of resazurin to resorufin is quantified by ratiometric absorptimetry at 570/610 nm and plotted as a function of the concentration of compounds, from

which IC_{50} values are derived by conventional four-parameter logistic curve-fitting using GraphPad Prism.

5.3. Experimental Procedures

5.3.1. Chapter 2: HIV Protease Targeted Synthesis of Conformationally-Rigid Hydroxy-ethylene Dipeptide Isosteres: A Combinatorial approach

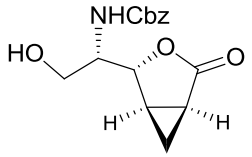


2.1

(*S*)-Benzyl 2,2-dimethyl-4-((1*R*,2*R*,5*S*)-4-oxo-3-oxabicyclo[3.1.0]hexan-2-yl)oxazolidine-3-carboxylate (2.1). To a stirred solution of the aminobutenolide **1** (1.5 g, 4.8 mmol) in anhydrous diethyl ether (70 mL) was added $Pd(OAc)_2$ (0.106g, 10 mol %) and cooled to 0 ^oC, followed by the addition of freshly prepared diazomethane solution in ether [prepared from 5 g of commercially available *N*-methyl-*N'*-nitrosoguanidine (NNMG) as described below]. The resulting solution was allowed to attain room temperature, and stirring continued for 24 h. A second lot of diazomethane (prepared from 5 g of MNNG) was added to the reaction mixture and stirred for an additional 24 h. Excess diazomethane was removed by bubbling nitrogen into the reaction mixture for 15 min. The reaction mixture was filtered over celite, and the residue washed with ether (4 x 10 mL). The combined filtrate was concentrated under vacuum, and the crude product purified by flash column chromatography (hexanes : EtOAc = 1 : 3) to afford the cyclopropane ring-fused bicyclic lactone **2.1** as a white solid (1.19g, 75 %): $[\alpha]_D$ - 3.8 (*c* 0.6, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.41-7.29 (m, 5H), 5.31-4.91 (m, 2H), 4.41-4.21 (m, 1H), 4.18-3.91 (m, 3H) 2.21-2.01 (m, 1H) 1.81-1.41 (m, 7H) 1.31-1.01 (m, 1H), 0.91-0.68 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 175.6, 175.4, 155.1, 152.4, 135.7, 128.7, 128.6, 128.5, 128.4, 128.2, 95.1, 94.7, 82.2, 79.7, 67.7, 67.2, 65.6,

64.7, 60.5, 59.9, 29.7, 27.5, 26.8, 24.4, 22.8, 20.3, 19.8, 17.4, 12.4, 12.3; **HRMS** (ESI-TOF) *m/z*: [M+H] + Calcd for $C_{18}H_{22}NO_5$ 332.1498; Found 332.1484.

[CAUTION: Diazomethane (CH_2N_2) is an explosive and a highly toxic gas. Explosions may occur if the substance is dried and undiluted. All operations involving diazomethane should be carried out in an efficient fume hood following appropriate precaution]. To a biphasic solution of KOH (20 g) in H_2O (50 mL) and ether (120 mL) at 0 °C was added *N*-methyl-*N'*-nitrosoguanidine (MNNG, 5 g) in one lot. The organic layer turned bright yellow. The ethereal layer was separated into an ice-cooled Erlenmeyer flask containing KOH pellets. The aqueous layer was carefully washed with ether (3 x 10 mL), and the ethereal layers were combined for use in the cyclopropanation reaction as described above].



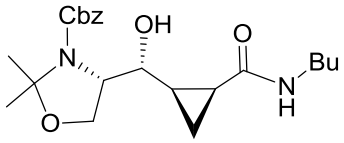
2.2

Benzyl ((*S*)-2-hydroxy-1-((1*R*,2*R*,5*S*)-4-oxo-3-oxabicyclo[3.1.0]hexan-2-yl)ethyl)-carbamate (2.2). The cyclopropane ring-fused bicyclic lactone **2.1** (0.03 g, 0.091 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. TMSOTf (0.067 mL, 0.039 mmol) was added to the solution and continued stirring at 0 °C for 6 h. The reaction was quenched with saturated aq. $NaHCO_3$ solution, and extracted with EtOAc (3 x 10 mL). The combined extracts washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (hexanes:EtOAc = 1:4) to afford the aminoalcohol **2.2** as a viscous liquid (0.025 g, 81 %): $[\alpha]_D$ - 0.32 (c 0.655, CH_3OH); **^{1}H NMR** (500 MHz, DMSO-d6): δ 7.42-7.36 (m, 5H), 5.18 (brs, 2H), 4.91-4.81 (m, 1H), 4.52-4.48 (m, 1H), 3.81-3.61 (m, 1H), 3.59-3.41 (m, 2H), 2.18-2.11 (m, 1H), 2.01-1.97 (m, 1H), 1.28-1.20 (m, 1H), 0.85-0.75 (m, 1H); ^{13}C NMR

(125.8 MHz, $CDCl_3$) 175.7, 156.2, 135.5, 126.8, 126.4, 126.2, 78.7, 65.1, 58.8, 54.5, 18, 15.8, 10.0; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{15}H_{18}NO_5$ 293.1185; Found 293.1203.

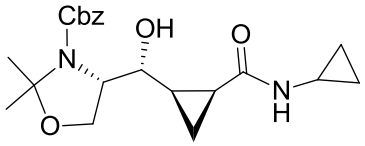
General procedure for the synthesis of cyclopropyl carboxamide analogs 3.1a-h.

A solution of the bicyclic lactone **2.1** (1 equiv.) in *i*-propanol (10% solution w/v), and the respective amine (**a-h**: 4 equiv.) was refluxed for 5-18 h (tlc monitoring). The reaction mixture was concentrated under vacuum and the residue purified by flash chromatography (hexanes : EtOAc = 9/1 to 1/2) affording the cyclopropane carboxamides **3.1a-h.**



3.1a

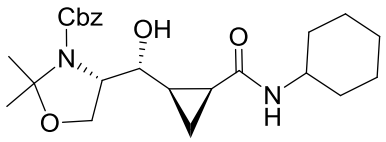
(*S*)-Benzyl 4-((*R*)-((1*R*,2*S*)-2-(butylcarbamoyl)cyclopropyl)(hydroxy)methyl)-2,2-dimethyl-oxazolidine-3-carboxylate (3.1a). Obtained as white solid (54 mg, 87 %): ^{1}H NMR (400 MHz, $CDCl_3$; mixture of rotamers): δ 7.41-7.28 (brs, 5H), 6.21-6.05 (m, 0.5H), 5.71-5.51 (m, 0.5H), 5.21-5.01 (m, 2H), 4.61-4.42 (m, 0.5H), 4.41-3.55 (m, 4.5 H), 3.31-3.08 (m, 2H), 2.15-2.04 (m, 0.5H), 1.65-1.11 (m, 13H), 0.99-0.81 (m, 3H), 0.75-0.66 (m, 0.5H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 172.9, 172.6, 154.1, 152.7, 136.2, 136.1, 128.6, 128.5, 128.2, 128.1, 128, 127.9, 94.4, 94.1, 70.9, 70.7, 67.2, 66.8, 65.2, 62.2, 61.2, 39.6, 31.4, 29.6, 27.3, 26.7, 24.5, 23.5, 23.3, 23, 19.9, 19.8, 13.6, 10.7; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{33}N_2O_5$ 405.2389; Found 405.2378.



3.1b

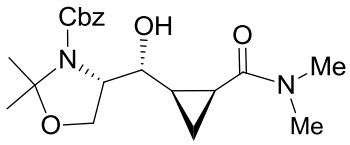
(*S*)-Benzyl 4-((*R*)-((1*R*,2*S*)-2 (cyclopropylcarbamoyl)cyclopropyl)(hydroxy) methyl)-2,2-di-methyloxazolidine-3-carboxylate (3.1b). Obtained as a white solid (0.9 g, 82%): **^{1}H NMR** (500

MHz, $CDCl_3$; mixture of rotamers) δ 7.41-7.29 (m, 5H), 6.09 and 5.52 (2s, 0.5H and 0.5H), 5.18-4.91 (m, 2H), 4.44-4.36 (brs, 0.5H), 4.21-3.80 (m, 3.5H), 3.73-3.64 (brs, 1H), 2.69 & 2.52 (2s, 1H), 1.71-1.41 (m, 6H), 1.40-1.00 (m, 3H), 0.99-0.81 (m, 1H), 0.71-0.61 (m, 2H), 0.51-0.25 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 174.8, 174.4, 154.2, 152.8, 136.3, 136.2, 128.7, 128.6, 128.4, 128.2, 128, 94.5, 94.2, 70.9, 70.6, 67.6, 67.3, 66.9, 66.6, 65.3, 64.3, 62.3, 61.2, 30.9, 30.6, 29.7, 27.4, 27.2, 26.8, 24.5, 24.3, 23.9, 23.8, 23.5, 23, 22.8, 22.6, 19.8, 19.6, 19.1, 6.7, 6.5; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{29}N_2O_5$ 389.2076; Found 389.2065.



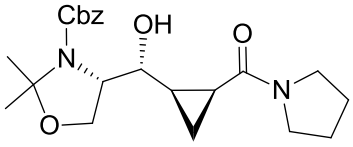
3.1c

(*S*)-Benzyl 4-((*R*)-((1*R*,2*S*)-2-(cyclohexylcarbamoyl)cyclopropyl)(hydroxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate (3.1c). Obtained as a white solid (0.12 g, 99 %): **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers): δ 7.34 (brs, 5H), 5.71 (brs, 0.5H) and 5.41 (brs, 0.5H), 5.21-4.95 (m, 2H), 4.45 (brs, 0.5H), 4.29-3.88 (m, 3.5H), 3.79-3.62 (m, 2H), 1.95-1.76 (m, 2H), 1.75-1.01 (m, 17.5H), 0.79-0.61 (m, 0.5H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 172.1, 171.7, 154.2, 152.8, 136.4, 136.3, 128.7, 128.6, 128.4, 128.2, 94.6, 94.2, 71.2, 67.3, 66.8, 65.3, 64.4, 62.3, 61.2, 48.7, 33.1, 32.9, 29.7, 25.5, 24.8, 23.5, 23.3, 23, 20.2, 20, 10.8; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{35}N_2O_5$ 431.2546; Found 431.2526.



3.1d

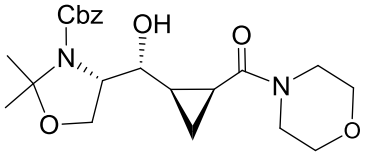
(*S*)-Benzyl 4-((*R*)-((1*R*,2*S*)-2-(dimethylcarbamoyl)cyclopropyl)(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (3.1d). Obtained as a white solid (0.118 g, 80 %): **^{1}H NMR** (400 MHz, $CDCl_3$; mixture of rotamers): δ 7.41-7.29 (m, 5H), 5.21-5.01 (m, 2H), 4.88 (brs, 0.5H), 4.55 (brs, 0.5H) 4.29-4.01 (m, 2H), 3.96-3.86 (m, 1H), 3.51-3.31 (m, 1H), 3.21 and 3.01 (2s, 3H), 2.99 and 2.85 (2s, 3H), 1.71-1.41 (m, 7.5H), 1.31-1.18 (m, 0.5H), 0.99-0.81 (m, 1H), 0.71-0.51 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 172.1, 171.7, 154.1, 152.7, 136.3, 128.6, 128.2, 128, 94.5, 94.2, 72.9, 72.6, 67.2, 66.8, 65.4, 65.2, 62.2, 60.9, 37.6, 37.5, 35.7, 35.5, 27.3, 26.7, 24.6, 23.1, 23, 17.6, 11, 10.9; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{29}N_2O_5$ 377.2076; Found 377.2066.



3.1e

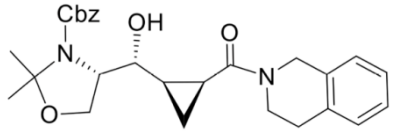
(*S*)-Benzyl 4-((*R*)-hydroxy((1*R*,2*S*)-2-(pyrrolidine-1-carbonyl)cyclopropyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (3.1e) Obtained as a white solid (320 mg, 88%): **^{1}H NMR** (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.41-7.29 (m, 5H), 5.21-5.09 (m, 2H), 5.04-4.89 (m, 0.5H), 4.41-4.19 (m, 0.5H), 4.28-4.05 (m, 2H), 3.95-3.85 (m, 1H), 3.61-3.59 (m, 1H), 3.58-3.31 (m, 4H), 1.98-1.75 (m, 4H), 1.71-1.41 (m, 7.5H), 1.41-1.21 (m, 0.5H), 1.11-0.89 (m, 1H), 0.81-0.65 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 171.2, 170.5, 154.1, 152.7, 136.3, 136.2, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 94.4, 94.1, 72.3, 71.8, 67.2, 66.8, 66.7, 65.8, 65.4, 65.3, 64.9, 64.1, 62.2, 61, 46.8, 46, 45.9, 45.7, 27.9, 27.4, 26.7, 26, 25, 24.6, 23.4,

23.2, 23, 22.6, 18.2, 11, 10.8; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{31}N_2O_5$ 403.2233; Found 403.2237.



3.1f

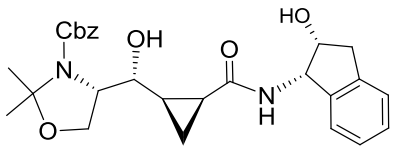
(*S*)-Benzyl4-((*R*)-hydroxy((1R,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl)methyl)-2,2-dimethyl oxazolidine-3-carboxylate (3.1f). Obtained as a white solid (73 mg, 52 %): **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers) δ 7.41-7.29 (brs, 5H), 5.21-5.01 (m, 2H) 4.65-4.58 (m, 0.5H), 4.29-4.01 (m, 2.5H), 3.97-3.88 (m, 1H), 3.71-3.31 (m, 10H), 1.71-1.41 (m, 6H) 1.38-1.21 (m, 1H), 1.01-0.89 (m, 1H), 0.71-0.61 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 170.5, 170.1, 154.2, 152.6, 136.1, 128.5, 128.2, 128, 94.5, 94.3, 72.9, 72.6, 67.3, 66.8, 66.7, 66.6, 65.1, 64.3, 62.2, 60.8, 46.2, 42.3, 42.2, 30.6, 29.7, 27.1, 26.7, 24.6, 23, 22.8, 21, 19.1, 17.2, 17, 16.8, 14.1, 10.7, 10.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{31}N_2O_6$ 419.2182; Found 419.2174.



3.1g

(*S*)-Benzyl 4-((*R*)-hydroxy((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquinoline-2 carbonyl) cyclopropyl)methyl)-2,2-dimethyl oxazolidine-3-carboxylate (3.1g). Obtained as a light brown solid (0.35 g, 72 %): ^{1}H NMR (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.48-7.05 (m, 9H), 5.28-5.05 (m, 2H), 4.91-4.61 (m, 2H), 4.02-3.71 (m, 6H), 3.59-3.31 (m, 1H), 3.01-2.75 (m, 2H), 1.85-1.77 (m, 0.5H), 1.68-1.54 (m, 7H), 1.48-1.33 (m, 0.5H), 1.05-0.95 (m, 1H), 0.71-0.61 (m, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 169.2, 169, 152, 136.6, 136.3, 135.1,

134.9, 133.6, 133.4, 128.4, 128.3, 128.1, 127.9, 126.4, 126.2, 126.1, 93.3, 93.1, 69, 68.9, 66.2, 66, 64.9, 64.4, 63.9, 61.9, 61.1, 46.4, 44.1, 42.8, 28.7, 28.2, 26.9, 26.1, 24.6, 24.2, 24.1, 23.9, 22.9, 16.5, 16.3; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{33}N_2O_5$ 465.2390; Found 465.2231.



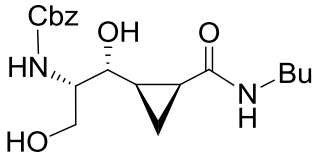
3.1h

(*S*)-Benzyl 4-((*R*)-hydroxy((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl) carbamoyl)cyclopropyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (3.1h). Obtained as a brown solid (200 mg, 69 %): **^{1}H NMR** (400 MHz, CD_3OD; mixture of rotamers) δ 7.48-7.15 (m, 9H), 5.31-5.19 (m, 1H), 5.17-5.01 (m, 2H), 4.61-4.51 (m, 1H), 4.18-3.91 (m, 3H), 3.82-3.61 (m, 1H), 3.51-3.41 (m, 1H), 3.18-3.01 (m, 1H), 2.94-2.85 (m, 1H), 1.91-1.71 (m, 1H), 1.68-1.38 (m, 6H), 1.31-1.21 (m, 1H), 1.09-1.01 (m, 1H), 0.81-0.61 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 174.6, 174.1, 155.5, 154.5, 141.9, 137.7, 95.6, 74.1, 71.5, 71.1, 68.4, 68.1, 66.9, 66.2, 63.4, 62.7, 59.5, 59.3, 40.1, 27.7, 26.9, 25.8, 25.5, 24.9, 23.4, 19.3, 19.1, 11.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{33}N_2O_6$ 481.2339 ; Found 481.2342.

General procedure for acetonide deprotected *N*-Cbz-aminodiol derivatives (4.1a-h):

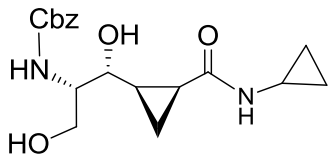
To a stirred, ice-cooled solution of cyclopropyl carboxamides **3.1a-3.1h** (1 equiv.) in anhydrous CH_2Cl_2 (10% solution w/v) under an inert atmosphere was added TMSOTf (2.2 equiv.) through a syringe. The resulting solution was stirred at the same temperature for 3-4 h (monitored by TLC), followed by quenching the reaction mixture by addition of a saturated aqueous $NaHCO_3$ solution. After stirring the mixture for 5 min, the organic layer was separated and the aqueous layer was saturated with solid NaCl and extracted with CH_2Cl_2 (three times).

The combined organic layers were dried over anhydrous Na_2SO_4. Concentration of the solvent under reduced pressure and column chromatographic purification of the residue (10 % MeOH in EtOAc) provided the pure acetonide-cleaved cyclopropyl aminodiol derivatives **4.1a-h.**



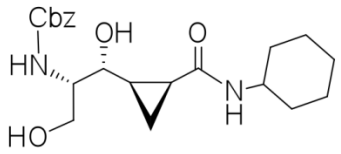
4.1a

Benzyl ((1*R*,2*S*)-1-((1*R*,2*S*)-2-(butylcarbamoyl)cyclopropyl)-1,3-dihydroxypropan-2-yl)-carbamate (4.1a). Obtained as a white solid (100 mg, 96%): **^{1}H NMR** (500 MHz, CD_3OD) δ 7.48-7.30 (m, 5H), 5.21-5.05 (dd, *J* = 12.5 Hz, 2H), 3.81-3.24 (m, 4H), 3.21-3.05 (m, 2H), 1.61-1.55 (m, 1H), 1.55-1.41 (m, 2H), 1.38-1.28 (m, 3H), 1.07-0.99 (m, 1H), 0.95-0.88 (t, *J* = 7.5 Hz, 3H), 0.87-0.89 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD): δ 174.5, 159.1, 138.6, 129.6, 129.2, 129.1, 129, 71.2, 67.6, 62.9, 59.7, 40.6, 32.7, 25.3, 21.3, 19.4, 14.3, 11.3; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{29}N_2O_5$ 365.2076; Found 365.2082.



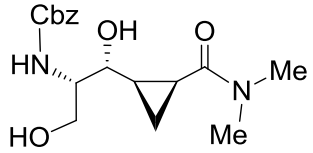
4.1b

Benzyl ((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclopropylcarbamoyl)cyclopropyl)-1,3-dihydroxypropan-2-yl)carbamate (4.1b). Obtained as a colorless liquid (71 mg, 97 %): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.38-7.21 (m, 5H), 5.21-5.01 (dd, *J* = 12.5 Hz, 2H), 3.80-3.61 (m, 4H), 2.67-2.58 (m, 1H), 1.58-1.49 (m, 1H), 1.38-1.21 (m, 1H), 1.11-1.01 (m, 1H), 0.91-0.81 (m, 1H), 0.71-0.61 (m, 2H), 0.56-0.41 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.2, 159.2, 138.6, 129.6, 129.4, 129.2, 129.1,129.0, 71.1, 67.7, 62.9, 59.7, 25.5, 23.7, 19.1, 11.9, 6.7, 6.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{18}H_{25}N_2O_5$ 349.1764; Found 349.1760.



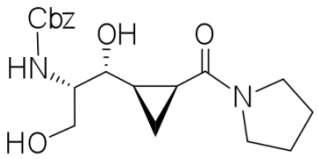
4.1c

Benzyl((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclohexylcarbamoyl)cyclopropyl)-1,3-dihydro-xypropan-2-yl)carbamate (4.1c). Obtained as a white solid (273 mg, 75 %): **^{1}H NMR** (500 MHz, CD_3OD) δ 7.48-7.21 (m, 5H), 5.19-5.01 (dd, *J* = 12.5 Hz, 2H), 3.81-3.51 (m, 5H), 1.91-1.81 (m, 2H), 1.78-1.68 (m, 2H), 1.61-1.51 (m, 2H), 1.41-1.11 (m, 6H), 1.05-0.95 (m, 1H), 0.91-0.79 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 173.6, 159.1, 138.6, 129.6, 129.1, 123.2, 71.2, 63.3, 60.3, 34.1, 26.8, 26.3, 25.2, 19.5, 14.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{31}N_2O_5$ 391.2233; Found 391.2245.



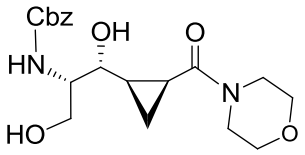
4.1d

Benzyl ((1*R*,2*S*)-1-((1*R*,2*S*)-2-(dimethylcarbamoyl)cyclopropyl)-1,3-ihydroxypropan-2yl) carbamate (4.1d). Obtained as a white solid (424 mg, 91%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.49-7.31(m, 5H), 5.21-5.02 (dd, *J* = 12.5 Hz, 2H), 3.68-3.51 (m, 3H), 3.28-3.19 (m, 1H), 3.18 (s, 3H), 2.91 (s, 3H), 1.91-1.81 (m, 1H), 1.49-1.38 (m, 1H), 1.11-1.01 (m, 1H), 0.91-0.78 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 173.7, 159.1, 138.6, 129.6, 129.3, 129.1, 72.3, 67.6, 62.8, 59.6, 38.1, 36.3, 24.9, 17.8, 11.1; ^{13}C NMR (125.8 MHz, CD_3OD) **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{17}H_{25}N_2O_5$ 337.1764; Found 337.1777.



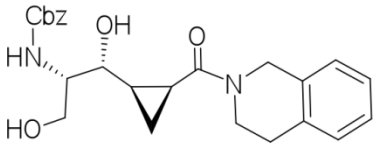
4.1e

Benzyl((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(pyrrolidine-1 carbonyl)cyclopropyl) propan-2-yl)carbamate (4.1e). Obtained as a white solid (298 mg, 81 %): **^{1}H NMR** (500 MHz, CD_3OD) δ 7.49-7.28 (m, 5H), 5.21-5.02 (dd, *J* = 12.5 Hz, 2H), 3.79-3.69 (m, 3H), 3.68-3.51 (m, 2H), 3.45-3.35 (m, 3H), 2.03-1.75 (m, 5H), 1.51-1.41 (m, 1H), 1.18-1.11 (m, 1H), 0.91-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 172.4, 159.1, 138.7, 129.6, 129.4, 129.3, 129.1, 129, 71.7, 68.1, 63.2, 59.6, 48.1, 47.4, 27, 25.6, 25.1, 18.8, 11.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{27}N_2O_5$ 363.1920; Found 363.1920.



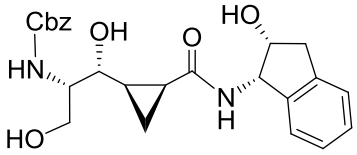
4.1f

Benzyl ((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl) propan-2-yl)carbamate (4.1f). Obtained as a white solid (302 mg, 87%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.49-7.31 (m, 5H), 5.21-5.02 (dd, *J* = 12.5 Hz, 2H), 3.81-3.61 (m, 10H), 3.45-3.39 (m, 1H), 3.21-3.11 (m, 1H), 1.91-1.85 (m, 1H), 1.49-1.35 (m, 1H), 1.19-1.11 (m, 1H), 0.91-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 172.2, 159.1, 138.6, 129.6, 129.3, 129.1, 72.3, 62.8, 60.1, 67.8, 67.7, 67.6, 62.8, 59.7, 47.7, 47.6, 43.9, 25.1, 17.7, 11; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{27}N_2O_6$ 379.1869; Found 379.1876.



4.1g

Benzyl((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl) cyclopropyl)propan-2-yl)carbamate (4.1g). Obtained as a light brown solid (125 mg, 89 %): ^{1}H NMR (400 MHz, MeOD) δ 7.45-7.21 (m, 5H), 7.19-7.05 (m, 4H), 5.51 (brs, 1H), 5.18-5.01 (m, 2H), 4.81-4.71 (m, 0.5H), 4.61-4.48 (m, 0.5H), 3.99-3.81 (m, 1H), 3.80-3.61 (m, 4H), 2.95-2.75 (m, 2H), 2.11-1.91 (m, 1H), 1.51-1.41 (m, 1H), 1.21-1.09 (m, 2H), 0.95-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 172.9, 158.5, 138.2, 136.1, 135.8, 134.4, 134, 129.6, 129.5, 129.4, 129, 128, 127.7, 127.4, 127.3, 67.7, 64.3, 63.9, 48.1, 45.4, 44.5, 41.4, 35.7, 30.1, 29.4, 28.6, 28.2, 14.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{29}N_2O_5$ 425.2055; Found 425.2077.



4.1h

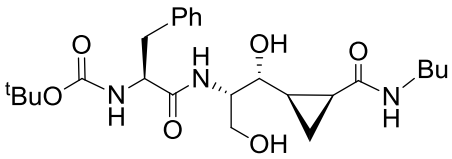
Benzyl ((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamoyl)cyclopropyl)propan-2-yl)carbamate (4.1h). Obtained as a white solid (190 mg, 63 %): **^{1}H NMR** (400 MHz, DMSO-d6) δ 8.15 (brs, 1H), 7.41-7.25 (m, 5H), 7.21-7.15 (m, 4H), 6.91 (brs, 1H), 5.18-5.11 (m, 1H), 5.04-4.95 (m, 2H), 4.82-4.78 (m, 1H), 4.61-4.41 (m, 3H), 3.91-3.71 (m, 3H), 3.20 (brs, 1H), 3.04-2.96 (m, 1H), 2.81-2.78 (m, 1H), 1.91-1.71 (m, 1H), 1.38-1.21 (m, 1H), 1.05-0.91 (m, 1H), 0.81-0.71 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 174.2, 156.6, 140.9, 139.9, 136.3, 128.5, 128.4, 128.2, 128.1, 127, 125.3, 124.3, 73.1, 69.3, 67.1,

62.5, 58.3, 56.3, 38.9, 24.1, 20.1, 10.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{29}N_2O_6$ 441.2026; Found 441.2017.

General procedure for *N*-Cbz hydrogenolysis of amino diol derivatives (4.1a-h) and subsequent peptide coupling towards synthesis of a combinatorial library of dipeptide isosteres (4.2aA-4.2hF).

Step 1: Cyclopropyl carboxamide aminodiol derivative (**4.1a-4.1h**, 1.0 equiv.) was dissolved in methanol (10% solution w/v) and 10% Pd-C (10 mol %) was added under nitrogen blanket. Degassed the reaction mixture twice and flushed with hydrogen. Stirred the mixture under H_2 atmosphere at rt until disappearance of the starting material (monitored by TLC). The reaction mixture was filtered through a celite pad and the residue was washed with methanol (5x5 mL). The combined filtrate was concentrated under vacuum and dried under high vacuum pump for 2 h. The crude amine thus obtained was used directly for the next reaction.

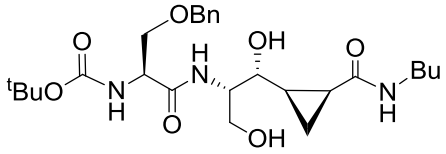
The amine as obtained above was dissolved in anhydrous DMF (10% solution w/v) and the respective *N*-Boc- aminoacid (**A-F**, 1.2 equiv) was added to it. To the resulting solution was added EDCI (1.5 equiv.) and the mixture stirred at rt overnight. The volatiles were removed under high vacuum and the crude product purified by column chromatography (CH_2Cl_2 : MeOH + 1% NH_4OH = 9 : 1) to obtain *cis*-1,2-disubstituted cyclopropane dipeptide isosteres **4.2aA-4.2hF** respectively. In a combinatorial approach, 6 types of Boc-protected aminoacids were utilized for peptide coupling (**A-F**).



4.2aA

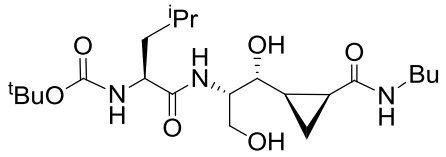
***tert*-Butyl((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(butylcarbamoyl)cyclopropyl)-1,3-dihydroxypropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2aA)**. Obtained as a white powder (68

mg, 51 %): **^{1}H NMR** (500 MHz, CD_3OD) δ 7.38-7.11 (m, 5H), 4.41-4.28 (m, 1H), 4.01-3.91 (m, 1H), 3.81-3.59 (m, 3H), 3.31-3.05 (m, 3H), 2.91-2.71 (t, 1H), 1.61-1.42 (m, 3H**),** 1.41-1.15 (m, 12H), 1.11-0.99 (m, 1H), 0.95-0.78 (m, 4H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 174.6, 157.8, 138.9, 130.7, 130.6, 129.5, 127.8, 80.8, 80.7, 70.6, 62.6, 57.9, 40.7, 39.5, 32.7, 28.8, 28.5, 25.3, 21.2, 19.6, 14.3, 11.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{40}N_3O_6$ 478.2917; Found 478.2900.



4.2aB

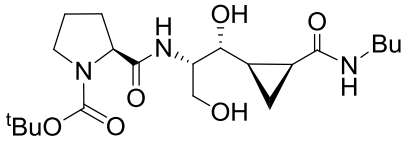
***tert*-Butyl ((*S*)-3-(benzyloxy)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-butylcarbamoyl)cyclopropyl)-1,3-di -hydroxypropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2aB)**. Obtained as a white solid (139 mg, 74 %): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41-7.21 (m, 5H), 6.95-6.85 (m, 1H), 6.05-5.91 (m, 1H), 5.48-5.38 (m, 1H), 4.99-4.85 (m, 1H), 4.61-4.41 (m, 2H), 4.31-4.21 (m, 1H), 4.05-3.71 (m, 4H), 3.70-3.51 (m, 2H), 3.31-3.15 (m, 2H), 3.01-2.81 (m, 1H)**,** 1.51-1.49 (m, 3H), 1.51-1.41 (m, 9H), 1.41-1.28 (m, 3H), 1.27-1.09 (m, 1H), 1.01-0.91 (m, 1H), 0.89-0.81 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 173.5, 170.2, 155.6, 137.3, 128.5, 127.9, 127.8, 80.3, 73.5, 70.9, 70.5, 69.8, 62.4, 55.1, 54.8, 39.8, 34.6, 31.4, 28.23, 23.5, 20.1, 13.7, 10.7; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{42}N_3O_7$ 508.3023; Found 508.3015.



4.2aC

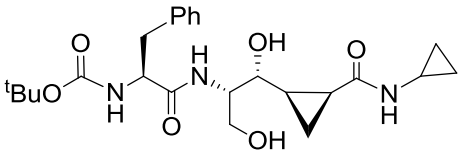
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(butylcarbamoyl)cyclopropyl)-1,3-dihydroxypropan -2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2aC)**. Obtained as a white solid (117

mg, 71 %): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.92 (brs, 1H), 6.01-6.11 (m, 1H), 4.68-5.15 (m, 2H), 3.95-4.15 (m, 3H), 3.61-3.81 (m, 2H), 3.41-3.52 (brs, 1H), 3.05-3.41 (m, 2H), 1.71-1.91 (m, 4H), 1.58-1.78 (m, 4H), 1.41-1.52 (m, 9H), 1.21-1.41 (m, 1H), 1.12-1.20 (m, 1H), 0.97-1.08 (m, 1H), 0.81-0.97 (m, 9H); **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 173.4, 172.8, 155.9, 80, 69.5, 62.3, 55.1, 54.8, 41.5, 41.2, 31.4, 28.3, 25.2, 21.6, 19.6, 13.7, 11.0; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{42}N_3O_6$ 444.3074; Found 444.3077.



4.2aD

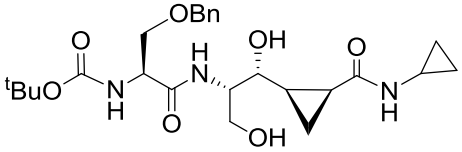
(*S*)-*tert*-Butyl 2-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(butylcarbamoyl)cyclopropyl)-1,3-dihydroxy propan -2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2aD). Obtained as a white solid (32 mg, 66 %): **^{1}H NMR** (500 MHz, CD_3OD; mixture of rotamers) δ 4.28-4.11 (m, 1H), 4.01-3.89 (m, 1H), 3.78-3.61 (m, 3H), 3.52-3.31 (m, 2H), 3.21-3.01 (m, 2H), 2.31-2.09 (m, 1H), 2.04-1.78 (m, 3H), 1.69-1.51 (m, 1H), 1.51-1.25 (m, 14H), 1.11-1.01 (m, 1H), 0.99-0.81 (m, 4H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers): δ 175.5, 174.5, 156.6, 81.8, 70.6, 62.7, 62.6, 58.0, 40.7, 35.9, 32.9, 31.6, 28.9, 21.2, 14.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{38}N_3O_6$ 428.2761; Found 428.2748.



4.2bA

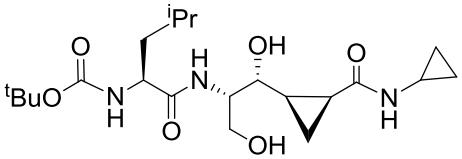
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclopropylcarbamoyl)cyclopropyl)-1,3-dihydroxy propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2bA)**. Obtained as a white solid (79 mg, 92%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.39-7.09 (m, 5H), 4.39-4.25 (m, 1H), 3.95-

3.85 (m, 1H), 3.79-3.65 (m, 2H), 3.19-3.02 (m, 1H), 2.91-2.71 (m, 1H), 2.64-2.55 (m, 1H), 2.42-2.30 (m, 1H), 1.85-1.61 (m, 1H), 1.51-1.49 (m, 9H), 1.25-1.11 (m, 3H), 0.72-0.61 (m, 2H), 0.50-0.40 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.4, 174.5, 157.8, 138.9, 130.5, 129.6, 127.9, 80.9. 70.6, 70.3, 62.6, 62.5, 58.2, 58.0, 57.8, 45.5, 45.2, 44.4, 39.6, 39.4, 39.3, 38.8, 36.7, 36.0, 29.2, 28.8, 28.6, 25.6, 23.6, 19.3, 16, 15, 11.7,6.7, 6.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{36}N_3O_6$ 462.2604; Found 462.2601.



4.2bB

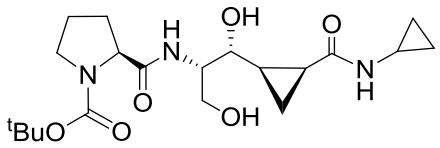
***tert*-Butyl((*S*)-3-(benzyloxy)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclopropylcarbamoyl) cyclopropyl)-1,3-dihydroxypropan-2-yl)amino)-1-oxopropan-2-yl)carbamate(4.2bB)**. Obtained as a white solid (82 mg, 60%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.41-7.29 (m, 5H), 4.51 (s, 2H), 4.32-4.24 (m, 1H), 4.01-3.89 (m, 1H), 3.81-3.61 (m, 5H), 2.64-2.57 (m, 1H), 1.51-1.47 (m, 1H), 1.45 (s, 9H), 1.31-1.21 (m, 1H), 1.09-1.01 (m, 1H), 0.91-0.85 (m, 1H), 0.71-0.61 (m, 2H), 0.52-0.39 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.4, 172.9, 157.9, 139.4, 130.1, 129.5, 129.4, 129.2, 129.1, 129, 128.9, 126.4, 81.1, 74.4, 71.3, 70.4, 62.6, 57.9, 56.4, 28.8, 25.6, 23.6, 19.4, 19.3, 11.7, 8.7, 8.0; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{38}N_3O_7$ 492.2710; Found 492.2693.



4.2bC

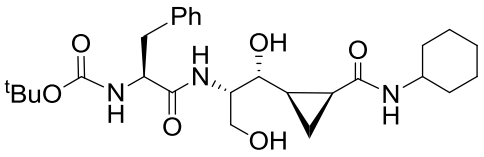
***tert*-butyl((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclopropylcarbamoyl)cyclopropyl)-1,3-dihydroxy propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2bC)**. Obtained as a white solid

(32 mg, 66%): **^{1}H NMR** (500 MHz, CD_3OD) δ 4.11-4.01 (m, 1H), 3.95-3.85 (m, 1H), 3.75-3.61 (m, 3H), 2.68-2.58 (m, 1H), 1.74-1.61 (m, 1H), 1.58-1.49 (m, 3H**),** 1.48-1.38 (m, 9H), 1.41-1.28 (m, 1H), 1.11-1.03 (m, 1H), 0.99-0.91 (m, 7H), 0.71-0.62 (m, 2H), 0.54-0.43 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.3, 158.1, 80.8, 70.6, 62.6, 57.8, 55.1, 42.4, 28.9, 26.1, 25.7, 23.7, 22, 19.2, 12.0, 6.8, 6.5; **HRMS** (ESI-TOF) *m/z*: [M+H] + Calcd for $C_{21}H_{38}N_3O_6$ 428.2761; Found 428.2742.



4.2bD

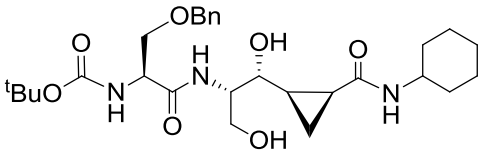
(*S*)-*tert*-Butyl2-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclopropylcarbamoyl)cyclopropyl)-1,3-dihydroxy-propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2bD). Obtained as a white solid (26 mg, 23%): **^{1}H NMR** (500 MHz, CD_3OD; mixture of rotamers) δ 4.25-4.18 (m, 1H), 4.01-3.91 (m, 1H), 3.85-3.65 (m, 3H), 3.55-3.38 (m, 2H), 2.69-2.59 (m, 1H), 2.29-2.15 (m, 1H), 2.01-1.81 (m, 3H), 1.61-1.55 (m, 1H), 1.49-1.41 (m, 9H), 1.39-1.28 (m, 1H), 1.17-1.09 (m, 1H), 1.03-0.95 (m, 1H), 0.73-0.65 (m, 2H), 0.58-0.45 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers) δ 176.4, 176.2, 175.6, 175.4, 156.6, 156.7, 156.3, 81.7, 81.5, 70.7, 70.3, 62.8, 62.7, 61.9, 57.9, 57.4, 48.1, 32.9, 32.8, 31.7, 30.6, 28.9, 25.7, 25.6, 24.7, 23.6, 19.4, 19.3, 11.8, 11.7, 6.7, 6.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{34}N_3O_6$ 412.2448; Found 412.2441.



4.2cA

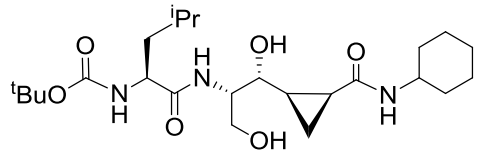
***tert*-Butyl((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclohexylcarbamoyl)cyclopropyl)-1,3-dihydroxy-propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2cA)**. Obtained as a white solid

(75 mg, 53%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.35-7.29 (m, 5H), 4.37-4.21 (m, 1H), 3.97-3.85 (m, 1H), 3.81-3.51 (m, 4H), 3.19-3.05 (m,1H), 2.89-2.71 (m, 1H), 1.99-1.79 (m, 2H), 1.77-1.65 (m, 2H), 1.63-1.49 (m,2H), 1.35 (s, 9H), 1.33-1.11 (m, 6H), 1.05-0.95 (m, 1H), 0.94-0.79 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 174.5, 174.4, 173.8, 173.7, 157.8, 138.9, 130.6, 129.6, 127.8, 80.9, 80.8, 70.7, 70.5, 62.6, 62.5, 58.0, 57.9, 50.1, 39.6, 39.4, 34.1, 33.9, 28.8, 26.9, 26.3, 25.3, 19.7, 11.5 **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{42}N_3O_6$ 504.3074; Found 504.3078.



4.2cB

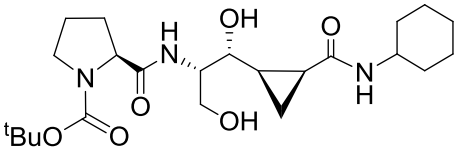
***tert*-Butyl(*S*)-1-(benzyloxy)-2-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclohexylcarbamoyl) cyclopropyl)-1,3-dihydroxypropan-2-yl)amino)-2-oxoethyl)carbamate (4.2cB)**. Obtained as a white solid (66 mg, 54%): **^{1}H NMR** (500 MHz, CD_3OD) δ 7.41-7.25 (m, 5H), 4.53-4.45 (m, 2H), 4.33-4.25 (m, 1H), 3.95-3.85 (m, 1H), 3.78-3.55 (m, 6H), 1.91-1.81 (m, 2H), 1.79-1.68 (m, 2H), 1.67-1.51 (m, 2H), 1.49-1.41 (m, 9H), 1.38-1.11 (m, 6H), 1.11-1.01 (m, 1H), 0.95-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 173.8, 172.9, 157.9, 139.4, 129.5, 129, 128.9, 81.1, 74.3, 71.3, 70.6, 62.6, 57.9, 56.5, 50, 34.1, 33.9, 28.8, 26.8, 26.3, 25.3, 19.7, 11.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{28}H_{44}N_3O_7$ 534.3179; Found 534.3167.



4.2cC

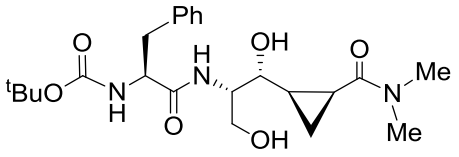
***tert*-Butyl((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclohexylcarbamoyl)cyclopropyl)-1,3-dihydroxy-propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2cC)**. Obtained as a white solid

(81 mg, 74%): **^{1}H NMR** (500 MHz, CD_3OD) δ 4.15-4.05 (m, 1H), 3.95-3.85 (m, 1H), 3.81-3.51 (m, 4H) , 1.95-1.81 (m, 2H), 1.79-1.71 (m, 3H), 1.67-1.58 (m, 2H), 1.56-1.48 (m, 2H), 1.49-1.41 (m, 9H), 1.38-1.29 (m, 4H), 1.25-1.15 (m, 3H), 1.11-1.03 (m, 1H), 0.99-0.89 (m, 7H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.1, 173.7, 158.11, 80.8, 70.6, 62.7, 57.9, 55.1, 50.2, 42.4, 34.1, 33.9, 28.9, 26.9, 26.3, 26.1, 25.5, 25.4, 23.7, 22.1, 19.6, 11.7; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{44}N_3O_6$: 470.3230; Found 470.3211.



4.2cD

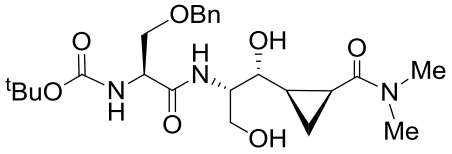
(*S*)-*tert*-Butyl 2-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(cyclohexylcarbamoyl)cyclopropyl)-1,3-dihydroxy-propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2cD). Obtained as a white solid (74 mg, 70%): **^{1}H NMR** (400 MHz, CD_3OD; mixture of rotamers) δ 4.29-4.21 (m, 1H), 4.03-3.95 (m, 1H), 3.81-3.61 (m, 4H) 3.58-3.41 (m, 2H), 2.31-2.18 (m, 1H), 2.15-1.85 (m, 4H), 1.81-1.61 (m, 3H), 1.51-1.41 (m, 9H), 1.40-1.29 (m, 3H), 1.27-1.16 (m, 3H), 1.15-1.09 (m, 1H), 1.03-0.97 (m, 1H), 0.95-0.88 (m, 2H), **^{13}C NMR** (125.8 MHz, CD_3OD) δ 175.5, 175.4, 173.8, 173.6, 156.6, 156.3, 81.7, 81.5, 70.9, 70.4, 62.7, 61.9, 58, 57.4, 50.2, 48.1, 34.1, 33.9, 32.8, 28.8, 26.8, 26.3, 23.8, 21.7, 19.8, 19.6, 11.9, 11.6, 11.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{40}N_3O_6$ 454.2917; Found 454.2904.



4.2dA

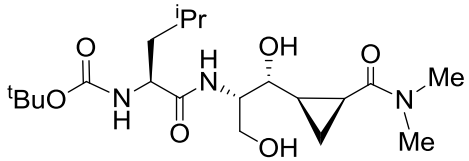
***tert*-Butyl ((S)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(dimethylcarbamoyl)cyclopropyl)-1,3-dihydroxypropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2dA)**. Obtained as a white solid

(69 mg, 49%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.41-7.11 (m, 5H), 4.41-4.21 (m, 1H), 4.09-3.78 (m, 1H), 3.81-3.61 (m, 2H), 3.41-3.31 (m, 1H), 3.29-3.09 (m, 4H), 2.92 (brs, 3H), 2.71-2.68 (m, 1H), 1.95-1.81 (m, 1H), 1.31 (brs, 10H), 1.11-1.01 (m, 1H), 0.91-0.81(m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 174.6, 173.8, 157.9, 138.9, 130.7, 130.5, 129.6, 127.8, 80.8, 71.8, 62.5, 57.8, 39.5, 38.2, 36.3, 28.7, 25.1, 18, 11.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{36}N_3O_6$ 450.2604; Found 450.2594.



4.2dB

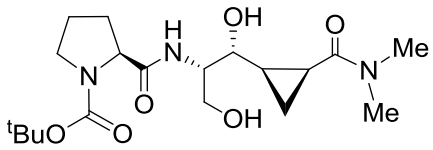
***tert*-Butyl ((S)-3-(benzyloxy)-1-(((1R,2S)-1-((1R,2S)-2-(dimethylcarbamoyl)cyclo propyl)-1,3-dihydroxypropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2dB)**. Obtained as a white solid (87 mg, 58%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.41-7.11 (m, 5H), 4.61-4.49 (m, 2H), 4.38-4.28 (m, 1H), 4.05-3.95 (m, 1H), 3.81-3.61 (m, 4H), 3.38 (brs, 1H), 3.18(s, 3H), 2.92 (s, 3H), 1.81-1.78 (m, 1H), 1.45 (brs, 10H), 1.15-1.01 (m, 1H), 0.96-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 173.8, 172.9, 157.9, 139.4, 129.6, 129.3, 128.9, 81.1, 79.6, 74.3, 71.6, 71.5, 62.5, 57.9, 56.4, 38.2, 36.3, 28.9, 25.1, 17.9, 14.6, 11.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{38}N_3O_7$: 480.2710; Found 480.2718.



4.2dC

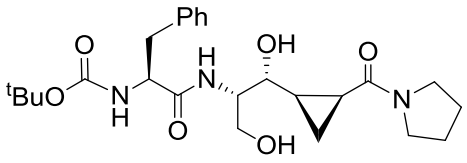
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(dimethylcarbamoyl)cyclopropyl)-1,3-dihydroxy-propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2dC)**. Obtained as a white solid (59 mg, 45%): **^{1}H NMR** (400 MHz, CD_3OD) δ 4.19-4.02 (m, 1H), 4.01-3.91 (m, 1H), 3.75-3.61

(m, 2H), 3.31-3.22 (m, 1H), 3.21 (s, 3H), 2.92 (s, 3H), 2.01-1.89 (m, 1H), 1.79-1.61 (m, 1H), 1.58-1.41 (m, 12H), 1.18-1.03 (m, 1H), 0.99-0.85 (m, 7H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.0, 173.8, 158.1, 80.8, 71.7, 62.6, 57.8, 55.1, 42.9, 38.2, 36.3, 28.8, 26.1, 25.3, 23.7, 22.0, 17.9, 11.71; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{38}N_3O_6$ 416.2761; Found 416.2755.



4.2dD

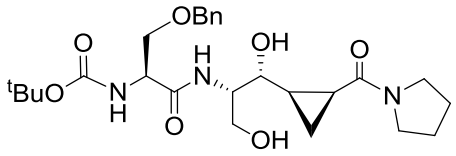
(*S*)-*t*-Butyl 2-(((1*R*,2*S*)-1-((1*R*,2*S*)-2-(dimethylcarbamoyl)cyclopropyl)-1,3-dihydroxyprop-an-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2dD). Obtained as a white solid (54 mg, 43%): **^{1}H NMR** (400 MHz, CD_3OD; mixture of rotamers) δ 7.81 (brs, 1H), 4.21-4.18 (m, 1H), 3.95-3.81 (m, 1H), 3.81-3.61 (m, 2H), 3.51-3.31 (m, 2H), 3.30-3.21 (m, 1H), 3.18 (brs, 3H), 2.91 (s, 3H), 2.31-2.11 (m, 1H), 1.98-1.65 (m, 4H), 1.49-1.31 (brs, 9H), 1.18-1.05 (m, 1H), 1.01-0.91 (m, 1H), 0.89-0.78 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers) δ 175.6, 175.4, 173.8, 156.6, 156.3, 81.7, 72.0, 71.5, 62.7, 62.6, 61.9, 61.8, 57.9, 57.4, 48.0, 38.1, 36.3, 32.8, 31.7, 28.9, 25.6, 25.4, 25.2, 24.8, 23.9, 18.0, 17.8, 14.6, 11.6, **HRMS** (ESI-TOF) *m/z*: [M+H] + Calcd for $C_{19}H_{34}N_3O_6$ 400.2448; Found 400.2434.



4.2eA

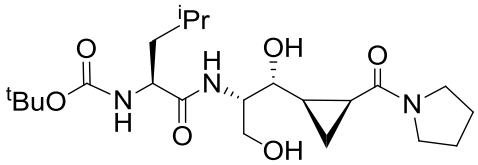
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(pyrrolidine-1-carbonyl) cyclopropyl) propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2eA)**. Obtained as a white solid (63 mg, 53%): **^{1}H NMR** (400 MHz, CD_3OD; mixture of rotamers) δ 7.31-7.09 (m, 5H), 4.38-4.21 (m, 1H), 3.95-3.85 (m, 1H), 3.78-3.61 (m, 2H), 3.51-3.45 (m, 1H), 3.44-3.31 (m, 2H), 3.15-

3.01 (m, 1H), 2.81-2.71 (m, 1H), 2.01-1.71 (m, 5H), 1.51-1.43 (m, 1H), 1.41-1.21 (m, 10H), 1.21-1.01 (m, 1H), 0.98-0.81 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers) δ 174.6, 174.5, 157.8, 138.9, 130.5, 129.6, 129.5, 127.8, 80.9, 71.3, 71.1, 62.6, 62.4, 57.9, 57.5, 57.8, 48.1, 47.4, 39.5, 39.4, 35.8, 32.9, 28.8, 27, 26.4, 25.6, 25.3, 23.9, 18.9, 14.6, 12; **HRMS** (ESI-TOF) *m/z*: [M+H] + Calcd for $C_{25}H_{38}N_3O_6$ 476.2761; Found 476.2761.



4.2eB

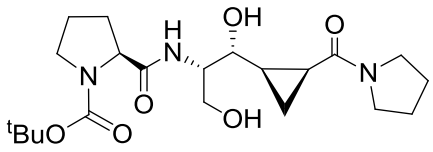
***tert*-Butyl ((*S*)-1-(benzyloxy)-2-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(pyrrolidine-1-carbonyl)cyclopropyl)propan-2-yl)amino)-2-oxoethyl)carbamate (4.2eB)**. Obtained as a white solid (112 mg, 80%): **^{1}H NMR** (500 MHz, CD_3OD, mixture of rotamers) δ 7.43-7.28 (m, 5H), 4.57-4.42 (m, 2H), 4.28-4.23 (m, 1H), 4.01-3.91 (m, 1H), 3.78-3.61 (m, 5H), 3.53-3.31 (m, 4H), 2.01-1.78 (m, 4H), 1.73-1.65 (m, 1H), 1.49-1.35 (m, 10H), 1.15-1.03 (m, 1H), 0.93-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD, mixture of rotamers) δ 172.9, 172.6, 157.9, 139.4, 129.6, 129.5, 129.1, 129, 128, 81.1, 74.3, 71.3, 70.1, 62.6, 57.8, 57.7, 56.4, 48.2, 47.4, 28.8, 27.0, 25.5, 25.3, 18.8, 11.5; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{40}N_3O_7$ 506.2866; Found 506.2851.



4.2eC

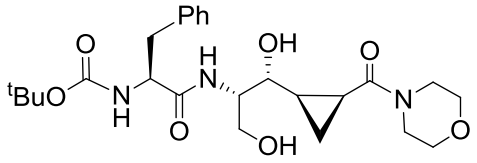
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(pyrrolidine-1-carbonyl)cyclopropyl)propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2eC)**. Obtained as a white solid (94 mg, 78%): **^{1}H NMR** (400 MHz, CD_3OD, mixture of rotamers) δ 4.15-4.01 (m, 1H), 3.98-3.85 (m, 1H), 3.81-3.61 (m, 3H), 3.59-3.48 (m, 1H), 3.47-3.31 (m, 3H), 2.01-1.75 (m, 5H), 1.73-

1.61 (m, 1H), 1.57-1.51 (m, 2H), 1.38 (brs, 10H), 1.18-1.11 (m, 1H), 1.11-0.81 (m, 7H); **^{13}C NMR** δ (125.8 MHz, CD_3OD, mixture of rotamers) δ 175.9, 172.4, 158.1, 80.7, 71.2, 62.6, 57.7, 55, 48.1, 47.3, 42.4, 28.9, 27, 26.1, 25.5, 25.4, 23.7, 22, 18.9, 11.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{40}N_3O_6$: 441.2917; Found 442.2929.



4.2eD

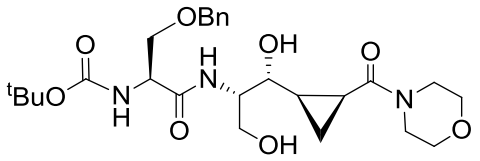
(*S*)-*tert*-Butyl 2-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(pyrrolidine-1-carbonyl) cyclopropyl) propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2eD). Obtained as a white solid (81 mg, 70%): **^{1}H NMR** (500 MHz, CD_3OD; mixture of rotamers) δ 4.28-4.18 (m, 1H), 3.99-3.89 (m, 1H), 3.85-3.65 (m, 3H), 3.61-3.29 (m, 6H), 2.31-2.01 (m, 1H), 2.11-1.78 (m, 8H), 1.61-1.31 (brs, 10H), 1.19-1.11 (m, 1H), 1.01-0.91 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers) δ 175.5, 175.4, 172.5, 172.3, 156.6, 156.3, 81.6, 81.5, 71.5, 71, 62.8, 62.6, 61.8, 57.9, 57.4, 48.1, 48, 47.4, 32.8, 31.6, 28.9, 27, 25.5, 25.4, 24.7, 18.9, 18.8, 11.5, 11.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{36}N_3O_6$: 426.2617; Found 426.2617.



4.2fA

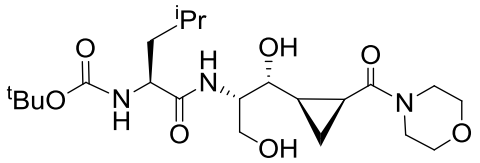
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl) propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2fA).** Obtained as a white solid (32 mg, 66%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.38-7.18 (m, 5H), 4.49-4.47 (m, 1H), 4.05-3.95 (m, 1H), 3.81-3.61 (m, 9H), 3.50-3.19 (m, 3H), 2.91-2.79 (m, 1H), 1.98-1.81 (m, 1H), 1.41-1.13 (m, 11H), 1.03-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 171.7, 169.4, 155, 136.2, 127.8,

126.8, 125, 78.1, 68.9, 66.2, 65.1, 64.9, 59.7, 55.1, 55.0, 44.8, 41.1, 36.7, 26.1, 23.9, 22.5, 14.9, 8.6; **HRMS** (ESI-TOF) *m/z*: [M+Na] Calcd for $C_{25}H_{37}N_3O_7Na$ 514.2529; Found 514.2526.



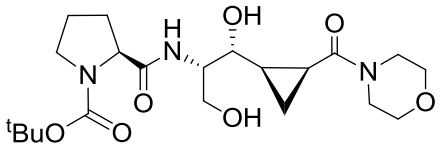
4.2fB

***tert*-Butyl ((*S*)-3-(benzyloxy)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2fB)**. Obtained as a white solid (69 mg, 56%): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41-7.01 (m, 6H), 5.04 (brs, 1H), 4.92 (brs, 1H), 4.58-4.43 (m, 2H), 4.48-4.34 (m, 1H), 4.09-3.90 (m, 2H), 3.88-3.80 (m, 1H), 3.74-3.57 (m, 10H), 3.51-3.41 (m, 2H), 1.71-1.51 (m, 1H), 1.35 (brs, 9H), 1.28-1.18 (m, 1H), 0.99-0.91 (m, 1H), 0.89-0.73 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 171.4, 170.8, 156.4, 137.9, 128, 127.5, 127.4, 79.6, 72.8, 70.2, 69.8, 66.3, 66.2, 60.1, 56.4, 56.2, 54.9, 46.1, 42.3, 27.4, 23.8, 23.7, 16.1, 10; **HRMS** (ESI-TOF) *m/z*: [M+Na] Calcd for $C_{26}H_{39}N_3O_8Na$ 544.2635; Found 545.2651.



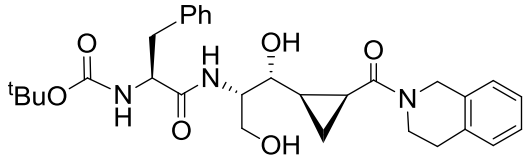
4.2fC

***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl) propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4.2fC)**. Obtained as a white solid (48 mg, 45%): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.16 (brd, *J* = 8.8 Hz, 1H), 5.41-5.21 (m, 1H), 4.15-3.81 (m, 3H), 3.77-3.58 (m, 8H), 3.50-3.39 (m, 2H), 1.79-1.51 (m, 3H), 1.49-1.41 (m, 2H), 1.42 (s, 9H), 1.11-0.99 (m, 1H), 0.93-0.71 (m, 7H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 174.4, 170.7, 156.6, 79.3, 70.2, 66.3, 61, 56.3, 53.5, 46.1, 42.4, 40.1, 27.4, 24.6, 23.9, 22.2, 21.7, 16.1, 10; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{40}N_3O_7$ 458.2866; Found 458.2834.



4.2fD

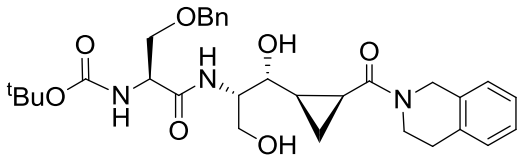
(*R*)-*tert*-Butyl 2-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(morpholine-4-carbonyl)cyclopropyl)-propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4.2fD). Obtained as a white solid (107 mg, 63%): **^{1}H NMR** (400 MHz, CD_3OD; mixture of rotamers) δ 4.38-4.21 (m, 1H) 4.04-3.96 (m, 1H), 3.95-3.61 (m, 8H), 3.59-3.41 (m, 3H), 3.40-3.21 (m, 2H), 2.61-2.35 (m, 1H), 2.01-1.71 (m, 4H)**,** 1.48-1.38 (m, 9H), 1.24-1.18 (m, 2H), 1.01-0.81 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD; mixture of rotamers) δ 174.7, 172.7, 169.4, 169.2, 153.8, 153.5, 78.8, 69.3, 68.8, 67.2, 65.1, 64.9, 59.9, 59.7, 59.2, 59, 57.9, 55.2, 54.6, 52.3, 44.8, 41, 37.8, 34, 30, 28.9, 26.1, 22.8, 22.6, 22.5, 22.3, 22, 21, 15, 14.8, 12.2, 12.3, 8.7, 8.6; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{21}H_{35}N_3O_7Na$: 464.2373; Found 464.2317.



4.2gA

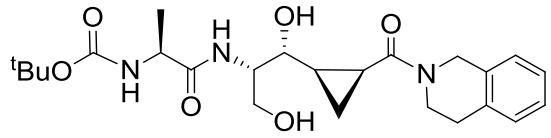
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroiso-quinoline-2-carbonyl)cyclopropyl)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4.2gA)**. **^{1}H NMR** (500 MHz, DMSO-d6; mixture of rotamers). Obtained as a white solid (66 mg, 42%): δ 7.65 (brs, 1H), 7.49 and 7.30 (2s, 9H), 7.04 (brs, 1H), 5.02 (s, 1H), 4.88-4.73 (m, 1H), 4.39 (brs, 1H), 4.02-3.90 (m, 2H), 3.88-3.64 (m, 3H), 3.39-2.85 (m, 4H), 2.12 (brs, 1H), 1.58-1.29 (m, 11H), 1.34-1.20 (m, 1H), 0.98-0.82 (m, 1H); **^{13}C NMR** (125.8 MHz, DMSO-d6; mixture of rotamers) δ 171.2, 169.5, 155.1, 138.3, 135.1, 129.2, 128.3, 127.9, 78, 69.4, 60.4, 55.9, 44, 46.5,

42.8, 37.7, 28.2, 28.1, 27.7, 23.9, 16.6, 9.8; **HRMS** (ESI-TOF) *m/z*: Found 538.2914 $[M + H]^+$ Calcd for $C_{30}H_{40}N_3O_6$: 538.2917).



4.2gB

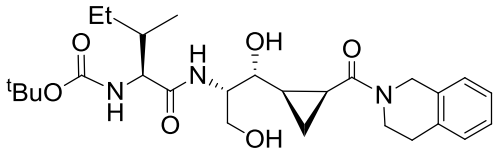
***t*-Butyl((*S*)-3-(benzyloxy)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopropyl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2gB)**. Obtained as a white solid (80 mg, 46%): **^{1}H NMR** (400 MHz, DMSO-d6; mixture of rotamers). δ 7.65-7.55 (m, 1H), 7.41-7.25 (m, 5H), 7.23-7.11 (m, 4H), 6.91-6.81 (m, 1H), 4.81-4.78 (m, 1H), 4.72-4.66 (m, 1H), 4.65-4.41 (m, 4H), 4.35-4.25 (m, 1H), 3.81-3.71 (m, 2H), 3.71-3.41 (m, 5H), 3.15-3.01 (m, 1H), 2.99-2.80 (m, 1H), 2.78-2.71 (m, 1H), 1.89-1.81 (m, 1H), 1.51-1.21(m, 10H), 1.05-0.95 (m, 1H), 0.81-0.74 (m, 1H); **^{13}C NMR** (125.8 MHz, DMSO-d6; mixture of rotamers) δ 169.4, 169.3, 155.1, 138.2, 135.1, 134.9, 133.6, 133.5, 128.3, 128.1, 127.4, 127.3, 126.4, 126.2, 126, 78.2, 71.8, 70.1, 69.2, 60.4, 55.9, 54.3, 46.5, 44, 42.8, 28.7, 28.1, 23.9, 16.2, 9.8; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{31}H_{41}N_3O_7Na$ 590.2842; Found 590.2904.



4.2gE

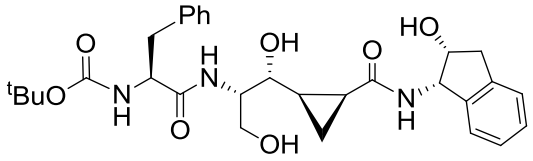
***t*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopropyl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2gE)**. Obtained as a white solid (55 mg, 44%): **^{1}H NMR** (500 MHz, DMSO-d6; mixture of rotamers) δ 7.47-7.40 (m, 1H), 7.25-7.13 (brs, 4H), 6.78-6.76 (m, 1H), 4.85 (brs, 1H), 4.70-4.44 (m, 3H), 4.11-3.72 (m, 3H), 3.68-3.40 (m, 2H), 3.21-3.02 (m, 2H), 2.98-2.72 (m, 2H), 1.98-1.88 (m, 1H), 1.35 (s, 9H),

1.25-1.15 (m, 4H), 1.11-1.07 (m, 1H), 0.83-0.76 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 172.7, 171.0, 156.4, 134.8, 134, 132.8, 132.3, 128.7, 128.3, 127.1, 126.7, 126.6, 126.5, 126.1, 65.9, 62.3, 54.3, 50.6, 47.6, 44.7, 43.7, 40.5, 29.3, 28.4, 28.3, 18.4, 18.1, 17.9, 15.3, 11.0, 10.9; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{24}H_{35}N_3O_6Na$: 484.2423; Found 484.2415.



4.2gF

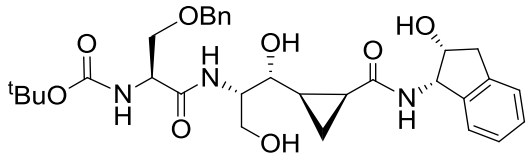
***t*-butyl((2*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquin-oline-2-carbonyl)cyclopropyl)propan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (4.2gf)**.White solid (70 mg, 45%): **^{1}H NMR** (400 MHz, DMSO-d6; mixture of rotamers) δ 7.51-7.35 (m, 1H), 7.29-7.05 (m, 4H), 6.91-6.75 (m, 1H), 4.91-4.75 (m, 1H), 4.71-4.38 (m, 3H), 3.91-3.76 (m, 3H), 3.71-3.59 (m, 1H), 3.54-3.41 (m, 2H), 3.21-3.01 (m, 1H), 2.99-2.79 (m, 1H), 2.78-2.71 (m, 1H), 1.99-1.88 (m, 1H), 1.81-1.61 (m, 1H), 1.51-1.31 (m, 10H), 1.15-0.95 (m, 3H), 0.91-0.69 (m, 7H); **^{13}C NMR** (125.8 MHz, DMSO-d6; mixture of rotamers) δ 171, 169.5, 155.3, 135.1, 134.9, 133.7, 128.3, 126.4, 126.2, 126, 78, 69.5, 69.4, 64.9, 60.5, 59.3, 55.8, 46.5, 44, 42.8, 36.6, 28.7, 28.1, 24.1, 24, 16.5, 16.2, 15.5, 15.1, 11.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{42}N_3O_6$: 504.3074; Found 504.2923.



4.2hA

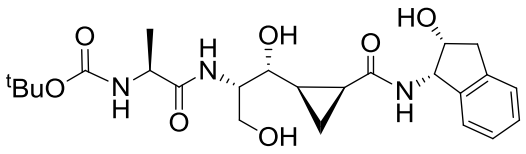
***tert*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamoyl)cyclopropyl)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carba-**

mate (4.2hA). Obtained as a white solid (80 mg, 60%): **^{1}H NMR** (400 MHz, DMSO-d6) δ 8.25-8.15 (m, 1H), 7.75-7.65 (m, 1H), 7.41-7.11 (m, 9H), 6.91-6.81 (m, 1H), 5.21-5.05 (m, 1H), 4.91-4.78 (m, 1H), 4.74-4.65 (m, 1H), 4.61-4.51 (m, 1H), 4.50-4.46 (m, 1H), 4.31-4.11 (m, 1H), 3.91-3.71 (m, 1H), 3.69-3.41 (m, 3H), 3.05-2.96 (m, 2H), 2.85-2.61 (m, 2H), 1.91-1.75 (m, 1H), 1.41-1.15 (m, 9H), 1.14-0.99 (m, 2H), 0.89-0.71 (m, 1H); **^{13}C NMR** (125.8 MHz, DMSO-d6) δ 171.8, 171.4, 155.1, 141.6, 141, 138.3, 129.4, 129.2, 127.9, 127.3, 126.2, 126, 124.7, 124.2, 77.9, 72, 68.5, 64.9, 60.6, 57.5, 55.8, 55.6, 37.7, 28.1, 27.7, 24.1, 17.6, 15.1, 10.2; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{30}H_{40}N_3O_7$: 554.2866; Found 554.2845.



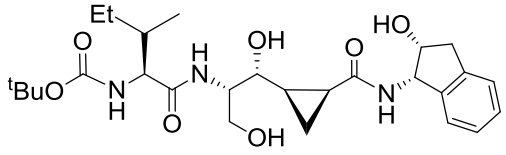
4.2hB

***tert*-Butyl ((*S*)-3-(benzyloxy)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamoyl)cyclopropyl)propan-2-yl)amino)-1-oxopropan-2-yl)-carbamate (4.2hB)**. Obtained as a white solid (80 mg, 57%): **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.21-8.11 (m, 1H), 7.71-7.55 (m, 1H), 7.45-7.26 (m, 5H), 7.22-7.11 (m, 4H), 6.88-6.71 (m, 1H), 5.21-5.11 (m, 1H), 4.89 (s, 1H), 4.70 (s, 1H), 4.59-4.41 (m, 4H), 4.39-4.21 (brs, 1H), 3.85-3.71 (m, 1H), 3.61-3.41 (m, 5H), 3.09-2.99 (m, 1H), 2.81-2.71 (m, 1H), 1.84-1.75 (m, 1H), 1.42-1.31 (brs, 9H), 1.27-1.11 (m,1H), 1.03-0.96 (m, 1H), 0.81-0.65 (m, 1H)**; ^{13}C NMR** (125.8 MHz, DMSO-d6) δ 171.8, 169.4, 155.1, 141.6, 140.9, 138.2, 128.1, 127.4, 127.3, 124.7, 124.3, 78.2, 72, 71.8, 70.1, 68.4, 60.5, 57.5, 55.7, 54.3, 28.1, 24.1, 17.5, 10.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{31}H_{42}N_3O_8$ 584.2972; Found 584.2952.



4.2hE

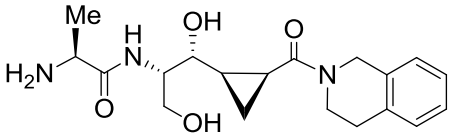
***t*-Butyl ((*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamoyl)cyclopropyl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate (4.2hE)**. Obtained as a white solid (60 mg, 55%): **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.21-8.11 (m,1H), 7.61-7.41 (m, 1H), 7.31-7.11 (m, 4H), 6.87-6.75 (m, 1H), 5.21-5.11 (m, 1H), 4.82 (s, 1H), 4.74-4.65 (m, 1H), 4.63-4.41 (m, 2H), 4.05-3.91 (m, 1H), 3.81-3.71 (m, 1H), 3.62-3.45 (m, 3H), 3.05-2.91 (m, 1H), 2.85-2.71 (m, 1H), 1.89-1.71 (m, 1H), 1.44-1.32 (brs, 9H), 1.31-1.11 (m, 4H), 1.01-0.95 (m, 1H), 0.89-0.75 (m, 1H)**; ^{13}C NMR** (125.8 MHz, DMSO-d6) δ 172.3, 171.8, 154.8, 141.6, 140.9, 127.3, 126.2, 124.7, 124.2, 78.1, 72, 68.5, 60.6, 57.5, 55.5, 54.8, 49.7, 28.1, 24.2, 18.6, 17.5, 10.3; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{36}N_3O_7$ 478.2553; Found 478.2550.



4.2hF

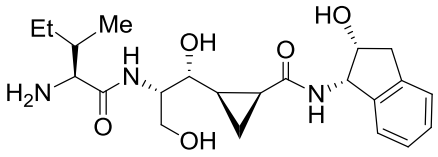
***tert*-Butyl ((2*S*,3*S*)-1-(((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(((1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamoyl)cyclopropyl)propan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (4.2hF).** Obtained as a white solid (61 mg, 50%): **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.31-8.11 (m, 1H), 7.61-7.41 (m, 1H), 7.31-7.11 (m, 4H), 6.81-6.61 (m, 1H), 5.21-5.05 (m, 1H), 4.81-4.75 (m, 1H), 4.74-4.65 (m, 1H), 4.63-4.41 (m, 2H), 3.91-3.77 (m, 2H), 3.76-3.71 (m, 3H), 3.11-2.95 (m, 1H), 2.89-2.71 (m, 1H), 1.92-1.80 (m, 1H), 1.72-1.68 (m, 1H), 1.59-1.28 (m, 10H), 1.29-1.18 (m, 1H), 1.11-0.95 (m,2H), 0.91-0.75 (m, 7H); **^{13}C NMR** (125.8 MHz, DMSO-

d6) δ 171.8, 171.2, 155.3, 141.6, 140.9, 127.3, 126.1, 124.7, 124.2, 78.1, 72, 68.5, 60.7, 59.2, 57.6, 55.6, 36.5, 28.1, 24.2, 17.5, 15.4, 11.1, 10.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{42}N_3O_7$: 520.3023; Found 520.3003.



5.1 (as TFA salt)

(*S*)-2-Amino-N-((1*R*,2*S*)-1,3-dihydroxy-1-((1*R*,2*S*)-2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopropyl)propan-2-yl)propanamide (as TFA salt) **(5.1)**. *Cis*-1,2-disubstituted cyclopropane dipeptide isostere **4.2gE** (40 mg) was dissolved in trifluoroacetic acid (5 mL) and stirred at rt for 2 h. The reaction mixture was then subjected to vacuum and triturated with anhydrous diethyl ether to obtain the trifluoacetic acid salt of *cis*-1,2-disubstituted cyclopropane dipeptide isostere **5.1** as a hygroscopic solid (26 mg, quant.). **^{1}H NMR** (500 MHz, D_2O) δ 7.31-7.11 (m, 4H), 4.95-4.85 (m, 2H), 4.61-4.41 (m, 1H), 4.05-3.85 (m, 2H), 3.84-3.65 (m, 2H), 3.61-3.41 (m, 1H), 3.19-3.01 (m, 1H), 2.91-2.71 (m, 2H), 2.18-2.01 (m, 1H), 1.81-1.41 (m, 4H), 1.19-1.01 (m, 2H)**; ^{13}C NMR** (125.8 MHz, D_2O) δ 171.9, 163.1, 162.8, 135.2, 135, 132.9, 132.7, 128.3, 126.9, 126.5, 126.4, 126.2, 117.4, 115.1, 70.2, 60.5, 56.7, 56.6, 49.2, 47.2, 44.8, 43.7, 41.0, 28.4, 27.7, 22.9, 17.5, 17.2, 16.8, 16.5, 9.9; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{28}N_3O_4$: 362.2080; Found 362.2075.

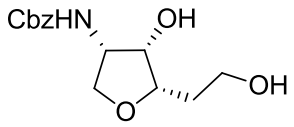


5.2 (as TFA salt)

(1*S*,2*R*)-2-((1*R*,2*S*)-2-((2*S*,3*S*)-2-Amino-3-methylpentanamido)-1,3-dihydroxypropyl)-N-(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)cyclopropanecarboxamide (as TFA salt) **(5.2).** As

described above in synthesis of trifluoroacetic acid salt of dipeptidic isostere **5.1**, a similar protocol was utilized for Boc-deprotection of *cis*-1,2-disubstituted cyclopropane dipeptide isostere **4.2hF** to afford *cis*-1,2-disubstituted cyclopropane dipeptide isostere **5.2** as a hygroscopic solid (30 mg, quant.). **^{1}H NMR** (500 MHz, D_2O) δ 7.41-7.18 (m, 4H), 5.24 (brs, 1H), 4.52 (brs, 1H), 4.13 (brs, 1H), 3.91-3.78 (m, 2H), 3.71-3.48 (m, 2H), 3.21-3.02 (m, 1H), 2.91-2.71 (m, 1H), 2.01-1.81 (m, 2H), 1.61-1.31 (m, 2H), 1.29-1.01 (m, 3H), 1.01-0.79 (m, 6H); **^{13}C NMR** (125.8 MHz, D_2O) δ 174.4, 169.6 140.3, 139.6, 128.2, 127, 125.3, 123.9, 73.3, 69.2, 60.5, 58.1, 57.9, 56.7, 38.4, 36.1, 23.7, 23, 18.2, 14.2, 10.6, 10.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{34}N_3O_5$: 420.2498; Found 420.2482.

5.3.2. Chapter 3. Total Synthesis and Structure–Activity Relationship Studies of the Cytotoxic Anhydrophytosphingosine Jaspine B

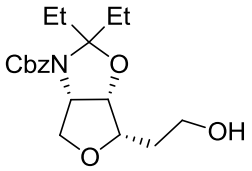


28.1

Benzyl ((3*S*,4*S*,5*S*)-4-hydroxy-5-(2-hydroxyethyl)tetrahydrofuran-3-yl)carbamate (28.1). **Step1**: To a stirred solution of the bicyclic lactone **6** (1.5 g, 5.4 mmol) in anhydrous dichloromethane (25 mL) at -78 °C was added DIBAL-H dropwise (1M in toluene; 6.5 mL, 6.5 mmol) over 10 min.[28] After stirring for another 1.5 h at the same temperature, the reaction was quenched by adding methanol (1.5 mL) and allowed to attain rt. To the resulting solution, ethylacetate (40 mL), and saturated aq. sodium potassium tartarate solution (10 mL) was added and stirring continued for another 1 h. The organic layer was separated, and the aq. Layer extracted with ethylacetate (3 x 25 mL). The combined extract dried over anhydrous Na_2SO_4 and

solvent evaporated on a rotary evaporator to obtain 1.55 g of the crude lactol, which was used as such for the next reaction.

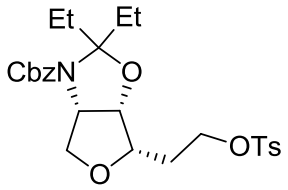
Step 2 : The product from step 1 (1.5 g) was dissolved in ethanol (20 mL), the solution cooled to 0 °C (ice-bath) and powdered $NaBH_4$ (420 mg, 11.11 mmol) added to it in small portions over a period of 5 min. After stirring at 0 °C for 15 min, the reaction was quenched by adding crushed ice and the resulting mixture extracted thoroughly with ethylacetate (8 x 30 mL). The combined extract was dried (anhydrous. Na_2SO_4), concentrated, and the residue purified by column chromatography (hexanes : EtOAc = 1:1 to 1:2) to afford the pure diol **28.1** as a white solid (1.2 g, 77% over two steps); mp = 105-107 °C; $[\alpha]_D$ 14.4 (*c* 0.4, CH_3OH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45-7.30 (m, 5H), 5.53 (brs, 1H, exchangeable with D_2O), 5.12 (s, 2H), 4.50-4.35 (m, 1H), 4.21 (s, 1H), 4.12-3.96 (m, 2H), 3.95-3.85 (brs, 1H), 3.75-3.67 (m, 1H), 3.66 (t, 1H, *J* = 10 Hz), 3.37 (brs, 1H, exchangeable with D_2O), 2.25 (brs, 1H, exchangeable with D_2O) 2.10-1.71 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 158, 136.3, 128.6, 128.2, 128.1, 81.9, 71.4, 69.9, 66.9, 59.5, 53.9, 31.2; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{20}NO_5$ 282.1341; Found 282.1334.



28.2

(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-(2-hydroxyethyl)tetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (28.2). An ice-cooled solution of the diol **28.1** (2 g, 7.1 mmol), 3-pentanone (12 mL) and freshly prepared 3,3-dimethoxypentane[268] (20 mL) was treated with a catalytic amount of BF_3•OEt_2 (0.1 mL). After stirring the resulting solution at the same temperature for 3 h, the

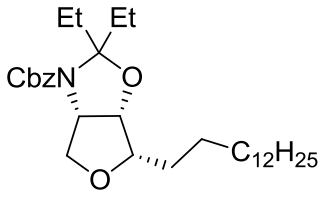
reaction was quenched with triethylamine (0.5 mL) and concentrated under vacuum. The crude residue was purified by flash chromatography (hexanes : EtOAc = 4:1 to 1:1), affording the corresponding oxazolidine derivative **28.2** as a viscous liquid (2g, 81%): $[\alpha]_D$ 71 (*c* 1.8, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.45-7.25 (m, 5H), 5.21-5.05 (m, 2H), 4.71-4.41 (m, 2H), 4.18 and 4.01 (2m, 1H), 3.91-3.74 (m, 2H), 3.73-3.55 (m, 2H), 2.21-1.66 (m, 7H), 0.92-0.71 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$ mixture of rotamers) δ 152.9, 151.6, 136.5, 136.1, 128.6, 128.2, 128.1, 128.0, 127.7, 102.4, 101.6, 82.0, 81.9, 81.5, 80.9, 73.8, 73.3, 67.4, 66.7, 64.0, 62.8, 60.8, 60.7, 31, 30.9, 30.8, 30.3, 29.6, 29.3, 8.1, 7.4; **HRMS** (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{19}H_{27}NO_5Na$ 372.1787; Found 372.1826.



28.3

(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-(2-(tosyloxy)ethyl)tetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (**28.3**). To an ice-cooled solution of the oxazolidine alcohol **28.2** (2 g, 5.73 mmol) in anhydrous dichloromethane (30 mL) were added *p*- toluenesulfonyl chloride (9.85 g, 9 mmol), pyridine (0.9 mL, 11.1 mmol), and a catalytic amount of 4-DMAP (30 mg). After stirring the resulting solution at rt for 48 h, the reaction was quenched with 10% aq. HCl solution (50 mL). The organic layer was separated, and the aqueous layer extracted once with dichloromethane (50 mL). The combined organic layer was washed with brine (1x50 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. Purification of the crude residue by flash chromatography (hexanes : EtOAc = 4:1 to 2:1) afforded the pure tosylate **28.3** as a viscous liquid (2.6 g, 90%); $[\alpha]_D$ 48.8 (*c* 0.6, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.81 (d, *J* = 8.24 Hz, 2H), 7.43-7.31 (m, 7H), 5.21-5.05 (m, 2H), 4.60 – 4.46 (m, 2H), 4.29-

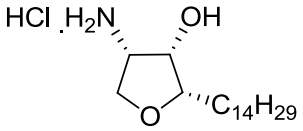
4.14 (m, 2H), 4.21 and 3.94 (2d, J = 10.0 Hz and 8.2 Hz, 1H), 3.60-3.41 (m, 2H), 2.47 (s, 3H), 2.11-1.61 (m, 6H), 0.90-0.73 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 152.9, 151.6, 144.8, 136.5, 136.1, 132.9, 130, 128.6, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 102.3, 101.5, 80.7, 73.7, 73.1, 68.0, 67.4, 66.7, 64.1, 63.0, 30.9, 30.1, 29.6, 29.1, 28.0, 21.6, 21.6, 8.0, 7.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{34}NO_7S$ 504.2056; Found 504.2093.



28.4

(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-tetradecyltetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (28.4). To a cooled mixture (-78 °C) of the tosylate **28.3** (250 mg, 0.5 mmol) and finely powdered CuI (190 mg, 1 mmol) in dry THF (8 mL) was added dodecyl magnesium bromide (1M soln. in ether, 3.4 mL, 3.4 mmol) dropwise over a period of 10 min. After stirring at -78°C for 30 min, the reaction mixture was warmed to 0°C (ice-bath) and stirring continued for another 1.5 h. The reaction was quenched with 5 mL of satd. NH_4Cl solution, and extracted with ethyl acetate (5x25 mL). The combined extract washed sequentially with water and brine, dried over anhydrous Na_2SO_4 and solvent removed under vacuum. Purification of the crude residue by flash chromatography (hexanes : EtOAc = 9 : 1) afforded the fully protected Jaspine B derivative **28.4** as a viscous liquid (180 mg, 72%): $[\alpha]_D$ 65.7 (*c* 0.6, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.46-7.30 (m, 5H), 5.22-5.06 (m, 2H), 4.56-4.50 (2s, 2H), 4.09 and 4.01 (2d, J = 10.75 Hz and 10.5 Hz, 1H), 3.70-3.54 (m, 1H), 3.44-3.34 (m, 1H), 2.19-1.98 (m, 1H), 1.97-1.71 (m, 5H), 1.48-1.15 (m, 24H), 0.95-0.81 (m, 9H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 153.0, 151.7, 136.6, 136.2, 128.6, 128.5, 128.2, 128.1, 128.0, 127.7,

102.1, 101.3, 83.5, 83.4, 81.1, 80.5, 73.6, 73, 67.4, 66.6, 64.1, 63.0, 31.9, 29.7, 29.6, 29.5, 29.4, 28.1, 26.3, 22.7, 14.1, 8.1, 7.4; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{26}H_{52}NO_4$ 502.3846; Found 502.3846.



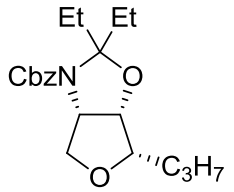
Jaspine B

Jaspine B hydrochloride. A solution of the *N,O*-protected Jaspine B derivative **28.4** (85 mg, 0.169 mmol) in 50% aq. HCl (10 mL) was refluxed for 16 h. The reaction mixture was cooled to rt and extracted once with ether (10 mL) to remove any organic soluble impurities. The aqueous layer was then concentrated in a rotary evaporator and the residual oily liquid kept under high vacuum overnight to afford the desired jaspine B hydrochloride salt as a white solid (44 mg, 77%): $[\alpha]_D$ 2.5 (*c* 0.46, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD) δ 4.25 (br s, 1H), 3.95-3.84 (m, 2H), 3.83-3.75 (m, 1H), 3.73-3.66 (m, 1H), 1.72-1.57 (m, 2H), 1.51-1.20 (m, 24H), 0.88 (t, *J* = 5.6 Hz, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 84.4, 71.0, 69.0, 54.1, 33.1, 30.9, 30.8, 30.7, 30.5, 29.7, 23.8, 14.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{18}H_{38}NO_2$ 300.2903; Found 300.2879.

General procedure for the synthesis of C2 alkyl analogs 29.1-29.3

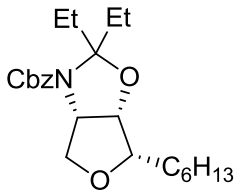
To a cooled mixture (-78°C) of the tosylate **28.3** (1 equiv.) and finely powdered CuI (2 equiv.) in dry THF (8 mL) was added the respective Grignard reagents (7 equiv.) dropwise over a period of 10 min. After stirring at -78°C for 30 min, the reaction mixture was warmed to 0°C (ice-bath) and stirring continued for additional 12 h – 16 h (monitoring by TLC). Subsequently, the reaction was quenched with 5 mL of satd. NH_4Cl solution and extracted with ethyl acetate (5x25 mL). The combined extract washed sequentially with water and brine, dried over

anhydrous Na_2SO_4 and solvent removed under vacuum. Purification of the crude residue by flash chromatography (hexanes : EtOAc = 9 : 1) afforded the corresponding C2 alkyl analogs **29.1-29.3**.



29.1

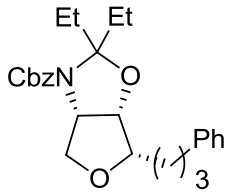
(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-propyltetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (**29.1**). Obtained as a viscous liquid (72 mg, 82%): $[\alpha]_D$ 90 (*c* 1.1, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.41-7.21 (m, 5H), 5.21-5.11 (m, 2H), 4.61-4.53 (m, 1.4H), 4.51-4.45 (m, 0.6H), 4.15-4.06 (m, 0.4H), 4.05-3.98 (m, 0.6H), 3.69-3.59 (m, 1H), 3.45-3.38 (m, 1H), 2.07-1.71 (m, 6H), 1.54-1.41 (m, 2H), 1.01-0.95 (m, 3H), 0.91-0.81 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 152.9, 151.6, 136.6, 136.1, 128.6, 128.5, 128.1, 128.0 127.6, 102.0, 101.3, 83.2, 83.1, 80.5, 73.5, 73.0, 67.3, 66.6, 64.1, 62.9, 30.9, 30.3, 30.2, 30.1, 29.5, 29.3, 19.6, 14.3, 8.08, 8.07, 7.4; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{30}NO_4$ 348.2175; Found 348.2165.



29.2

(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-hexyltetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (29.2). Obtained as a viscous liquid (63 mg, 66%): $[\alpha]_D$ 82 (*c* 0.85, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.41-7.29 (m, 5H), 5.21-5.11 (m, 2H), 4.61-4.53 (m, 1.4H), 4.51-4.49 (m, 0.6H), 4.18-4.11 (m, 0.4H) 4.01-3.91 (m, 0.6H) 3.71-3.51 (m, 1H), 3.42-3.31 (m,

1H), 2.40-1.70 (m, 6H), 1.49-1.23 (m, 8H), 0.95-0.71 (m, 9H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 152.9, 151.6, 136.6, 136.2, 128.5, 128.4, 128.1, 128.06, 128.0, 127.6, 102.0, 101.3, 83.4, 81.1, 80.5, 73.5, 73.0, 67.3, 66.6, 64.1, 62.9, 31.7, 30.9, 30.3, 29.7, 29.5, 29.4, 29.3, 28.08, 26.3, 22.5, 14.09, 8.07, 7.4; **HRMS** (ESI-TOF) *m/z*: M+H Calcd for $C_{23}H_{36}NO_4$ 390.2644; Found 390.2661.

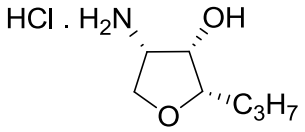


29.3

(3a*S*,6*S*,6a*S*)-Benzyl 2,2-diethyl-6-(3-phenylpropyl)tetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (29.3). The crude product **29.3** was purified by column chromatography (ethyl acetate/hexane) to obtain the product as a viscous liquid (63 mg, 67%): $[\alpha]_D$ 88.4 (*c* 0.5, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.31-7.12 (m, 10H) 5.22-5.11 (m, 2H), 4.62-4.51 (m, 1.4H), 4.51-4.45 (m, 0.6H), 4.18-4.11 (m, 0.4H), 4.05-3.96 (m, 0.6H), 3.71-3.55 (m, 1H), 3.48-3.35 (m, 1H), 2.78-2.61 (m, 2H), 2.15-1.93 (m, 1H), 1.91-1.71 (m, 7H), 0.91-0.75 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 152.9, 151.6, 142.2, 136.6, 136.1, 130.5, 129.5, 129.3, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 127.2, 126.9, 126.1, 125.9, 125.7, 102.1, 101.3, 83.2, 83.2, 81.1, 80.5, 73.6, 73.0, 67.3, 66.6, 65.2, 64.1, 62.9, 35.9, 35.8, 30.8, 30.3, 29.7, 29.5, 29.4, 29.0, 28.7, 28.1, 27.9, 27.8, 27.7, 27.7, 8.11, 8.09, 7.94, 7.47; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{26}H_{33}NO_4Na$ 424.2603; Found 424.2542.

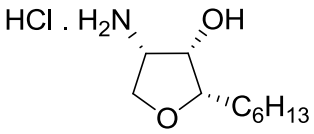
General procedure for global deprotection of the acetonide protected C2 alkyl analogs 29.1-29.3.

The *N,O*- protected C2 alkylated analogs (**29.1-29.3**) were refluxed in a solution of 50% aq. HCl for 12–16 h (monitored by TLC). The reaction mixture was cooled to rt. and extracted once with dichloromethane (10 mL) to remove any organic soluble impurities. The aqueous layer was then concentrated in a rotary evaporator and the residual oily liquid kept under high vacuum overnight to afford the desired Jaspine B C2 alkyl analogs **29.4-29.6** as their hydrochloride salts.



29.4

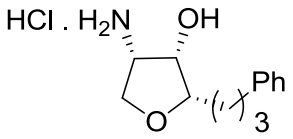
(2*S*,3*S*,4*S*)-4-Amino-2-propyltetrahydrofuran-3-ol (HCl salt) **(29.4)**. Obtained as a hygroscopic solid (31 mg, 82%); $[\alpha]_D$ 7.7 (*c* 0.6, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 4.29 (brs, 1H), 3.98-3.89 (m, 2H), 3.88-3.81 (m, 1H), 3.79-3.72 (m, 1H), 1.72-1.61 (m, 2H), 1.59-1.41 (m, 2H), 1.05-0.95 (m, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 81.8, 68.6, 66.7, 52.1, 28.6, 18.2, 12.3; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_7H_{16}NO_2$ 146.1181; Found 146.1153.



29.5

(2*S*,3*S*,4*S*)-4-Amino-2-hexyltetrahydrofuran-3-ol (HCl salt) **(29.5)**. Obtained as a hygroscopic solid (35 mg, 83%); $[\alpha]_D$ 5.48 (*c* 1.35, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 4.21-4.15 (m, 1H), 3.91-3.78 (m, 2H), 3.77-3.68 (m, 1H), 3.67-3.59 (m, 1H), 1.64-1.51 (m, 2H), 1.41-1.19 (m, 8H), 0.89-0.76 (m, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 83.2, 69.7, 67.8, 53.2, 31.8, 29.4,

28.6, 26.0, 22.5, 13.3; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{22}NO_2$ 188.1651; Found 188.1635.

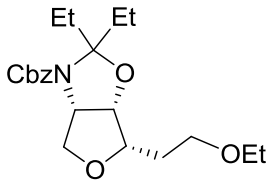


29.6

(2*S*,3*S*,4*S*)-4-Amino-2-(3-phenylpropyl)tetrahydrofuran-3-ol (HCl salt) **(29.6)**. Obtained as a hygroscopic solid (60 mg, 90%); $[\alpha]_D$ 10.4 (*c* 0.86, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 7.39-7.09 (m, 5H), 4.41-4.21 (m, 1H), 4.11-3.75 (m, 4H), 2.81-2.61 (m, 2H), 1.94-1.61 (m, 4H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 143.5, 129.7, 129.4, 129.3, 126.7, 84.2, 70.8, 69, 54.3, 36.9, 29.3, 29.1; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{13}H_{20}NO_2$ 222.1494; Found 222.1475.

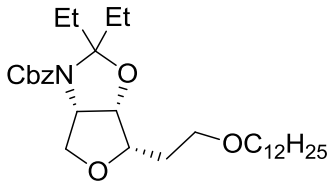
General procedure for Jaspine B C2 Ether analogs 30.1-30.3.

To an ice-cooled solution of the oxazolidine alcohol **28.2** (1 equiv.) in THF (10% solution w/v) was added potassium *tert*-butoxide (3 equiv, 1 M solution in THF) and stirred for 30 min. The respective alkyl halides (1.2 equiv.) were then added to the ice-cooled solution with stirring. The reaction mixture was allowed to attain rt, and stirring continued for another 6-16 h (monitored by tlc). The reaction was then quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc (3x). The combined organic layers washed with brine and dried over anhydrous Na_2SO_4. Removal of solvent under vacuum and purification of the residue by flash chromatography (EtOAc:hexanes = 1:9) afforded the C2 alkyloxy analogs **30.1-30.3**.



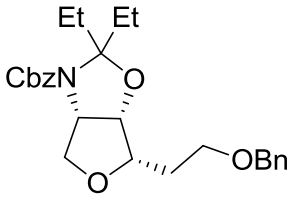
30.1

(3a*S*,6*S*,6a*S*)-Benzyl 6-(2-ethoxyethyl)-2,2-diethyltetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (30.1). Obtained as a syrupy liquid (75 mg, 70%); $[\alpha]_D$ 79.3 (c 0.38, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers): δ 7.41-7.29 (m, 5H), 5.21-5.09 (m, 2H), 4.63-4.45 (m, 2H), 4.18-4.09 (m, 0.4H) 4.03-3.95 (m, 0.6H), 3.68-3.55 (m, 4H), 3.51-3.45 (m, 2H), 2.15-2.01 (m, 3H), 1.99-1.69 (m, 3H), 1.21-1.15 (m, 3H), 0.98-0.75 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) 152.9, 151.6, 136.6, 136.1, 128.5, 128.0, 127.6, 102.1, 101.3, 81.2, 80.6, 80.2, 73.6, 73.0, 67.5, 67.4, 67.3, 66.6, 66.1, 64.1, 63, 30.9, 30.2, 29.7, 29.6, 29.2, 28.5, 15.2, 8.1, 7.4, 7.3; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for : $C_{21}H_{32}NO_5$: 378.2280; Found 378.2278.



30.2

(3a*S*,6*S*,6a*S*)-Benzyl 6-(2-(dodecyloxy)ethyl)-2,2-diethyltetrahydrofuro[3,4-d]oxazole-3(2H) carboxylate (30.2). Obtained as a viscous liquid (70 mg, 79%): $[\alpha]_D$ 59.25 (*c* 0.675, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers) δ 7.41-7.29 (m, 5H), 5.21-5.09 (m, 2H), 4.63-4.55 (m, 1.4H), 4.53-4.49 (m, 0.6H), 4.15-4.11 (m, 0.4H), 4.05-3.95 (m, 0.6H), 3.71-3.51 (m, 4H), 3.51-3.35 (m, 2H), 2.19-1.95 (m, 3H), 2.01-1.71 (m, 3H), 1.41-1.21 (m, 20 H), 0.95-0.81 (m, 9H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) 152.96, 151.65, 136.61, 136.17, 128.57, 128.56, 128.08, 128.04, 127.66, 102.12, 101.35, 81.22, 80.63, 80.34, 73.59, 73.03, 71.07, 71.05, 67.73, 67.66, 67.38, 66.67, 64.18, 63.02, 32.83, 31.93, 30.99, 30.28, 29.73, 29.68, 29.64, 29.56, 29.51, 29.46, 29.37, 29.26, 28.53, 26.19, 25.76, 22.70, 14.14, 8.10, 7.41; **HRMS** (ESI-TOF) *m/z*: [M+H] + Calcd for : $C_{31}H_{52}NO_5$: 518.3845; Found 518.3857.

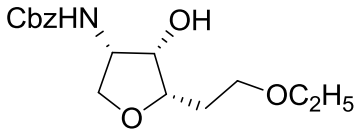


30.3

(3a*S*,6*S*,6a*S*)-Benzyl 6-(2-(benzyloxy)ethyl)-2,2-diethyltetrahydrofuro[3,4-d]oxazole-3(2H)-carboxylate (30.3). Obtained as a viscous liquid (120 mg, 70%); $[\alpha]_D$ 40.36 (*c* 0.55, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$; mixture or rotamers): δ 7.39-7.29 (m, 10H), 5.21-5.11 (m, 2H), 4.59-4.56 (m, 1H), 4.55-4.45 (m, 3H), 4.16-4.13 (m, 0.4H), 4.12-3.98 (m, 0.6H), 3.69-3.58 (m, 3H), 2.18-2.14 (m, 3H), 2.11-1.71 (m, 4H), 0.91-0.86 (m, 3H), 0.83-0.78 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture or rotamers) 152.9, 151.6, 138.4, 136.6, 136.1, 128.6, 128.5, 128.3, 128.1, 127.6, 127.6, 127.5, 102.1, 101.3, 81.1, 80.6, 80.3, 73, 61, 73.0, 72.9, 67.9, 67.4, 67.3, 66.6, 64.2, 63.0, 31, 30.3, 29.6, 29.3, 28.5, 25.6, 8.1, 7.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{34}NO_5$ 440.2437; Found 440.2433.

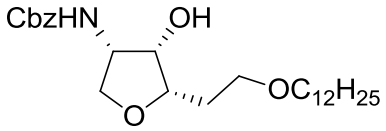
General procedure for the synthesis of acetonide deprotected C2 alkyloxy N-Cbz-Jaspine B analogs 30.4-30.6.

An ice-cooled solution of TFA:H_2O (excess) was added to the *N,O*-oxazolidine C2 alkyloxy analogs **30.1-30.3** and stirred at room temperature for 3-4 h. After completion of reaction (monitored by TLC), the reaction mixture was neutralized by cautiously pouring into a saturated solution of $NaHCO_3$. The resulting solution was extracted thoroughly with chloroform (5x). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Removal of solvent under vacuum and purification of the residue by flash chromatography afforded the acetonide deprotected C2 ether analogs **30.4-30.6**.



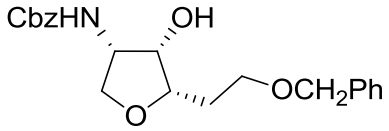
30.4

Benzyl ((3*S*,4*S*,5*S*)-5-(2-ethoxyethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (30.4). Obtained as a white solid (48 mg, 79%): $[\alpha]_D$ 13.45 (*c* 0.55, $CDCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.48-7.29 (s, 5H), 5.65-5.51 (m, 1H), 5.21-5.01 (m, 2H), 4.48-4.31 (m, 1H) 4.14 (s, 1H), 4.09-4.01 (m, 1H), 4.0-3.91 (m, 1H), 3.84 (s, 1H) 3.71-3.61 (m, 2H), 3.58-3.41 (m, 2H), 3.40-3.31 (m, 1H), 2.21-2.01 (m,1H), 1.99-1.81 (m, 1H), 1.31-1.11 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 156.2, 136.4, 128.5, 128.1, 82.1, 71.1, 69.9, 66.8, 66.8, 66.7, 53.8, 29.9, 15; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{24}NO_5$ 310.1654; Found 310.1645.



30.5

Benzyl ((3*S*,4*S*,5*S*)-5-(2-(dodecyloxy)ethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (30.5). Obtained as a fluffy solid (50 mg, 76%); $[\alpha]_D$ 7.7 (*c* 0.45, $CHCl_3$): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45-7.25 (m, 5H), 5.61-5.51 (m, 1H), 5.14 (s, 2H), 4.48-4.31 (m, 1H) 4.18-4.11 (m, 1H) 4.09-4.02 (m, 1H), 4.01-3.91 (m, 1H), 3.87 (s, 1H) 3.68-3.55 (m, 2H), 3.51-3.33 (m, 3H) 2.21-2.05 (m, 1H), 1.95-1.81 (m, 1H), 1.41-1.21 (m, 20H), 0.95-0.85 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 156.23, 136.46, 128.53, 128.13, 82.15, 71.75, 71.12, 70.33, 70.0, 67.04, 66.82, 53.88, 31.92, 29.91, 29.65, 29.61, 29.54, 29.51, 29.38, 29.36, 26.09, 22.71, 14.14; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{26}H_{44}NO_5$ 450.3219; Found 450.3225.

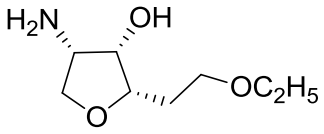


30.6

Benzyl ((3*S*,4*S*,5*S*)-5-(2-(benzyloxy)ethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (30.6) Obtained as a white solid (55 mg, 85%): $[\alpha]_D$ 5.6 (*c* 0.52, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41-7.21 (m, 10H), 5.61-5.51 (m, 1H), 5.13 (brs, 2H), 4.61-4.51 (m, 2H) 4.45-4.31 (m, 1H), 4.21-4.11 (m, 1H) 4.09-3.91 (m, 2H), 3.77-3.47 (m, 4H), 2.21-2.05 (m, 1H), 2.02-1.89 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 156.2, 137.1, 136.4, 128.6, 128.5, 128.4, 128.1, 127.8, 127.6, 81.9, 73.74, 71.1, 69.9, 66.8, 66.7, 60.4, 54.2, 50.8, 21.0, 14.2, 14.1; **HRMS** (ESI-TOF) *m/z*:, $[M + H]^+$ Calcd for $C_{21}H_{26}NO_5$: 372.1811; Found 372.1805.

General procedure for hydrogenolysis of the *N*-Cbz-protected C2 ether analogs 30.4-30.6

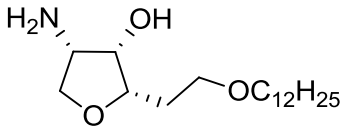
The *N*-Cbz-protected analogs **30.4-30.6** were dissolved in methanol (1% solution w/v) and 10% Pd-C (10% wt) added to it. Flushed the reaction mixture with hydrogen (twice) and stirred under a hydrogen atmosphere for 3-10 h (monitored by TLC). The reaction mixture was filtered through a celite pad and the residue washed with methanol (3x). Removal of solvent under vacuum, and subjecting the residue to high vacuum overnight provided the respective pure products **30.7-30.9**.



30.7

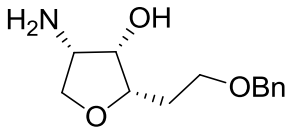
(2*S*,3*S*,4*S*)-4-Amino-2-(2-ethoxyethyl)tetrahydrofuran-3-ol (30.7). Obtained as a viscous liquid (20 mg, 89%): $[\alpha]_D$ 8.2 (*c* 1.15, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD) δ 3.99-3.88 (m, 1H), 3.87-3.79 (m, 2H), 3.61-3.31 (m, 6H), 1.91-1.73 (m, 2H), 1.21-1.05 (m, 3H); **^{13}C NMR**

(125.8 MHz, CD_3OD) δ 81.4, 73.5, 72.1, 68.5, 67.3, 66.0, 56.0, 31.08, 15.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_8H_{18}NO_3$: 176.1287; Found 176.1281.



30.8

(2*S*,3*S*,4*S*)-4-Amino-2-(2-(dodecyloxy)ethyl)tetrahydrofuran-3-ol (30.8). Obtained as a viscous liquid (25 mg, 89%): $[\alpha]_D$ 8.8 (*c* 0.54, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD) δ 4.31-4.21 (m, 1H), 3.95-3.75 (m, 4H), 3.61-3.51 (m, 2H), 3.45-3.35 (m,2H), 1.98-1.81 (m, 2H), 1.61-1.51 (m, 2H), 1.41-1.21 (m, 18H), 0.92-0.81 (m, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 79.0, 69.5, 68.4, 66.4, 66, 51.70, 30.4, 28.0, 27.9, 27.8, 27.6, 24.6, 21.0, 11.7; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{18}H_{38}NO_3$ 316.2852; Found 316.2843.



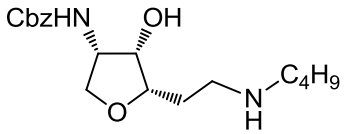
30.9

(2*S*,3*S*,4*S*)-4-Amino-2-(2-(benzyloxy)ethyl)tetrahydrofuran-3-ol (30.9). Obtained as a viscous liquid (21 mg, 82%): $[\alpha]_D$ 9.6 (*c* 1.2, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 7.38-7.31 (m, 4H), 7.30-7.21 (m, 1H), 4.55-4.45 (m, 2H), 4.05-3.95 (m, 1H), 3.93-3.81 (m, 2H), 3.78-3.58 (m, 2H), 3.55-3.48 (m, 1H), 3.46-3.38 (m, 1H), 2.11-1.81 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 137.0, 126.7, 126.2, 126.0, 78.7, 71.7, 71.3, 70.8, 69.5, 65.7, 53.3, 27.0; **HRMS** (ESI-TOF) *m/z*:, $[M+H]^+$ Calcd for $C_{13}H_{20}NO_3$ 238.1443; Found 238.1441.

General procedure for synthesis of *N*-Cbz protected Jaspine B C2-alkylamine analogs 31.1-31.3

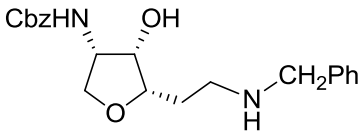
To a stirred, ice-cooled solution of the bicyclic lactol **26.1** (1 equiv.) in dry THF (10% solution w/v) was added $NaBH(OAc)_3$ (1.2 equiv.) followed by the respective alkyl amines (1

equiv.). The reaction mixture was allowed to attain room temperature and stirring continued for another 2-3 hours. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The aqueous layer was saturated with solid NaCl and re-extracted with EtOAc (2x). The combined extract washed sequentially with water and brine, and dried over anhydrous Na_2SO_4. Solvent was removed under vacuum, and the crude product purified by flash chromatography (MeOH: DCM + 0.1% NH_4OH = 5:95) affording the C2-alkylamino tetrahydrofuran derivatives **31.1-31.3**.



31.1

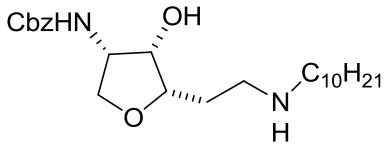
Benzyl((3*S*,4*S*,5*S*)-5-(2-(butylamino)ethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (31.1). Obtained as a light brown solid (50%): $[\alpha]_D$ 18 (*c* 0.5, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.49-7.29 (m, 5H), 5.71-5.61 (m, 1H), 5.11 (brs, 2H), 4.45-4.31 (m, 1H), 4.13-4.03 (m, 2H), 4.02-3.99 (m, 1H), 3.71-3.61 (m, 1H), 2.95-2.85 (m, 1H), 2.75-2.51 (m, 3H), 2.04-1.81 (m, 3H), 1.52-1.41 (m, 2H), 1.41-1.31 (m, 2H), 0.94-0.85 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 156.3, 136.5, 128.5, 128.0, 82.1, 70.4, 70.1, 66.7, 54.2, 43.2, 31.7, 20.3, 13.9; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for : $C_{18}H_{29}N_2O_4$: 337.2127; Found 337.2111.



31.2

Benzyl((3*S*,4*S*,5*S*)-5-(2-(benzylamino)ethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (31.2). Obtained as a light brown solid (140 mg, 39%): $[\alpha]_D$ 18 (*c* 0.5, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.49-7.25 (m, 10H), 5.75-5.61 (m, 1H), 5.12 (brs, 2H), 4.41-4.21 (m,1H), 4.15-3.91 (m, 4H), 3.85-3.68 (m, 2H), 3.67-3.59 (m, 1H), 2.91-2.81 (m, 1H), 2.61-2.48 (m, 1H), 2.01-

1.81 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 154.2, 136.3, 134.5, 126.8, 126.7, 126.5, 126.5, 126.5, 126.3, 126.2, 126.1, 126.1, 125.6, 80.1, 68.4, 68.1, 64.7, 52.2, 51.7, 40.9, 27.6; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for : $C_{21}H_{27}N_2O_4$: 371.1971; Found 371.1942.

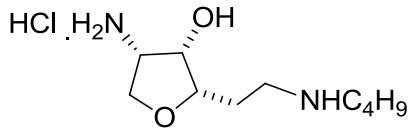


31.3

Benzyl((3*S*,4*S*,5*S*)-5-(2-(dodecylamino)ethyl)-4-hydroxytetrahydrofuran-3-yl)carbamate (31.3). Obtained as a light brown solid (95 mg, 50%): $[\alpha]_D$ 10.4 (*c* 0.5, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.49-7.31 (m, 5H), 5.71-5.61(m, 1H), 5.14 (brs, 2H), 4.41-4.31 (m, 1H), 4.11-4.02 (m, 2H), 4.01-3.91 (m, 1H), 3.71-3.61 (m, 1H), 2.95-2.81 (m, 1H), 2.71-2.51 (m, 3H), 1.99-1.85 (m, 2H), 1.51-1.42 (m, 2H), 1.41-1.21 (m, 15H), 0.95-0.81 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 156.3, 136.5, 128.4, 128.0, 82.1, 70.4, 70.1, 66.7, 54.2, 49.5, 43.3, 31.8, 29.6, 29.5, 29.4, 29.3, 27.1, 22.6, 14.1; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{41}N_2O_4$ 421.3066; Found 421.3027.

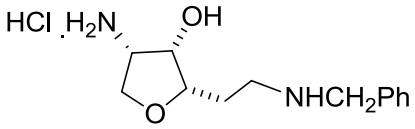
General procedure for the synthesis of C2-alkyl amine anlogs 31.4-31.6

A solution of the *N*-Cbz protected alkyl amine (**31.1-31.3**) was refluxed in 6 M HCl solution (excess) for 12-16 hours. After completion of reaction (monitored by TLC), the reaction mixture was extracted with dichloromethane (once) to remove any organic impurities and the aqueous layer concentrated under vacuum. The resulting product was subjected to high vacuum overnight to afford the pure Jaspine B C2 alkyl amine analogs **31.4-31.6** as the corresponding HCl salts.



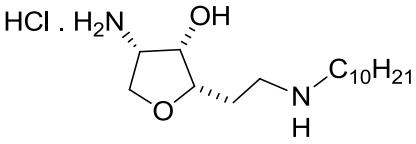
31.4

(2*S*,3*S*,4*S*)-4-Amino-2-(2-(butylamino)ethyl)tetrahydrofuran-3-ol hydrochloride (HCl salt) **(31.4)**. Obtained as a light brown hygroscopic solid (32 mg, 80%): $[\alpha]_D$ 13.6 (*c* 0.44, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 4.45-4.35 (m, 1H), 4.01-3.81 (m, 4H), 3.25-3.15 (m, 2H), 3.01-2.91 (m, 2H), 2.21-2.11 (m, 1H), 2.05-1.95 (m, 1H), 1.81-1.65 (m, 2H), 1.51-1.35 (m, 2H), 1.05-0.95 (m, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 81.8, 71.1, 69.5, 54.2, 47.0, 30.3, 29.3, 26.9, 26.1, 25.5, 20.91, 14.0; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{23}N_2O_2$: 203.1760; Found 203.1745.



31.5

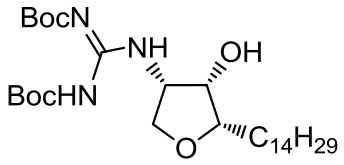
(2*S*,3*S*,4*S*)-4-Amino-2-(2-(benzylamino)ethyl)tetrahydrofuran-3-ol (HCl salt) **(31.5)**. Obtained as a light brown solid (40mg, 57%): $[\alpha]_D$ - 22.7 (*c* 0.43, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.61-7.41 (m, 5H), 4.34 (brs, 1H), 4.28-4.12 (m, 2H), 4.01-3.75 (m, 4H), 3.21-3.11 (m, 2H), 2.21-2.09 (m, 1H), 2.05-1.91 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 132.5, 131.1, 130.7, 130.5, 130.3, 81.8, 71.1, 69.5, 54.2, 52.4, 46.6, 26.7; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for : $C_{13}H_{21}N_2O_2$: 237.1603; Found 237.1604.



31.6

(2*S*,3*S*,4*S*)-4-Amino-2-(2-(decylamino)ethyl)tetrahydrofuran-3-ol (HCl salt) **(31.6).** Obtained as a light brown hygroscopic solid (43 mg, 78%): $[\alpha]_D$ 2.76 (*c* 1.12, CH_3OH); **^{1}H NMR** (500 MHz, CD_3OD) δ 4.36 (brs, 1H), 3.97-3.88 (m, 4H), 3.18 (brs, 2H), 3.0 (brs, 2H), 2.18-2.07 (m, 1H), 2.05-1.95 (m, 1H), 1.69 (brs, 2H), 1.30 (brs, 14H), 0.89 (brs, 3H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 79.1, 68.4, 66.8, 51.5, 44.2, 30.4, 28.0, 27.9, 27.8, 27.6, 25.0, 24.6, 24.2, 21.1, 11.8; **HRMS** (ESI-TOF) *m/z*: Found 287.2687 $[M+H]^+$ Calcd for $C_{16}H_{35}N_2O_2$: 287.2699).

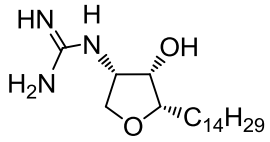
Synthesis of C4-*N,N'*-di-Boc-guanidine analog of Jaspine B (32.2)



32.2

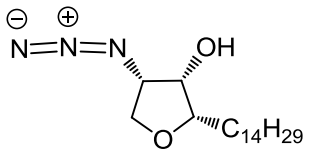
To a stirred solution of Jaspine B (25 mg, 83.19 mmol) in 1,4-dioxane (2 mL) at room temperature was added *N*,*N*'-*Di*-Boc-*N*''-triflylguanidine (**32.1**)[269] (93.5 mg, 249.6 mmol), followed by triethylamine (24 mg, 249.6 mmol).[8] After stirring for 6 h, the reaction mixture was concentrated and the residue partitioned between chloroform and water. The organic layer was separated, and the aqueous layer extracted with $CHCl_3$ (3x10 mL). The combined organic layers dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue is purified by flash chromatography (EtOAc : hexanes = 12 : 88) to afford the product **32.1** as a white solid (24 mg, 58 %): $[\alpha]_D$ 6.3 (*c* 0.19, CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 11.48 (brs, 1H), 8.91 (brs, 1H), 4.89-4.71 (m, 1H), 4.28-4.11 (m, 2H), 3.88-3.75 (m, 1H), 3.71-3.58 (m, 1H), 1.95 (brs, 1H), 1.71-1.61 (m, 2H), 1.59-1.56 (m, 2H), 1.54-1.48 (m, 15H), 1.39-1.21 (m, 25H), 0.93-0.71 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 163.46, 156.20, 152.98, 83.31, 82.06, 79.45, 72.07,

70.21, 54.03, 31.93, 29.71, 29.69, 29.68, 29.66, 29.57, 29.52, 29.37, 28.93, 28.29, 28.06, 22.70, 14.14; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{29}H_{56}N_3O_6$: 542.4169; Found 542.4123.



32.3 (as TFA Salt)

1-((4*S*,5*S*)-4-Hydroxy-5-tetradecyltetrahydrofuran-3-yl)guanidine (32.3). The *N,N*'-di-boc-guanidine Jaspine B was dissolved in a premixed solution of DCM and TFA (2 mL, 1:1) and stirred at rt for 4h. The reaction mixture is then concentrated and the residue flushed with dichloromethane (2x3 mL) under high vacuum. The resulting residue was triturated with hot dichloromethane:hexane (15 mL, 2:1). Drying of the product under high vacuum afforded the C4-guanidino Jaspine B analog (**32.3**) as a white solid (19 mg, 90%); $[\alpha]_D$ 2.2 (*c* 0.008, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 4.25-4.15 (m, 2H), 4.08-3.99 (m, 1H), 3.85-3.75 (m, 1H), 3.71-3.55 (m, 1H), 1.71-1.58 (m, 2H), 1.51-1.18 (m, 24H), 0.95-0.85 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 157.2, 82.6, 78, 70.3, 68.7, 54.3, 31.6, 31.3, 29.4, 29.3, 29.2, 29, 28.7, 25.7, 22.3, 22.2, 13; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for : $C_{19}H_{40}N_3O_2$: 342.3121; Found 342.3106.

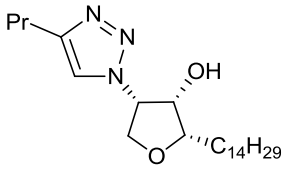


33.1

(2*S*,3*S*,4*S*)-4-azido-2-tetradecyltetrahydrofuran-3-ol (33.1) **Step 1:** To an ice-cooled solution of NaN_3 (157 mg, 2.42 mmol) in water (1 mL) was added dichloromethane (1 mL), followed by trifluoromethanesulfonic anhydride[270] (339 mg, 1.2 mmol) under vigorous stirring. After stirring for 2 h at 0 °C, the organic phase was separated and the aqueous layer extracted with

dichloromethane (3x2 mL). The combined organic layers are washed with saturated aqueous $NaHCO_3$ solution, water, brine, and dried over anhydrous Na_2SO_4.

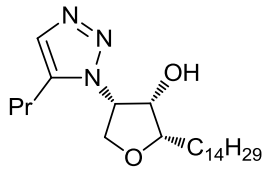
Step 2: To a solution of Jaspine B hydrochloride salt (45 mg, 0.134 mmol) in dichloromethane (1 mL) was added triethylamine (40.5 mg, 0.401 mmol) at rt. Cooled the solution to 0 °C (ice-bath) and treated dropwise with the triflyl azide (as synthesized above in Step 1). The reaction mixture was allowed to attain room temperature and stirred for 2h. The reaction was quenched by saturated aqueous $NaHCO_3$ solution (3 mL) and the organic layer separated. The aqueous layer was extracted with dichloromethane (3x3 mL), the combined extract washed with brine, and dried over anhydrous Na_2SO_4. Removal of solvent under vacuum and purification of the residue by flash chromatography (EtOAc : hexanes = 1 : 9) afforded the C4-Azido-Jaspine B (**33.1**) (34 mg, 71%) as a white shiny solid. $[\alpha]_D$ 11.8 (*c* 0.5, $CHCl_3$); IR (Neat) 3330, 2916, 2848, 2102 cm^{-1}; ^1H NMR (400 MHz, CDCl3) δ 4.26-4.09 (m, 2H), 4.04-3.95 (m, 1H), 3.92-3.73 (m, 2H), 2.17-1.95 (m, 1H), 1.71-1.57 (m, 2H), 1.46-1.16 (m, 24H), 0.92-0.85 (m, 3H).**^{13}C NMR** (125.8 MHz, CDCl3) δ 133.9, 125.0, 82.1, 72.5, 68.4, 63.7, 31.9, 31.6, 29.7, 29.6, 29.5, 29.4, 28.9, 26.1, 22.7, 22.6, 14.1.



34.1

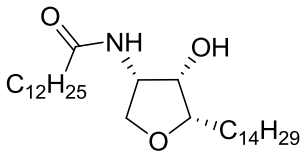
(2*S*,3*S*,4*S*)-4-(4-propyl-1H-1,2,3-triazol-1-yl)-2-tetradecyltetrahydrofuran-3-ol (34.1). To the C-4-azido Jaspine B (**33.1**) (40 mg, 0.123 mmol) was added a solution of t-BuOH and H_2O (2 mL, 1:1), followed by addition of 1-pentyne (18.4 mg, 0.27 mmol), $CuSO_4$ (15.4 mg, 0.0984 mmol), and L-Sodium Ascorbate (10 mg, 0.0490 mmol). The resulting mixture was stirred at room temperature for 20 h. After completion of the reaction, the reaction mixture was

concentrated under vacuum, and residue purified by flash chromatography (EtOAc : Hexanes = 15 : 85) to afford the 4-(1,4-disubstituted-triazolo) Jaspine B **(34.1)** as a white powder (26 mg, 67%). $[\alpha]_D$ 14.8 (*c* 0.28, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 5.31-5.18 (m, 1H), 4.61-4.41 (m, 1H), 4.31-4.11 (m, 2H), 4.03-3.89 (m, 1H), 3.61 (brs, 1H), 2.69-2.54 (m, 2H), 1.81-1.62 (m, 5H), 1.41-1.19 (m, 22H), 0.97-0.93 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 147.7, 121.1, 83, 71.8, 68.5, 63.5, 31.9, 29.7, 29.6, 29.5, 29.3, 28.9, 27.6, 26.1, 22.7, 22.4, 14.1, 13.7. **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{43}N_3O_2$ 394.3355; Found 394.3423.



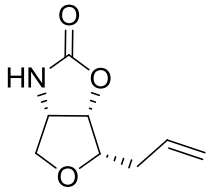
34.2

(2*S*,3*S*,4*S*)-4-(5-propyl-1H-1,2,3-triazol-1-yl)-2-tetradecyltetrahydrofuran-3-ol (34.2). To a stirred solution of C-4-azido Jaspine B (**33.1**) (15 mg, 0.046 mmol) in anhydrous THF (2 mL) was added $RuCp^*Cl(PPh_3)_2$ (3 mg, 0.004 mmol),[217] followed by addition of 1-pentyne (33 mg, 0.46 mmol). After refluxing the mixture for 24 h, solvent was removed under vacuum, and the residue purified by flash chromatography (EtOAc : hexanes = 1 : 4) to afford the 4-(1,4-disubstituted-triazolo) Jaspine B **34.2** as a light brown solid (11 mg, 61%). $[\alpha]_D$ 14.25 (*c* 0.4, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 (brs, 1H), 4.99-4.79 (m, 1H), 4.51-4.38 (m, 2H), 4.32-4.01 (m, 1H), 3.99-3.81 (m, 1H), 3.80-3.58 (m, 1H), 2.79-2.68 (m, 2H), 1.91-1.51 (m, 4H), 1.61-1.18 (m, 20H), 1.17-1.03 (m, 4H), 1.0-0.80 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 147.7, 121.4, 83.5, 72.2, 68.6, 60.4, 31.9, 29.7, 29.6, 29.4,28.8, 26, 25.2, 22.7, 25.2, 22.7, 21.5, 14.1, 13.8. **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{44}N_3O_2$ 394.3434; Found 394.3382.



35.1

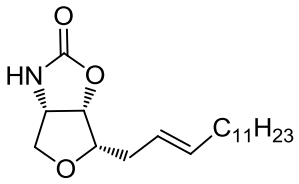
N-((3*S*,4*S*,5*S*)-4-hydroxy-5-tetradecyltetrahydrofuran-3-yl)tridecanamide (35.1). To a room temperature mixture of Jaspine B hydrochloride salt (30 mg, 0.1 mmol) in a biphasic solution of saturated aqueous $NaHCO_3$ and EtOAc (4 mL, 1:1) was added palmitoyl chloride (137 mg, 0.5 mmol) and stirred for 1 h. The organic layer was separated and the aqueous layer extracted with EtOAc (3x5 mL). The combined extract washed with brine and dried over anhydrous Na_2SO_4. Removal of solvent under vacuum and purification of the residue by flash chromatography (EtOAc : hexanes = 1 : 4) afforded the Jaspine B carboxamide analog **35.1** as a white greasy solid (24 mg, 47%): **^{1}H NMR** (400 MHz, CD_3OD) δ 4.24 (brs, 1H), 3.96-3.86 (m, 2H), 3.43-3.35 (m, 1H), 3.22-3.17 (m, 1H), 2.35-2.22 (m, 2H), 1.70-1.57 (m, 4H), 1.35 (brs, 42H), 0.93-0.84 (m, 6H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.3, 83, 69.5,67.6, 53, 29.2, 29, 28.8, 28.3, 25.8, 22.3, 13.0. **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{31}H_{61}NO_3Na$ 518.4549; Found 518.4549.



36.1

(3a*S*,6*S*,6a*S*)-6-allyltetrahydrofuro[3,4-d]oxazol-2(3H)-one (36.1). To a stirred solution of methyltriphenylphosphonium bromide (2 g, 5.62 mmol) in anhydrous THF (40 mL) at – 78 °C was added potassium tertiary butoxide (1M soln. in THF, 5 mL, 5 mmol) dropwise and the resulting solution stirred for 2 h (solution turned yellow-orange)[28]. A solution of the lactol **26.1** (0.35 g, 1.25 mmol in 40 mL anhydrous THF) was then added dropwise to the above Wittig ylide

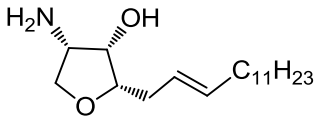
solution. After stirring for 1 h at – 78 °C, the reaction mixture was allowed to attain room temperature and stirring continued for an additional 16 h. The reaction was quenched by addition of a saturated ammonium chloride solution (5 mL), organic layer was separated and the aqueous layer extracted with EtOAc (2x10 mL). The combined extracts dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (EtOAc) to afford the C3-allyl-substituted bicyclic oxazolidine **36.1** as a white crystalline solid (77mg, 43 %): $[\alpha]_D$ 67.45 (c 0.55; CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.01-5.91 (M, 1H), 5.85-5.71 (m, 1H), 5.31-5.01 (m, 2H), 4.91-4.71 (m, 1H), 4.51-4.31 (m, 1H), 4.21-4.11 (m, 1H), 4.01-3.81 (m, 1H), 3.80-3.73 (M, 1H), 2.51-2.21 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ159.0, 132.6, 118.4, 83.5, 83.2, 73, 56.4, 35.2; **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_8H_{11}NO_3Na$: 192.0637; Found 192.0735.



36.2

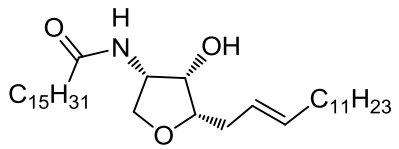
(3a*S*,6*S*,6a*S*)-6-((E)-tetradec-2-en-1-yl)tetrahydrofuro[3,4-d]oxazol-2(3H)-one (36.2). To a solution of the C2-allyl-substituted bicyclic oxazolidine **36.1** (50 mg, 0.35 mmol) in anhydrous dichloromethane (10 mL) was added 1-tridecene (0.318 g, 1.75 mmol) followed by Grubbs' second-generation catalyst (5 mg, 0.005 mmol). After refluxing for 3 h, the reaction mixture was concentrated under vacuum, and the residue purified by flash chromatography to afford the cross-metathesis product **36.2** as a white solid (43 mg, 67%): $[\alpha]_D$ 30 (*c* 0.75, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.18-5.91 (m, 1H), 5.71-5.62 (m, 1H), 5.50-5.40 (m, 1H), 4.99-4.91 (m, 1H), 4.51-4.31 (m, 1H), 4.01-3.92 (m, 1H), 3.61-3.42 (m, 1H), 2.71-2.51 (m, 2H), 2.21-1.98 (m, 2H). 1.41-1.11 (m, 19H), 0.96-0.85 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 159.6, 134.5,

124.2, 83.1, 80.8, 73.5, 57.1, 32.6, 31.9, 31.4, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 29.1, 22.7, 14.1. **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{19}H_{33}NO_3Na$ 346.2358; Found 346.2336.



36.3

(2*S*,3*S*,4*S*)-4-amino-2-((E)-tetradec-2-en-1-yl)tetrahydrofuran-3-ol (36.3). The bicyclic oxazolidine derivative **36.2** (53 mg, 0.16 mmol) was dissolved in a premixed solution of aqueous 1 M KOH:Ethanol (12 mL, 1:1) and the solution refluxed for 3 h. After removal of solvent under vacuum the residue was purified by flash chromatography to afford the C-2 alkenyl Jaspine B analog **36.3** as a white solid (37 mg, 76%): $[\alpha]_D$ 5.58 (*c* 1.2, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD) δ 5.65-5.41 (m, 2H), 4.21-4.10 (m, 1H), 4.01-3.88 (m, 1H), 3.83-3.61 (m, 3H), 2.51-2.21 (m, 2H), 2.20-1.97 (m, 2H), 1.61-1.19 (m, 18H), 0.99-0.81 (m, 3H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ132.7, 125.6, 83.0, 70.2, 68.8, 53.5, 32.3, 32.0, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0, 28.8, 22.3, 13.0. **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{18}H_{36}NO_2$: 298.2746; Found 298.2696.

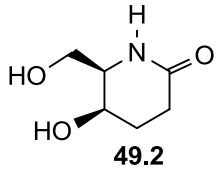


36.4

N-((3*S*,4*S*,5*S*)-4-hydroxy-5-((E)-tetradec-2-en-1-yl)tetrahydrofuran-3-yl)palmitamide (36.4). To a room temperature solution of the C-2 alkenyl Jaspine B analog **36.3** in a biphasic mixture of saturated aqueous $NaHCO_3$ and EtOAc (4 mL, 1:1) was added palmitoyl chloride (82 mg, 0.3 mmol) with stirring. After stirring for 30 min, the organic layer was separated and the aqueous layer extracted with EtOAc (3x5 mL). The combined extracts washed with brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was subjected to flash chromatography to afford Jaspine B-Ceramide hybrid **36.4** as a white greasy solid (14 mg, 40%):

$[\alpha]_D$ - 49.3(*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18-6.01 (m, 1H), 5.71-5.55 (m, 1H), 5.51-5.40 (m, 1H), 4.66-4.56 (m, 1H), 4.21-4.01 (m, 2H), 3.93-3.81 (m, 1H), 3.67-3.57 (m, 1H), 2.49-2.30 (m, 2H), 2.26-2.19 (m, 2H), 2.09-1.95 (m, 2H), 1.73-1.49 (m, 5H), 1.47-1.01 (m, 40H), 0.91-0.71 (m, 6H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ173.4, 134.1, 124.9, 81.6, 71.9, 70.7, 52.5, 36.7, 32.6, 32.5, 31.9, 30.9, 29.7 ,29.6, 29.5, 29.4, 29.3, 29.2, 25.7, 22.7, 14.2, 14.1. **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{34}H_{65}NO_3Na$ 558.4862; Found 558.4815.

5.3.3. Chapter 4: A Rapid Approach Towards Diastereoselective Synthesis of Azacarbohydrate Scaffolds of Biological Significance



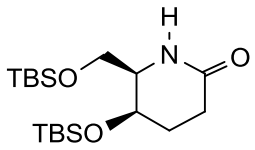
49.2

(5*R*,6*R*)-5-Hydroxy-6-(hydroxymethyl)piperidin-2-one (49.2). Step 1: To an ice-cooled solution of aminobutenolide **1** (400 mg, 1.26 mmol) in CH_2Cl_2 was added HCOOH [(99 %): 8 mL)] and stirred at the same temperature for 2 h. A second lot of HCOOH (3 mL) was added to the reaction mixture and stirring continued at 0° C for another 60 min. The reaction mixture was diluted with ethanol (5 mL) and excess solvent and HCOOH was removed under high vacuum at rt to afford the acetonide deprotected unsaturated lactone **49.1** as a viscous liquid, which was used as such for the next reaction.

Step 2: The amino alcohol **49.1** as obtained above (Step 1) was dissolved in methanol (10 mL) and catalytic $Pd(OH)_2$ (30 mg, 0.21 mmol) added to it. After flushing twice with hydrogen, the reaction mixture was stirred under hydrogen atmosphere for 18 h. After completion of reaction (monitored by IR), the reaction mixture was filtered over a Celite pad and the residue washed with hot methanol (4 x10 mL). The combined filtrate was concentrated under vacuum to afford

the free amine as a viscous liquid, which was directly used for the next reaction without any further purification.

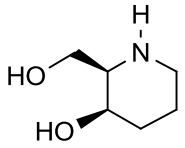
Step 3: The amino alcohol from the above reaction (Step 2) was dissolved in ethanol (10 mL) and a catalytic amount of NaOMe (2 drops) added to it. After stirring the resulting mixture at room temperature for 1 hour, solvent was removed under vacuum, and the residue subjected to flash chromatography (MeOH : CH_2Cl_2 = 1 : 4 + 1 mL of NH_4OH) to afford the pieperidinone diol **49.2** as a greasy white solid (140 mg, 76%, 3 steps): $[\alpha]_D$ - 0.316 (*c* 0.655, CH_3OH); IR (Neat) 3307, 1639, 1409, 1066 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d6): δ 7.05 (brs, 1H), 5.76 (brs, 1H), 5.09-4.89 (m, 1H), 4.84-4.72 (m, 1H), 3.85-3.61 (m, 1H), 3.18-3.08 (m, 2H), 2.32-2.18 (m, 1H), 2.17-2.03 (m, 1H), 1.89-1.74 (m, 1H), 1.70-1.59 (m, 1H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 173.3, 63.9, 62.5, 60.2, 27.3, 26.5; **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_6H_{11}NO_3Na$ 168.0637; Found 168.0613.



49.3

(5*R*,6*R*)-5-((*tert*-butyldimethylsilyl)oxy)-6-(((*tert*-butyldimethylsilyl)oxy)methyl) Piperidine 2-one (49.3). To an ice-cooled solution of the piperidinone diol **49.2** (150 mg, 1.03 mmol) in anhydrous DMF (6 mL) was added with stirring, TBDMSCl (1.086 g, 7.21 mmol) and imidazole (0.595 mg, 8.75 mmol). The reaction mixture was allowed to attain room temperature, and stirring continued for 4h. The reaction was quenched by adding ice-cold water (5 mL) and diluted with Et_2O (10 mL). The two layers were separated, and the aqueous layer extracted with Et_2O (3x5 mL). The combined extract dried over anhydrous Na_2SO_4, solvent removed under vacuum, and the residue purified by flash chromatography (EtOAc : hexanes = 3 : 7) to afford the Di-TBS protected piperidinone derivative **49.3** as a white fluffy solid (300 mg, 81%); **^{1}H**

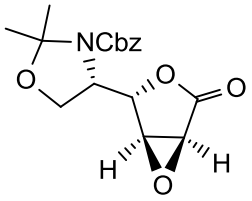
NMR (400 MHz, $CDCl_3$): δ 5.94 (s, 1H), 3.88-3.79 (m, 1H), 3.75-3.65 (m, 1H), 3.45-3.38 (m, 1H), 3.35-3.28 (m, 1H), 2.61-2.46 (m, 1H), 2.39-2.23 (m, 1H), 1.91-1.75 (m, 2H), 0.98-0.78 (m, 18H), 0.11-0.03 (m, 12H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 171.3, 66.3, 66.1, 64.7, 64.6, 64.4, 60.7, 60.3, 29, 28.2, 28.1, 27.3, 26.3, 26.1, 25.3, 25.1, 24.1, 18.2, 17.8, -3.9, -4.5, -4.9, -5.0, -5.5, -6; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{39}NO_3Si_2Na$ 396.2366; Found 396.2263.



49.5 (as HCl salt)

(2R,3R)-2-(hydroxymethyl) piperidin-3-ol hydrochloride (49.5). **Step 1**: To a solution of the Di-TBS protected piperidinone derivative **49.3** (55mg, 0.147 mmol) in anhydrous THF (4 mL) was added $BH_3.Me_2S$ (112 mg/140 mL, 1.47 mmol) and the solution refluxed for 3 h. After quenching the excess $BH_3.SMe_2$ with methanol (3 mL), the reaction mixture was concentrated under vacuum to afford the corresponding piperidine derivative **49.4** as a syrupy liquid (35 mg). The residue was directly continued for the next step without any further purification.

Step 2: The O-Di-TBS piperidine as obtained from the above reaction (Step 1) was dissolved in methanol (4 mL) and conc. HCl (5 drops) added to it. After refluxing for 3 h, solvent was removed under vacuum, and the residue triturated with ether to afford the 2,3,4-trideoxyazacarbohydrate analog **49.5** as its HCl salt (12 mg, 55%, 2 steps): $[\alpha]_D$ – 66.8 (*c* 0.6, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD) δ 3.92-3.85 (m, 1H), 3.82-3.75 (m, 1H), 3.69-3.59 (m, 1H), 3.26-3.19 (m, 1H), 2.98-2.82 (m, 2H), 2.12-2.02 (m, 1H), 2.01-1.89 (m, 1H), 1.81-1.61 (m, 1H), 1.61-1.41 (m, 1H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 64.5, 62.4, 57.9, 43.3, 31.0, 20.5; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{14}NO2$: 132.1025; Found 132.0944.

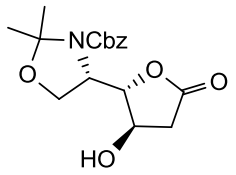


50.1

(*S*)-Benzyl 2,2-dimethyl-4-((1S,2S,5S)-4-oxo-3,6-dioxabicyclo[3.1.0]hexan-2-yl)oxazolidine-3-carboxylate (**50.1**). Step 1: To an ice-cooled solution of the aminobutenolide **1** (500 mg, 1.57 mmol) in pyridine (10 mL) was added with stirring NaOCl [(5.3 w/v %) 0.47 g/8.8 mL)].[258] The resulting solution was stirred at 0 °C for 1 h and allowed to reach rt over an additional 2h. The mixture was then poured onto CH_2Cl_2 - water (4:1) and aqueous 1 M $NaHCO_3$ solution (24 mL) added to it. The bicarbonate layer was separated, washed with ethyl acetate (once), acidified to pH 1-2, and extracted thoroughly with ethyl acetate (3x20 mL). The combined extract dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the crude product (600 mg, quantitative) as a viscous liquid. The epoxy carboxylic acid thus obtained was used as such for the next reaction.

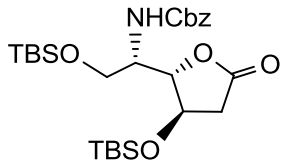
Step 2: The epoxy acid (600 mg, 1.812 mmol) from the above reaction (Step1) was dissolved in anhydrous CH_2Cl_2 and cooled to 0 °C in an ice-bath. To this solution was added EDCI (520 mg, 2.718 mmol) followed by DMAP (cat.) and stirred at the same temperature for 2h. The reaction was quenched with cold water and the organic layer was separated. The aqueous layer was saturated with solid NaCl and extracted (2x10 mL) with CH_2Cl_2. The combined extract was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue on purification by flash chromatography (EtOAc : hexanes = 2 : 3) afforded the bicyclic epoxy lactone **50.1** as a syrupy liquid (410 mg, 76 %): $[\alpha]_D$ - 48.6 (*c* 0.485, CH_3OH); IR (Neat) 2985, 2939, 1794, 1696 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$; mixture of rotamers): δ 7.29-7.48 (m, 5H), 4.99-5.39 (m, 2H), 4.31-4.51 (m, 1.5H), 3.91-4.21 (m, 3.5 H), 3.30 (2 brs, 1H), 1.51-1.81 (m, 6H); ^{13}C NMR (125.8

MHz, $CDCl_3$; mixture of rotamers) δ 169.8, 154.2, 135.5, 128.8, 95.4, 95, 79.2, 68.1, 67.7, 65.4, 64.7, 58, 57.5, 56.9, 56.4, 49.7, 49.3, 27.9, 27, 24.2, 22.7, **HRMS** (ESI-TOF) *m/z*: Found 356.1018 $[M + Na]^+$ Calcd for $C_{17}H_{19}NO_6Na$: 356.1110).



50.2

(*S*)-Benzyl 4-((2*S*,3*R*)-3-hydroxy-5-oxotetrahydrofuran-2-yl)-2,2-dimethyloxazolidine-3-car -boxylate (50.2). To a mixture of diphenyl diselenide (750 mg, 2.4 mmol) in EtOH (3 mL), $NaBH_4$ (186 mg, 4.92 mmol) was added in batches at 0 °C. After stirring at room temperature for 15 min, the solution was treated with acetic acid (0.36 mL, 6 mmol).[262] After 10 min, a solution of the epoxy lactone **50.1** (200 mg, 0.6 mmol) in THF:EtOH (4 mL, 1:1) was added to the PhSeH reagent prepared above and allowed to warm to room temperature. After stirring for 30 min, the reaction mixture was diluted with EtOAc, purged with air for 5 min and then concentrated under vacuum. The residue was purified by flash chromatography (EtOAc : hexanes = 1 : 1) to give the β-hydroxy lactone **50.2** as a syrupy liquid (170 mg, 80%): $[\alpha]_D$ 18.37 (*c* 0.8, CH_2Cl_2); **^{1}HNMR** (400 MHz, $CDCl_3$; mixture of rotamers): δ 7.29-7.41 (m, 5H), 4.95-5.38 (m, 2H), 4.42 and 4.68 (2 brs, 1H), 4.09-4.28 (m, 2H), 3.89-4.09 (m. 3H), 2.79-3.01 (m, 1H), 2.51-2.68 (m, 1H), 1.55-1.71 (m, 6H) **^{13}C NMR** (125.8 MHz, $CDCl_3$; mixture of rotamers) δ 174.9, 154.4, 135.5, 128.7, 128.5, 128.3, 128.1, 94.8, 86.4, 69.6, 68, 65, 58.7, 37.2, 36.8, 27.7, 27.5, 27, 24.3, 24.1; **HRMS** (ESI-TOF) *m/z*: Found 358.1287 $[M + Na]^+$ Calcd for $C_{17}H_{21}NO_6Na$: 358.1267).

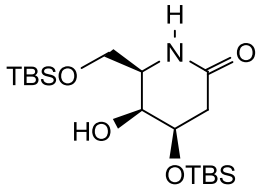


50.3

Benzyl((S)-2-((*t*-butyldimethylsilyl)oxy)-1-((2S,3R)-3-((*tert*-butyldimethylsilyl)oxy)-5-oxo-tetrahydrofuran-2-yl)ethyl)carbamate (50.3). **Step 1:** β-hydroxy lactone **50.2** (280 mg, 0.835 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to 0 °C. To this ice-cold solution added (99 %) HCOOH (10 mL) and continued stirring at 0 °C for 14 h. Diluted reaction mixture with ethanol (3 mL) and concentrated under high vacuum at rt. The residue was adsorbed to silica and subjected to flash chromatography to afford the corresponding acetonide deprotected dihydroxy lactone as a viscous liquid (174 mg, 70%): $[\alpha]_D$ 18.4 (*c* 0.8; CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.41-7.29 (m, 5H), 5.71 (brs, 1H), 5.10 (s, 2H), 4.59-4.41 (m, 1H), 4.38-4.29 (m, 1H), 3.95-3.65 (m, 3H), 2.92-2.79 (m, 1H), 2.61-2.41 (m, 1H), 1.99-1.71 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 176.9, 157.1, 136, 128.6, 128, 86.5, 68.5, 67.2, 60.9, 52.9, 36.9; **HRMS** (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{14}H_{17}NO_6Na$ 318.0954; Found 318.0921.

Step 2: To a room temperature solution of the above dihydroxy lactone (40 mg, 0.136 mmol) in anhydrous DMF (1 mL) was added imidazole (55.5 mg, 0.816 mmol), and TBDMSCl (102.5 mg, 0.68 mmol) and stirred for 14 h. After quenching the reaction with ice water (4 mL), the solution was extracted with EtOAc (3x10 mL), the combined extract washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue on purification by flash column chromatography (EtOAc : hexanes = 1 : 4) afforded the O-Di -TBS protected lactone **50.4** (60 mg, 75 %, 2 steps) as a shiny white solid; $[\alpha]_D$ 23.3 (*c* 0.88; CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.49-7.31 (m, 5H), 5.39-5.29 (m, 1H), 5.18-5.02 (m, 2H), 4.59-4.47 (m, 1H,), 4.38-4.29 (m, 1H), 3.83-3.75 (m, 1H), 3.71-3.61 (m, 1H), 3.61-3.48 (m, 1H), 2.99-2.85 (m, 1H), 2.49-2.38

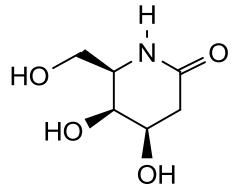
(m, 1H), 0.99-0.79 (m, 30H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 175.5, 156.4, 136, 128.6, 128.4, 86.2, 69.5, 67.3, 61.3, 52.7, 37.6, 25.8, 25.6, 18.2, 17.8, -4.8, -5, -5.5; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{26}H_{45}NO_6Si_2Na$: 546.2683; Found 546.2631.



50.4

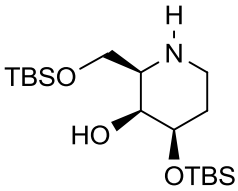
(4*R*,5*S*,6*R*)-4-((*tert*-butyldimethylsilyl)oxy)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-hydroxypiperidin-2-one (50.4) **Step 1**: The O-di-TBS protected lactone **50.3** (250 mg, 0.47 mmol) was dissolved in ethanol (10 mL) and $Pd(OH)_2$ (10 mg, 10 mol %) added to it. Degassing of the reaction mixture with hydrogen was followed by stirring of the reaction mixture under hydrogen atmosphere for 16 h. After completion of reaction, the mixture was filtered through celite, and the residue washed with warm ethanol (5x5 mL). The combined filtrate is concentrated under vacuum to afford the free amine which was used for the next reaction without any further purification.

Step 2: The amino lactone obtained from the previous reaction (Step 1) was dissolved in methanol (15 mL) and a catalytic amount of sodium methoxide added to it. After stirring the reaction mixture at room temperature for 8 h, solvent was removed under vacuum. Purification of the residue by flash chromatography (hexanes : EtOAc = 3 : 2) afforded the lactam **50.4** as a white solid (120 mg, 60%): IR (Neat) 2957, 1652 cm^{-1}; $[\alpha]_D$ - 94.9 (*c* 0.28; CH_2Cl_2); **^{1}H NMR** (400 MHz, CD_3OD) δ 5.9 (s, 1H), 4.21-4.14 (m, 1H), 3.91-3.79 (m, 1H), 3.71-3.45 (m, 3H), 2.55 (brs, 2H), 2.14 (brs, 1H, exchangeable with D_2O), 0.99-0.81 (m, 18H), 0.21-0.05 (m, 12H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 169.5, 69, 68.3, 65, 55.4, 37.7, 25.8, 18.2, -4.5, -4.8, -5.4, -5.5; **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{39}NO_4Si_2Na$: 412.2315; Found 412.2323.



50.5

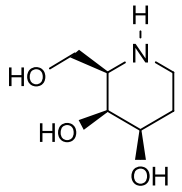
(4*R*,5*S*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2-one (**50.5**). A solution of the di-TBS lactam **50.4** (30 mg, 0.079 mmol) in methanol (4 mL) was treated with a few drops of conc. HCl (5 drops) and the reaction mixture stirred at room temperature for 3h. Removal of solvent under vacuum afforded the trihydroxy lactam **50.5** as a greasy solid (10 mg, 95%); $[\alpha]_D$ - 29.27 (*c* 0.75; CH_3OH); **^{1}H NMR** (400 MHz, D_2O): δ 4.21-4.17 (m, 1H), 3.98-3.89 (m, 1H), 3.80-3.66 (m, 2H), 3.55-3.49 (m, 1H), 2.71-2.61 (m, 1H), 2.51-2.41 (m, 1H); **^{13}C NMR** (125.8 MHz, D_2O) δ 173.5, 66.1, 61.3, 55.4, 35.9; **HRMS** (ESI-TOF) *m/z*: Found 162.0774 [M+H] + Calcd for $C_6H_{12}NO_4$: 162.0766).



50.6

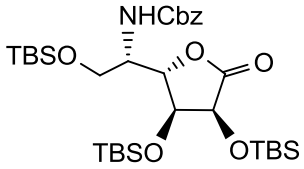
(2*R*,3*S*,4*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-2-(((*t*-butyldimethylsilyl)oxy)methyl) piperidin-3-ol (50. 6). Di-*O*TBS piperidinone **50.4** (60 mg, 0.154 mmol) was dissolved in anhydrous THF (6 mL) and $BH_3.SMe_2$ (117 mg/0.146 mL, 1.54 mmol) added to it. After refluxing for 2 h, excess $BH_3.SMe_2$ was quenched with methanol (3 mL), and solvents removed under vacuum. Subjecting the residue to flash chromatography (EtOAc : hexanes = 5 : 95) afforded the O-Di-TBS piperidine **50.6** as a greasy solid (32 mg, 55%): $[\alpha]_D$ - 52.3 (*c* 0.6, CH_2Cl_2); IR (Neat) 3508, 3245, 2950, 2896, 1461 cm^{-1} ; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.58-4.48 (m, 1H), 4.18-4.09 (m, 1H), 3.96-3.88 (m, 1H), 3.71-3.51 (m, 2H), 3.21-2.91 (m, 2H), 2.70-2.62 (m, 1H), 1.94-1.83 (m, 2H), 1.64-1.75 (m, 1H), 0.75-0.99 (m, 18H), 0.02-0.21 (m, 12H); **^{13}C NMR** (125.8 MHz,

CD_3OD) δ 67.7, 67.1, 62.9, 57.6, 46.7, 31.1, 25.9, 25.7, 18.4, 18, -4.4, -5, -5.3, -5.6; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{18}H_{42}NO_3Si_2$: 376.2703; Found 376.2668.



50.7 (4-*epi*-fagomine)
(as HCl salt)

(2R,3S,4R)-2-(hydroxymethyl)piperidine-3,4-diol (**50.7**). A solution of di-TBS piperidine **50.6** (15 mg, 0.038 mmol) in methanol (5 mL) and conc. HCl (5 drops) was refluxed for 3 h. Removal of solvent and drying of the product under high vacuum afforded 4-epi-fagomine (**50.7**) as its hydrochloride salt (5.5 mg, 79%): $[\alpha]_D$ - 7.3 (*c* 0.3, CH_3OH); **^{1}H NMR** (400 MHz, CD_3OD): δ 4.11-3.91 (m, 1H), 3.89-3.75 (m, 2H), 3.71-3.61 (m, 1H), 3.41-3.15 (m, 2H), 3.11-2.95 (m, 1H), 2.11-1.71 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 66.6, 65.5, 58, 56.2, 38.1, 27.8; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_6H_{14}NO_3$: 148.0974; Found 148.0968.

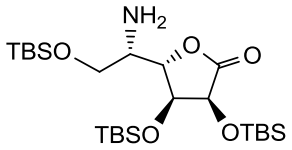


51.2

Benzyl((*S*)-1-((2*S*,3*S*,4*S*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-5-oxotetrahydrofuran-2-yl)-2-((*tert*-butyldimethylsilyl)oxy)ethyl)carbamate (51.2). **Step 1**: To an ice-cooled solution of acetonide diol **51.1** (90 mg, 0.256 mmol)[26] in CH_2Cl_2 (4 mL) was added HCOOH (99%, 4 mL) and the solution stirred at the same temperature for 5h. After removal of solvent under vacuum, the residue was purified by flash chromatography (MeOH: CH_2Cl_2 = 4:96) to afford the acetonide deprotected triol as a white solid (75 mg, 95%): **^{1}H NMR** (400 MHz, CD_3OD) δ 7.41-7.18 (m, 5H), 5.21-5.01 (m, 2H), 4.65-3.99 (m, 2H), 4.38-4.18 (m, 2H), 3.75-3.71 (brs, 1H),

3.65-3.61 (m, 2H); **^{13}C NMR** (125.8 MHz, CD_3OD) δ 176.4, 157.4, 136.7, 128.3, 127.8, 127.6, 83.6, 69, 68.4, 66.6, 60.6, 52.9; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{18}NO_7$ 312.1083; Found 312.1085.

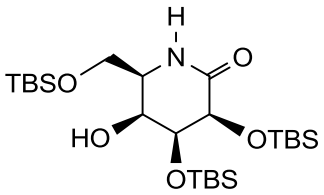
Step 2: To a room temperature solution of the triol **51.1** (0.24 g, 0.77 mmol) in anhydrous DMF (5 mL) was added imidazole (0.42 g, 6.16 mmol) and TBDMSCl (0.812 g, 5.39 mmol) sequentially. After stirring for 2 h, the reaction was quenched by ice water (10 mL) and extracted with EtOAc (3x20 mL). The combined extract washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (hexanes: EtOAc = 95 : 5) to afford the tri-*O*-TBS protected lactone **51.2** as a white solid (320 mg, 77%, 2 steps): **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 5H), 5.38 (d, *J* = 8.8 Hz, 1H), 5.24 and 5.05 (2d, *J* = 12 Hz, 2H), 4.62 (d, *J* = 4.4 Hz, 1H), 4.29-4.19 (m, 2H), 3.91-3.81 (m, 1H), 3.69-3.61 (m, 1H), 3.58-3.51 (m, 1H), 0.99-0.77 (m, 27 H), 0.22-0.01(m, 18H); **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 174.6, 156.4, 136.1, 128.7, 128.5, 82.8, 71.1, 69.8, 67.3, 61.2, 52.2, 25.8, 25.6, 18.4, 18.2, 18.1, -4.6, -4.8, -5.2, -5.5**;** **HRMS** (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{59}NO_5Si_3Na$ 644.3599; Found 644.3571.



51.3

(3*S*,4*S*,5*S*)-5-((*S*)-1-Amino-2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3,4-bis((*tert*-butyldimethyl-silyl)oxy)dihydrofuran-2(3H)-one (51.3). The trisilylated lactone **51.2** (220 mg, 0.52 mmol) was dissolved in anhydrous ethanol (5 mL) and $Pd(OH)_2$ (10 mg, 13 mol%) added to it. After degassing the mixture with hydrogen, the reaction mixture was stirred under hydrogen atmosphere at room temperature for 19 h. The reaction mixture was filtered over celite bed and

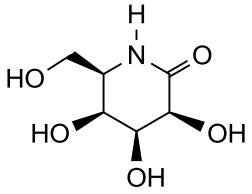
the residue was washed thoroughly with EtOAc (3x10 mL). The combined filtrate was concentrated under vacuum and the residue purified by flash chromatography (hexanes : EtOAc = 3 : 2) to afford the free amino lactone **51.3** as a white solid (160 mg, 63%): $[\alpha]_D$ -6.6 [*c* 0.45, CH_2Cl_2]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.50 (s, 2H), 4.16 (d, *J* = 8.4 Hz, 1H), 3.69-3.58 (m, 2H), 2.91-2.75 (m, 1H), 1.56 (brs, 2H, exchangeable with D_2O), 0.99-0.81 (m, 27H), 0.21-0.05 (m, 18H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 174.7, 86, 71.2, 70.3, 64.4, 53.3, 25.9, 25.8, 26.7, 18.4, 18.2, 18.1, -4.5, -4.6, -4.8, -4.9, -5.5; **HRMS** (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{54}NO_5Si_3$: 520.3310; Found 520.3169.



51.4

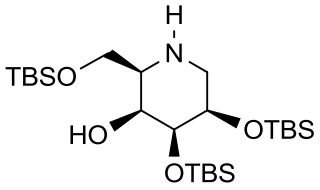
(3*S*,4*S*,5*S*,6*R*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-6-(((*tert*-butyldimethylsilyl)oxy) methyl)-5-hydroxypiperidin-2-one (**51.4**). A solution of the amino lactone **51.3** (35 mg, 0.067 mmol) in anhydrous methanol (2 mL) and a catalytic amount of NaOMe (1 drop) was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) and subjected to rotatory evaporation under vacuum to remove methanol. The residue thus obtained was taken up in CH_2Cl_2 (25 mL) and washed with water and brine. After drying over anhydrous Na_2SO_4 and removal of solvent under vacuum, the residue was purified by flash chromatography (hexanes : EtOAc = 9 : 1) to afford the piperidinone **51.4** as a white solid (24 mg, 69%): IR (Neat) 2952, 2929, 2910, 2858, 1674 cm^{-1}; $[\alpha]_D$ - 78.7 (*c* 0.24, H_2O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.92 (s, 1H, exchangeable with D_2O), 4.20 (s, 1H), 3.98 (s, 1H), 3.89-3.81 (m, 1H), 3.79-3.61 (m, 2H), 3.52-3.41 (m, 1H), 3.37 (s, 1H, exchangeable with D_2O), 0.99-0.77 (m, 27H), 0.18-0.05 (m, 18H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 172.4, 73.4, 69.3, 69.2, 64, 57.1, 27.4,

26.4, 25.4, 25.3, 24.3, 18.4, 18.3, 18, -3.6, -4.2, -4.3, -4.5, -4.9, -5.4; **HRMS** (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{24}H_{53}NO_5Si_3Na$ 542.3129; Found 542.3065.



51.5

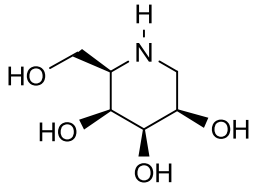
(3*S*,4S,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidin-2-one (**51.5**). A solution of the *O*-Tri-TBS piperidinone **51.4** (350 mg, 0.67 mmol) in methanol (10 mL) and conc. HCl (5 drops) was refluxed for 6h. After removal of solvent under vacuum, the polyhydroxy lactam **51.5** was obtained as a light brown solid (108 mg, 86%): $[\alpha]_D$ - 78.8 (*c* 0.9, CH_2Cl_2); IR (Neat) 3274, 3220, 1650 cm^{-1}; **^{1}H NMR** (400 MHz, D_2O): δ 4.28-4.21 (m, 2H), 4.01-3.98 (m, 1H), 3.81-3.69 (m, 2H), 3.52-3.43 (m, 1H); **^{13}C NMR** (125.8 MHz, D_2O) δ 173.9, 71.6, 68.1, 65.1, 60.3, 55.6; **HRMS** (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_6H_{11}NO_5Na$ 200.0535; Found 200.0535.



51.6

(2*R*,3*S*,4*S*,5*R*)-4,5-bis((*tert*-butyldimethylsilyl)oxy)-2-(((*tert*-butyldimethylsilyl)oxy)methyl) piperidin-3-ol (51.6). A solution of the tri-*O*TBS lactam **51.4** (60 mg, 0.115 mmol) in anhydrous THF (4 mL) and BH_3•DMS (52 mg/0.065 mL, 0.69 mmol) was refluxed vigorously for 2h. After cooling to room temperature, the reaction mixture was quenched with methanol (3 mL) and solvent removed under vacuum. The residue was purified by flash chromatography (hexanes: EtOAc = 85:15) to afford the piperidine **51.6**. (34 mg, 59%) as a viscous liquid: $[\alpha]_D$ - 24.9 (*c* 0.9, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.41-4.31 (m, 1H), 4.10 (s, *J*=10.4 Hz, 1H), 3.83

(d, *J*=10.4 Hz, 1H), 3.78-3.55 (m, 3H), 3.28-3.02 (m, 2H), 2.98-2.81 (m, 1H), 1.89-1.51 (m, 1H), 0.99-0.71 (m, 27H), 0.31-0.25 (m, 18H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 73.9, 69, 67.8, 60.4, 56.8, 51.9, 29.7, 26.1, 26, 18.5, 18.2, 18.1, -2.6, -3.9, -4.3, -4.7, -5, -5.4; **HRMS** (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{56}NO_4Si_3$ 506.3517; Found 506.3499.



51.7 (*talo*-DNJ)
(as HCl salt)

(2*R*,3*S*,4*R*,5*R*)-2-(Hydroxymethyl) piperidine-3,4,5-triol (51.7). To a solution of the tri-O-TBS piperidine **51.6** (30 mg, 0.0593 mmol) in methanol (4 mL) was added conc. HCl (5 drops) and the solution refluxed for 6 h. Removal of solvent and drying of the product under high vacuum afforded *talo*-deoxynojirimycin (**51.7**) as its HCl salt (12 mg, 100%): $[\alpha]_D$ - 13.6 (*c* 0.7, CH_3OH); **^{1}H NMR** (400 MHz, MeOD): δ 4.02-3.96 (m, 1H), 3.81-3.70 (m, 3H), 3.71-3.61 (m, 1H), 3.28-3.18 (m, 1H), 3.11-2.92 (m, 2H); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 71.9, 67.2, 66.4, 59.1, 56.6, 43.4; **HRMS** (ESI-TOF) *m/z*: (ES-) (M-H) Calcd for $C_6H_{12}NO_4$ 162.0766; Found 162.0783.

5.4 References

(1) Coppola, G. M.; Schuster, H. F.: *Asymmetric synthesis: construction of chiral molecules using amino acids.*; A Wiley-Interscience Publication East Hanover, NJ, 1987. pp. p 1-7, 127-145.

(2) Hanessian, S.; Giroux, S.; Merner, L. B.: *Design and Strategy in Organic Synthesis From the Chiron Approach to Catalysis*; Wiley-VCH: Germany, 2012. pp. 171-191.

(3) Stauffer, C. S.; Datta, A. Stereoselective synthesis of a novel carbamoyl oxybiotin. *Tetrahedron Lett.* **2005**, *46*, 6469-6471.

(4) Mohapatra, D. K.; Datta, A. Di-tert-Butyl Pyrocarbonate Mediated Cyclodehydration of N-Acyl Amino Acids into Functionalized Oxazoles and Acylanthranils#. *Synlett* **1996**, *1996*, 1129-1130.

(5) Ravi Kumar, J. S.; Datta, A. Efficient Conversion of (S)-Methionine into (R)-Garner Aldehyde. *Tetrahedron Lett.* **1997**, *38*, 6779-6780.

(6) Ravi Kumar, J. S.; Datta, A. First syntheses of (2S, 3S)- and (2S, 3R)-m-prenyl-β-hydroxytyrosine derivatives: Bioactive amino acid fragment of a substance P antagonist novel cyclic heptapeptide. *Tetrahedron Lett.* **1997**, *38*, 473-476.

(7) Veeresha, G.; Datta, A. Stereoselective synthesis of (−)-N-Boc-statine and (−)-N-Boc-Norstatine. *Tetrahedron Lett.* **1997**, *38*, 5223-5224.

(8) Varadarajan, S.; Mohapatra, D. K.; Datta, A. Studies towards the total syntheses of solandelactones: Stereoselective synthesis of the cyclopropane - lactone segment. *Tetrahedron Lett.* **1998**, *39*, 1075-1078.

(9) Veeresa, G.; Datta, A. Stereoselective synthesis of the antifungal antibiotic (+)-preussin. *Tetrahedron* **1998**, *54*, 15673-15678.

(10) Veeresa, G.; Datta, A. Stereoselective synthesis of chloramphenicol from D-serine. *Tetrahedron Lett.* **1998**, *39*, 8503-8504.

(11) Veeresa, G.; Datta, A. Stereoselective synthesis of (2S, 3R)-N-Boc-3-hydroxyglutamic acid. *Tetrahedron Lett.* **1998**, *39*, 3069-3070.

(12) Veeresa, G.; Datta, A. Stereoselective Synthesis of Polyoxamic Acid from (R)-Phenylglycine. *Tetrahedron Lett.* **1998**, *39*, 119-122.

(13) Kiran Kumar, K.; Datta, A. Stereoselective total synthesis of (+)-azimic acid. *Tetrahedron* **1999**, *55*, 13899-13906.

(14) Ravi Kumar, J. S.; Datta, A. Stereoselective synthesis of N-Boc-galantinic acid ethyl ester. *Tetrahedron Lett.* **1999**, *40*, 1381-1384.

(15) Ravi Kumar, J. S.; Roy, S.; Datta, A. Stereoselective synthesis of novel cyclopropyl analogues of known cysteine protease inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 513-514.

(16) Datta, A.; Kumar, J. S. R.; Roy, S. Stereoselective total synthesis of(-)-deoxoprosophylline. *Tetrahedron* **2001**, *57*, 1169-1173.

(17) Liang, N.; Datta, A. Stereoselective total synthesis of cis- and trans-3-hydroxypipecolic acid. *J. Org. Chem.* **2005**, *70*, 10182-10185.

(18) Liang, N.; Srinivas, P.; Datta, A. A stereoselective route to cis-2-phenyl-3-piperidinol. *Tetrahedron Lett.* **2005**, *46*, 7221-7223.

(19) Stauffer, C. S.; Datta, A. Efficient synthesis of (S,S)-ethambutol from l-methionine. *Tetrahedron* **2002**, *58*, 9765-9767.

(20) Khalaf, J. K.; Datta, A. An efficient and highly stereocontrolled route to bulgecinine hydrochloride. *J. Org. Chem.* **2004**, *69*, 387-390.

(21) Khalaf, J. K.; Datta, A. Stereoselective route to the ezoaminuroic acid core of the ezomycins. *J. Org. Chem.* **2005**, *70*, 6937-6940.

(22) Khalaf, J. K.; Datta, A. A concise, asymmetric synthesis of (2R,3R)-3-hydroxyaspartic acid. *Amino Acids* **2008**, *35*, 507-510.

(23) Datta, A.; Kumar, J. S. R.; Roy, S. Stereoselective total synthesis of (−)-deoxoprosophylline. *Tetrahedron* **2001**, *57*, 1169-1173.

(24) Datta, A.; Veeresa, G. A stereoselective route to hydroxyethylamine dipeptide isosteres. *J Org Chem* **2000**, *65*, 7609-7611.

(25) Stauffer, C. S.; Bhaket, P.; Fothergill, A. W.; Rinaldi, M. G.; Datta, A. Total synthesis and antifungal activity of a carbohydrate ring-expanded pyranosyl nucleoside analogue of nikkomycin B. *J. Org. Chem.* **2007**, *72*, 9991-9997.

(26) Bhaket, P.; Stauffer, C. S.; Datta, A. Complex peptidyl nucleoside antibiotics: efficient syntheses of the glycosyl nucleoside amino acid cores. *J. Org. Chem.* **2004**, *69*, 8594-8601.

(27) Mohapatra, D. K.; Datta, A. Stereoselective Synthesis of a Key Precursor of Halicholactone and Neohalicholactone. *J. Org. Chem.* **1998**, *63*, 642-646.

(28) Bhaket, P.; Morris, K.; Stauffer, C. S.; Datta, A. Total Synthesis of Cytotoxic Anhydrophytosphingosine Pachastrissamine (Jaspine B). *Org. Lett.* **2005**, *7*, 875-876.

(29) Garner, P. Stereocontrolled addition to a penaldic acid equivalent: an asymmetric of threo-β-hydroxy-L-glutamic acid. *Tetrahedron Lett.* **1984**, *25*, 5855-5858.

(30) Passiniemi, M.; Koskinen, A. M. P. Garner's aldehyde as a versatile intermediate in the synthesis of enantiopure natural products. *Beilstein J. Org. Chem.* **2013**, *9*, 2641-2659.

(31) Evans, D. A.; Bartroli, J.; Shih, T. L. Enantioselective aldol condensations. 2. Erythro-selective chiral aldol condensations via boron enolates. *J. Am. Chem. Soc.* **1981**, *103*, 2127-2129.

(32) Enders, D.: *Alkylation of Chiral Hydrazones*; J.D. Morrison ed.; Academic Press: Orlanda, FL, 1984; Vol. 3. pp. 275-339.

(33) Job, A.; Janeck, C. F.; Bettray, W.; Peters, R.; Enders, D. The SAMP-/RAMP-hydrazone methodology in asymmetric synthesis. *Tetrahedron* **2002**, *58*, 2253-+.

(34) Corey, E. J.; Bakshi, R. K.; Shibata, S. Highly enantioselective borane reduction of ketones catalyzed by chiral oxazaborolidines. Mechanism and synthetic implications. *J. Am. Chem. Soc.* **1987**, *109*, 5551-5553.

(35) Poon, K. W. C.; Liang, N.; Datta, A. De Novo Synthetic Route to a Combinatorial Library of Peptidyl Nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2008**, *27*, 389-407.

(36) Gottlieb, M. S.; Schroff, R.; Schanker, H. M.; Weisman, J. D.; Fan, P. T.; Wolf, R. A.; Saxon, A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **1981**, *305*, 1425-1431.

(37) Masur, H.; Michelis, M. A.; Greene, J. B.; Onorato, I.; Stouwe, R. A.; Holzman, R. S.; Wormser, G.; Brettman, L.; Lange, M.; Murray, H. W.; Cunningham-Rundles, S. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. *N. Engl. J. Med.* **1981**, *305*, 1431-1438.

(38) Siegal, F. P.; Lopez, C.; Hammer, G. S.; Brown, A. E.; Kornfeld, S. J.; Gold, J.; Hassett, J.; Hirschman, S. Z.; Cunningham-Rundles, C.; Adelsberg, B. R.; et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N. Engl. J. Med.* **1981**, *305*, 1439-1444.

(39) Kared, H.; Lelievre, J. D.; Donkova-Petrini, V.; Aouba, A.; Melica, G.; Balbo, M.; Weiss, L.; Levy, Y. HIV-specific regulatory T cells are associated with higher CD4 cell counts in primary infection. *AIDS* **2008**, *22*, 2451-2460.

(40) Niu, M. T.; Stein, D. S.; Schnittman, S. M. Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment intervention in humans and animal retrovirus infections. *J. Infect. Dis.* **1993**, *168*, 1490-1501.

(41) Daar, E. S.; Little, S.; Pitt, J.; Santangelo, J.; Ho, P.; Harawa, N.; Kerndt, P.; Glorgi, J. V.; Bai, J.; Gaut, P.; Richman, D. D.; Mandel, S.; Nichols, S.; Los Angeles County Primary, H. I. V. I. R. N. Diagnosis of primary HIV-1 infection. Los Angeles County Primary HIV Infection Recruitment Network. *Ann. Intern. Med.* **2001**, *134*, 25-29.

(42) http://www.who.int/gho/hiv/en/.

(43) Antiretroviral drugs used in the treatment of HIV infection. http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm.

(44) Progress report 2011: Global HIV/AIDS response. http://www.who.int/hiv/pub/progress_report2011/en/index.html.

(45) HIV/AIDS Statistics and Surveillance - AIDS Surveillance - General Epidemiology(through 2007). http://www.cdc.gov/hiv/topics/surveillance/resources/slides/epidemiology/index.htm.

(46) Mocroft, A.; Ledergerber, B.; Katlama, C.; Kirk, O.; Reiss, P.; d'Arminio Monforte, A.; Knysz, B.; Dietrich, M.; Phillips, A. N.; Lundgren, J. D.; Euro, S. s. g. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet* **2003**, *362*, 22-29.

(47) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 7096-7100.

(48) Mehellou, Y.; De Clercq, E. Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go? *J. Med. Chem.* **2010**, *53*, 521-538.

(49) Dunn, B. M.; Goodenow, M. M.; Gustchina, A.; Wlodawer, A. Retroviral proteases. *Genome Biol* **2002**, *3*, REVIEWS3006.

(50) Vondrasek, J.; Wlodawer, A. HIVdb: a database of the structures of human immunodeficiency virus protease. *Proteins* **2002**, *49*, 429-431.

(51) De Clercq, E. Strategies in the design of antiviral drugs. *Nat Rev Drug Discov* **2002**, *1*, 13-25.

(52) Wlodawer, A.; Gustchina, A. Structural and biochemical studies of retroviral proteases. *Biochim. Biophys. Acta* **2000**, *1477*, 16-34.

(53) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A.; Scolnick, E. M.; Sigal, I. S. Active human immunodeficiency virus protease is required for viral infectivity. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 4686-4690.

(54) Kovalevsky, A. Y.; Chumanevich, A. A.; Liu, F.; Louis, J. M.; Weber, I. T. Caught in the Act: the 1.5 A resolution crystal structures of the HIV-1 protease and the I54V mutant reveal a tetrahedral reaction intermediate. *Biochemistry* **2007**, *46*, 14854-14864.

(55) Chen, X.; Tropsha, A. Relative binding free energies of peptide inhibitors of HIV-1 protease: the influence of the active site protonation state. *J. Med. Chem.* **1995**, *38*, 42-48.

(56) Tyndall, J. D.; Pattenden, L. K.; Reid, R. C.; Hu, S. H.; Alewood, D.; Alewood, P. F.; Walsh, T.; Fairlie, D. P.; Martin, J. L. Crystal structures of highly constrained substrate and hydrolysis products bound to HIV-1 protease. Implications for the catalytic mechanism. *Biochemistry* **2008**, *47*, 3736-3744.

(57) Torbeev, V. Y.; Mandal, K.; Terechko, V. A.; Kent, S. B. Crystal structure of chemically synthesized HIV-1 protease and a ketomethylene isostere inhibitor based on the p2/NC cleavage site. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4554-4557.

(58) Hyland, L. J.; Tomaszek, T. A., Jr.; Roberts, G. D.; Carr, S. A.; Magaard, V. W.; Bryan, H. L.; Fakhoury, S. A.; Moore, M. L.; Minnich, M. D.; Culp, J. S.; et al. Human immunodeficiency virus-1 protease. 1. Initial velocity studies and kinetic characterization of reaction intermediates by 18O isotope exchange. *Biochemistry* **1991**, *30*, 8441-8453.

(59) Hyland, L. J.; Tomaszek, T. A., Jr.; Meek, T. D. Human immunodeficiency virus-1 protease. 2. Use of pH rate studies and solvent kinetic isotope effects to elucidate details of chemical mechanism. *Biochemistry* **1991**, *30*, 8454-8463.

(60) Polgar, L.; Szeltner, Z.; Boros, I. Substrate-dependent mechanisms in the catalysis of human immunodeficiency virus protease. *Biochemistry* **1994**, *33*, 9351-9357.

(61) Porter, D. J.; Hanlon, M. H.; Furfine, E. S. HIV-1 protease: characterization of a catalytically competent enzyme-substrate intermediate. *Biochemistry* **2002**, *41*, 1302-1307.

(62) Szeltner, Z.; Polgar, L. Rate-determining steps in HIV-1 protease catalysis. The hydrolysis of the most specific substrate. *J. Biol. Chem.* **1996**, *271*, 32180-32184.

(63) Wlodawer, A.; Vondrasek, J. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249-284.

(64) Mahalingam, B.; Louis, J. M.; Hung, J.; Harrison, R. W.; Weber, I. T. Structural implications of drug-resistant mutants of HIV-1 protease: high-resolution crystal structures of the mutant protease/substrate analogue complexes. *Proteins* **2001**, *43*, 455-464.

(65) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; et al. Rational design of peptide-based HIV proteinase inhibitors. *Science* **1990**, *248*, 358-361.

(66) Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; et al. L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 4096-4100.

(67) Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X. P.; et al. ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 2484-2488.

(68) Kempf, D. J.; Sham, H. L.; Marsh, K. C.; Flentge, C. A.; Betebenner, D.; Green, B. E.; McDonald, E.; Vasavanonda, S.; Saldivar, A.; Wideburg, N. E.; Kati, W. M.; Ruiz, L.; Zhao, C.; Fino, L.; Patterson, J.; Molla, A.; Plattner, J. J.; Norbeck, D. W. Discovery of ritonavir, a potent inhibitor of HIV protease with high oral bioavailability and clinical efficacy. *J. Med. Chem.* **1998**, *41*, 602-617.

(69) Kaldor, S. W.; Kalish, V. J.; Davies, J. F., 2nd; Shetty, B. V.; Fritz, J. E.; Appelt, K.; Burgess, J. A.; Campanale, K. M.; Chirgadze, N. Y.; Clawson, D. K.; Dressman, B. A.; Hatch, S. D.; Khalil, D. A.; Kosa, M. B.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, S. H.; Su, K. S.; Tatlock, J. H. Viracept (nelfinavir mesylate, AG1343): a potent, orally bioavailable inhibitor of HIV-1 protease. *J. Med. Chem.* **1997**, *40*, 3979-3985.

(70) Waters, L.; Nelson, M. Why do patients fail HIV therapy? *Int J Clin Pract* **2007**, *61*, 983-990.

(71) Deeks, S. G.; Lewin, S. R.; Havlir, D. V. The end of AIDS: HIV infection as a chronic disease. *The Lancet* **2013**, *382*, 1525-1533.

(72) Sham, H. L.; Kempf, D. J.; Molla, A.; Marsh, K. C.; Kumar, G. N.; Chen, C. M.; Kati, W.; Stewart, K.; Lal, R.; Hsu, A.; Betebenner, D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Saldivar, A.; Wideburg, N.; Chen, X.; Niu, P.; Park, C.; Jayanti, V.; Grabowski, B.; Granneman, G. R.; Sun, E.; Japour, A. J.; Leonard, J. M.; Plattner, J. J.; Norbeck, D. W. ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease. *Antimicrob. Agents Chemother.* **1998**, *42*, 3218-3224.

(73) Bold, G.; Fassler, A.; Capraro, H. G.; Cozens, R.; Klimkait, T.; Lazdins, J.; Mestan, J.; Poncioni, B.; Rosel, J.; Stover, D.; Tintelnot-Blomley, M.; Acemoglu, F.; Beck, W.; Boss, E.; Eschbach, M.; Hurlimann, T.; Masso, E.; Roussel, S.; Ucci-Stoll, K.; Wyss, D.; Lang, M. New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development. *J. Med. Chem.* **1998**, *41*, 3387-3401.

(74) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. Crystal structure of HIV-1 protease in complex with VX-478, a potent and orally bioavailable inhibitor of the enzyme. *J. Am. Chem. Soc.* **1995**, *117*, 1181-1182.

(75) Furfine, E. S.; Baker, C. T.; Hale, M. R.; Reynolds, D. J.; Salisbury, J. A.; Searle, A. D.; Studenberg, S. D.; Todd, D.; Tung, R. D.; Spaltenstein, A. Preclinical pharmacology and pharmacokinetics of GW433908, a water-soluble prodrug of the human immunodeficiency virus protease inhibitor amprenavir. *Antimicrob. Agents Chemother.* **2004**, *48*, 791-798.

(76) Turner, S. R.; Strohbach, J. W.; Tommasi, R. A.; Aristoff, P. A.; Johnson, P. D.; Skulnick, H. I.; Dolak, L. A.; Seest, E. P.; Tomich, P. K.; Bohanon, M. J.; Horng, M. M.; Lynn, J. C.; Chong, K. T.; Hinshaw, R. R.; Watenpaugh, K. D.; Janakiraman, M. N.; Thaisrivongs, S. Tipranavir (PNU-140690): a potent, orally bioavailable nonpeptidic HIV protease inhibitor of the 5,6-dihydro-4-hydroxy-2-pyrone sulfonamide class. *J. Med. Chem.* **1998**, *41*, 3467-3476.

(77) Ghosh, A. K.; Kincaid, J. F.; Cho, W.; Walters, D. E.; Krishnan, K.; Hussain, K. A.; Koo, Y.; Cho, H.; Rudall, C.; Holland, L.; Buthod, J. Potent HIV protease inhibitors incorporating high-affinity P2-ligands and (R)-(hydroxyethylamino)sulfonamide isostere. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 687-690.

(78) Ryom, L.; Mocroft, A.; Kirk, O.; Worm, S. W.; Kamara, D. A.; Reiss, P.; Ross, M.; Fux, C. A.; Morlat, P.; Moranne, O.; Smith, C.; Lundgren, J. D.; Group, D. A. D. S. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J. Infect. Dis.* **2013**, *207*, 1359-1369.

(79) Scherzer, R.; Estrella, M.; Li, Y.; Choi, A. I.; Deeks, S. G.; Grunfeld, C.; Shlipak, M. G. Association of tenofovir exposure with kidney disease risk in HIV infection. *AIDS* **2012**, *26*, 867-875.

(80) Tourret, J.; Deray, G.; Isnard-Bagnis, C. Tenofovir Effect on the Kidneys of HIV-Infected Patients: A Double-Edged Sword? *Journal of the American Society of Nephrology* **2013**, *24*, 1519-1527.

(81) Hunt, P. W.; Martin, J. N.; Sinclair, E.; Bredt, B.; Hagos, E.; Lampiris, H.; Deeks, S. G. T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy. *J. Infect. Dis.* **2003**, *187*, 1534-1543.

(82) Neuhaus, J.; Jacobs, D. R., Jr.; Baker, J. V.; Calmy, A.; Duprez, D.; La Rosa, A.; Kuller, L. H.; Pett, S. L.; Ristola, M.; Ross, M. J.; Shlipak, M. G.; Tracy, R.; Neaton, J. D. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J. Infect. Dis.* **2010**, *201*, 1788-1795.

(83) Casado, J. L. Liver toxicity in HIV-infected patients receiving novel second-generation nonnucleoside reverse transcriptase inhibitors etravirine and rilpivirine. *AIDS Rev* **2013**, *15*, 139-145.

(84) Fernandez-Montero, J. V.; Eugenia, E.; Barreiro, P.; Labarga, P.; Soriano, V. Antiretroviral drug-related toxicities – clinical spectrum, prevention, and management. *Expert Opinion on Drug Safety* **2013**, *12*, 697-707.

(85) Das, K.; Arnold, E. HIV-1 reverse transcriptase and antiviral drug resistance. Part 1. *Current Opinion in Virology* **2013**, *3*, 111-118.

(86) Pérez, L.; Kourí, V.; Alemán, Y.; Abrahantes, Y.; Correa, C.; Aragonés, C.; Martínez, O.; Pérez, J.; Fonseca, C.; Campos, J.; Álvarez, D.; Schrooten, Y.; Dekeersmaeker, N.; Imbrechts, S.; Beheydt, G.; Vinken, L.; Soto, Y.; Álvarez, A.; Vandamme, A.-M.; Van Laethem, K. Antiretroviral drug resistance in HIV-1 therapy-naive patients in Cuba. *Infection, Genetics and Evolution* **2013**, *16*, 144-150.

(87) Antiretroviral Therapy Cohort, C. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* **2008**, *372*, 293-299.

(88) Bhaskaran, K.; Hamouda, O.; Sannes, M.; Boufassa, F.; Johnson, A. M.; Lambert, P. C.; Porter, K.; Collaboration, C. Changes in the risk of death after HIV seroconversion compared with mortality in the general population. *JAMA* **2008**, *300*, 51-59.

(89) Gupta, R.; Hill, A.; Sawyer, A. W.; Pillay, D. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. *Clin Infect Dis* **2008**, *47*, 712-722.

(90) Walensky, R. P.; Paltiel, A. D.; Losina, E.; Mercincavage, L. M.; Schackman, B. R.; Sax, P. E.; Weinstein, M. C.; Freedberg, K. A. The survival benefits of AIDS treatment in the United States. *J. Infect. Dis.* **2006**, *194*, 11-19.

(91) Ferrer, E.; Podzamczer, D.; Arnedo, M.; Fumero, E.; McKenna, P.; Rinehart, A.; Perez, J. L.; Barbera, M. J.; Pumarola, T.; Gatell, J. M.; Gudiol, F.; Combine Study, T. Genotype and phenotype at baseline and at failure in human immunodeficiency virus-infected antiretroviral-naive patients in a randomized trial comparing zidovudine and lamivudine plus nelfinavir or nevirapine. *J. Infect. Dis.* **2003**, *187*, 687-690.

(92) Condra, J. H.; Petropoulos, C. J.; Ziermann, R.; Schleif, W. A.; Shivaprakash, M.; Emini, E. A. Drug resistance and predicted virologic responses to human immunodeficiency virus type 1 protease inhibitor therapy. *J. Infect. Dis.* **2000**, *182*, 758-765.

(93) Yin, P. D.; Das, D.; Mitsuya, H. Overcoming HIV drug resistance through rational drug design based on molecular, biochemical, and structural profiles of HIV resistance. *Cell. Mol. Life Sci.* **2006**, *63*, 1706-1724.

(94) Eliel, E. L. W., S.H.; Mander, L.N.: *Stereochemistry of Organic Compounds*; Wiley: New York, 1994. pp. 1267 pp.

(95) Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Baker, W. R.; Condon, S. L.; deLara, E.; Rosenberg, S. H.; Spina, K. P.; Stein, H. H.; Cohen, J.; et al. 1,2,3-trisubstituted cyclopropanes as conformationally restricted peptide isosteres: application to the design and synthesis of novel renin inhibitors. *J. Med. Chem.* **1992**, *35*, 1710-1721.

(96) Baker, W. R.; Jae, H.-S.; Martin, S. R.; Condon, S. L.; Stein, H. H.; Cohen, J.; Kleinert, H. D. Conformationally restricted peptide isosteres. 2.1 Synthesis and in vitro potency of dipeptide renin inhibitors employing a 2-alkylsulfonyl-3-phenylcyclopropane

carboxamide as a P3 amino acid replacement. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1405-1410.

(97) Martin, S. F.; Dorsey, G. O.; Gane, T.; Hillier, M. C.; Kessler, H.; Baur, M.; Matha, B.; Erickson, J. W.; Bhat, T. N.; Munshi, S.; Gulnik, S. V.; Topol, I. A. Cyclopropane-derived peptidomimetics. Design, synthesis, evaluation, and structure of novel HIV-1 protease inhibitors. *J. Med. Chem.* **1998**, *41*, 1581-1597.

(98) Hillier, M. C.; Davidson, J. P.; Martin, S. F. Cyclopropane-derived peptidomimetics. design, synthesis, and evaluation of novel Ras farnesyltransferase inhibitors. *J. Org. Chem.* **2001**, *66*, 1657-1671.

(99) Martin, S. F.; Dwyer, M. P.; Hartmann, B.; Knight, K. S. Cyclopropane-derived peptidomimetics. Design, synthesis, and evaluation of novel enkephalin analogues. *J. Org. Chem.* **2000**, *65*, 1305-1318.

(100) Davidson, J. P.; Martin, S. F. Use of 1,2,3-trisubstituted cyclopropanes as conformationally constrained peptide mimics in SH2 antagonists. *Tetrahedron Lett.* **2000**, *41*, 9459-9464.

(101) Watanabe, M.; Kobayashi, T.; Hirokawa, T.; Yoshida, A.; Ito, Y.; Yamada, S.; Orimoto, N.; Yamasaki, Y.; Arisawa, M.; Shuto, S. Cyclopropane-based stereochemical diversity-oriented conformational restriction strategy: Histamine H3 and/or H4 receptor ligands with the 2,3-methanobutane backbone. *Org. Biomol. Chem.* **2012**, *10*, 736-745.

(102) Zhang, H.; Tückmantel, W.; Eaton, J. B.; Yuen, P.-w.; Yu, L.-F.; Bajjuri, K. M.; Fedolak, A.; Wang, D.; Ghavami, A.; Caldarone, B.; Paterson, N. E.; Lowe, D. A.; Brunner, D.; Lukas, R. J.; Kozikowski, A. P. Chemistry and Behavioral Studies Identify Chiral Cyclopropanes as Selective α4β2-Nicotinic Acetylcholine Receptor Partial Agonists Exhibiting an Antidepressant Profile. *J. Med. Chem.* **2011**, *55*, 717-724.

(103) Campbell, J. E.; Englund, E. E.; Burke, S. D. Synthesis and Structure of Preorganized, C3-Symmetric Trilactam Scaffolds with Convergently Oriented (S)-Acetylthiomethyl Appendages. *Org. Lett.* **2002**, *4*, 2273-2275.

(104) Ghosh, A. K.; Cappiello, J.; Shin, D. Ring-closing metathesis strategy to unsaturated γ- and δ-lactones: Synthesis of hydroxyethylene isostere for protease inhibitors. *Tetrahedron Lett.* **1998**, *39*, 4651-4654.

(105) Doyle, M. P. Catalytic methods for metal carbene transformations. *Chem. Rev. (Washington, DC, U. S.)* **1986**, *86*, 919-939.

(106) Davies, H. M. L. Asymmetric Synthesis Using Rhodium-Stabilized Vinylcarbenoid Intermediates. *Aldrichim. Acta* **1997**, *30*.

(107) Doyle, M. P.; Protopopova, M. N. New aspects of catalytic asymmetric cyclopropanation. *Tetrahedron* **1998**, *54*, 7919-7946.

(108) Herndon, J. W. Applications of carbene complexes toward organic synthesis. *Coord. Chem. Rev.* **2000**, *206–207*, 237-262.

(109) Morandi, B.; Carreira, E. M. Iron-Catalyzed Cyclopropanation in 6 M KOH with in Situ Generation of Diazomethane. *Science* **2012**, *335*, 1471-1474.

(110) Mastronardi, F.; Gutmann, B.; Kappe, C. O. Continuous Flow Generation and Reactions of Anhydrous Diazomethane Using a Teflon AF-2400 Tube-in-Tube Reactor. *Org. Lett.* **2013**, *15*, 5590-5593.

(111) Illa, O.; Rodríguez-García, C.; Acosta-Silva, C.; Favier, I.; Picurelli, D.; Oliva, A.; Gómez, M.; Branchadell, V.; Ortuño, R. M. Cyclopropanation of Cyclohexenone by Diazomethane Catalyzed by Palladium Diacetate: Evidence for the Formation of Palladium(0) Nanoparticles. *Organometallics* **2007**, *26*, 3306-3314.

(112) Li, Y.; Huang, J.-S.; Zhou, Z.-Y.; Che, C.-M. Isolation and X-ray Crystal Structure of an Unusual Biscarbene Metal Complex and Its Reactivity toward Cyclopropanation and Allylic C−H Insertion of Unfunctionalized Alkenes. *J. Am. Chem. Soc.* **2001**, *123*, 4843-4844.

(113) Tomilov, Y. V.; Dokitchev, V. A.; Dzhemilev, U. M.; Nefedov, O. M. Catalytic decomposition of diazomethane as a general method for the methylenation of chemical compounds. *Russ. Chem. Rev.* **1993**, *62*, 799.

(114) Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. Synthesis of four diastereomeric L-2-(carboxycyclopropyl)glycines. Conformationally constrained L-glutamate analogs. *J. Org. Chem.* **1991**, *56*, 4167-4176.

(115) Shimamoto, K.; Ohfune, Y. New routes to the syntheses of cis-α-(carboxycyclopropyl)glycines from L-glutamic acid. conformationally restricted analogues of the excitatory neurotransmitter L-glutamic acid. *Tetrahedron Lett.* **1989**, *30*, 3802-3804.

(116) Ghazi, M. New Method of Pure Diazomethane Preparation. *Synth. Commun.* **1992**, *22*, 2853-2857.

(117) Hoffmann, H. M. R.; Otte, A. R.; Wilde, A.; Menzer, S.; Williams, D. J. Isolation and X-Ray Crystal Structure of a Palladacyclobutane: Insight into the Mechanism of Cyclopropanation. *Angew. Chem.* **1995**, *34*, 100-102.

(118) Mattson, R. J.; Catt, J. D.; Denhart, D. J.; Deskus, J. A.; Ditta, J. L.; Higgins, M. A.; Marcin, L. R.; Sloan, C. P.; Beno, B. R.; Gao, Q.; Cunningham, M. A.; Mattson, G. K.; Molski, T. F.; Taber, M. T.; Lodge, N. J. Conformationally restricted homotryptamines. 2. Indole cyclopropylmethylamines as selective serotonin reuptake inhibitors. *J. Med. Chem.* **2005**, *48*, 6023-6034.

(119) Poon, K. W.; Lovell, K. M.; Dresner, K. N.; Datta, A. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) assisted facile deprotection of N,O-acetonides. *J. Org. Chem.* **2008**, *73*, 752-755.

(120) Hanessian, S.: *Total Synthesis of Natural Products: The 'Chiron' Approach*; Pergamon Press, 1983; Vol. 1.

(121) Martín-Vilà, M.; Hanafi, N.; Jiménez, J. M.; Alvarez-Larena, A.; Piniella, J. F.; Branchadell, V.; Oliva, A.; Ortuño, R. M. Controlling π-Facial Diastereoselectivity in the 1,3-Dipolar Cycloadditions of Diazomethane to Chiral Pentenoates and Furanones: Enantioselective Stereodivergent Syntheses of Cyclopropane Hydroxy Acids and Didehydro Amino Acids. *J. Org. Chem.* **1998**, *63*, 3581-3589.

(122) Sheehan, J.; Cruickshank, P.; Boshart, G. Notes- A Convenient Synthesis of Water-Soluble Carbodiimides. *J. Org. Chem.* **1961**, *26*, 2525-2528.

(123) American Cancer Society : Cancer Facts & Figures 2013. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2013/index.

(124) World Health Organization: Cancer Fact Sheet. http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

(125) Hanahan, D.; Weinberg, Robert A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, 646-674.

(126) Newman, D. J. Natural products as leads to potential drugs: An old process or the new hope for drug discovery? *J. Med. Chem.* **2008**, *51*, 2589-2599.

(127) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. Impact of Natural Products on Developing New Anti-Cancer Agents. *Chem. Rev. (Washington, DC, U. S.)* **2009**, *109*, 3012-3043.

(128) Kuroda, I.; Musman, M.; Ohtani, I. I.; Ichiba, T.; Tanaka, J.; Gravalos, D. G.; Higa, T. Pachastrissamine, a Cytotoxic Anhydrophytosphingosine from a Marine Sponge, Pachastrissa sp. *J. Nat. Prod.* **2002**, *65*, 1505-1506.

(129) Ledroit, V.; Debitus, C.; Lavaud, C.; Massiot, G. Jaspines A and B: two new cytotoxic sphingosine derivatives from the marine sponge Jaspis sp. *Tetrahedron Lett.* **2003**, *44*, 225-228.

(130) Salma, Y.; Lafont, E.; Therville, N.; Carpentier, S.; Bonnafe, M. J.; Levade, T.; Genisson, Y.; Andrieu-Abadie, N. The natural marine anhydrophytosphingosine, Jaspine B, induces apoptosis in melanoma cells by interfering with ceramide metabolism. *Biochem. Pharmacol.* **2009**, *78*, 477-485.

(131) Canals, D.; Mormeneo, D.; Fabriàs, G.; Llebaria, A.; Casas, J.; Delgado, A. Synthesis and biological properties of Pachastrissamine (jaspine B) and diastereoisomeric jaspines. *Bioorg. Med. Chem.* **2009**, *17*, 235-241.

(132) Klionsky, D. J. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 931-937.

(133) Degenhardt, K.; Mathew, R.; Beaudoin, B.; Bray, K.; Anderson, D.; Chen, G.; Mukherjee, C.; Shi, Y.; Gelinas, C.; Fan, Y.; Nelson, D. A.; Jin, S.; White, E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* **2006**, *10*, 51-64.

(134) Yoo, H.; Lee, Y. S.; Lee, S.; Kim, S.; Kim, T.-Y. Pachastrissamine from Pachastrissa sp. Inhibits Melanoma Cell Growth by Dual Inhibition of Cdk2 and ERK-mediated FOXO3 Downregulation. *Phytother. Res.* **2012**, *26*, 1927-1933.

(135) Gomez-Gutierrez, J. G.; Souza, V.; Hao, H. Y.; de Oca-Luna, R. M.; Dong, Y. B.; Zhou, H. S.; McMasters, K. M. Adenovirus-mediated gene transfer of FKHRL1 triple mutant efficiently induces apoptosis in melanoma cells. *Cancer Biology & Therapy* **2006**, *5*, 875-883.

(136) Stoica, B. A.; Movsesyan, V. A.; Lea, P. M. t.; Faden, A. I. Ceramide-induced neuronal apoptosis is associated with dephosphorylation of Akt, BAD, FKHR, GSK-3beta, and induction of the mitochondrial-dependent intrinsic caspase pathway. *Mol. Cell. Neurosci.* **2003**, *22*, 365-382.

(137) Ekoff, M.; Kaufmann, T.; Engström, M.; Motoyama, N.; Villunger, A.; Jönsson, J.-I.; Strasser, A.; Nilsson, G. The BH3-only protein Puma plays an essential role in cytokine deprivation–induced apoptosis of mast cells. *Blood* **2007**, *110*, 3209-3217.

(138) Skurk, C.; Maatz, H.; Kim, H.-S.; Yang, J.; Abid, M. R.; Aird, W. C.; Walsh, K. The Akt-regulated Forkhead Transcription Factor FOXO3a Controls Endothelial Cell Viability through Modulation of the Caspase-8 Inhibitor FLIP. *J. Biol. Chem.* **2004**, *279*, 1513-1525.

(139) Maceyka, M.; Payne, S. G.; Milstien, S.; Spiegel, S. Sphingosine kinase, sphingosine-1-phosphate, and apoptosis. *Biochim. Biophys. Acta* **2002**, *1585*, 193-201.

(140) Sakai, T.; Koezuka, Y. Glycolipid derivatives as therapeutic agents. *Expert Opin. Ther. Pat.* **1999**, *9*, 917-930.

(141) Radin, N. S. Designing anticancer drugs via the achilles heel: ceramide, allylic ketones, and mitochondria. *Bioorg. Med. Chem.* **2003**, *11*, 2123-2142.

(142) Reynolds, C. P.; Maurer, B. J.; Kolesnick, R. N. Ceramide synthesis and metabolism as a target for cancer therapy. *Cancer Lett* **2004**, *206*, 169-180.

(143) Hannun, Y. A.; Obeid, L. M. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 139-150.

(144) Bartke, N.; Hannun, Y. A. Bioactive sphingolipids: metabolism and function. *J. Lipid Res.* **2009**, *50*, S91-S96.

(145) Smith, W. L.; Merrill, A. H. Sphingolipid Metabolism and Signaling Minireview Series. *J. Biol. Chem.* **2002**, *277*, 25841-25842.

(146) Futerman, A. H.; Hannun, Y. A. The complex life of simple sphingolipids. *EMBO Rep.* **2004**, *5*, 777-782.

(147) Hannun, Y. A.; Bell, R. M. Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. *Science* **1989**, *243*, 500-507.

(148) Saddoughi, S.; Song, P.; Ogretmen, B.: Roles of Bioactive Sphingolipids in Cancer Biology and Therapeutics. In *Lipids in Health and Disease*; Quinn, P., Wang, X., Eds.; Subcellular Biochemistry; Springer Netherlands, 2008; Vol. 49; pp 413-440.

(149) Modrak, D. E.; Gold, D. V.; Goldenberg, D. M. Sphingolipid targets in cancer therapy. *Mol. Cancer Ther.* **2006**, *5*, 200-208.

(150) Padron, J. M. Sphingolipids in anticancer therapy. *Curr. Med. Chem.* **2006**, *13*, 755-770.

(151) Ogretmen, B.; Hannun, Y. A. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat. Rev. Cancer* **2004**, *4*, 604-616.

(152) Zheng, W.; Kollmeyer, J.; Symolon, H.; Momin, A.; Munter, E.; Wang, E.; Kelly, S.; Allegood, J. C.; Liu, Y.; Peng, Q.; Ramaraju, H.; Sullards, M. C.; Cabot, M.; Merrill, A. H., Jr. Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. *Biochim. Biophys. Acta* **2006**, *1758*, 1864-1884.

(153) Brodesser, S.; Sawatzki, P.; Kolter, T. Bioorganic Chemistry of Ceramide. *Eur. J. Org. Chem.* **2003**, *2003*, 2021-2034.

(154) Merrill, A. H. Sphingolipid and Glycosphingolipid Metabolic Pathways in the Era of Sphingolipidomics. *Chem. Rev. (Washington, DC, U. S.)* **2011**, *111*, 6387-6422.

(155) Maceyka, M.; Milstien, S.; Spiegel, S. Sphingosine-1-phosphate: the Swiss army knife of sphingolipid signaling. *J. Lipid Res.* **2009**, *50*, S272-S276.

(156) Takabe, K.; Paugh, S. W.; Milstien, S.; Spiegel, S. "Inside-Out" Signaling of Sphingosine-1-Phosphate: Therapeutic Targets. *Pharmacological Reviews* **2008**, *60*, 181-195.

(157) Hait, N. C.; Oskeritzian, C. A.; Paugh, S. W.; Milstien, S.; Spiegel, S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. *Biochim. Biophys. Acta* **2006**, *1758*, 2016-2026.

(158) Cuvillier, O.; Pirianov, G.; Kleuser, B.; Vanek, P. G.; Coso, O. A.; Gutkind, S.; Spiegel, S. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature* **1996**, *381*, 800-803.

(159) Taha, T. A.; Mullen, T. D.; Obeid, L. M. A house divided: ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. *Biochim. Biophys. Acta* **2006**, *1758*, 2027-2036.

(160) Huwiler, A.; Zangemeister-Wittke, U. Targeting the conversion of ceramide to sphingosine 1-phosphate as a novel strategy for cancer therapy. *Critical Reviews in Oncology/Hematology* **2007**, *63*, 150-159.

(161) Gulbins, E. Regulation of death receptor signaling and apoptosis by ceramide. *Pharmacol. Res.* **2003**, *47*, 393-399.

(162) Pettus, B. J.; Chalfant, C. E.; Hannun, Y. A. Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta* **2002**, *1585*, 114-125.

(163) Radin, N. S. Apoptotic death by ceramide: will the real killer please stand up? *Medical Hypotheses* **2001**, *57*, 96-100.

(164) Haimovitz-Friedman, A.; Kolesnick, R. N.; Fuks, Z. Ceramide signaling in apoptosis. *British Medical Bulletin* **1997**, *53*, 539-553.

(165) Obeid, L.; Linardic, C.; Karolak, L.; Hannun, Y. Programmed cell death induced by ceramide. *Science* **1993**, *259*, 1769-1771.

(166) Delgado, A.; Fabrias, G.; Bedia, C.; Casas, J.; Abad, J. L. Sphingolipid Modulation: A Strategy for Cancer Therapy. *Anti-Cancer Agents Med. Chem.* **2012**, *12*, 285-302.

(167) Génisson, Y.; Levade, T.; Ballereau, S.; Andrieu-Abadie, N. Alteration of Ceramide 1-*O*-Functionalization as a Promising Approach for Cancer Therapy. *Anti-Cancer Agents Med. Chem.* **2012**, *12*, 316-328.

(168) Radin, N. S. Killing tumours by ceramide-induced apoptosis: a critique of available drugs. *Biochem. J.* **2003**, *371*, 243-256.

(169) Senchenkov, A.; Litvak, D. A.; Cabot, M. C. Targeting ceramide metabolism--a strategy for overcoming drug resistance. *J. Natl. Cancer Inst.* **2001**, *93*, 347-357.

(170) Radin, N. S. Killing cancer cells by poly-drug elevation of ceramide levels: a hypothesis whose time has come? *Eur. J. Biochem.* **2001**, *268*, 193-204.

(171) Samsel, L.; Zaidel, G.; Drumgoole, H. M.; Jelovac, D.; Drachenberg, C.; Rhee, J. G.; Brodie, A. M.; Bielawska, A.; Smyth, M. J. The ceramide analog, B13, induces apoptosis in prostate cancer cell lines and inhibits tumor growth in prostate cancer xenografts. *Prostate* **2004**, *58*, 382-393.

(172) Selzner, M.; Bielawska, A.; Morse, M. A.; Rudiger, H. A.; Sindram, D.; Hannun, Y. A.; Clavien, P. A. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. *Cancer Res.* **2001**, *61*, 1233-1240.

(173) Bai, A.; Meier, G. P.; Wang, Y.; Luberto, C.; Hannun, Y. A.; Zhou, D. Prodrug modification increases potassium tricyclo[5.2.1.0(2,6)]-decan-8-yl dithiocarbonate (D609) chemical stability and cytotoxicity against U937 leukemia cells. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 1051-1059.

(174) Meng, A.; Luberto, C.; Meier, P.; Bai, A.; Yang, X.; Hannun, Y. A.; Zhou, D. Sphingomyelin synthase as a potential target for D609-induced apoptosis in U937 human monocytic leukemia cells. *Exp. Cell Res.* **2004**, *292*, 385-392.

(175) Abraham, E.; Davies, S. G.; Roberts, P. M.; Russell, A. J.; Thomson, J. E. Jaspine B (pachastrissamine) and 2-epi-jaspine B: synthesis and structural assignment. *Tetrahedron: Asymmetry* **2008**, *19*, 1027-1047.

(176) Abraham, E.; Brock, E. A.; Candela-Lena, J. I.; Davies, S. G.; Georgiou, M.; Nicholson, R. L.; Perkins, J. H.; Roberts, P. M.; Russell, A. J.; Sanchez-Fernandez, E. M.; Scott, P. M.; Smith, A. D.; Thomson, J. E. Asymmetric synthesis of N,O,O,O-tetra-acetyl d-lyxo-phytosphingosine, jaspine B (pachastrissamine), 2-epi-jaspine B, and deoxoprosophyllinevialithium amide conjugate addition. *Org. Biomol. Chem.* **2008**, *6*, 1665-1673.

(177) van den Berg, R. J. B. H. N.; Boltje, T. J.; Verhagen, C. P.; Litjens, R. E. J. N.; van der Marel, G. A.; Overkleeft, H. S. An Efficient Synthesis of the Natural Tetrahydrofuran Pachastrissamine Starting from d-ribo-Phytosphingosine. *J. Org. Chem.* **2005**, *71*, 836-839.

(178) Lee, T.; Lee, S.; Kwak, Y. S.; Kim, D.; Kim, S. Synthesis of Pachastrissamine from Phytosphingosine: A Comparison of Cyclic Sulfate vs an Epoxide Intermediate in Cyclization. *Org. Lett.* **2007**, *9*, 429-432.

(179) Sudhakar, N.; Kumar, A. R.; Prabhakar, A.; Jagadeesh, B.; Rao, B. V. The first synthesis of the anhydrophytosphingosine pachastrissamine (jaspine B) from Garner's aldehyde. *Tetrahedron Lett.* **2005**, *46*, 325-327.

(180) Passiniemi, M.; Koskinen, A. M. P. Stereoselective total synthesis of pachastrissamine (jaspine B). *Tetrahedron Lett.* **2008**, *49*, 980-983.

(181) Reddy, L. V. R.; Reddy, P. V.; Shaw, A. K. An expedient route for the practical synthesis of pachastrissamine (jaspine B) starting from 3,4,6-tri-O-benzyl-d-galactal. *Tetrahedron: Asymmetry* **2007**, *18*, 542-546.

(182) Du, Y.; Liu, J.; Linhardt, R. J. Stereoselective Synthesis of Cytotoxic Anhydrophytosphingosine Pachastrissamine (Jaspine B) from d-Xylose. *J. Org. Chem.* **2005**, *71*, 1251-1253.

(183) Liu, J.; Du, Y.; Dong, X.; Meng, S.; Xiao, J.; Cheng, L. Stereoselective synthesis of jaspine B from d-xylose. *Carbohydr. Res.* **2006**, *341*, 2653-2657.

(184) Yoshimitsu, Y.; Inuki, S.; Oishi, S.; Fujii, N.; Ohno, H. Stereoselective Divergent Synthesis of Four Diastereomers of Pachastrissamine (Jaspine B). *J Org Chem* **2010**, *75*, 3843-3846.

(185) Ghosal, P.; Ajay, S.; Meena, S.; Sinha, S.; Shaw, A. K. Stereoselective total synthesis of Jaspine B (Pachastrissamine) utilizing iodocyclization and an investigation of its cytotoxic activity. *Tetrahedron: Asymmetry* **2013**, *24*, 903-908.

(186) Bae, H.; Jeon, H.; Baek, D. J.; Lee, D.; Kim, S. Stereochemically Reliable Syntheses of Pachastrissamine and Its 2-epi-Congener via Oxazolidinone Precursors from an Established Starting Material N-tert-Butoxycarbonyl-Protected Phytosphingosine. *Synthesis* **2012**, *44*, 3609-3612.

(187) Du, Y.; Liu, J.; Linhardt, R. J. Stereoselective synthesis of cytotoxic anhydrophytosphingosine pachastrissamine (jaspine B) from D-xylose. *J. Org. Chem.* **2006**, *71*, 1251-1253.

(188) Ramana, C. V.; Giri, A. G.; Suryawanshi, S. B.; Gonnade, R. G. Total synthesis of pachastrissamine (jaspine B) enantiomers from D-glucose. *Tetrahedron Lett.* **2007**, *48*, 265-268.

(189) Ribes, C.; Falomir, E.; Carda, M.; Marco, J. A. Stereoselective synthesis of pachastrissamine (jaspine B). *Tetrahedron* **2006**, *62*, 5421-5425.

(190) Prasad, K. R.; Chandrakumar, A. Stereoselective synthesis of cytotoxic anhydrophytosphingosine pachastrissamine [jaspine B]. *J. Org. Chem.* **2007**, *72*, 6312-6315.

(191) Ichikawa, Y.; Matsunaga, K.; Masuda, T.; Kotsuki, H.; Nakano, K. Stereocontrolled synthesis of cytotoxic anhydrosphingosine pachastrissamine by using [3.3] sigmatropic rearrangement of allyl cyanate. *Tetrahedron* **2008**, *64*, 11313-11318.

(192) Yakura, T.; Sato, S.; Yoshimoto, Y. Ennantioselective synthesis of pachastrissamine (Jaspin B) using Dirhodium(II)-Catalyzed C-H amination and asymmetric dihydroxylation as key steps. *Chem. Pharm. Bull.* **2007**, *55*, 1284-1286.

(193) Génisson, Y.; Lamandé, L.; Salma, Y.; Andrieu-Abadie, N.; André, C.; Baltas, M. Enantioselective access to a versatile 4-oxazolidinonecarbaldehyde and application to the synthesis of a cytotoxic jaspine B truncated analogue. *Tetrahedron: Asymmetry* **2007**, *18*, 857-864.

(194) Salma, Y.; Ballereau, S.; Maaliki, C.; Ladeira, S.; Andrieu-Abadie, N.; Genisson, Y. Flexible and enantioselective access to jaspine B and biologically active chain-modified analogues thereof. *Org. Biomol. Chem.* **2010**, *8*, 3227-3243.

(195) Enders, D.; Terteryan, V.; Pale¿ek, J. í. Asymmetric Synthesis of Jaspine B (Pachastrissamine) via an Organocatalytic Aldol Reaction as Key Step. *Synthesis* **2008**, *2008*, 2278-2282.

(196) Dhand, V.; Chang, S.; Britton, R. Total Synthesis of the Cytotoxic Anhydrophytosphingosine Pachastrissamine (Jaspine B). *J. Org. Chem.* **2013**, *78*, 8208-8213.

(197) Venkatesan, K.; Srinivasan, K. V. A novel stereoselective synthesis of pachastrissamine (jaspine B) starting from 1-pentadecanol. *Tetrahedron: Asymmetry* **2008**, *19*, 209-+.

(198) Rao, G. S.; Chandrasekhar, B.; Rao, B. V. Total synthesis of the acetyl derivatives of lyxo-(2R,3R,4R)-phytosphingosine and (-)-jaspine B. *Tetrahedron: Asymmetry* **2012**, *23*, 564-569.

(199) Azuma, H.; Tamagaki, S.; Ogino, K. Stereospecific Total Syntheses of Sphingosine and Its Analogues from l-Serine. *J. Org. Chem.* **2000**, *65*, 3538-3541.

(200) Liu, J.; Jian, T.; Guo, L.; Atanasova, T.; Nargund, R. P. Preparation of 3,4-fused-spiro[furan-5(5H),4′-piperidin]-2-one. *Tetrahedron Lett.* **2009**, *50*, 5228-5230.

(201) Inuki, S.; Yoshimitsu, Y.; Oishi, S.; Fujii, N.; Ohno, H. Ring-Construction/Stereoselective Functionalization Cascade: Total Synthesis of Pachastrissamine (Jaspine B) through Palladium-Catalyzed Bis-cyclization of Propargyl Chlorides and Carbonates. *J. Org. Chem.* **2010**, *75*, 3831-3842.

(202) Campbell, A. D.; Raynham, T. M.; Taylor, R. J. K. A Simplified Route to the (R)-Garner Aldehyde and (S)-Vinyl Glycinol. *Synthesis* **1998**, *1998*, 1707-1709.

(203) Jeon, H.; Bae, H.; Baek, D. J.; Kwak, Y.-S.; Kim, D.; Kim, S. Syntheses of sulfur and selenium analogues of pachastrissamine via double displacements of cyclic sulfate. *Org. Biomol. Chem.* **2011**, *9*, 7237-7242.

(204) Liu, J.; Du, Y. G.; Dong, X. M.; Meng, S. C.; Xiao, J. J.; Cheng, L. J. Stereoselective synthesis of jaspine B from D-xylose. *Carbohydr. Res.* **2006**, *341*, 2653-2657.

(205) Defaye, J.; Ratovelomanana, V. Solvolyse d'esters sulfoniques dans la se´rie du glucofuranose. Acce`s aux de´rive´s du 2,5-anhydro-L-idose et du 2,5-anhydro-L-iditol. *Carbohydr. Res.* **1971**, *17*, 57-65.

(206) Yu, H. W.; Zhang, H. Y.; Yang, Z. J.; Min, J. M.; Ma, L. T.; Zhang, L. H. Studies on the syntheses and biological activities of isonucleosides. *Pure Appl. Chem.* **1998**, *70*, 435-438.

(207) Ohira, S. Methanolysis of Dimethyl (1-Diazo-2-Oxopropyl)Phosphonate - Generation of Dimethyl (Diazomethyl)Phosphonate and Reaction with Carbonyl-Compounds. *Synth. Commun.* **1989**, *19*, 561-564.

(208) Muller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. An improved one-pot procedure for the synthesis of alkynes from aldehydes. *Synlett* **1996**, 521-&.

(209) Ichikawa, Y. Evolution, development and personal experience in studies of the allyl cyanate-to-isocyanate rearrangement. *Synlett* **2007**, 2927-2936.

(210) Yakura, T.; Sato, S.; Yoshimoto, Y. Enantioselective synthesis of pachastrissamine (jaspin B) using dirhodium(II)-catalyzed C-H amination and asymmetric dihydroxylation as key steps. *Chemical & pharmaceutical bulletin* **2007**, *55*, 1284-1286.

(211) Amatore, M.; Beeson, T. D.; Brown, S. P.; MacMillan, D. W. C. Enantioselective Linchpin Catalysis by SOMO Catalysis: An Approach to the Asymmetric α-Chlorination of Aldehydes and Terminal Epoxide Formation. *Angew. Chem.* **2009**, *48*, 5121-5124.

(212) Rives, A.; Ladeira, S.; Levade, T.; Andrieu-Abadie, N.; Génisson, Y. Synthesis of Cytotoxic Aza Analogues of Jaspine B. *J. Org. Chem.* **2010**, *75*, 7920-7923.

(213) Jayachitra, G.; Sudhakar, N.; Anchoori, R. K.; Rao, B. V.; Roy, S.; Banerjee, R. Stereoselective Synthesis and Biological Studies of the C2 and C3 Epimer and the Enantiomer of Pachastrissamine (Jaspine B). *Synthesis* **2010**, *2010*, 115-119.

(214) Gao, Z.-X.; Wang, M.; Wang, S.; Yao, Z.-J. Efficient Synthesis of 4-Amido-N5-acetyl-4-deoxyneuraminic Acid and Its Application to the C-4 Modification of Sialic Acids. *Org. Lett.* **2009**, *11*, 3678-3681.

(215) Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54*, 2529-2591.

(216) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. Copper(I)-Catalyzed Synthesis of Azoles. DFT Study Predicts Unprecedented Reactivity and Intermediates. *J. Am. Chem. Soc.* **2004**, *127*, 210-216.

(217) Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. Ruthenium-Catalyzed Azide−Alkyne Cycloaddition: Scope and Mechanism. *J. Am. Chem. Soc.* **2008**, *130*, 8923-8930.

(218) Stutz, A. E.: *Iminusugars as Glycosidase Inhibitors. Nojirimycin and Beyond*; Wiley-VCH: Weinheim, 1999.

(219) Compain, P.; Martin, O. R.: *Iminosugars. From Synthesis to Therapeutic Applications*; Wiley-VCH: Weinheim, 2007.

(220) Winchester, B.; Fleet, G. W. J. Amino-sugar glycosidase inhibitors: versatile tools for glycobiologists. *Glycobiology* **1992**, *2*, 199-210.

(221) Somsak, L.; Nagya, V.; Hadady, Z.; Docsa, T.; Gergely, P. Glucose analog inhibitors of glycogen phosphorylases as potential antidiabetic agents: recent developments. *Curr. Pharm. Des.* **2003**, *9*, 1177-1189.

(222) Karlsson, G. B.; Butters, T. D.; Dwek, R. A.; Platt, F. M. Effects of the imino sugar N-butyldeoxynojirimycin on the N-glycosylation of recombinant gp120. *J. Biol. Chem.* **1993**, *268*, 570-576.

(223) Groopman, J. E. Management of the hematologic complications of human immunodeficiency virus infection. *Rev Infect Dis* **1990**, *12*, 931-937.

(224) Weiss, M.; Hettmer, S.; Smith, P.; Ladisch, S. Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. *Cancer Res.* **2003**, *63*, 3654-3658.

(225) Butters, T. D.; Dwek, R. A.; Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders. *Chem Rev* **2000**, *100*, 4683-4696.

(226) Miglitol. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a601079.html (accessed 09/01/2010.

(227) Abian, O.; Alfonso, P.; Velazquez-Campoy, A.; Giraldo, P.; Pocovi, M.; Sancho, J. Therapeutic Strategies for Gaucher Disease: Miglustat (NB-DNJ) as a Pharmacological Chaperone for Glucocerebrosidase and the Different Thermostability of Velaglucerase Alfa and Imiglucerase. *Mol. Pharm.* **2011**, *8*, 2390-2397.

(228) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. Recent Developments of Transition-State Analogue Glycosidase Inhibitors of Non-Natural Product Origin. *Chem. Rev. (Washington, DC, U. S.)* **2002**, *102*, 515-554.

(229) Pearson, M. S. M.; Mathé-Allainmat, M.; Fargeas, V.; Lebreton, J. Recent Advances in the Total Synthesis of Piperidine Azasugars. *Eur. J. Org. Chem.* **2005**, *2005*, 2159-2191.

(230) Davis, B. G. A silver-lined anniversary of Fleet iminosugars: 1984-2009, from DIM to DRAM to LABNAc. *Tetrahedron: Asymmetry* **2009**, *20*, 652-671.

(231) Storer, R. H., G.;. Seglin Technology: A Major Source of Novel Small-Molecule Drugs. *Biopharma: Innovations in Pharmaceutical Technology*, 64-68.

(232) Fleet, G. W. J.; Son, J. C.; Green, D. S. C.; di Bello, I. C.; Winchester, B. Synthesis from d-mannose of 1,4-dideoxy-1,4-imino-l-ribitol and of the α-mannosidase inhibitor 1,4-dideoxy-1,4-imino-d-talitol. *Tetrahedron* **1988**, *44*, 2649-2655.

(233) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. Potent competitive inhibition of α-galactosidase and α-glucosidase activity by 1,4-dideoxy-1,4-iminopentitols: syntheses of 1,4-dideoxy-1,4-imino-d-lyxitol and of both enantiomers of 1,4-dideoxy-1,4-iminoarabinitol. *Tetrahedron Lett.* **1985**, *26*, 3127-3130.

(234) Fleet, G. W. J.; Smith, P. W. Enantiospecific syntheses of deoxymannojirimycin, fagomine and 2r,5r-dihydroxymethyl-3r,4r-dihydroxypyrrolidine from D-glucose. *Tetrahedron Lett.* **1985**, *26*, 1469-1472.

(235) Fleet, G. W. J.; Carpenter, N. M.; Petursson, S.; Ramsdena, N. G. Synthesis of deoxynojirimycin and of nojirimycin δ-lactam. *Tetrahedron Lett.* **1990**, *31*, 409-412.

(236) Goujon, J.-Y.; Gueyrard, D.; Compain, P.; Martin, O. R.; Asano, N. Ring-opening reactions of iminosugar-derived aziridines: application to the general synthesis of α-1-C-substituted derivatives of fagomine. *Tetrahedron: Asymmetry* **2003**, *14*, 1969-1972.

(237) Ravinder, M.; Reddy, T. N.; Mahendar, B.; Rao, V. J. Stereoselective synthesis of 1-deoxynojirimycin, D-glucono-delta-lactam and D-altrono-delta-lactam from a common chiral intermediate derived from D-mannitol. *Arkivoc* **2012**, 287-302.

(238) Takahata, H.; Banba, Y.; Ouchi, H.; Nemoto, H. Concise and Highly Stereocontrolled Synthesis of 1-Deoxygalactonojirimycin and Its Congeners Using Dioxanylpiperidene, a Promising Chiral Building Block. *Org. Lett.* **2003**, *5*, 2527-2529.

(239) Dondoni, A.; Merino, P.; Perrone, D. Totally chemical synthesis of azasugars via thiazole intermediates. Stereodivergent routes to (-)-nojirimycin, (-)-mannojirimycin and their 3-deoxy derivatives from serine. *Tetrahedron* **1993**, *49*, 2939-2956.

(240) Kang, M.; Park, J.; Konradi, A. W.; Pedersen, S. F. Synthesis of 1,4-Diaminocyclitols From l-Serine Methyl Ester. *J. Org. Chem.* **1996**, *61*, 5528-5531.

(241) Rengasamy, R.; Curtis-Long, M. J.; Ryu, H. W.; Oh, K. Y.; Park, K. H. Stereoselective Synthesis of L-Deoxyaltronojirimycin from L-Serine. *Bull. Korean Chem. Soc.* **2009**, *30*, 1531-1534.

(242) Kummeter, M.; Kazmaier, U. Synthesis of Monocyclic and Bicyclic Imino Sugars. *Eur. J. Org. Chem.* **2003**, *2003*, 3330-3334.

(243) Haukaas, M. H.; O'Doherty, G. A. Synthesis of d- and l-Deoxymannojirimycin via an Asymmetric Aminohydroxylation of Vinylfuran. *Org. Lett.* **2001**, *3*, 401-404.

(244) Boglio, C.; Stahlke, S.; Thorimbert, S.; Malacria, M. A Stereoselective Route toward Polyhydoxylated Piperidines. A Total Synthesis of (±)-Deoxymannojirimycin. *Org. Lett.* **2005**, *7*, 4851-4854.

(245) McAlpine, I. J.; Armstrong, R. W. Stereoselective synthesis of a tricyclic guanidinium model of cylindrospermopsin. *Tetrahedron Lett.* **2000**, *41*, 1849-1853.

(246) Schürer, S. C.; Blechert, S. A versatile synthesis of substituted tetrahydropyridines. *Tetrahedron Lett.* **1999**, *40*, 1877-1880.

(247) Stecko, S.; Jurczak, M.; Panfil, I.; Furman, B.; Grzeszczyk, B.; Chmielewski, M. Synthesis of iminosugars via 1,3-dipolar cycloaddition reactions of nitrones to α,β-unsaturated sugar aldonolactones. *C. R. Chim.* **2011**, *14*, 102-125.

(248) Burland, P. A.; Coisson, D.; Osborn, H. M. I. Rapid Synthesis of Carbohydrate Derivatives, Including Mimetics of C-Linked Disaccharides and C-Linked Aza

Disaccharides, Using the Hetero-Diels−Alder Reaction. *J. Org. Chem.* **2010**, *75*, 7210-7218.

(249) Imashiro, R.; Uehara, H.; Barbas, C. F. One-Pot Enantioselective Syntheses of Iminosugar Derivatives Using Organocatalytic anti-Michael−anti-Aza-Henry Reactions. *Org. Lett.* **2010**, *12*, 5250-5253.

(250) Ruiz, M.; Ruanova, T. M.; Blanco, G.; Nunez, F.; Pato, C.; Ojea, V. Diastereoselective synthesis of piperidine imino sugars using aldol additions of metalated bislactim ethers to threose and erythrose acetonides. *J. Org. Chem.* **2008**, *73*, 2240-2255.

(251) Malik, G.; Guinchard, X.; Crich, D. Asymmetric Synthesis of Polyhydroxylated N-Alkoxypiperidines by Ring-Closing Double Reductive Amination: Facile Preparation of Isofagomine and Analogues. *Org. Lett.* **2012**, *14*, 596-599.

(252) Yokoyama, H.; Otaya, K.; Kobayashi, H.; Miyazawa, M.; Yamaguchi, S.; Hirai, Y. Palladium(II)-Catalyzed Cyclization of Urethanes and Total Synthesis of 1-Deoxymannojirimycin. *Org. Lett.* **2000**, *2*, 2427-2429.

(253) Knight, J. G.; Tchabanenko, K. Total synthesis of deoxymannojirimycin and d-mannolactam via carbonylation of 5-vinyloxazolidin-2-ones. *Tetrahedron* **2003**, *59*, 281-286.

(254) Louvel, J.; Chemla, F.; Demont, E.; Ferreira, F.; Pérez-Luna, A. Synthesis of (−)-Swainsonine and (−)-8-epi-Swainsonine by the Addition of Allenylmetals to Chiral α,β-Alkoxy Sulfinylimines. *Org. Lett.* **2011**, *13*, 6452-6455.

(255) van den Nieuwendijk, A. M. C. H.; van den Berg, R. J. B. H. N.; Ruben, M.; Witte, M. D.; Brussee, J.; Boot, R. G.; van der Marel, G. A.; Aerts, J. M. F. G.; Overkleeft, H. S. Synthesis of Eight 1-Deoxynojirimycin Isomers from a Single Chiral Cyanohydrin. *Eur. J. Org. Chem.* **2012**, 3437-3446.

(256) Becker.W, P. E. *Biochem. Z* **1963**, *337*.

(257) Zandbergen, P.; Vandennieuwendijk, A. M. C. H.; Brussee, J.; Vandergen, A. A One-Pot Reduction-Transimination-Reduction Synthesis of N-Substituted Beta-Ethanolamines from Cyanohydrins. *Tetrahedron* **1992**, *48*, 3977-3982.

(258) Jakubowski, A. A.; Guziec, F. S.; Sugiura, M.; Tam, C. C.; Tishler, M.; Omura, S. Total Syntheses of (+/-)-Cerulenin, (+/-)-Tetrahydrocerulenin, and Related-Compounds. *J. Org. Chem.* **1982**, *47*, 1221-1228.

(259) Cardellach, J.; Font, J.; Ortuno, R. M. Highly Stereo and Regiocontrolled Synthesis of Both Racemic and Optically-Active (-)-Blastmycinone. *Tetrahedron Lett.* **1985**, *26*, 2815-2816.

(260) Ortuno, R. M.; Alonso, D.; Cardellach, J.; Font, J. Enantiomeric Beta-Angelica Lactone Epoxides - Their Syntheses from Suitable Chiral Precursors and Their Use in the Preparation of Blastmycinone. *Tetrahedron* **1987**, *43*, 2191-2198.

(261) Sakai, N.; Ohfune, Y. Total Synthesis of Galantin-I - Acid-Catalyzed Cyclization of Galantinic Acid. *J. Am. Chem. Soc.* **1992**, *114*, 998-1010.

(262) Ding, F.; Jennings, M. P. Total Synthesis of (−)-Dactylolide and Formal Synthesis of (−)-Zampanolide via Target Oriented β-C-Glycoside Formation. *J. Org. Chem.* **2008**, *73*, 5965-5976.

(263) Churruca, F. t.; Fousteris, M.; Ishikawa, Y.; von Wantoch Rekowski, M.; Hounsou, C.; Surrey, T.; Giannis, A. A Novel Approach to Indoloditerpenes by Nazarov Photocyclization: Synthesis and Biological Investigations of Terpendole E Analogues. *Org. Lett.* **2010**, *12*, 2096-2099.

(264) Nociari, M. M.; Shalev, A.; Benias, P.; Russo, C. A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. *J. Immunol. Methods* **1998**, *213*, 157-167.

(265) Hamid, R.; Rotshteyn, Y.; Rabadi, L.; Parikh, R.; Bullock, P. Comparison of alamar blue and MTT assays for high through-put screening. *Toxicol. in Vitro* **2004**, *18*, 703-710.

(266) Shahan, T. A.; Siegel, P. D.; Sorenson, W. G.; Kuschner, W. G.; Lewis, D. M. A Sensitive New Bioassay for Tumor-Necrosis-Factor. *J. Immunol. Methods* **1994**, *175*, 181-187.

(267) Ahmed, S. A.; Gogal, R. M.; Walsh, J. E. A New Rapid and Simple Nonradioactive Assay to Monitor and Determine the Proliferation of Lymphocytes - an Alternative to [H-3] Thymidine Incorporation Assay. *J. Immunol. Methods* **1994**, *170*, 211-224.

(268) Schmid, C. R.; Bradley, D. A. 2,3-O-(3-Pentylidene)-D-Glyceraldehyde and 2,3-O-(3-Pentylidene)-L-Glyceraldehyde - Convenient Glyceraldehyde Surrogates Obtained Via a Novel Periodate-Based Oxidation System. *Synthesis-Stuttgart* **1992**, 587-590.

(269) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. Diprotected triflylguanidines: A new class of guanidinylation reagents. *J. Org. Chem.* **1998**, *63*, 3804-3805.

(270) Yan, R. B.; Yang, F.; Wu, Y. F.; Zhang, L. H.; Ye, X. S. An efficient and improved procedure for preparation of triflyl azide and application in catalytic diazotransfer reaction. *Tetrahedron Lett.* **2005**, *46*, 8993-8995.