SYNTHESIS AND BIOLOGICAL EVALUATION OF ANALOGUES OF A GLYCOSYLTRANSFERASE INHIBITOR

by

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DEDICATION

This dissertation is dedicated to my mother and father, Cynda and Peter, for giving me a chemistry set at the age of 9 and teaching me to think, "why not me," instead of "why me." Also, this dissertation is dedicated to my sister, Mikaela, and my girlfriend, Brooke, for their respective perseverance and courage. Finally, to all of those who may read this someday, think big and fear little.
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ABSTRACT

Glycosphingolipids (GSLs) have been found to be involved in a myriad of cellular function, including the following: cell-cell communication, cell adhesion and proliferation, neuronal growth and repair, immune response and tumor progression (metastasis). To study the cellular effects of GSL depletion, Radin developed the first known inhibitors of the enzyme responsible for the initial glycosylation of the lipid portion (ceramide) of the glycosphingolipid. This inhibitor, PDMP (1-phenyl 2-decanoyl amino 3-morpholino 1-propanol), not only suppresses the initial glycosylation, but also inhibits the formation of all preceding glycosphingolipids and causes the accumulation of ceramide, an active participant in cellular apoptosis (or programmed cell death).

Using a developed procedure, PDMP analogues with truncations about the aromatic region were synthesized. Aromatic PDMP analogues were more potent than the lead compound when tested against isolated embryonic cells from the Manduca sexta. Later procedures developed allowed for the manufacturing of head group analogue, which allowed for the generation of a water-soluble PDMP analogue. A new synthetic protocol, using a commercially available advanced intermediate, permitted the production of enantiomerically pure PDMP in five steps with an overall yield of 50%. As these compounds gain more medicinal attention, we hope to use PDMP and analogues, combined with the Manduca sexta as a model system, to gain a greater understanding of GSL functionality and cellular malfunctions—including cancer metastasis.
Chapter 1. Introduction to Glycosphingolipids & Metabolites

The lipid bilayer, created mostly of glycerol-based phospholipids, displays a wide variety of cellular and structural functions including providing a permeable barrier of cells and sub-cellular organelles. The model of membrane structure (Figure 1, adapted from proposed membrane structure by Singer and Nicholson) shows that the major subclasses of lipids exist on the cellular surface including: glycoproteins (GP), glycolipids (glycosphingolipids, GSL), cholesterol and sphingomyelin (SM, not pictured).

Figure 1. Fluid Mosaic of the Lipid Bilayer

One of the components of the plasma membrane is glycosphingolipids. The carbohydrate moiety, varying from simple to complex, extends into the aqueous media, while the lipo-portion serves as an anchor—embedded in the lipid bilayer. Though a thorough understanding of the GSLs’ functionalities is still
enigmatic, GSLs are known to be generally involved in the following: ligands for viral/bacteria infection and phagocytosis, signal transduction, and cell proliferation/differentiation.

The *de novo* synthesis of GSLs is initiated by the synthesis of lipophilic ceramide (Figure 1.1) on the membranes of the endoplasmic reticulum. The synthesis begins with the condensation of L-serine with palmitoyl coenzyme A (CoA), which is catalyzed by serine palmitoyltransferase (SPT), to give 3-ketosphinganine. Due to the low activity of SPT, this condensation is considered the rate determine step in the GSL biosynthesis process. 3-Ketosphinganine is reduced to D-erythro-sphinganine by 3-ketosphinganine reductase (NADPH-dependent). The resulting D- erythro-sphinganine is N-acylated (acyl chain lengths vary from C_{16} to C_{24}) by sphinganine N-acyltransferase to give dihydroceramide. Finally, dihydroceramide is oxidized to ceramide via dihydroxyceramide desaturase.
Ceramide, once synthesized, is an active intermediate in a multitude of metabolic pathways (Figure 1.2), including the generation of sphingomyelin, glycosphingolipids and sphingosine. Sphingosine is an intermediate towards sphingolipid degradation.
Figure 1.2. Active ceramide
Ceramide can be converted to sphingomyelin (Figure 1.3) via a pathway known as the sphingomyelin (SM) cycle.\(^\text{16}\) Phosphatidylcholine converts ceramide to sphingomyelin by transfer of the phosphatidylcholine head group.\(^\text{17}\) The byproduct of this transfer, diacyl glycerol (DAG),\(^\text{18}\) promotes protein kinase C.\(^\text{19}\) Sphingomyelin can be converted to ceramide via acidic and neutral sphingomyelinase.\(^\text{20}\)

\[
\begin{align*}
\text{phosphatidyl choline} & \xrightarrow{\text{sphingomyelin synthase}} \text{diacyl glycerol (DAG)} \\
\text{ceramide} & \xrightarrow{\text{sphingomyelinase}} \text{sphingomyelin}
\end{align*}
\]

\textbf{Figure 1.3. The Synthesis of Sphingomyelin}

The remaining degradation of ceramide can be found in \textbf{Figure 1.4}.\(^\text{21}\) Removal of the \(N\)-acyl group provides sphingosine,\(^\text{22}\) which is not considered as an intermediate in the \textit{de novo} synthesis of ceramide, though sphingosine can be \(N\)-acylated to generate ceramide.\(^\text{23}\) Sphingosine is a known inhibitor of protein kinase C.\(^\text{24}\) Phosphorylation of sphingosine yields sphingosine 1-phosphate (S 1-P), which has mitogenic properties.\(^\text{25}\) Similarly, ceramide-1-phosphate has been
found to mediate cell survival and to be involved in synaptic vesicular fusion in neuronal cells.\textsuperscript{26} S 1-P binds to Edg-1 receptor, which is coupled to an inhibitory G protein, activates mitogen-activated protein kinase (MAPK).\textsuperscript{27} S 1-P can also be metabolized in a retroaldol-fashion providing ethanolamine phosphate and hexadec-2-enal.\textsuperscript{28}

\[
\begin{align*}
\text{sphingosine 1-phosphate} & \xrightarrow{\text{ceramidase}} \text{sphingosine} \\
\text{hexadec-2-enal} & + \xrightarrow{\text{sphingosine \ 1-phosphate-Lyase}} \text{sphingosine 1-phosphate}
\end{align*}
\]

**Figure 1.4. Degradation of ceramide**

Another role for active ceramide to participate in is the generation of all GSL’s. Once ceramide synthesis is complete, the lipid is transferred, via vesicular transport, to the cisternae of the Golgi Apparatus, where the glycosyltransferase enzymes are located.\textsuperscript{29} On the cytosolic surface of the Golgi, the initial carbohydrate attached to the ceramide moiety is predominantly glucose via the glycosyltransferase (GlcT) enzyme (Figure 1.5).\textsuperscript{30} Little is currently
known about the enzyme besides its location and the sugar donor is uridine diphosphate glucose (UDP-Glc).\textsuperscript{31}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure15.png}
\caption{Glycosylation of ceramide}
\end{figure}

The glycosylated product (GlcCer) serves as the precursor to predominantly all GSL's, albeit there are cases where the initial carbohydrate is galactose. GlcCer, in the presence of galactosyltransferase (GalT), is glycosylated by UDP-galactose, forming lactosylceramide (Figure 1.6). The GalT enzyme and the remaining glycosylating enzymes of GSLs exist in the lumen of the ER-Golgi,\textsuperscript{32} indicating that there must be a mechanism to "flip" the GlcCer into the lumen of the ER-Golgi.\textsuperscript{33}

\begin{figure}
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\includegraphics[width=0.8\textwidth]{figure16.png}
\caption{Formation of Lactosylceramide}
\end{figure}

This intermediate (LacCer) is the precursor for all complex GSLs including the Gangio Series, Lacto series, Neolacto Series and Globo Series (Figure 1.7).\textsuperscript{34} Once the desired GSL has been synthesized, the GSL is delivered to the cellular surface.\textsuperscript{35} Once the desired expression/function is complete, the GSL reenters the cell via vesicular transport or "coated pits."\textsuperscript{36} These coated pits
deliver GSL to the lysosomal compartment. In lysosomes, the majority of carbohydrates are removed to regenerate Cer or GlcCer. Cer (or GlcCer) can then be glycosylated to form a new GSL or metabolized (Figure 1.4). The recycling of GSLs is diagramed in Figure 1.8. Several known diseases stem from deficiencies in GSL metabolism and storage.

Figure 1.7. Complex GSL series
Originally, GSLs were believed to be ubiquitously located on the membrane surface.\textsuperscript{42} However, recent research has found that GSLs will group themselves together in what are known as GSL rafts (Figure 1.9) or glycolipid-enriched microdomains (GEMs).\textsuperscript{43} These rafts also contain sphingomyelin (SM), ceramide (Cer), cholesterol and GPI (glycophosphodiylinositol)-linked proteins.\textsuperscript{44}
Rafts can form either prematurely in the late Golgi, or self-assemble once delivered to the surface. Although GSLs compose only ~5% of the total lipid population, GEMs can represent anywhere from 13-50% of the surface area in a given cell. GEMs are implicated in many signaling processes, though the raft, itself, is not sufficient for signaling. Raft formation involvement in the signaling process may simply be influencing the transportation of signals. GEMs also appear to be involved in protein sorting in both biosynthetic and endocytotic pathways.

The driving force for the formation of the rafts is currently unknown. The raft structure maybe stabilized by two forces: 1) hydrogen-bonding between the
cholesterol hydroxyl group and the amide portion of the sphingolipid and 2) the van der Waals interactions between the hydrocarbon units. Cholesterol also fills the voids present in the hydrocarbon chains caused by the bulky sugar moieties—providing tighter packing of the lipophilic portion of the raft. Disruptions in the cholesterol concentrations, using such drugs as methyl-β-cyclodextrin, generally results in disruption of raft-mediated cellular functions.

The actual role of SM and GSLs in these rafts is currently unknown. Studies with mutant CHO (Chinese Hamster Ovary) cells that are defective in SM biosynthesis (without Cer accumulation) found that SM had no effect on the steady-state level of cellular cholesterol and its distribution to the plasma membrane. However, the lower levels of SM caused disruption in the localization of cholesterol in rafts, which enhanced cellular cholesterol efflux to extracellular acceptors of cholesterol. These findings are consistent with other findings that cholesterol-SM interactions are stronger than cholesterol-phospholipid interactions.

Some investigations into GSL functionality have ventured into determining the cellular responses to GSL depletion. Mutant GSL-deficient CHO cells (without Cer accumulation) were found to be hypersensitive to phosphotidylinositol-specific phospholipase, which releases various GPI-anchored proteins from the plasma membrane by cleavage at the GPI-anchor. In these mutant GSL-deficient cells, depletion of GSLs did not affect any of the following: normal cellular functioning, cholesterol/SM concentrations and
cholesterol efflux to extracellular acceptors. Using a known inhibitor of GSL biosynthesis, D-threo-PDMP (Chapter 2), studies have determined that T cells devoid of GSLs are still capable of signal transduction via T cell receptor,\textsuperscript{59} albeit cells were also hypersensitive to phosphotidylinositol-specific phospholipase. The removal of GSLs had no effect on cholesterol and SM levels, nor GEM integrity in T cells.\textsuperscript{60} In the latter case, authors did not comment on the cellular ceramide levels, which can increase in the presence of D-threo-PDMP.\textsuperscript{61}

As new technologies became available, new findings have led to a better, more specific understanding of the significance of particular GSL's. The simplest GSL, GlcCer, has been determined to be essential for embryogenesis.\textsuperscript{62} Genetically modified mice, whose GlcCer synthase is knocked-out, die as early as the 19\textsuperscript{th} day of embryogenesis.\textsuperscript{63} Intracutaneous administration of GlcCer in hairless mouse epidermis stimulated epidermal DNA synthesis and hence epidermal mitogenesis.\textsuperscript{64} Moreover, intraperitoneal injections of GlcCer into rats induces tissue hypertrophy, including 19-24\% increase in liver mass.\textsuperscript{65}

GlcCer has also been found to be involved in cellular multidrug resistance.\textsuperscript{66} GlcCer levels are consistently increased in several multidrug-resistant cancer cell lines.\textsuperscript{67} It has been demonstrated that some sensitive cells acquire drug resistance by overexpressing GlcCer synthase.\textsuperscript{68} Inhibition of GlcCer generation has been found to result in increased sensitivity to adriamycin in multidrug resistant MCF-7 breast cancer cells.\textsuperscript{69}
Lactosylceramide (LacCer), generated by the attachment of galactose to GlcCer (see Figure 1.6), has been found to stimulate aortic smooth muscle cell proliferation by five fold. The enzyme responsible for this glycosylation, β1,4-GalT-4, has been found to be overexpressed in several tumors in rats induced by adenocarcinoma cells. LacCer, resulting from the hydrolysis of gangliosides, has been claimed to inhibit apoptosis by increasing Bcl-2 (an anti-apoptosis peptide) and decreasing caspase expression (a pro-apoptosis peptide).

As for the complex GSLs, which stem from LacCer (especially sialic acid-bearing GSLs called gangliosides), the plot seems to thicken. Mono-sialic acid ganglioside, GM1 (Figure 1.10), added exogenously inhibited cell outgrowth in human epidermoid carcinoma cell lines (KB and A431) and mouse Swiss 3T3 cells. GM1 has also been shown to compete with GPI-AP (glycosylphosphatidylinositol-anchored proteins) for raft occupation in a supported model membrane monolayer. Exogenously added GM1 can displace GPI-APs from the lipid microdomain in living cells. Taken together, alterations in GSL expression can have drastic effects on cellular growth and dynamics.
However, GM1 has also been reported to prevent apoptotic cell death in growth factor-deprived neuronal PC12 cells by promoting nerve growth factor (NGF)-induced TrkA dimerization.\textsuperscript{78} It has been shown that exogeneous addition of GM1 can act similar to exogeneous addition of S 1-P (sphingosine-1-phosphate) and protected cells from ceramide-induced apoptosis\textsuperscript{79} (ceramide-induced apoptosis will be discussed in greater detail in a later chapter). Later work found that GM1 enhanced S 1-P production by activating sphingosine kinase.\textsuperscript{80} GM1 is currently ongoing clinical trials as a therapeutic agent for promoting nerve regeneration in Alzheimer's disease.\textsuperscript{81}

Ganglioside GD3 (Figure 1.11), on the other hand, has been identified as a pro-apoptotic agent.\textsuperscript{82} Apoptosis is a natural process in which a cascade of events ultimately leads to cellular death via a mechanism(s) currently being elucidated.\textsuperscript{83} Though the mechanism of apoptosis is enigmatic, apoptosis is believed to result from activation of proteases and nucleases in the nucleus to
destroy chromatin and, finally, DNA without affecting the integrity of the cellular membrane (unlike necrosis).

Figure 1.11. Ganglioside GD3

Ceramide and metabolites have gained an immense amount of scientific interest since ceramide was found to be involved in cellular apoptosis. The medicinal value of understanding apoptosis is crucial when one considers that malfunctions in apoptosis can lead to dire consequences: too much apoptosis maybe at fault in Parkinson’s Disease, Alzheimer’s Disease and stroke damage, while too little apoptosis may cause cancer and autoimmune diseases. Currently, these malfunctions in apoptosis are attributed to parameters/thresholds that control increases/decreases in apoptosis, rather than faultiness in the machinery necessary for apoptosis.

Although the mechanistic understanding of apoptosis is in an infancy stage, Cer generation/accumulation has gained acceptance as a contributor to inducing cellular apoptosis. Many studies have revealed that in times of cellular stress (including apoptosis), that Cer is generated via either increased de novo
synthesis or activation of sphingomyelinase. The increased levels of Cer can cause all of the following: activation of either a Cer-activated kinase, a cytosolic Cer-activated protein phosphatase, PKC-ζ, NF-κB and release of cytochrome C or a reactive oxygen species (ROS) from the mitochondria. Given all of the possible activities, Cer is considered to be a second messenger in apoptosis.

However, ceramide can also be considered a modulator of membrane structure and dynamics that lead to apoptosis. Cer can increase rigidization of cellular membranes in rafts and increase permeability of mitochondrial outer membranes for a number of small proteins, including cytochrome C, a well-accepted trigger of apoptosis. Given that ceramide has a role in apoptosis, whether as a second messenger or modulator of membrane dynamics/structure, one can see the importance of synthesis and development of compounds that increase the levels of endogeneous ceramide.

Scientist have found that apoptosis (Figure 1.12) is triggered by the binding of an external ligand (e.g. Fas or tumor necrosis factor-alpha, TNF-α) to the cellular surface. Once bound, this agent promotes the hydrolysis of surface-bound-sphingomyelin (SM) to ceramide (Cer) by activating acid SMase (acid sphingomyelinase). Cer is rapidly converted to GD3, which enters the cell presumably via actin-dependent endosomal vesicles. Cer can be generated via two ways—increase in de novo synthesis or activation of sphingomyelinase—both of which can induce apoptosis. Conversely, it
appears that only newly synthesized GD3, not GD3 generated from complex GSL degradation, is involved in apoptosis.\textsuperscript{104}

Figure 1.12. Possible Mechanism for Ceramide-induced Apoptosis\textsuperscript{105}

Once GD3 enters the cell, it is delivered to the mitochondria and interacts with the mitochondria surface—causing an oxidative burst and mitochondrial swelling.\textsuperscript{106} The mitochondrial swelling causes the release of \textit{intra}-mitochondrial elements such as cytochrome C and/or ROS (reactive oxygen species).\textsuperscript{107} This release maybe caused by GD3-induced: decrease in mitochondrial membrane potential ($\Delta\Psi_m$)—causing an increase in the permeability of the inner mitochondrial membrane,\textsuperscript{108} opening of the mitochondrial permeability transition
pore or interactions with complex III of the mitochondrial electron transport chain.\textsuperscript{109}

Given the immense involvement of GSLs in biological systems, it seems imperative to gain a greater understanding of GSLs functionality. In earlier attempts to gain a greater understanding of biological behaviors in mammals, scientists have found that studying species of the insect world can help gain insight.\textsuperscript{111} While the systems within the human can be complex and difficult to monitor, the insect existence is simpler and more predictable. For example, while simpler organisms, like insects, contain approximately 1200 neurons in their brain, the human brain contains on the order of $10^{10}$ neurons.\textsuperscript{112} Prior studies on the nervous systems of insect species have provided a greater understanding of nervous system plasticity in humans.\textsuperscript{113} Research on insect species also benefit from the shorter life and the ease of reproduction of the specimen and the ability to obtain reproducible results from insect testing(s). The *Manduca sexta* (or *M. sexta*, Sphinx Moth or hawkmoth), in particular, has well-studied life cycle and larger size, making it a favorite insect of study in the field of development biology.\textsuperscript{114}

The *M. sexta* is a holometabolous insect,\textsuperscript{115} meaning the initial phenotype bears little resemblance to the final. The life cycle of the *M. sexta* is pictorial depicted in Figure 1.13.\textsuperscript{116} The *M. sexta* emerges from the egg as a larva (commonly known as worm or caterpillar) to feed and grow. The larva, which exists as five different stages or instars, metamorphoses into a pupa (cocoon)
and undergoes extensive reorganization of the body plan; it then emerges as an adult (moth) and reproduces.

Figure 1.13. The life cycle of the *M. sexta*

The entire process of metamorphosis in the *M. sexta* has been well studied. Several hormones control metamorphosis including: the juvenile hormone (JH), prothoracicotropic hormone (PTTH), among others. JH maintains tissues in the larval stage, which hence prevents maturation in the form of metamorphosis. JH (Figure 1.14), an extremely lipophilic molecule, requires a
carrier protein to ensure property deliver and avoid degradation. This carrier protein also protects JH from general esterases, which can hydrolyze the hormone, but cannot defend JH from the JH-specific esterase. Release of the JH-specific esterase metabolizes the hormone via either ester-hydrolysis (major pathway) or hydration of the epoxide (minor pathway). After metamorphosis, JH levels rise again to promote the formation of sexual organs.

![Chemical structure of JH](image)

**Figure 1.14.** JH isolated from cecropia moth *Hyalophora cecropia*

As JH product levels decrease, prothoracicotropic hormone (PTTH), a neuropeptide produced in neurosecretory cells, is released into the blood and travels to the prothoracic glands. Once PTTH arrives to these glands, PTTH activates the release two types of ecdysteroids—α-ecdysone and 20-hydroxy-α-ecdysone (**Figure 1.15**). The more active compound, 20-hydroxy-α-ecdysone, activates disconnect of larva neuronal and muscular networks and connection of the new neuronal muscular developments of the moth.

![Ecdysteroid structures](image)

**Figure 1.15.** Ecdysteroid structures
During this metamorphic stage, clusters of cells that were "put aside" during embryogenesis now proceed to develop, producing structures such as the adult antenna, wings, and compound eyes; and internal structures such as muscles are reorganized to suit the requirements of the adult form of the insect.\(^4\) The nervous system also is reorganized. Sensory receptor axons arriving from newly formed sensory structures like the eye and the antenna impose new components that pattern the circuitry in the primary sensory regions of the brain;\(^1\) branching structures of some neurons are changed, \(^1\) neurons are added to the system, and some neurons die, their presence no longer needed in the adult.\(^2\) The adult lives about a week, during which time it mates and lays eggs.

Though the hormones involved in metamorphosis of the \textit{M. sexta} are known, little is known about GSLs involvement in not only metamorphosis, but also embryogenesis. To date, GSLs have not been isolated and characterized in the \textit{M. Sexta}. However, GSLs have been isolated in other insect species and we have observed evidence that supports the presence of GSLs in the \textit{M. sexta}. Abeytunga et al.\(^2\) have isolated two varieties of ceramides (Cers) and sphingomyelins (SMs) molecules (Figure 1.16).
Figure 1.16. Isolated SMs and Cers in the *M. sexta*

Lipid rafts were also isolated from brains of the *M. sexta*, and the association of these novel SMs with rafts was confirmed. This finding suggests that *Manduca* SMs may participate in the formation of lipid rafts, which is consistent with their function in vertebrates. Interestingly, the ratio of each SM, mono unsaturated: doubly unsaturated, varies depending on the stage of development. In 5-day-old 5th instar larvae, the ratio of mono unsaturated: doubly unsaturated is 5:1; while in the stage 12 metamorphosing adults (pupae) the ratio was 10:1. Changes in corresponding ceramide ratios, mono unsaturated: doubly unsaturated, have not been observed.
In two species of Calliphoridae, the *Lucilia caesar* (green-bottle fly) and the *Calliphora vicina* (blowfly), GSLs have been isolated.\(^{124}\) The GSLs that exist in invertebrates known as arthrosides (or Arthro series, **Figure 1.17**) because the carbohydrate sequence found in invertebrates resembles the series first encountered in species of the Arthropodes.\(^{125}\) The Mollu series has been isolated from the Mollusks.\(^{126}\)

**Figure 1.17.** GSLs of Vertebrates and Invertebrates

These arthrosides bear some resemblance to the GSLs of mammalian systems. GlcCer is a common intermediate in both vertebrates and
invertebrates. Studies with GlcCer have shown that GlcCer is necessary for embryogenesis in mammals and causes hypertrophy in human liver cells.

As stated earlier in this chapter, complex GSLs, which stem from GlcCer, in mammalian systems are involved cell-cell communication, cell adhesion and proliferation, neuronal growth and repair, immune response and tumor progression (metastasis). We envision that the majority of functionalities of the GSLs in the vertebrates are also true for the arthrosides in the invertebrates. We also believe that studying the effects of arthroside perturbation in the M. sexta could help elucidate GSL-functionality relationships that would also apply to mammalian systems. Since the vertebrate and invertebrate GSLs have GlcCer as a common intermediate, we envision that by blocking the formation of GlcCer (using enzyme inhibitors) could provide a greater understanding of GSL functionality (via GSL depletion) in the vertebrate and invertebrate systems. Ultimately, our findings could lead to human relevance, including cancer metastasis and neuronal repair.

While it’s difficult to determine the concentration of drug necessary to exhibit medicinal and behavioral effects in vivo, it is easier to determine minimum dosages required to block neurite extensions in vitro. Our work will first focus on determining these minimum dosages and generating analogues that have greater potency. We will also want to see if inhibition is reversible to observe if GSL levels fluctuate during these times.
Gaucher's disease is a genetic disorder where GlcCer accumulates due to ineffective GlcCer glycosidase. The excess GlcCer is transferred to macrophages—causing hypertrophy of organs where these storage cells reside, the liver, lymph nodes and the spleen. Infiltration of the bone marrow by these storage cells can result in painful bone damage. In order to help patients with Gaucher's disease, Radin addressed the need to develop an inhibitor of the glycosylceramide synthase enzyme (GlcCer synthase, Figure 2.1). It was envisioned that by inhibiting the GlcCer synthase enzyme, one could reduce GlcCer formation.

![Figure 2.1. The glycosylation of ceramide by the GlcCer synthase enzyme](image)

At the time, little was known about the active site of the GlcCer synthase enzyme. Original inhibitors used were the N-acyl version of the amines in Figure 2.2 resembling the substrate, ceramide. A decanoyl chain replaced the longer N-acyl chain found on natural ceramide (C_{14}-C_{22}). The decanoyl chain length provided enough lipophilicity to serve as a glycosyltransferase-inhibitor.
The sphingosine backbone (long alkenyl moiety) was replaced with a phenyl ring. The phenyl ring not only retained the lipophilicity of the substrate, but also provided the "bend" in the backbone of ceramide produced by the trans-double bond. While the natural D-erythro ceramide analogues (2.2) served as glycone acceptors, the D-threo (2.1), and to a lesser extent the L-threo, served as competitive inhibitors. Replacement of the primary hydroxyl group with a hydrogen atom (2.3) provided significant inhibition, but removal of benzylic alcohol (2.4) displayed ineffective inhibition in brain homogenates from young rats.

As studies on the GlcCer synthase enzyme increased, interest also increased on studying enzymes responsible for attaching carbohydrates to substrates. These enzymes are collectively known as glycosyltransferases (or GTases). Studies on the folding patterns of all known GTases have revealed that GTases adopt only two folds. Rather generically, one fold is involved in nucleotide binding, while the other fold is for acceptor binding. The nucleotide
binding region of GTases is known to possess strong structural restraint on the coordination of a metal ion (Mn⁺⁺ in most cases)\(^{141}\) by two phosphate oxygen atoms from the sugar donor.\(^{142}\) Most GTases, including the GTase responsible for the glycosylation of ceramide, are "inverting enzymes" (α-donor; β-product) at the anomeric position.\(^{143}\) The transition state of these GTases possesses considerable oxocarbenium-ion-like character and is general base catalyzed (by Asp or Glu acid residues).\(^{144}\) The transition state of the glycosylation of ceramide was deduced to look similar to the transition state in Figure 2.3.

Later enzyme inhibition studies elucidated the following four properties of the GlcT enzyme active site: 1) an anionic moiety may bind to the active form of glucose, 2) oxygen-binding region oriented toward the third carbon (allylic position) of ceramide, 3) narrow region that binds alkyl chain of the fatty acid
chain and 4) less narrow region that binds the hydrocarbon chain of the sphingoid base.\textsuperscript {145}

Replacing the primary hydroxyl group with a morpholine moiety was found to increase potency. This increase in inhibition was attributed to the cationic property of the amine group and its attraction to the anionic moiety of the active site. When the electronics of the primary position were changed to anionic (carboxylate), the inhibition was severely decreased. Radin proposed that the positive charge of the protonated amine mimicked the oxonium ion, which forms during the transition state of the GlcCer synthase enzyme (Figure 2.4).

Initial testing found that DL-keto amine (Figure 2.5) was able to completely deactivate the GlcCer synthase enzyme at 300 μM within one hour.\textsuperscript {146} Kinetic studies revealed DL-keto amine irreversibly inhibited the GlcT enzyme by
either forming a covalent linkage to the active site or binding elsewhere on the enzyme, making the enzyme thermally unstable or catalytically inactive.\textsuperscript{147}

![DL-keto amine](image)

\textbf{Figure 2.5.} DL-keto amine

When the keto DL-morpholino compound was administered to mice \textit{in vivo}, the GlcCer levels in the liver dropped by 48\% within two hours. Combined with the decrease in GlcCer levels was an increase in ceramide levels. After 24 hours, however, the GlcCer levels returned to concentrations similar to the concentrations of the control group. Another drawback to the utilization of DL-keto amine was the morpholino ketone was also found to inhibit monoamine oxidase,\textsuperscript{148} which caused behavioral abnormalities.

In search of an analogue to DL-keto amine that did not inhibit monoamine oxidase, Radin found that the reduction of the morpholino ketone with sodium borohydride provided four isomers of the amino alcohol, known as PDMP (1-phenyl 2-decanoyl amino 3-morpholino propanol) (\textbf{Figure 2.6}).\textsuperscript{149}
Using a mixture of the four isomers, PDMP proved to be more potent than the morpholino ketone. Unlike the DL-keto amine, PDMP inhibited the GlcT enzyme in a reversible fashion. When lyophilized mouse brain was employed as the source of GlcCer synthase, 38 µM PDMP produced 84% inhibition compared to 34% inhibition provided by the morpholino ketone at the same concentration.\textsuperscript{150} In addition, PDMP did not inhibit monoamine oxidase.\textsuperscript{151}

Early tests showed that only the \textit{threo} diastereomers, particularly the D-enantiomer, had the most inhibition activity.\textsuperscript{152} Later testing showed that the L-\textit{threo} stereoisomer also had enzymatic activity.\textsuperscript{153} D-\textit{threo}-PDMP exhibits a mixed inhibitor profile relative to ceramide and is noncompetitive for the glycosyl donor.\textsuperscript{154} The apparent \( K_i \) is 0.7 µM.\textsuperscript{155}

\textit{In vivo} mice studies with radioactive D-\textit{threo}-\textsuperscript{3}H]PDMP (typically 80 mg/kg) showed that much of the radioactive D-\textit{threo}-PDMP entered the blood.
and organs very rapidly, but concentrations dropped to nearly zero in three hours.\textsuperscript{156} In order to increase the effectiveness of D-*threo*-PDMP, piperonyl butoxide was co-administered to block the oxidation of D-*threo*-PDMP by \textit{P}-450 cytochrome.\textsuperscript{157} Due to the presence of piperonyl butoxide, high levels of D-*threo*-PDMP were reported for at least 16 hours, but the entire drug was gone by 24 hours. In studies with B16 melanoma cells, though D-*threo*-PDMP caused a reduction of GlcCer levels after just 2.5 minutes of exposure, the effect of D-*threo*-PDMP on GSL suppression diminished in 2 hours after the removal of the drug media.\textsuperscript{158} The fast effects of D- *threo*-PDMP on GlcCer levels suggest that D-*threo*-PDMP, not a metabolite, produces the observed alterations.\textsuperscript{159}

As the testing with D-*threo*-PDMP increased, it was observed that D-*threo*-PDMP not only blocked GlcCer formation, but also depleted cells of all GSLs where GlcCer served as the precursor, including sialic acid-containing GSL's known as gangliosides.\textsuperscript{160} This inhibitor was specific for the GlcCer synthase. PDMP does not affect GalCer synthase.\textsuperscript{161} By impeding the glycosylation of ceramide, PDMP causes an increase in \textit{endogeneous} ceramide (167% increase compared to untreated cells),\textsuperscript{162} which can induce apoptosis.\textsuperscript{163} In addition, the level of ceramide metabolites, sphingosine, increased by 167%.\textsuperscript{164} Other metabolites of ceramide, sphingomyelin and diacyl glycerol (DAG) are also in larger abundance in PDMP-treated cells.\textsuperscript{165} With higher concentrations of PDMP, sphingomyelin can decrease in concentration.\textsuperscript{166} Though PDMP blocks glycolipid formation, PDMP does not inhibit glycoprotein synthesis. PDMP can,
however, slow protein and DNA synthesis, apparently by hampering protein transport through the Golgi apparatus. Studies with fluorescent analogue of PDMP demonstrated that most cell-associated PDMP was sequestered in lysosomes, while the concentration at the Golgi complex was relatively low.

Though PDMP causes intracellular changes, it appears to have minimal long-term effect on cell morphology and physiology, including: microtubule and ER structure, mitochondrial function and endocytosis. The following short-term phenotypic changes/effects of PDMP-treated 3T3 cells were observed: 1) loss of cobblestone appearance at cell density saturation and development of fibroblastic appearance with partial overlapping of cell; 2) reduction of cell growth rate: enhanced production of lactic acid; 3) enhanced rate of glucose transport and 4) higher incidence of large colony formation with infiltrating appearance in soft agar. All of these alterations were reversed after the administration of gangliosides except rate of cell growth and colony formation with infiltrating appearance in soft agar.

Cells exposed to PDMP have decreased PKC-activity; whether this observation is due to increased levels of ceramide, sphingosine or PDMP itself is currently unknown. Interestingly, when the morpholine head group is replaced with a pyrrolidine head group and the decanoyl N-acyl chain was replaced with a more lipophilic, palmitoyl, chain, the drug was able to inhibit the formation of GSLs at lower concentrations. However, this analogue, D-threo-PPPP, does not cause ceramide accumulation and has no effect on cellular growth and
embryogenesis. These findings suggest a possible second mode of action by D-threo-PDMP and/or begin to explain which of the observable changes in cellular behavior by D-threo-PDMP are due to ceramide accumulation or GSL depletion.

In the most recent PDMP "news," administration of D- or L-threo-PDMP (0.5 to 20 μM) to human carcinoma cells caused cellular blebbing and DNA condensation. These cellular changes are typical of Caspase-3-induced apoptosis, where cleavage of targeted proteins leads to disassembly of the chromosomes and the break down of the nucleus. These inhibitors were found to induce apoptosis in carcinoma cells after 6 hours—damaging DNA (via Caspase-3 activation) between 24 and 48 hours after treatment. New studies should hopefully not only gain insight to the mechanisms of apoptosis and cancer cell’s immunity to apoptosis, but also determine if D- or L-threo-PDMP is selective—that is, can the inhibitor(s) selectively cause cancer cells to undergo apoptosis in the presence of normal cells?
Chapter 3. Compounds which Disrupt Ceramide Synthesis/Metabolism

There are a multitude of molecules that researchers have found that induce cellular apoptosis or thwart cellular growth by affecting "ceramide homeostasis." Table 3.1 provides a comprehensive list of substances that elevate cellular ceramide concentration and induce apoptosis. Herein, molecules, which disrupt ceramide synthesis/metabolism, will be discussed.

**Table 3.1. Substances which effect ceramide metabolism/synthesis**

<table>
<thead>
<tr>
<th>Drug or metabolite</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMP and analogues</td>
<td>Inhibits GSL formation</td>
</tr>
<tr>
<td>N-Butyl deoxyoajirimycin</td>
<td>Inhibits GlcCer synthesis</td>
</tr>
<tr>
<td>Buthionine sulfoximine</td>
<td>Inhibits GSH&lt;sup&gt;a&lt;/sup&gt; syn., speeding Cer syn.</td>
</tr>
<tr>
<td>N-Oleoyl ethanolamine</td>
<td>Inhibits acid ceramidase and GlcCer synthase, stimulates SMase&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>threo-sphinganine</td>
<td>Inhibits phosphorylation of sphingolipids.</td>
</tr>
<tr>
<td>N,N-Dimethyl sphingosine</td>
<td>Inhibits sphingosine kinase</td>
</tr>
<tr>
<td>Dexamthasone</td>
<td>Stimulates SMase</td>
</tr>
<tr>
<td>PSC 833 (Valspodar)</td>
<td>Speeds Cer de novo synthesis/SMase</td>
</tr>
<tr>
<td>Dietary β-sitosterol</td>
<td>Stimulates Cer formation</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Activates SMase</td>
</tr>
<tr>
<td>Compound</td>
<td>Effect</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>D-erythro-MAPP</td>
<td>Inhibits ceramidase</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Inhibits GlcCer synthase</td>
</tr>
<tr>
<td><em>Trans</em> retinoic acid</td>
<td>Slows GlcCer synthesis</td>
</tr>
<tr>
<td>RU486</td>
<td>Inhibits ceramide glycosylation</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Inhibits GlcCer synthase</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>Promotes SMases</td>
</tr>
<tr>
<td>THC</td>
<td>Speeds SMase</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>Stimulates Cer <em>de novo</em> synthesis</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>Produces elevated Cer</td>
</tr>
<tr>
<td>Arabinofuranosylcytosine</td>
<td>Stimulates SMase</td>
</tr>
<tr>
<td>Phorbol diester</td>
<td>Stimulates Cer <em>de novo</em> synthesis</td>
</tr>
</tbody>
</table>

*aGSH, glutathione; bSMase, sphingomyelinase*

Given ceramide's apoptotic activities, analogues of natural ceramide have been studied. Due to natural ceramide's poor solubility, studies used the more hydrophilic C₂-ceramide (Figure 3.1). C₂-ceramide was able to cause some cellular behaviors similar to natural ceramide including: activation of caspase, DNA fragmentation, release of cytochrome-C, activation of retinoblastoma-gene product (Rb) and inactivation of PKC. However, C₂-ceramide was not able to mimic natural Cer's ability to induce NF-κB and members of the MAP
kinase family.\textsuperscript{181} Though C\textsubscript{2}-ceramide has medicinal value as an inducer of apoptotic activities, one must be wary of deducing that cell behavior changes by C\textsubscript{2}-ceramide are identical to or mimic natural ceramide.\textsuperscript{182}

![Figure 3.1. C\textsubscript{2}-ceramide](image)

However, C\textsubscript{2}-ceramide requires a concentration too high to be developed as a potential anti-proliferative agent (IC\textsubscript{50} value of 31.6 \textmu M).\textsuperscript{183} Macchina et al.\textsuperscript{184} synthesized a variety of conformationally constrained ceramide analogues (Figure 3.2, 3.1-3.4) and tested their potency against CCRF-CEM cells. Analogues 3.1 and 3.2 were found to have stronger IC\textsubscript{50} values (1.7 and 7.9, respectively) than C\textsubscript{2}-ceramide and induce apoptosis in a caspase-3 manner.\textsuperscript{185} Analogues 3.3 and 3.4, on the other hand, lacked potency.

![Figure 3.2. Conformationally constrained ceramide analogues.](image)
Another analogue of ceramide that has gained interest is the 3,4: 6,7-dialkenyl ceramide 3.5 (Figure 3.3). The doubly-unsaturated ceramide has been isolated from natural sources, including Manduca sexta. Bittman has shown that the doubly-unsaturated ceramide is more potent than the mono-unsaturated ceramide. At 30 µM, ceramide analogue 3.5 had 10% viability, while natural ceramide had 50%. Shang-U Kim has recently accomplished the synthesis of this molecule.

Another ceramide-like molecule that thwarts cellular growth by affecting ceramide levels is 1-methylthiodihydroceramide (3.6, Figure 3.4). Compound 3.6, at 10 µM, caused a decrease in de novo ceramide biosynthesis by 90% in primary cultured cerebellar neurons. Initial tests showed that the decrease in ceramide levels was not due inhibition of ceramide precursors. Instead, the reduced level of ceramide was attributed to the 2.5-fold increase in the activity of sphinganine kinase (leading to sphinganine degradation). Depletion of sphinganine levels impeded axonal growth in cultured hippocampal neurons. Interestingly, the inhibition was observed with both the threo- and erythro-isomer of 3.6. The free base of 3.6 had no effect on growth. The synthesis of the threo
isomer of 3.6 could be accomplished using commercially available S-methyl D-cysteine and established Polt methodology (see later chapters).

Figure 3.4. 1-Methylthiodihydroceramide 3.6 in action

In addition to inhibitors of the GlcCer synthase, inhibitors of the Cer synthase have gained attention. The Cer synthase enzyme is responsible for the N-acylation of either sphinganine (3.7) or sphingosine (3.8) to form ceramide (3.9).²
Therefore, Cer synthase is involved in *de novo* synthesis (from sphinganine) and in the recycling pathway (from sphingosine). The most prominent inhibitor of the Cer synthase is a family of microbial secondary metabolites known as fumonisins. Specifically, fumonisin B₁ (FB₁, Figure 3.6) has been studied the most extensively because it is responsible for a number of diseases of veterinary animals and humans.
FB₁ inhibits Cer synthase in vitro in the $K_i$ range of 0.05 to 0.1 μM.\(^{196}\) Enzymatic studies have led researchers to believe that FB₁ inhibits by interacting with the binding sites for both substrates (sphingolipid and fatty acyl-CoA).\(^{197}\) FB₁ shows rapid depletion of sphingolipid mass (via sphingosine/sphinganine decomposition)\(^{198}\) in cells undergoing rapid membrane synthesis, \(^{199}\) while FB₁ displays limited effect in cells where lipid turnovers is slow.\(^{200}\) Both FB₁ and PDMP have been reported to cause shortening of axon plexus and less axonal branching in hippocampal neurons.\(^{201}\)

Polyhydroxylated piperidines (e.g. compound 3.11, Figure 3.7), also known as iminosugars or aza-sugars, have long been known as inhibitors of
glycosidase enzymes. In the presence of the glycosidase enzyme the secondary amine is considered protonated, making it isoelectronic with the oxonium ion.

\[\text{HO-} \text{HO-} \text{HO}\]

\[\text{NS} \rightleftharpoons \text{OH} \; \text{OH}\]

**Figure 3.7.** Glycosidase Enzyme: Intermediates, Products and Inhibitors

Researchers later found that placing alkyl groups on the ring nitrogen caused inhibition of N-linked glycoprotein processing and glycosyltransferase activity. \(^{204}\) N-butyl-deoxynojirimycin (NB-DNJ, 3.12), like PDMP, blocked the formation of GlcCer and all proceeding GSLs at 50 µM (Figure 3.8). \(^{205}\) Inhibition of the GlcCer synthase by NB-DNJ was determined to be competitive for ceramide \((K_i=7.4 \; \mu M)\) and non-competitive for the UDP-glucose, indicating ceramide mimicry as inhibitory activity. \(^{206}\) NB-DNJ is also a potent inhibitor of HIV replication *in vitro*. \(^{207}\) NB-DNJ has also gained attention as treatment for Gaucher’s disease among other glycolipid storage disorders. \(^{208}\)
After early encouraging findings, a plethora of NB-DNJ analogues were produced and tested.\textsuperscript{209} Increasing the length of the \textit{N}-alkyl chain caused an increase in potency, but also resulted in an increase in cell lysis and death (3.13, Figure 3.9).\textsuperscript{32} Cellular toxicity could be overcome by decreasing lipophilicity with oxygen-substituted alkyl chains (3.14).\textsuperscript{32} Esterification of the alcohol caused a decrease in potency (3.15). Interestingly, the galactose analogue of NB-DNJ (3.16, \textit{C}_4-epimer of 3.12) selectively inhibited the GSL biosynthesis and no effect on the \textit{N}-linked glycoprotein processing.

Recently, Guerrera and Ladisch have shown that 50 \textmu M NB-DNJ reduced MEB4 (murine melanoma tumor) cells GlcCer synthase activity by 70\%.\textsuperscript{210} The observed decrease in GlcCer synthase activity also slowed ganglioside synthesis.
and ganglioside "shedding" (cancerous cells' ability to eradicate ganglioside from their cellular surface, which may explain cancerous cells' immunosuppression) by 61% and 37%, respectively. Partial ganglioside depletion caused a delay in tumor onset but not in tumor incidence, possibly due to the rapid recovery of gangliosides after 48 hours of exposure to NB-DNJ.

Though NB-DNJ does block the formation of GSLs by inhibiting the GlcCer synthase enzyme, this inhibition does not result in the increase of endogeneous ceramide. Unlike PDMP, the NB-DNJ inhibition of GlcCer synthase by NB-DNJ does not cause any of the following: suppression of cellular growth, apoptosis or thwart embryogenesis. This problem/feature of NB-DNJ allows researchers to study solely the cellular effects of GSL depletion, without the complications of ceramide accumulation and/or cellular viability.

One final molecule of "apoptotic interest" are analogues of a natural product from *Isodon excisus* (3.17) Hergenrother et al. found that analogues could selectively induce apoptosis in cancer cell lines (HL-60 and U-937), but not in non-cancerous cell lines (Figure 3.10). After screening a combinatorial library of analogues of 3.17, the most potent analogue (3.18) had an IC₅₀ value of 44 μM in cancerous cell lines. Tests with 3.18 was against non-cancerous cell lines revealed that 3.18 had an IC₅₀ value greater than 1000 μM. The authors did not propose any mechanisms as to how 3.18 induced apoptosis selectively in cancerous cells, yet practically inert in non-cancerous cells. This author speculates that the observed induction of apoptosis is related to ceramide
accumulation because of the structural similarities between 3.18 and compounds that were found to inhibit the GlcCer synthase enzyme.\textsuperscript{217}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure310.png}
\caption{Inducers of Apoptosis selectively in cancerous cell lines}
\end{figure}
Chapter 4. Prior syntheses of D-threo-PDMP

Radin first synthesized PDMP (1-phenyl 2-decanoyl amino 3-morpholino 1-propanol) starting with 2-bromo acetophenone (4.1, Scheme 4.1).\textsuperscript{218} Amination of 2-bromo acetophenone with hexamethylene tetramine, followed by acylation provided keto amide 4.2. Hydroxymethylation with formalin provides alcohol 4.3. Deacylation and re-acylation with decanoyl chloride provides 3-hydroxy-1-phenyl-oxo-2-amide (4.4). Reduction of the ketone 4.4 with sodium borohydride gave a mixture of diastereomers as racemic mixtures of diol 4.5.\textsuperscript{219} The methodology that delivers the morpholine substitution at the 3-position and the yields for each reaction were not provided. Original screenings used mixtures of stereoisomers,\textsuperscript{220} though later assays used enantiomerically pure D-threo-PDMP that was purified by recrystallization or HPLC.\textsuperscript{221}

![Scheme 4.1. The original synthesis of PDMP](image)

As investigations into D-threo-PDMP increased, synthetic approaches toward enantiomerically pure D-threo-PDMP (and analogues) increased. Carson et al.\textsuperscript{222} developed methodology that was applicable towards an alkenyl analogue, which gave a greater resemblance to ceramide and better inhibition
(Scheme 4.2). Aldol condensation of oxazolidinone 4.6 with unsaturated aldehyde 4.7 in the presence of zinc chloride and LDA gave an inseparable, equal mixture of 4.8a and 4.8b in 60% yield. The mixture of bromo alcohols was converted to their corresponding azides 4.9a (42% yield) and 4.9b (46% yield), which were separable using flash chromatography. Hydroxyl protection, followed by reductive removal of the "chiral auxiliary," provided alcohol 4.10a in 46% yield. Alcohol 4.10a was activated as the triflate and aminated to deliver morpholino azide 4.11a in 76% yield over two steps. Azide reduction, amine acylation, and silyl-group removal (80% yield over three steps) completed the synthesis of the D-threo-PDMP alkenyl analogue 4.12, in approximately 8% overall yield (from 4.6).
Scheme 4.2. Synthesis of D-threo-PDMP alkenyl analogue

Jimbo et al.\textsuperscript{223} began their synthesis of D-threo-PDMP with N-Cbz D-serine (Scheme 4.3). N-Cbz D-serine (4.13) was treated with 8 equivalents phenyl magnesium bromide in THF to afford the amino ketone 4.14 in 85% yield. Reduction of the ketone with diisobutylaluminiumhydride gave the threo-amino diol 4.15 in 80% yield (no diastereoselectivity provided). Selective mesylation of the primary alcohol, followed by morpholine displacement provided morpholino alcohol 4.16 in 83% yield. The final two steps, amino deprotection followed by N-
acylation, went in only 9% yield, but did provide desired compound 4.17. The overall yield was 5%.

Scheme 4.3. Generation of D-threo-PDMP from N-Cbz D-serine

Husain et al.\textsuperscript{224} began their synthesis of D-\textit{threo}-PPPP, an analogue of D-\textit{threo}-PDMP, with a well-known D-serine derivative, Garner's aldehyde (4.18, Scheme 4.4).\textsuperscript{225} Alkyl addition to Garner's aldehyde \textsuperscript{226} has also been well documented to provide high diastereomeric excess depending on choice of solvent and C-nucleophile.\textsuperscript{227} Aryl addition to 4.18 with appropriate Grignard reagent in the presence of copper iodide in THF: dimethylsulfide gave alcohol 4.19 in 64-74% yield with 20:1 syn:anti selectivity. Removal of the acetonide, followed by mesylation and displacement made available pyrrolidino alcohol 4.20 in 40% yield. Acid-catalyzed deprotection of BOC group, then \textit{N}-acylation using palmitoyl chloride delivered D-\textit{threo}-PPPP 4.21 in an overall yield of 26%.
Scheme 4.4. D-threo-PPPP from D-Garner's Aldehyde.

A novel approach to enantiomerically pure D-threo-PDMP involved highly regioselective nucleophilic opening of non-activated aziridine-2-methanols (Scheme 4.5). Aziridine alcohol **4.22** is treated with trimethylsilyl iodide in acetonitrile to generate the iodo-intermediate **4.23**. Addition of morpholine to the iodo-intermediate affords the morpholino amino alcohol **4.24** in 99% yield. Reduction and N-acylation provide D-threo-PDMP (4.25) in 85% yield (from 4.22).

Scheme 4.5. Regioselective aziridine opening and displacement
Chapter 5. Stereoselective reductive alkylations of amino acid derivatives

After reviewing the medicinal value of D-threo-PDMP, it is clear that PDMP is an attractive molecule for synthetic chemists. Prior syntheses exist in the literature (see Chapter 4). As with most synthetic strategies, it is desirable to: start with commercially available, preferably cheap, starting materials and use a limited number of steps/reactions to achieve the target molecule. For the generation of PDMP, the most recent strategies start with D-serine, which provides the amino stereocenter of D-threo-PDMP. This chiral center can be used for chirality transfer to deliver the desired threo-product. This chapter will review the original total synthesis of D- and L-threo-PDMP from D- or L-serine achieved by Mitchell et al.

The retro-synthesis of D-threo-PDMP (5.1) is located in Scheme 5.1. It was originally conceived that a variety of cyclic amino analogues of 5.1 could be arrived at by nucleophilic displacement of tosylate 5.2, where P could either be the N-acyl chain (which is present in D-threo-PDMP) or the remainder of an amino-protecting group. Tosylate 5.2 could be generated from its corresponding β-amino alcohol 5.3. The stereoselective incorporation of the phenyl group could be achieved by the tandem reductive alkylation procedure developed by Polt et al., using D-serine derivative 5.4.
To initiate the synthesis of D-threo-PDMP and analogues (Scheme 5.2), D-Serine was treated with thionyl chloride in methanol (at lower temperatures to prevent epimerization) to provide methyl ester 5.5 in quantitative yield. Hydrochloride salt 3 was treated with commercially available benzophenone imine in dichloromethane (or acetonitrile) to give imine 5.6 in 75% yield. Recrystallization of 5.6 (hot EtOAc: cold hexanes) provides enantiomerically enrichment of the D-isomer.\textsuperscript{234} \textsuperscript{13}C NMR studies have shown that 5.6 predominantly exists in the “closed” oxazolidine tautomer.\textsuperscript{235} However, this tautomer population issue does not inhibit the silylation of 5.4 with tert-butyl dimethylsilyl chloride and imidazole in DMF. The primary hydroxyl protection reaction provides imino ester 2 in 90% yield (70% yield over three steps).
Scheme 5.2. Generation of D-serine derivative 5.4

O'Donnell\textsuperscript{238} developed the generation of the benzophenone imine-protection procedure for amino acids. This imine-protection protocol is extremely versatile in organic syntheses for many reasons including the following: good thermal stability, enhanced tolerance of moisture, stability during chromatography over silica gel and high yields \textit{without epimerization} and their tendency to crystallize.\textsuperscript{5} This Schiff base also features various modes of removal, mildly acidic conditions (aqueous PPTS) and hydrogenation.\textsuperscript{237} Another clear advantage of the imine-protection is the absence of the acidic hydrogen, which is present when using carbamate (e.g. N-BOC or N-Cbz) protection Scheme 5.s.\textsuperscript{238} The presence of an acidic hydrogen from either of these protection groups can complicate alkylation attempts of amino acid derivatives.\textsuperscript{239} Compound 5.4 has been used to synthesize many molecules of synthetic interest other than D-threo-PDMP, including: amino sugars,\textsuperscript{240} aza-sugars,\textsuperscript{241} and alkaloids.\textsuperscript{242}
Earlier attempts at performing tandem reductive-alkylations with imino esters (e.g. 5.4) to provide diastereomerically-enriched β-imino alcohols (e.g. 5.7) used diisobutylaluminiumhydride (DIBAL) as the reducing agent (Scheme 5.3), followed by phenylmagnesium bromide as the alkylating agent.

Rather surprisingly, DIBAL reacted rather slowly with imino esters at lower temperatures (-78° C). Activation of the reducing agent was achieved by the addition of Grignard reagent—presumably due to the formation of the “ate complex” (Scheme 5.4). To use DIBAL as the reducing agent required two equivalents of hydride source, which provided significant amounts of overreduced primary alcohol 5.8 under these tandem reductive alkylation conditions.
In order to reduce the formation of overreduced product, the reducing agent was changed to a 1:1 mixture of diisobutylaluminiumhydride: triisobutylaluminium (TRIBAL) solution. This reducing agent, pentaisobutylaluminiumhydride (or DIBAL-TRIBAL), also was rather inert at lower temperatures. Using DIBAL-TRIBAL caused a 20-30% increase in yield of the desired secondary alcohol products. Compound 5.9 was isolated in 65-70% yield (d.r. 8: 1 threo: erythro).

The observed threo-stereoselectivity is presumably achieved through Cram-chelation controlled delivery of sequential nucleophiles to the ester group of compound 5.4 (Scheme 5.6). Initially, the aluminum complex (abbreviated "M") chelates to the carbonyl oxygen and the imine nitrogen in a bidentate manner (I). This chelation has been detected in \(^1\)H NMR studies and is believed to have an observable yellow color. The addition of the carbon nucleophile (e.g. Ph\(^-\)) forms the kinetically active "ate" complex (II). This activated aluminum species delivers the hydride from the less-hindered face, the Re-face, of the carbonyl (III). To the resulting mixed acetal IV, the excess phenyl anion displaces the methoxy group in an \(S_N2\) manner (V) to provide VI. Using bulkier ester groups (e.g. t-butyl) in these reductive-alkylations have provided higher threo-selectivities. Authors proposed that this enhancement of
stereoselective using bulkier esters strengthens the argument for the $S_N2$ displacement mechanism. If no enhancement of stereoselectivity was observed, Peterson et al. have concluded that the aluminum acetal may decompose—forming an aldehyde in an $n$ $S_N1$-like mechanism. Mixed acetal intermediate $IV$ has been trapped as the silyl acetal. Addition of water provides hydroxy-imine $VII$, which predominantly exists in the oxazolidine tautomer $VIII$. 
Scheme 5.6. Proposed Reductive Alkylation Mechanism
In these reductive alkylations, the solvent used for the carbon nucleophile has a dramatic effect on the stereoselectivity of the reaction. The solvent effect on stereoselectivity is summarized in Table 5.1. Organolithiates (varying in chain length from propenyl to pentadecyl), generated in hexanes and/or hexanes: toluene mixtures, have been found to provide the highest stereoselectivity, 15: 1 threo: erythro. Grignard reagents in diethyl ether have consistently provided good stereoselectivities, 8: 1. However, when the solvent for the carbon nucleophile is switched to THF, the stereoselectivity drops to 3: 1 or even 1.7: 1.

This major decrease in stereoselectivity has been attributed to one, if not both, of the following: competitive chelation between the THF and aluminum species, hence decreasing Cram-controlled delivery of the nucleophiles and/or increased polarity causes an SN1-like mechanism, where the carbon nucleophile attacks the aldehyde intermediate instead of the mixed acetal. A combination of both possibilities is provided in Scheme 5.7. Coordination of THF to intermediate IV could decrease the chelation of the aluminum species with the carbonyl oxygen—causing the formation of aldehyde intermediate X. The excess phenyl anion could attack an aldehyde in confirmation X, providing the threo-product XI, or attack an aldehyde in confirmation XII to provide the erythro-product XI.
Table 5.1. Solvent Effect on Tandem Reductive Alkylation Stereoselectivities

<table>
<thead>
<tr>
<th>C-Nucleophile</th>
<th>Solvent</th>
<th>Threo: Erythro</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{R} \text{Li} ) (^{a})</td>
<td>Hexanes(^b)</td>
<td>( \geq 15: 1 )(^c)</td>
</tr>
<tr>
<td>( \text{MgBr} ) (^d)</td>
<td>Et(_2)O</td>
<td>8:1</td>
</tr>
<tr>
<td>( \text{MgBr} )</td>
<td>THF</td>
<td>( \leq 3:1 )(^e)</td>
</tr>
</tbody>
</table>

\(^{a}\)R = CH\(_3\) \( \rightarrow \) (CH\(_2\))\(_{12}\)CH\(_3\). \(^{b}\)Toluene: hexanes solvent mixtures gave similar selectivities, \(^{c}\)15: 1 stereoselectivities were observed when L-alanine was used, 20: 1 where observed in the case of L-serine, \(^{d}\)long chain saturated alkyl Grignard reagents (e.g. octadecylmagnesium bromide) failed to alkylate. \(^{e}\)3:1 stereoselectivities when phenylmagnesium bromide in THF was used, 1.7: 1 when vinylmagnesium bromide in THF was employed.
Separation of the diastereomeric products was achieved using flash chromatography. $^{13}$C NMR experiments revealed only a small imine carbon peak at 161 ppm. It was later realized that the threo-isomer exists almost exclusively.
(8: 1) in the "closed" oxazolidine tautomer 5.9b, rather than the "open" hydroxy-imine tautomer 5.9a, which explained the observed $^{13}$C NMR peak at 90 ppm. On the other hand, the erythro-isomer (5.10 a/b) exists in each tautomer equally (Scheme 5.8).

This difference in tautomeric ratios is presumably due to the steric interactions of the "closed" form of the erythro-isomer (5.10b). These steric interactions are absent in the threo-isomer. It is believed that the differences in observed polarity ($R_l$) can also be attributed to the differences in tautomeric ratios, where the oxazolidine is less polar than the hydroxy-imine. The advantages of this difference in polarity are: 1) using flash chromatography is a viable option for separation and 2) because the erythro-isomer is more likely to exist as the hydroxy-imine (5.10a), it is more prone to silica-gel-induced hydrolysis—leaving the amino alcohol—which is significantly more polar than the less-acid-sensitive threo-isomer.
With purified threo-product (5.9) in hand, the remaining synthesis of D-threo-PDMP (Scheme 5.9) began with the removal of the Schiff base with PPTS in aqueous THF to provide β-amino alcohol 5.3 in 72% yield. An initial attempt to introduce the morpholine head group was as follows: to acylate the amino group to provide amide 5.11, followed by silyl removal and tosylation to yield tosylate 5.12, and morpholine displacement. However, this strategy failed because of the competing intramolecular cyclization—resulting in the formation of oxazoline 5.13.
Scheme 5.9. Initial attempts at introducing the morpholino head group

To avoid this cyclization, another method was developed (Scheme 5.10). After the Schiff base removal of imine 5.9 using PPTS, the amino alcohol (5.3) was protected as the oxazolidinone, which "ties back" the nucleophilic amino group. Treatment of amino alcohol 5.3 with triphosgene (or 1,1'-diimidazole carbonyl) in THF provided carbamate 5.14 in 70% yield. Removal of the silyl group by aqueous HF gave hydroxyl carbamate 5.15 in 92% yield. The hydroxyl carbamate was treated with tosyl chloride in pyridine to furnish the tosylate 5.16 in 97% yield. The tosyl group was displaced with morpholine in refluxing THF to afford the morpholino carbamate 5.17 in 90% yield. Saponification of the carbamate with aqueous KOH in methanol provided amine 5.18 in 80% yield. Amine 5.18 was treated with para-nitro phenyl decanoate and HOBr in pyridine to deliver amide 5.1 in 90% yield. The overall yield of D-threo-PDMP from 5.4 was 20%. Tosylate 5.16 was found to be a valuable intermediate towards the synthesis of a variety of D-threo-PDMP head group analogues (Table 5.2). The
D-threo-PDMP aromatic analogues that I made and will discuss, in Chapters 4 & 5, were generated using this methodology.

Scheme 5.10. Total synthesis of D-threo-PDMP
Table 5.2. Generation of D-threo-PDMP head group analogues

\[
\begin{align*}
\text{5.16} & \xrightarrow{\text{i}} \text{5.17a-c} \xrightarrow{\text{ii}} \text{5.1a-c} \\
\text{i) HNR}_2 / \text{THF/ } \Delta; \text{ ii) a. KOH/ MeOH/ } \Delta; \text{ b. NO}_2\text{C}_6\text{H}_4\text{OC(OR), HOBt, pyridine}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Head group (HNR)</th>
<th>Yield (i)</th>
<th>Yield (ii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>93%</td>
<td>57%</td>
</tr>
<tr>
<td>b</td>
<td>77%</td>
<td>63%</td>
</tr>
<tr>
<td>c</td>
<td>70%</td>
<td>60%</td>
</tr>
</tbody>
</table>
Chapter 6. Reductive amination Conditions Towards Aza-sugar Synthesis

Due to the medicinal value of aza-sugars\textsuperscript{250} (nitrogen-in-the-ring sugars), multiple synthetic procedures have been developed. These developed procedures usually employ readily available starting materials, like glucose\textsuperscript{251} or serine.\textsuperscript{252} This chapter will focus on reductive amination conditions and the synthesis of aza-sugars using reductive amination conditions.\textsuperscript{253} For recent reviews on the significance and generation of aza-sugars, see Asano\textsuperscript{254} and Stutz,\textsuperscript{1} respectively.

A reductive amination, or reductive alkylation, reaction involves the condensation of an amine (Scheme 6.1)\textsuperscript{255} with carbonyl-bearing substrate (e.g. aldehydes and ketones) and also acetals and ketals in the presence of a reducing agent (Red. Ag.).\textsuperscript{256} The amine can vary from an ammonium salt to an aliphatic or aromatic amine.\textsuperscript{257} As for reducing agents, sodium cyanoborohydride is the best-known hydride source for reductive aminations,\textsuperscript{258} though sodium triacetoxyborohydride has gained popularity.\textsuperscript{259}

\begin{equation}
\begin{array}{c}
\text{R}^1 \text{R}_2 \text{H}^\text{N} \text{R}_3 \\
\text{R=alkyl} & \text{R}_2=\text{alkyl or aromatic} & \text{R}_3=\text{alkyl, aromatic or H} \\
\text{R}_1=\text{alkyl or H} & \\
\end{array}
\end{equation}

\textbf{Scheme 6.1.} Reductive amination; reagents and product

If the carbonyl substrate bears a second carbonyl (dialdehydes, diketones, ketoaldehydes, acetals and ketals) and the amine is a primary amine, a second
condensation/cyclizations is permitted—considering entropic factors. Once the intermolecular condensation has taken place, the intramolecular reaction is believed to proceed rather fast unless the one of the carbonyls is significantly less reactive or the amine is only weakly nucleophilic.

The proposed mechanism of the reductive amination is in Scheme 6.2. After the initial, reversible formation of the carbinolamine 6.1, a reversible dehydration takes place. The resulting iminium ion 6.2 is reduced irreversibly to provide amine 6.3. It has also been reported that the hydride attacks the carbinolamine.

\[
\begin{align*}
R \ O & \quad + \quad R_2 \ HN \ - \ R_3 \\
\text{6.1} & \\
\text{6.2} & \\
R_2 \ N \ - \ R_3 & \quad \rightarrow \quad R_2 \ N \ - \ R_3 \\
\text{6.3} & \\
\end{align*}
\]

Scheme 6.2. The Mechanism of a Reductive amination

The original reducing agent for reductive aminations was sodium cyanoborohydride (SCB), which was developed by Borch in 1971. The cyano ligand present on SCB reduces its hydridic reactivity, which increases its selectivity—reducing the iminium ion the presence of unreacted carbonyl
substrates. Using SCB in a reductive amination requires a pH in the range of 5-7 and an alcoholic solvent. Dehydrating agents (e.g. molecular sieves) promote iminium ion formation, which help facilitate successful product formation. Lewis acids (e.g. TiCl₄) can be employed to not only increase the reactivity of carbonyl substrates, but also serve as water scavengers. The common side product when using SCB is the cyanohydrin product.

Sodium triacetoxyborohydride (STB), the other popular reducing agent in reductive aminations, was introduced by Gribble in 1974. Though commercially available now, STB can be prepared by addition of sodium borohydride to acetic acid. The mechanism for reductive aminations using STB is currently unknown, though it may involve an amide intermediate. The advantages of using STB over SCB are as follows: STB can be used in aprotic solvents, STB works well with weakly basic amines and STB is less toxic to use and prepare. Using STB in less polar, aprotic solvents (dichloromethane, 1,2-dichloroethane, tetrahydrofuran and acetonitrile) allows for enhancement in selectivity of imminium ion reduction in the presence of carbonyl substrates. The main observed side-products of STB reductive aminations are N-acylation and N-ethylation.

As stated earlier, the amine in a reductive amination can vary from an ammonium salt to an aliphatic or aromatic amine. Interestingly, even strongly hindered amines (e.g. butylamine) and weakly nucleophilic nitrogens (e.g. sulfonamides and ureas) will react with perform reductive aminations. Primary
amines can dialkylate if excess carbonyl substrate is present. If dialkylations are undesired, changes in amine: aldehyde ratios and using stronger reducing agents (e.g. NaBH₄) can suppress the second alkylation from occurring by reducing the less reactive carbonyl before the amine can react.²⁷⁸

Dialkylation can also be observed if the carbonyl substrate bears a second carbonyl and the amine is a primary amine, a second condensation/cyclizations is permitted—considering entropic factors.¹¹ Once the intermolecular condensation has taken place, the intramolecular reaction is believed to proceed rather fast unless the one of the carbonyls is significantly less reactive or the amine is only weakly nucleophilic.²⁷⁹ Reductive aminations have been shown to deliver pyrrolidines and piperidines by employing various amines in the presence of dicarbonyl substrates and a reducing agent, usually either SCB or STB. Initially, the synthesis of simpler pyrrolidines and piperidines via reductive amination-cyclizations will be reviewed, then more sophisticated reductive amination-cyclizations, mainly in synthetic efforts towards aza-sugars will be discussed.

Studies on ant venom have revealed that 2,5-dialkylpyrrolidine 6.4 (Scheme 6.3) is a poisonous component in select species.²⁸⁰ Jones et al. studied the synthesis of the 2,5-substituted pyrrolidines using a reductive amination protocol.²⁸¹ Using a variety of diketone compounds (e.g. 6.5) in the presence of ammonium acetate, potassium hydroxide and SCB provided pyrrolidines (e.g. 6.6a & 6.6b) in 15-90% yield, with a cis:trans ratio of 1: 1.
Attempts to increase stereoselectivity and techniques for separation of isomers were not discussed. Other works by Manescalchi and Savoia have found superior ways to affect the cis:trans ratio with similar substrates.\textsuperscript{282}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {6.5};
\node at (1.5,0) {$\text{a, b}$};
\node at (3,0) {6.6a};
\node at (3.5,0) {$+$};
\node at (4,0) {6.6b};
\end{tikzpicture}
\end{center}

\textsuperscript{a)} NH$_4$OAc, NaCNBH$_3$, KOH, MeOH; \textsuperscript{b)} NaBH$_4$

\textbf{Scheme 6.3.} Reductive aminations towards 2,5-dialkylpyrrolidines

Another dicarbonyl source used in reductive amination is 2,5-hydroxy-tetrahydrofuran \textit{6.7}.\textsuperscript{283} Compound \textit{6.7} was synthesized by acid hydrolysis of 2,5-methoxy-tetrahydrofuran.\textsuperscript{284} Costello \textsuperscript{285} used \textit{6.7} in a reductive-amination methodology with alanine derivative \textit{6.8} to afford pyrrolidine \textit{6.9} (\textbf{Scheme 6.4}, yield not provided).
Scheme 6.4. Reductive-Amination with 1,4-butane dial hydrate 6.7

Now, we will shift our focus to reductive amination protocols towards the generation of aza-sugars. Beginning with D-mannitol (6.10, Scheme 6.5), which was benzylidene-protected to selectively form the 1,3: 4,6-di- O-benzylidene D-mannitol in 70% yield. Oxidation of 1,3:4,6-di- O-benzylidene D-mannitol with PCC delivered C₂-symmetric, cyclic hydrate 6.11 in 42% yield. Double reductive amination with benzylamine in the presence of sodium cyanoborohydride and acetic acid, followed by deprotection provided polyhydroxylated pyrrolidine 6.12 in 65% yield.

Scheme 6.5. Double reductive amination with diketone hydrate 6.11
Baxter and Reitz\textsuperscript{287} have employed glucose derivatives as sources of dicarbonyl carbohydrates. Commercially available 1,2-isopropylidene D-glucose (6.13) was oxidized using either sodium periodate or dibutyltin oxide, followed by hydrolysis to deliver dialdehyde 6.14\textsuperscript{288} or ketone 6.15, respectively (Scheme 6.6).

\begin{align*}
\text{Scheme 6.6. Oxidation and deprotection of diol 12}

\text{Dialdehyde 6.14 and ketone 6.15, under reductive amination conditions similar to Scheme 6.5, delivered piperidine polyols in modest yields (55-70%). Reductive amination with dialdehyde 6.14 provided amino triol 6.16 in 67\% yield (Scheme 6.7). The same reagents and conditions with ketone 6.15 gave 6.17 in high stereoselectivity (>95:5) at the C5-position (Scheme 6.7).}\textsuperscript{289}
\end{align*}
Another aza-sugar of medicinal value is homonojirimycin 6.18, which was originally isolated from the *Omphalea diandra* (Euphorbiaceae, as known as the Jamaican navel spurge).\(^{290}\) For the synthesis of \(\beta\)-homonojirimycin 6.18 (Scheme 6.8), Saavadra\(^{291}\) began with the alkylation of tetra-O-benzyl glucono-1,5-lactone 6.19 to provide alcohol 6.20 in 70% yield. Reduction with LAH gave diol 6.21 in 97% yield. Oxidation using TFAA-DMSO\(^{292}\) followed by reductive amination with ammonium formate and sodium cyanoborohydride provided amine 6.22 as the single stereoisomer in 50% yield over two steps.\(^{293}\) All attempts to use amines other than ammonium formate (including allyl- and benzylamine) under these same conditions failed to provide desired, reductive amination products. Deprotection delivered \(\beta\)-homonorijymycin 6.18 in 64% yield. Yokoyama employed similar oxidation/reductive amination protocols in their synthesis of C-aza-nucleosides.\(^{294}\)

\(\text{Scheme 6.7. Reductive aminations to provide aza-sugars 6.16 and 6.17}\)

\(a)\) BnNH\(_2\), NaBH\(_3\)CN, HOAc, MeOH

6.14 \[\rightarrow \text{a} \rightarrow \text{Bn} \]

6.15 \[\rightarrow \text{a} \rightarrow \text{Bn} \]

6.16

6.17

6.18

6.19

6.20

6.21

6.22

6.19
In efforts towards the generation of penta-substituted piperidines, Shankar et al.\cite{Shankar2020} converted diol 6.13 to dialdehyde 6.14 using the same conditions as Scheme 6.6. Dialdehyde 6.14 was treated with benzylamine in the presence of benzotriazole in water to provide condensation product 6.23 in 60% overall yield (Scheme 6.9). Compound 6.23 was subjected to a variety of nucleophiles, which provided the trans-axial-equatorial product 6.24a as the major product in most cases (Table 6.1).
Scheme 6.9. Condensation of dialdehyde 13 with benzotriazole, followed by nucleophilic displacement.

Table 6.1. Nucleophilic Displacement with High Diastereoselectivity

<table>
<thead>
<tr>
<th>Nucleophile (Source)</th>
<th>Yield</th>
<th>Selectivity (a: b: c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(^+) (NaBH(_4))</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>C(_2)H(_5) (EtMgBr)</td>
<td>60</td>
<td>3: 1: 1</td>
</tr>
<tr>
<td>CN(^-) (NaCN)</td>
<td>70</td>
<td>8: 2: 0</td>
</tr>
<tr>
<td>CH(_3)S(^-) (NaSCH(_3))</td>
<td>60</td>
<td>7.5: 2: 0.5</td>
</tr>
</tbody>
</table>

Obviously, the formation of aza-sugars is not limited to using reductive amination chemistry. The advantage to using this methodology is that reductive amination protocols require fewer equivalents of the amine.\(^{296}\) While reductive amination procedures require 1: 1 amine: aldehyde, displacement conditions require 6-10 equivalents of amine per one equivalents tosylate, mesylate or epoxide.\(^{297}\) Management of the amine is only an issue when the amine is
"precious." Garden-variety amines—benzyl amine, allyl amine and ammonium salts—can be used without conservation. Displacements have other disadvantages, including elimination product formation, harsher conditions (higher pressures or temperatures) and sometimes Lewis acid catalysts.48

Though the reductive amination conditions have advantages, examples exist in literature of direct displacements towards hydroxylated piperidines and pyrrolidines. In the synthesis of C2-symmetric tetrahydroxyazepanes by Wong et al.,298 treatment of bis-epoxide 6.25 with allylamine and acid-catalyst in attempts to form desired azepane 6.26 was complicated by the formation of piperidine 6.27 (Scheme 6.10). Optimal conditions to deliver 6.26 were excess nucleophile (12 equivalents) in the presence of perchloric acid (6 equivalents) in refluxing methanol.299 These conditions provided 7-endo-tet product 6.26: 6-endo-tet 6.27 in greater than 15: 1.

Masaki et al.300 converted 1,3:4,6-di-O-benzylidene D-mannitol 6.28 to the dimesylate 6.29 (Scheme 6.11) and attempted displacement has been reported. Attempts to acquire doubly displacement product 6.30 were successful in 65% yield. However, the reaction conditions required greater than 5 equivalents of
amine and temperatures of 135° C. Attempts to provide the doubly displaced product using fewer equivalents at lower temperatures or fewer equivalents in the presence of various bases could not afford the desired product.\textsuperscript{301} Also, purification of 6.30 was problematic due to the presence of unreacted amine in the reaction mixture.

\begin{center}
\begin{tikzpicture}

\node[draw, shape=circle] (a) at (0,0) {6.28};
\node[draw, shape=circle] (b) at (2,0) {6.29};
\node[draw, shape=circle] (c) at (4,0) {6.30};

\draw[->] (a) -- (b) node[above] {a)};
\draw[->] (b) -- (c) node[above] {b)};

\node[above] at (a) {a) MsCl, TEA, CH\textsubscript{2}Cl\textsubscript{2}, 90\% yield, c) C\textsubscript{10}H\textsubscript{19}NH\textsubscript{2}, 65 \% yield}
\end{tikzpicture}
\end{center}

**Scheme 6.11.** Attempts at the Double Displacement of mesylate 6.29
Chapter 7. The reasoning, synthesis and testing of a novel D-threo-PDMP with an ortho-methyl substituent.

As stated earlier, initial inhibition studies on the GlcCer synthase by Radin\textsuperscript{302} involved empirical structures similar to the substrate, ceramide, with the following alterations: 1) replacing the long alkenyl chain with a phenyl group, 2) shortening the amide chain length to ten carbons, 3) altering the primary hydroxyl to a morpholine head group and 4) changing the stereochemistry from natural erythro to threo provided an effective inhibitor, D-threo-PDMP, of the GlcCer enzyme in \textit{in vitro} studies (Figure 7.1).

![Figure 7.1. D-threo-PDMP, an inhibitor of the GlcCer synthase enzyme](image_url)

Radin observed that when small substituents (methyl or methoxyl) were placed on the lead molecule at the \textit{para}-position, little enhancement in inhibitory power was observed. Larger \textit{para}-substituents (chloro-, bromo- or nitro) on the phenyl ring of the inhibitor lead to decreases in potencies. Radin concluded that "the binding site of the enzyme can accommodate the relatively bulky side group, but the resultant effect does not look useful."

From the X-ray structure of D-threo-PPMP HCl monohydrate salt (Figure 7.2),\textsuperscript{303} we observed that the molecule has two preferred confirmations. In both
instances, the morpholine head group orients itself orthogonally from the aromatic moiety.

We envisioned that placing a small substituent, specifically a methyl group, at the ortho-position on the aromatic ring would favor one confirmation due to steric repulsions between the substituent and the N-decanoyl chain. In an attempt to determine the preferred confirmation of this D-threo-PDMP analogue, D-threo-TDMP ("T" for ortho-tolyl), MM3 modeling calculations were performed. Conformer 1 (Figure 7.3) was calculated to be the most stable, with the methyl group oriented away from the amide chain, which caused the morpholine head group to be perpendicular to the amide chain.

Figure 7.2. X-ray structure of D-threo-PPMP HCl monohydrate. Both structures are derived from a single crystal of PPMP HCl H2O. The H2O and Cl- have been removed for clarity. Two molecules appear in the unit cell, with the tail groups interpenetrating in rows.
Figure 7.3. Calculated (MM3) conformation of D-threo-TDMP based on PPMP. This structure was obtained from the PPMP coordinates (Figure 7.2) by substitution of an ortho hydrogen with –CH₃, and using the dihedral driver function to find the minimum energy rotamer about the ortho-tolyl-C axis.

In Figure 7.4, the steric energies of the bond rotation about the aromatic-methine (bearing hydroxyl group). Clearly, the ortho-methyl group causes an increase in steric energy when the methyl group approaches the decanoyl moiety.
Figure 7.4. The steric energies about the aromatic-methine bond rotation

It is predicted that this conformational bias would reveal a structure-activity relationship for inhibition. To test our hypothesis, we synthesized a D-threo-PDMP analogue with an ortho-methyl substituent (1-tolyl-2-amino decanoyl-3-morpholino-propan-1-ol or TDMP) starting from D-serine. To determine the potency of D-threo-PDMP versus D-threo-TDMP, MRRL-CH1 cells, an embryonic cell line from *Manduca sexta* (tobacco horn worm or Sphinx moth), were treated with each analogue. Because of its short life cycle, a relatively simple GSL series, well-characterized neuroanatomy and developmental profile,
the insect *M. sexta* is an ideal model system for the study of GSL roles in neural development. Moreover, since this insect has been extensively studied, drug effects can be observed at the cellular, tissue, morphological, and even behavioral studies on the adult moth become feasible.

Treatment of imino ester 7.1 (Scheme 7.1) with one equivalent of 0.5M solution of pentaisobutylaluminiumhydride (1:1 mixture of diisobutylaluminiumhydride (DIBAL-H): triisobutylaluminium (TRIBAL) in hexanes), followed by three equivalents of *ortho*-tolyl magnesium bromide provided *threo*-β-imino alcohol product 7.2 in 69% yield (d.r. *threo*: *erythro* 9:1). The *threo* adducts were isolated via column chromatography.

![Scheme 7.1. Tandem reductive-alkylation of D-serine imino-ester 2](image)

The O'Donnell Schiff Base of imine 7.2 (in Scheme 7.2) was removed with pyridinium *p*-toluenesulfonate in 70% yield (Scheme 7.2). Treatment of β-amino alcohol 7.3 with 1,1'-diimidazole carbonyl gave carbamate 7.4 in 80% yield. Removal of the silyl group by aqueous HF gave crude hydroxyl carbamate. The crude hydroxyl carbamate was treated with tosyl chloride in pyridine to furnish the tosylate 7.5 in 83% yield over two steps. The tosyl group was displaced with morpholine in refluxing THF to afford the morpholino carbamate 7.6 in 82% yield.
To complete the synthesis of D-threo-TDMP analogues (Scheme 7.3), morpholino carbamate 7.6 was saponified and sequentially acylated. Acylations utilized para-nitrophenyl esters from the desired carboxylic acids. These two steps provided amides 7.7a-c in 67% yield. The overall yield for these D-threo-PDMP analogues was 18%. The bis-oxadecanoic acid was prepared using protocols employed by Johnstone and Gravestock. We envisioned the palmoyl (D-threo-TPMP, 7.7b) analogue to increase lipophilicity and possibly increase potency. The bis-oxadecanoyl (D-threo-TbisoxaDMP, 7.7c) analogue was reasoned to increase hydrophilicity. Dwek et al. found that the cellular toxicity of long chain NBJ analogues could be overcome by decreasing lipophilicity with oxygen-substituted alkyl chains (see Chapter 3). Attempts at obtaining an X-ray structure of any tolyl analogues have not been fruitful as of yet.
In our analogue tests, MRRL-CH1 cells were originally exposed to D-threo-PDMP at 2, 5, 10, and 20 μM for up to two days. After two days of exposure to the analogues, the drug media was removed and fresh media was provided. Cells were allowed to grow for an addition two days. The objectives of the drug testing were the following: to compare the potencies of D-threo-PDMP to D-threo-TDMP, to establish the amount of time necessary for the drugs to take action, and to determine if the suppression of cellular growth was reversible after the removal of the drug.

In the 5-20 μM range, D-threo-PDMP had a limited effectiveness against cellular growth. No effects were observed at 5 and 10 μM and only moderate inhibition was observable at 20 μM. Images in Figure 7.5 reveal the modest suppression of D-threo-PDMP at 20 μM. After the removal of the drug, cells were able to grow healthy extensions.
To evaluate the potency of D-threo-TDMP, cells were exposed to the drug at 5, 10, and 20 µM for 48 hours. D-threo-TDMP, at these concentrations, was deemed cytotoxic to cells. Images in Figure 7.6 show the inhibition of cellular outgrowth at 1 µM. After the removal of the drug, cells were unable to produce healthy extensions. Not only were cells unable to develop healthy extensions, but also the cells apparently lost adhesion to the matrix and began to aggregate compared to the untreated cells. Even after the removal of the drug, viability was abolished.
The dosage concentrations were then reduced to 0.1, 0.25, 0.5 and 1.0 \( \mu \text{M} \) of D-threo-TDMP. After the cells were exposed to 0.5 \( \mu \text{M} \) of D-threo-TDMP, the effects were, once again, rapid. After 24 hours, although normal cellular morphology and membrane integrity were maintained, cells appeared to be unable to form neurite extensions, and showed reduced adhesion to their substrate. After removal of the D-threo-TDMP at 0.5 \( \mu \text{M} \); however, a majority of the cells were able to develop healthy extensions, similar to the neurite extensions observed in the untreated cells (Figure 7.7). At concentration lower than 0.5 \( \mu \text{M} \), suppression of outgrowth was modest at best.
Figure 7.7. Reversible inhibition of D-threo-TDMP at 0.5 μM

Analogues 7.7b and 7.7c were screened in the same manner as the decanoyl analogue at 0.1, 0.25, 0.5, 1.0 and 5.0 μM. The more lipophilic palmyo analogue (7.7b) appeared to render the same potency and reversibility as its decanoyl counterpart. The bis-oxadecanoyl analogue (7.7c); however, provided no
effectiveness at these concentrations. Efforts to determine effective dosages of the bis-oxadecanoyl and other analogues, with increased water solubility, are currently being investigated.

D-threo-PDMP has been shown to deplete cells of endogeneous glycolipids, which can suppress cellular outgrowth and reduce cellular adhesion. We have synthesized a novel D-threo-PDMP analogue, D-threo-TDMP, from D-serine that reduce cellular extensions at lower concentrations than D-threo-PDMP. The suppression of outgrowth is irreversible after the removal of the analogue at 1 \( \mu \text{M} \), however, reversible at 0.5 \( \mu \text{M} \). A complete understanding of the true effect(s) of D-threo-PDMP and its analogues is currently unclear. We hope that creating perturbations of GSL expression by treatment with D-threo-PDMP analogues at lower concentrations and by isolating GSL’s from the Manduca sexta (in progress)\(^\text{309}\), we can gain a greater understanding of the role of cell-surface carbohydrates.
Chapter 8. Ethereal Aromatic PDMP analogues

Given the biological utility and pharmacological potential of D-threo-PDMP, researchers have attempted to synthesize more potent inhibitors.\textsuperscript{310,311} Shayman \textit{et al.}\textsuperscript{312} recently reported that electron-rich aromatic D-threo-PDMP analogues gave increased inhibition of glucosylceramide synthase. D-threo-4'-hydroxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (D-4'-hydroxy-P4) and D-threo-(3',4'-ethylenedioxy)-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (D-3',4'-ethylenedioxy-P4) (Figure 8.1) inhibited the glucosylceramide synthase from Madin-Darby canine kidney (MDCK) cell homogenates at an IC\textsubscript{50} of 90 nM. However, these novel inhibitors did not significantly raise intracellular ceramide levels or inhibit cell growth.

Prior to Shayman’s work was reported, we initiated the synthesis and biological evaluation of D-threo-PDMP analogues (Figure 8.2, 8.1a-c) that bear ether substituents on the aromatic ring; specifically, 4-methoxy (8.1a), 4-tert-butoxy (8.1b), and 3,4-methylenedioxy (8.1c). The analogues were tested against...
embryonic MRRL-CH1 cells from *M. sexta* to determine their effectiveness in neurite outgrowth suppression and cytotoxicity. All ether-bearing analogues suppressed cellular growth at lower concentrations than the lead compound, D-threo-PDMP, and the suppressive effects were reversible at lower concentrations.

**Figure 8.2.** Oxygenated D-threo-PDMP analogues (8.1a-c)

Starting with the imino ester of D-serine\(^3\) (8.2), the aromatic ring (yields given in Table 8.1) was introduced in a stereoselective manner via a tandem reductive-alkylation reaction to provide the desired *threo*-isomer.\(^4\) The *threo* adducts 8.3a-c were isolated by column chromatography and elaborated as described previously.\(^5\) The lower yields of the tandem reductive alkylations with these aromatic Grignard reagents can be attributed to the solvent (THF)\(^6\) in the methylenedioxyphenyl (c) case. As for the methoxy (a) and tert-butoxy (b) cases, those Grignard reagents were generated by the author, which may explain the lower yield.
Table 8.1. Tandem reductive-alkylation with oxygenated aromatic nucleophiles

<table>
<thead>
<tr>
<th>Aromatic Ring</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="a" alt="Aromatic Ring" /></td>
<td>48%</td>
</tr>
<tr>
<td><img src="b" alt="Aromatic Ring" /></td>
<td>44%</td>
</tr>
<tr>
<td><img src="c" alt="Aromatic Ring" /></td>
<td>39%</td>
</tr>
</tbody>
</table>

Scheme 8.1. Synthesis of Ether-Substituted D-threo-PDMP Analogaues

The O'Donnell Schiff Base of imines 8.3a-c was removed with pyridinium p-toluenesulfonate in 70% yield in all cases. Treatment of β-amino alcohol 8.4a-c with 1,1'-diimidazole carbonyl gave the carbamate 8.5a-c in 55-64% yield. Removal of the silyl group was by aqueous HF gave crude hydroxyl carbamate. The crude
hydroxyl carbamate was treated with tosyl chloride in pyridine to furnish the tosylate 8.6a-c in 83% yield over two steps. The tosyl group was displaced with morpholine in refluxing THF to afford the morpholino carbamate 8.7a-c in >90% yield in all cases. Saponification and N-acylation completed the synthesis of the D-threo-PDMP analogues 8.1a-c in 61-68% yield over two steps. The overall yield from 8.2 for the ethereal D-threo-PDMP analogues was approximately 10%. Only the decanoyl amide chain lengths were generated because the C10 length seemed to have optimal potency and solubility in prior studies.

In our analogue tests, MRRL-CH1 cells were originally exposed to the D-threo-PDMP analogues at concentrations varying from 5 to 20 μM for 48 hours. In all cases, the analogues at these concentrations were deemed cytotoxic. This observation was intriguing because early testing had shown that D-threo-PDMP exhibited only weak inhibition at 20 μM (see ortho-tolyl analogue chapter). The dosage concentrations were then reduced to 0.5 and 1.0 μM for 48 hours. The results of the 24 hours exposure to the D-threo-PDMP analogues are illustrated in Figure 8.3. After 48 hours of exposure to the analogues, the drug media was removed and fresh media was provided. Cells were allowed to grow for an addition six days to see if the inhibition was reversible. The results are illustrated in Figure 8.4. The reversibility of each analogue is summarized in Table 8.2.
Figure 8.3. Effects of 0.5 μM D-three-PDMP analogues on neurite outgrowth of MRRL-cell line.
Figure 8.4. Six days after the removal of the D-threo-PDMP analogues
Table 8.2. Inhibitory effects of the analogues on MRRL-CH1 cell outgrowth.

<table>
<thead>
<tr>
<th>Analogue</th>
<th>5.0 μM</th>
<th>1.0 μM</th>
<th>0.5 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-threo-PDMP</td>
<td>No Effect</td>
<td>No Effect</td>
<td>No Effect</td>
</tr>
<tr>
<td>D-threo-ADMP (8.1a)</td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Reversible</td>
</tr>
<tr>
<td>D-threo-BDMP (8.1b)</td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Reversible</td>
</tr>
<tr>
<td>D-threo-PipDMP (8.1c)</td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Reversible</td>
</tr>
</tbody>
</table>

After the cells were exposed to 0.5 and 1.0 μM of the D-threo-PDMP ether analogues, the effects were rapid. After 24 hours, although normal cellular morphology and membrane integrity were maintained, cells appeared to be unable to form neurite extensions, and showed reduced adhesion to their substrate. Prior reports have stated that D-threo-PDMP effects on neurite extensions were not observed until 3 days after exposure.\(^{316}\) Cellular adhesion decreased, resulting in an increase in cellular aggregation compared to the untreated cells. The loss of adhesion may be a consequence of the decrease in endogenous ganglioside levels.\(^{317}\)

After removal of the D-threo-PDMP ethereal analogues, a majority of the cells were able to develop healthy extensions, similar to the neurite extensions observed in the untreated cells. At 1 μM, suppression of outgrowth was also observed, and recovery was reduced. Some recovery was observed where cells
were exposed to D-threo-ADMP (8.1a) and D-threo-PipDMP (8.1c). D-threo-BDMP (8.1b); however, appeared to be cytotoxic even at 1.0 µM. Treatment with 0.5 µM BDMP afforded the same level of inhibition as 1.0 µM of D-threo-ADMP and PipDMP. The potency of D-threo-BDMP was comparable to that of D-threo-TDMP and D-threo-TPMP.

We have synthesized three D-threo-PDMP ethereal analogues from D-serine that reduce cellular extensions at lower concentrations than D-threo-PDMP. Presumably, our analogues inhibit growth by the same mechanism as D-threo-PDMP, which is depletion of intracellular glycosphingolipids and increase in intracellular ceramide levels. The suppression of outgrowth is reversible after the removal of the drugs. Shayman et al.\(^3\) found that their novel D-threo-PDMP ethereal analogues, which have a pyrrolidine head group in place of the morpholine, depleted *endogenous* glycolipids *without* affecting cellular growth. This observation convolutes the understanding of the true effect(s) of D-threo-PDMP and its analogues and the role of cell-surface carbohydrates and one of the metabolites of GSLs, ceramide. These findings suggest that the effect of D-threo-PDMP and analogues, similar to the analogues reported herein, maybe functioning by causing increased *endogenous* levels of ceramide, not by depleting glycolipid biosynthesis. Our findings suggest that the inability of Shayman's ethereal analogues maybe a consequence of the head group (pyrrolidine instead of morpholine), not the alkoxy aromatic substituent.
Chapter 9. Abridged Synthesis of PDMP and Generation of a water soluble PDMP analogues

Given the medicinal value of PDMP, three unattractive features of PDMP keep it from being a serious drug candidate: 1) length of synthesis, 2) poor water solubility, 3) early *in vivo* studies with PDMP show that the drug is metabolized in the liver.\(^{318}\)

The original synthesis of racemic PDMP began with 2-aminoacetophenone, which required isomer separations (total yield unreported).\(^{319}\) Later synthetic schemes performed by Carson and Ganem \(^{320}\) utilized Evans' enantioselective aldol condensation with unsaturated aldehydes to deliver alkenyl D-*threo*-PDMP analogues in 8% overall yield.\(^{321}\) Other novel approaches towards D (or L)-*threo*-PDMP and analogues include the following: stereoselective alkylation of Garners aldehyde,\(^{322}\) stereoselective tandem reductive-alkylation of methyl ester derivatives from serine,\(^{323}\) and alkylation, followed by reduction, of *N*-carbobenzyloxy D-serine.\(^{324}\) All of these protocols require stereoisomer separation, greater than 10 steps and provide enantiomerically pure D-*threo*-PDMP in 20% overall yield at best.\(^{325}\)

Attempts to increase water solubility by shortening the amide chain length lead to inactive enzyme inhibitors.\(^{326}\) The minimal amide chain length with potency was hexanoyl, but that analogue was not only inferior to the potency of the decanoyl through hexadecanoyl amide chain length inhibitors, but also showed no water solubility.\(^{327}\)
In lieu of these inconveniences, we wanted to develop a method for producing PDMP that avoided isomer separations and moisture-sensitive alkylating reagents. Mostly, we wanted this procedure to have large-scale capability and flexibility to provide analogues bearing novel head groups and various N-acyl chain lengths. Ideally, the new method could provide a water-soluble PDMP analogue without loss of bioactivity.

After researching pro-drug molecules, it came to our attention that both (1S, 2S)- and (1R, 2R)-2-amino-1-phenyl 1,3-propanediol (9.1, Scheme 9.1) is a commercially available compound. We thought that either enantiomer of the amino diol served as an ideal starting material for the synthesis of enantiomerically pure PDMP—not only did 9.1 have the desired framework and functionality, but also the necessary threo-stereochemistry. With ample amounts of 9.1 on hand, we worked on shortening the synthesis of enantiomerically-pure PDMP and generating water-soluble analogues of PDMP in the L-isomer form first.

Compound 9.1 was treated with benzyl chloroformate in the presence of triethylamine in methanol to afford the N-carbobenzyloxy- product (9.1' not shown) in 88% yield. Treatment with tosyl chloride in pyridine gave regioselective tosylation of the primary alcohol to afford 9.2 in 79% yield. Jimbo reported selective primary mesylation of similar substrates in high yields. In our hands, the primary tosylate was not only more robust, but also
gave more reproducible results. The mesylate seemed to decomposed in organic solvents (e.g. CH$_2$Cl$_2$, CHCl$_3$) in a short time span (>30 minutes).

\[
\text{Scheme 9.1. Amine protection, selective primary tosylation}
\]

The primary tosylate product 9.2 was exposed to various secondary amines in either neat conditions or DMF (Scheme 9.2) to provide the displacement product(s) 9.3a-e. Yields are provided in Table 9.1.

\[
\text{Scheme 9.2. Nucleophilic Displacement of Tosylate 9.2}
\]

<table>
<thead>
<tr>
<th>Nucleophile (R$_2$NH)</th>
<th>Product</th>
<th>Isolated Yield (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morpholine</td>
<td>9.3a</td>
<td>86$^b$</td>
</tr>
<tr>
<td>1-Methyl Piperazine</td>
<td>9.3b</td>
<td>57$^b$</td>
</tr>
<tr>
<td>1-Hydroxyethyl piperazine</td>
<td>9.3c</td>
<td>60$^{c,d}$</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>9.3d</td>
<td>67$^b$</td>
</tr>
<tr>
<td>Piperidine</td>
<td>9.3e</td>
<td>70$^b$</td>
</tr>
</tbody>
</table>

$^a$Yields after purification via column chromatography. $^b$Reaction run under neat conditions. $^c$Reaction used DMF as solvent. $^d$Peracylated prior to chromatography.
Some of the compounds containing the head groups found in Table 9.1 have already been synthesized and tested as PDMP analogues. With pyrrolidin as a head group, that analogue was unable to inhibit cellular growth—presumably due to its inability to cause the accumulation of ceramide.\textsuperscript{331} Piperidine, as a head group on a PDMP analogue has been found to be inferior to the morpholino head group.\textsuperscript{332} As for the piperazine products, only 1-methyl piperazine has been generated, however, it has never been tested.\textsuperscript{333} Based on these findings, only products 9.3a-c were converted to PDMP analogues and tested against Manduca sexta embryonic cells.

This displacement scheme is limited to cyclic dialkyl amines. Attempts to use acyclic secondary amines, such as \textit{N}-methyl ethanolamine, diethanol amine, gave predominately imino ether 9.4 (Scheme 9.3). Compound 9.4 had been observed as a product in the displacement of attempted displacements of tosyl ceramide compounds (see Chapter 5). The most popularly used PDMP analogues contain the secondary cyclic amine as the head group. We envisioned PDMP analogues with \textit{N}-methyl ethanolamine and diethanol amine as the head group would provide a simple route to provide hydroxyl groups, which may enhance water solubility.
Scheme 9.3. Undesired products with acyclic amines as nucleophiles

In order to circumvent this problem, an oxazolidinone protection scheme (similar to the original D-threo-PDMP synthesis performed by Mitchell et al.\textsuperscript{334}) was employed instead of the carbobenzyloxy group (Scheme 9.4). Compound 9.1 was treated with benzylchloroformate and triethylamine in methanol, followed by regioselective silyl-protection gave 9.5\textsuperscript{335} in 73% yield. Compound 9.5 in the presence of n-butyl lithium in anhydrous THF afforded oxazolidinone 9.6\textsuperscript{10} in 75% yield. Removal of silyl protection, followed by tosylation provided tosylate 9.7 in 82% yield. Nucleophilic displacement of tosylate 9.7 provided acyclic amino products 9.8a-b in moderate yields (Table 9.2).

Scheme 9.4. Generation of tosyl oxazolidinone 9.7 and Displacement
Table 9.2. Nucleophilic Displacement of Tosylate 9.7 with Acyclic Amines

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Product</th>
<th>% yield&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>R=R'=CH₂OAc</td>
<td>9.8a</td>
<td>52</td>
</tr>
<tr>
<td>R= CH₂OAc, R'=H</td>
<td>9.8b</td>
<td>44</td>
</tr>
</tbody>
</table>

<sup>a</sup>Yields after purification via column chromatography.

Treatment of N-carbobenzyloxy compounds 9.3a-c and oxazolidinones 9.8a-b with KOH under refluxing conditions, followed by addition of decanoyl chloride<sup>336</sup> delivered D-threo-PDMP and analogues (Scheme 9.5) in moderate to good yields without epimerization of the benzylic alcohol moiety.<sup>337</sup> Yields are provided in Table 9.3.

![Diagram](attachment:image.png)

(a) KOH, MeOH: H₂O; (b) decanoic chloride, CH₂Cl₂.

**Scheme 9.5. Saponification and N-acylation**
Table 9.3. Yields of Saponification and N-acylation

<table>
<thead>
<tr>
<th>Compound</th>
<th>9.9a</th>
<th>9.9b</th>
<th>9.9c</th>
<th>9.9d</th>
<th>9.9e</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Yield</td>
<td>74</td>
<td>45</td>
<td>45</td>
<td>44</td>
<td>64</td>
</tr>
</tbody>
</table>

Compounds 9.9a-e were then tested against a Manduca sexta embryonic cell line.\textsuperscript{338} Original concentrations administered were 5, 10 and 20 μM of the drug. DMSO was employed as the drug vehicle. For comparison, L-threo-PDMP (9.9a) was determined to be moderately effective at inhibiting the neurite outgrowth at 20 μM (unpublished work). After 24 hours of exposure, images were recorded at 20 μM of L-threo-PDMP (9.9a), PDDEAP (9.9d) and PDMEAP (9.9e) (Figure 9.1). PDDEAP appears to superior inhibition potency at 20 μM. At lower concentrations, some suppression was observed by PDDEAP, though moderate. Compounds 9.9b and 9.9c showed moderate inhibition at 20 μM.
Figure 9.1. Inhibition by L-threo-PDMP and analogues after 24 h

After exposure for 48 hours, the drug media was removed. Cells were allowed to grow for an additional 48 hours. Images were recorded in the case of
exposure of PDDEAP and PDMEAP (Figure 9.2). After the removal of 20 μM 9.9b-e, the cells were able to recover and develop healthy extensions.

L-threo-PDDEAP, 48 h after removal  
L-threo-PDMEAP, 48 h after removal

Figure 9.2. Recovery of Growth After Removal of Inhibitors
Given the effectiveness of 20 μM, inhibition studies were performed using water as the drug vehicle. Attempts to dissolve and administer drugs 9.9a-e in water failed, except 9.9d. L-threo-PDDEAP had significant water solubility and was administered to cells. The results of cells exposed to 20 μM L-threo-PDDEAP using water as solvent were similar to the effects observed using DMSO as the drug vehicle—reversible growth inhibition. To date, no PDMP analogues have been successfully administered with water as the drug vehicle in the literature. Overcoming this obstacle, this PDMP analogue could lead to greater successes for the advancement of PDMP as a drug candidate.

In conclusion, though this synthetic development may not warrant much synthetic attention, the medicinal value could be immense. The other published synthetic route provide enantiomerically pure D- or L-threo-PDMP in greater than ten steps, less than 20% overall yield and require isomer separation and moisture-sensitive alkylating agents. Using this newly developed scheme, D- or L-threo-PDMP can be generated in five steps with an overall yield of 50% yield.

This methodology is also applicable to large-scale PDMP synthesis. In one week, five grams of D-amino diol 9.1 can be converted to three grams of D-threo-PDMP (9.9a), with only one silica gel column chromatography purification was required (purification of final product prior to acidification). We have been fortunate to receive financial contributions from Matreya, Inc. in exchange for quantities of D-threo-PDMP.
Compound 9.1 costs $14-20 per gram.\textsuperscript{339} In order to generated this compound via Poll’s developed scheme (Scheme 9.6), a researcher would have to: treat D-serine with thionyl chloride in methanol to provide methyl ester 5.5 (quantitative yield) without epimerization, treat hydrochloride salt 5.5 with commercially available benzophenone imine to give imine 5.6 (75% yield), recrystallize, protect hydroxy group with tert-butyl dimethylsilyl group to provide 5.4 (~90% yield), perform a successful tandem reductive alkylation (70% yield at best) to give 5.9 and remove the imine and silyl group (70% yield) to provide amino diol 9.1 in theoretically 42% overall yield. Though serine is cheap ($0.50-3 per gram)\textsuperscript{340} Benzophenone imine costs $4 per gram, \textsuperscript{341} benzophenone imine can made by the addition of phenylmagnesium bromide to benzonitrile, but the product can be difficult to distill/purify. The tandem reductive alkylation reaction and the separation of products can also be problematic and can give inconsistent results. Other expenses that also need to be factored into the total cost include: other reagents, materials and the cost of the lab worker. It is clear that starting with 9.1 has a financial advantage.
This newly developed route allows for a garden variety of head group PDMP analogues. Some of the head groups reported here are new, some have been reported and tested. Though the author was only able to generate several head group analogues, one can imagine the ability to make a combinatorial library of PDMP analogues by exposing tosylate 9.2 to an array of cyclic amines. One could also synthesize various N-acyl chain length analogues, plus modify the chain from linear to branched. One limitation in using 9.1 (and also 9.2) is the phenyl ring, but one could take a synthetic voyage into alkylations of (or adding other decorations to) the aromatic moiety if one desired such an endeavor.
Chapter 10. Efforts towards generating a polyhydroxylated piperidine PDMP head group analogue

Due to the lipophilic requirements (of the amide chain) for inhibition, we envisioned that modifications about the morpholino head group could give increased inhibition and water-solubility. As stated earlier, similar to PDMP (10.1, Figure 10.1), N-butyl deoxynorjimycin (NBJ; 10.2) has been utilized as a potent inhibitor of the GlcCer synthase enzyme (see Chapter 3). Kinetic studies on the inhibition by NB-DNJ were determined to be competitive for ceramide ($K_i=7.4 \mu M$) and non-competitive for the UDP-glucose, indicating ceramide mimicry as inhibitory activity. The only difference is NBJ did not cause the accumulation of ceramide, which may explain why imino sugar 10.2 did not cause the suppression of neurite outgrowth, nor inhibit embryogenesis as PDMP.

![Figure 10.1. Structures of known glucosyl transferase enzyme inhibitors](image)

Replacing the morpholine head-group with an imino sugar, similar to deoxynorjimycin, could provide not only a better resemblance to the GlcCer synthase transition state, but also increase water solubility. Not only that, but if NBJ inhibits by competing with ceramide for the active site, the lipophilic portion of PDMP could give greater resemblance to ceramide than the lipophilic portion
NBJ—the $n$-butyl group. The combination of these two features could lead to a superior drug 10.3 (Figure 10.2).

We originally hypothesized that using synthetic developments by Polt$^{344}$ and Razavi$^{345}$ one could deliver aza-sugar 10.4. These methodologies also provide aza-sugars in the piperidine variety. Pyrrolidine 10.4 could be employed in a displacement reaction with tosylate 9.7 to provide oxazolidinone 10.5 (Scheme 10.1).
Generation of pyrrolidine 10.4 started with the imino ester of L-serine 10.7 (Scheme 10.2). The vinyl moiety was introduced in a stereoselective manner via a tandem reductive-alkylation reaction to provide the desired threo-isomer 10.8. The threo adducts were isolated by column chromatography and elaborated as described previously. The low yield of the reductive alkylation has been attributed to the vinyl nucleophile solvent—THF. Alcohol 10.8 was esterified to provide pivylate 10.9 in 92% yield. The steric bulk of the pivoly ester provided high stereoselectivity of the dihydroxylation of 10.9 to afford diol 10.10 in 61% yield. Reduction and pivoly-removing with lithium borohydride provided benzhydryl amine 10.11 in 30% yield. Cyclization of 10.11 provided 10.12, which was acylated, followed by benzhydryl removal provided 10.4 in 3.0% overall yield. Unfortunately, attempts to displace tosylate 9.7 with pyrrolidine 10.4 did not provide desired displacement product (Scheme 10.3). This displacement failure has been attributed to two factors: low nucleophile:tosylate ratio (~1:1) because of the required chemistry to generate 10.4 and the bulkiness of the silyl group on the primary hydroxyl group.
Scheme 10.2. Synthesis of Aza-sugar 10.4

Scheme 10.3. Unsuccessful displacement of Tosylate 9.7 with Aza-sugar 10.4

Instead of pre-creating the aza-sugar, we shifted our efforts towards incorporating the hydroxylated pyrrolidine or piperidine head group analogue of PDMP (e.g. 10.13) using the retrosynthetic scheme in Scheme 10.4.
Scheme 10.4. Retrosynthesis of imino-sugar PDMP analogue 10.13

We envisioned that the imino sugar portion of 10.13 could be synthesized from a condensation of amine 10.14 and glucose-derivative 10.15, followed by reduction, could provide 10.16. Removal of the acetonide group would give acetal 10.17, which could ring-annulate to yield iminium 10.18. Reduction of iminium ion could deliver 10.19. Removal of the amine-protecting group,
followed by N-acylation could provide target molecule 10.13. Amine 10.14 could be arrived at from the displacement of tosylate 9.7.

Initially, we had three major objectives: 1) optimal condensation-reduction conditions with aldehyde 10.15, 2) generation of amine 10.14 with optimal amino-protection (determine whether using N-carbobenzyloxy- or oxazolidinone-protection would be superior), 3) develop a reductive amination protocol that could deliver a cyclized head group.

To determine the superior reducing agent (sodium cyanoborohydride or sodium triacetoxyborohydride), racemic α-methyl benzyl amine was treated with aldehyde 10.15, which was generated by sodium periodate oxidation of 1,2-diisopropylidene glucofuranoside (57% yield), and each reducing agent under their respective conditions (sodium triacetoxyborohydride in dichloroethane or sodium cyanoborohydride in MeOH with pH ~ 5). Using sodium triacetoxyborohydride in 1,2-dichloroethane afforded dialkyl amine product 10.20 in 87% yield (Scheme 10.5). Using the sodium cyanoborohydride/acetic acid/methanol protocol provided 10.20 in lower yields. Hence, sodium triacetoxyborohydride was employed as the reducing reagents.
As for determining the amino-protecting group for amine 10.14, for simplicity, *N*-carbobenzyloxy-protection was employed first. Similar to a protocol reported in Chapter 6, amino diol 9.1 was treated with benzyl chloroformate in the presence of triethylamine in methanol to afford the *N*-carbobenzyloxy-product (Scheme 10.6), which was converted to its corresponding tosyIate 9.2 (not shown). Tosylate was treated with sodium azide provided displaced product 10.21 in 36% overall yield.

Azide 10.21 was treated with triphenylphosphine in anhydrous THF to generate the phospho-imine 10.22 (Scheme 10.7). In the presence of aldehyde 10.15, followed by treatment with sodium triacetoxyborohydride, compound 10.23 was determined to be the major isolated product, according to mass spectroscopy. Compound 10.23 was found to be rather robust, surviving
aqueous washings and silica column chromatography. This type of cyclized product has been reported in the literature.\textsuperscript{352}

Scheme 10.7. Aza-wittig reaction with azide 10.21 and aldehyde 10.15

To circumvent this problem, azide 10.21 was treated with wet THF, creating the amine 10.24 (from phospho-imine hydrolysis) \textit{in situ}.\textsuperscript{353} Attempts to use aldehyde 10.15 under these same conditions, however, resulted in \textit{intra}molecular-cyclized product 10.25 as the major product (Scheme 10.8).\textsuperscript{354}

Scheme 10.8. Undesired \textit{intra}molecular-cyclization of amine 10.24

Due to the ineffectiveness of the \textit{N}-carbobenzyloxy-protecting scheme, an oxazolidinone protection scheme was used. The silyl-group of oxazolidinone 9.6 was removed to provide the primary alcohol. The primary alcohol was converted to its corresponding tosylate (9.7, not shown). The tosylate was treated with sodium azide to afford azide 10.26 in 75\% yield (over three steps, Scheme 10.9).
Treatment of azide 10.26 with anhydrous THF affords phospho-imine 10.27, in 87% yield. The phospho-imine is crystalline and surprisingly stable (Scheme 10.10), though decomposes in polar protic solvents. IR experiments reveal two stretches, which could be due to the phosphorous-nitrogen bond, occurring at 1441 and 1187 cm$^{-1}$. Zhmurova$^{355}$ reports the P-N stretch at 1385 cm$^{-1}$, while Bragin$^{356}$ reports 1239 cm$^{-1}$. The normal range for the P-N stretch is from 1140-1500.$^{357}$

Scheme 10.10. Generation of stable phospho-imine

Phospho-imine 10.27 was surprising unreactive in the presence of aldehyde 10.15, hence decomposition of 10.26 with triphenylphosphine in wet THF, generated amine 10.28, was necessary for reactivity. Using the new reductive-amination procedure, followed by acylation, gave amine 10.29 in 25% yield (Scheme 10.11). Attempts to isolate the reductive-amination product without acylation could only provide the free amine in 20% yield. Even with the
acylations, amine 10.29 was rather polar and could not be completely separated from triphenylphosphine oxide.

\[ \text{Scheme 10.11. Reductive Amination with amine 10.29} \]

Due to the low yields of these condensation attempts, we shifted our attention to another attractive source of carbohydrate chirality, D-mannitol. Even if these yields were higher, in all of these cases, removal of the 1,2-isopropylidene group on compound 10.15 (or 10.29) would require strongly acidic conditions,\(^{358}\) which could lead to decomposition of product. The following procedures employed were similar to protocols developed by Singh towards the synthesis of lentiginosine\(^{359}\) and muricatacin.\(^{360}\)

D-Mannitol was converted to its corresponding 1,2-5,6-bis-acetonide 10.30 in 67% yield (Scheme 10.12). Allylation with allyl bromide and sodium hydride provided 1,2-5,6-bis-acetonide 3,4-diallyl protected mannitol 10.31\(^{361}\) in 85% yield.\(^{362}\) Treatment of 10.31 with 5 equivalents of acetyl chloride in cold methanol provided 5,6-diol 10.32, which was oxidized with sodium periodate in dichloromethane: water to give aldehyde 10.33 in 81% yield over two steps (12% unreacted 10.31 was also isolated).
Treatment of aldehyde 10.33 with amine 10.28 (made available after the decomposition of azide 10.26 in wet THF) in the presence of sodium triacetoxyborohydride in dichloroethane gave desired product 10.34 in a disappointing 20% yield (Scheme 10.13). Once again, due to the polarity of 10.34, it was impossible to completely remove triphenylphosphine oxide via flash chromatography.
Scheme 10.13. Reductive-Amination with aldehyde 10.33

Since we could not isolate desired mono-condensation/reduction products, we changed our synthetic strategy. We envisioned that amine 10.28 in the presence of bis-aldehydes or bis-acetal and sodium triacetoxy borohydride could facilitate a condensation-reduction-cyclization-reduction reaction to provide product 10.34 in a similar fashion as developed by Shim, Natsuko and Costello (see Chapter 6).


Using a combination of procedures (from Scheme 10.13 and 10.14), treatment of azide 10.26 with triphenyl phosphine in aqueous THF, followed by
hydrated dial 10.35 and sodium triacetoxy borohydride in dichloroethane afforded pyrrolidino oxazolidinone 10.36 in 70% overall yield (Scheme 10.15). Carbamate 10.36 was saponified and N-acylated (Scheme 10.16) to provide L-threo-1-phenyl-2-palmitoyl amino 3-pyrrolidino 1-propanol (L-threo-P4) 10.37 in 56% yield. P4 is a more lipophilic and more potent inhibitor of the GlcCer synthase enzyme than PDMP. Though P4 is a strong suppressor of GSL formation, P4 does not cause the build-up of ceramide, like PDMP, and hence does not affect cellular growth or embryogenesis.

Scheme 10.15. Reductive-amination with azide 10.26 and dial 10.35

(a) KOH, MeOH; H2O;
(b) decanoic chloride, KOH, CH2Cl2

Scheme 10.16. Delivery of L-threo-P4
Next, we attempted to diversify our reductive amination cyclization protocol to employ similar protocols established by Zou and Reitz to provide dicarbonyl compounds similar to 10.38 (see Chapter 6). This procedure would avoid the acidic conditions needed to remove the acetonide of 10.15 when attached to 10.29, for example.

In our hands, D-mannitol (Scheme 10.17) was converted to 1,3:4,6-di-O-benzylidene D-mannitol in 56% yield. Oxidation of 1,3:4,6-di-O-benzylidene D-mannitol with PCC delivered C2-symmetric, cyclic hydrate 10.38 in 38% yield. However, the reproducibility of this oxidation was inconsistent. The use of various other oxidizing agents/conditions (NaIO4, TPAP and Swern) was equally problematic. Though problematic, when 10.38 was made available, attempts to perform reductive amination cyclizations similar to Scheme 10.17 to provide product 10.39 were unsuccessful.

Scheme 10.17. Double reductive amination with diketone hydrate 10.38.
Attempts to hydrolyze aldehyde 10.15 with acidified water and DOWEX® were unsuccessful to provide 10.40 in our hands. It seemed that the only time we were able to get the hydrolysis to take place, the product seemed to either decompose under the acidic conditions and high temperatures required and/or could not be purified \(\text{(Scheme 10.18)}\).

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{10.15} & \quad \text{a} \quad \text{10.40}
\end{align*}
\]

a) acidic conditions: HCl (aq); HCl (anhydrous); DOWEX

\textbf{Scheme 10.18. Attempted hydrolysis of acetonide 10.15}

After multiple attempts at performing reductive amination reactions with amine 10.28 and a variety of mono- and bis-aldehyde substrates, we realized that this methodology has potential to provide sophisticated PDMP analogues. Although attempts to deliver sophisticated head groups only provided desired products in low yields, the methodology does work and can be utilized and optimized in the future. The pyrrolidine head group can also be delivered by the displacement of tosylate 9.7 with pyrrolidine in DMF or neat. We had hoped that using similar conditions to provide the pyrrolidine product could work for complicated bis-aldehydes, but I was unsuccessful at achieving this feat.
Chapter 11. Synthesis of and Biological Studies with N-acyl norephidrine, a ceramidase inhibitor

Looking back at the ceramide cycle (Chapter 1) in Figure 11.1, we observe that ceramide can be metabolized to sphingosine by the ceramidase enzyme. Sphingosine, a known suppressor of protein kinase C, can be phosphorylated to provide sphingosine-1-phosphate (S1-P), which is a known promoter of growth. Sphingosine-1-phosphate can be broken-down in retroaldol fashion by S1-P lyase to hexadec-2-enal and ethanolamine phosphate.

![Chemical structures](image)

**Figure 11.1.** Degradation of sphingomyelin and resulting ceramide

Due to ceramide's involvement in cellular apoptosis and apoptosis's possible involvement in various diseases, attempts to cause an intracellular increase in ceramide levels have become a pharmaceutical passion. Human
Colon cancer cells have a greater than 50% decrease in cellular content of ceramide when compared with normal colon mucosa. Preliminary data have also shown that malignant cells with low ceramide levels are resistant to apoptosis. These findings clearly exemplify the need/interest in developing drug candidates that cause increases in ceramide.

As we have seen earlier, cellular exposure to D-threo-PDMP causes an increase in endogenous ceramide levels. Though this increase in intracellular ceramide can cause suppression of cellular growth, not until recently had PDMP-induced ceramide accumulation causing apoptosis been published. In most reports, if apoptosis is not observed, the suppression of cellular growth is short and reversible after the removal of the drug.

A new candidate for the increase in intracellular ceramide is D-erythro-myristoylamino phenyl propanol (D-MAPP, 11.1). Similar to PDMP, D-MAPP (Figure 11.2) was found to cause an intracellular increase in ceramide levels. Unlike PDMP, D-MAPP did not block the formation of GlCer (and other GSL's). D-MAPP bioactivity was attributed to its ability to block the metabolism of ceramide to sphingosine. Interestingly, the observed inhibition of the ceramidase enzyme was limited to the D-erythro isomer, while the L-erythro isomer was ineffective. The threo isomers were not tested. The ineffectiveness of the L-erythro isomer was attributed to its stereochemistry, which bears the stereochemistry of natural ceramide, and hence was metabolized.
Given the effect of D-MAPP on lipid metabolism, new analogues of D-erythro-MAPP have been developed and tested. Ceramidase inhibitor 11.2 (Figure 11.3) was found to be more potent than D-MAPP and to induce apoptosis in cultured human cancer cells at concentrations as low as 5 µM. Intriguingly, 11.2 did not affect the ceramide levels and hence did not induce apoptosis in normal cells at concentrations as high as 100 µM.

The straightforward synthesis of D-erythro-MAPP is simply the N-acylation of naturally occurring D-norephedrine. Though this approach allows for simple modifications of the amide region, little can be done with respect to manipulations to aromatic region, or the stereochemistry of the molecule. Hence, our attention focused on developing a synthetic protocol to deliver enantiomerically pure analogues of D-erythro-MAPP from D-alanine. We also envisioned that this work
would allow for the development of a procedure towards stereoselective
formation of erythro-products, converse to Pohl's reductive-alkylation protocol that
provides predominantly threo-adducts.\textsuperscript{387} One can foresee attempts to perform
asymmetric reductions of imine-protected ketone\textsuperscript{388} (e.g. 11.3 in Scheme 11.1)
to provide erythro-enriched products. Using various reducing agents, one could
use chelating or non-chelating hydride sources to affect the stereoselective
outcome.\textsuperscript{389}

Scheme 11.1. Asymmetric Reduction of Amino Ketones from Amino Acids

Beginning with D-amino acid derivatives 11.4a (from alanine) and b (from
serine), which were synthesized using a published procedure,\textsuperscript{390} were treated
with \textit{N}, O-dimethylhydroxyamine HCl in the presence of trimethylaluminium in
anhydrous THF (Scheme 11.2) to provide Weinreb amide product 11.5a\textsuperscript{391} and b
in 50-59% yield (Table 11.1, yield A).\textsuperscript{392} Alkylation of Weinreb amide 11.5a and
b with phenyl magnesium bromide in THF\textsuperscript{393} delivered ketone products 11.6a
and b in 85-95% yield (Table 11.1, yield B). One advantage with the imine
protection of Weinreb amide 11.5a and b is the absence of the exchangeable amino proton (e.g. H-NBOC), which can complicate alkylation strategies. Yields of this alkylation increased when triethylamine was added to the employed column chromatography solvent to suppress imine hydrolysis. Using IR, as the functional groups of this synthetic scheme changed, so did the carbonyl stretching frequencies (Table 11.2). Attempts to convert ester 11.4 to ketone 11.6 via Weinreb amide 11.5 in one pot were unsuccessful.

\[
\text{Scheme 11.2. Formation of phenyl ketone from known amino acid derivatives}
\]

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Carbonyl stretches (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ester (11.4a-b)</td>
<td>1737</td>
</tr>
<tr>
<td>Weinreb Amide (11.5a-b)</td>
<td>1662</td>
</tr>
<tr>
<td>Ketone (11.6a-b)</td>
<td>1686</td>
</tr>
</tbody>
</table>
With ketone 11.6a in hand (Scheme 11.3), removal of the imine via acid hydrolysis provided amine hydrochloride salt 11.7. Attempts to determine the enantiomeric purity of 11.7, by comparing the optical rotation of 11.7 to known compound cathinone HCl were unsuccessful. Development of HPLC purification protocol should be considered. Acylation of amine 11.7 using decanoyl chloride and triethylamine in dichloromethane provide keto amide 11.8 in 57% yield (over two steps).

In early acyl amino phenyl propanol studies, the myristoyl N-acyl chain length was determined to be the optimal length, based on growth inhibition studies of various lipophilic amides of 2-amino 1-phenyl 1,3-propane diol. When the primary hydroxyl group was removed, leaving a terminal methyl group as in D-erythro-MAPP, the N-acyl chain length was not re-evaluated. We chose the decanoyl chain for the amide portion of keto amide 11.8 in order to keep constant the lipophilicity of 11.8 and our PDMP analogues in the amide region, albeit later studies should include various amide chain lengths in order to determine optimal lipophilicity and solubility.
Our initial inhibition studies with analogues of D-erythro-MAPP used D-erythro-DAPP as our lead compound. DAPP, D-erythro-2-decanoyl amino-1-phenyl-propan-1-ol was synthesized by acylating commercially available (1S,2R)-(+)-norephedrine hydrochloride using similar conditions to Scheme 11.3. Analogue testing employed similar condition/protocols as used for all PDMP and analogue testings, using Manduca sexta embryonic cells. Cells were allowed to develop prior to drug contact, and then exposed to DAPP at 10, 20 and 40 μM for 48 hours. After 48 hours, DAPP was removed and cells were allowed to grow for an additional 48 hours. Images were recorded every 24 hours. DMSO was used as the drug vehicle and cells lacking drug contact were used as controls.

After 24 hours of drug contact, neurite growth of cells was suppressed at 10, 20 and 40 μM. Cells were unable to retain the healthy extensions that had developed prior to drug exposure (Figure 11.4). The observed suppression of outgrowth continued until after the removal of the drug. Once the drug was removed, the cells were able to growth healthy extensions at 10 μM. At higher concentration of D-DAPP, cells were unable to reestablish normal extensions. The ability of Manduca sexta embryonic cells to grow normal extensions after the removal of the drug is comparable to the reversible inhibition that has been observed with PDMP and analogue studies.
Figure 11.4. Reversible Inhibition of Neurite Outgrowth by DAPP

Given these findings, future tests should include keto amide 11.8 with similar concentrations as DAPP. Unfortunately, it appears as though keto amide 11.8 is an oil. Administration of an oil at controlled concentrations can be problematic. Exposing cells to cathinone HCl 11.7 and analogues is a possibility, but with 7 lacking appreciable lipophilicity, delivery of 11.7 to affect lipid
processes may not be plausible. Cathinone, the active agent in khat leaves, has been a known stimulate since at least the 13th century in Africa. Cathinone is considered a controlled substance in the United States of America, though, in moderation, cathinone acts much like caffeine.401

Another intriguing test would be exposing cells to a mixture of DAPP and PDMP (and analogues) to see if these compounds can function synergistically to cause intracellular increases of ceramide (Figure 11.5). If PDMP blocks the formation of GlcCer (and all resulting GSL’s from GlcCer), which hence causing a buildup of ceramide and DAPP inhibits the metabolism of ceramide (also causing the buildup of ceramide), the cocktail of PDMP and DAPP could provide an appreciable amount of intracellular ceramide.

![Figure 11.5. PDMP-DAPP Cocktail: Providing lethal ceramide buildup?](image)

Initial tests have shown that in order to inhibit similar neurite outgrowth, it would require 10 μM DAPP or 20 μM PDMP. These results suggest that a synergistic relationship is not possible, however, other tests would need to be required prior to abolishing this hypothesis.
EXPERIMENTALS

**General Information.** All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks. THF was distilled and deoxygenated over Ph$_2$C=O/K°. Dichloromethane and acetonitrile were distilled over CaH$_2$. All other solvents used were freshly distilled under an argon atmosphere before use. For flash chromatography, 400-230 mesh silica gel 60 (E. Merck no. 9385) was used. All compounds described were >95% pure by $^1$H and $^{13}$C NMR, and purity was confirmed by low-resolution fast atom bombardment (FAB) mass spectrometry. The $^1$H and $^{13}$C NMR spectra were obtained on a Varian 300 MHz spectrometer. Chemical shifts are reported in δ vs. Me$_4$Si in $^1$H spectra and vs. CDCl$_3$ in $^{13}$C spectra. Optical rotations were taken on a Jasco DIP-1000 polarimeter using the Na$_{D-}$line. All melting points were taken on a Hoover capillary melting point apparatus and are uncorrected.
(1R,2R)-(−)-2-Amino-N-(diphenylmethylene)-3-O-(tert-butyldimethylsilyl)-1-(o-methyl)-phenylpropane-1,3-diol (7.2). Protected-D-serine (7.1, 5.22 g, 13.12 mmol) was dissolved in dry CH2Cl2 and chilled to -78°C. To this solution was added iBu2AlH/iBu3Al (13.8 mmol of each in 30 mL of hexanes) dropwise over one hr. The solution turned yellow upon addition of iBu2AlH/iBu3Al. After addition iBu2Al2H is complete, ortho-tolylmagnesium bromide (20mL, 3 eq., 2M in Et2O, 39.4 mmol) was added dropwise over 30 min. Reaction is then allowed to warm to RT. Reaction flask was placed in an ice bath and the excess Grignard reagent was quenched by the slow addition of saturated NaHCO3. Solution was then diluted with CH2Cl2 and transferred to a separatory funnel. Organic layer was washed with saturated NaHCO3 twice. Organic layer was then dried with K2CO3 and filtered. Solvent was removed under reduced pressure and chromatographed with SiO2 (5% EtOAc: Hexanes, Rf = 0.4) yielded 3.78 g of pure threo product as a colorless oil in 69.0% yield (9:1 threo: erythro) [α]25D = −59° (c = 1.0, CHCl3). 1H NMR (300MHz, CDCl3): δ 7.8-7.1 (m, 20 H), 5.15 (d, J = 8.4 Hz, 1H), 3.95, (dd, J= 10.8, 4.4 Hz, 1H), 3.65 (dd, J = 10.5, 4.4 Hz, 1H), 3.15 (bs, 1H), 3.07 (bs, 1H), 2.39 (s, 3H), 0.91 (s, 9H), 0.03 (s, 6H). 13C NMR (75.0 MHz, CDCl3): δ 146, 145, 140, 128, 127, 126, 125, 100, 82, 68, 59, 25, 19, 18, -6.

(1R,2R)-(−)-2-Amino-3-O-(tert-butyldimethylsilyl)-1-(o-methyl)-phenylpropane-1,3-diol (7.3). Threo product 7.2 (0.97 g, 2.1 mmol) was dissolved in THF (10 mL) and H2O (2 mL) at RT. Pyridinium p-toluenesulfonate (1.0 g, 4.2 mmol, 2 equiv) was added and reacted for 5 hr. THF was removed under reduced pressure. The residual was dissolved in CH2Cl2 (30 mL). The organic solvent was washed with
saturated NaHCO₃ (3 x 30mL), dried over K₂CO₃. The crude product was then
chromatographed on silica gel (9:1 CHCl₃: MeOH, Rₖ = 0.5), which yielded an off-
white powder in 70% yield. Melting point: 80-82° C. [α]²⁵D = −18° (c = 0.8, CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 7.5-7.1 (m, 4 H), 4.95 (d, J = 4.2 Hz, 1H),
3.85 (dd, J = 10.1, 4.6 Hz, 1H), 3.72 (dd, J = 10.0, 5.4 Hz, 1H), 2.95 (ddd, J =
4.4, 5.1, 4.6, 1H), 2.25 (s, 3H), 0.91 (s, 9H), 0.03 (s, 6H). ¹³C NMR (75.0 MHz,

(4R,5R)-(+) -4-(tert-Butyldimethylsilyloxy)methyl)-5-(o-
Methyl)-phenyloxazolidin-2-one (7.4). Amino alcohol 7.3
(0.34 g, 1.15 mmol) was dissolved in dry THF (6 mL) under
argon. 1,1'-diimidazole carbonyl (0.22 g, 1.38 mmol, 1.2
equiv.) was added in one portion. Once deemed complete via
thin layer chromatography, solvent was removed under reduced pressure. The
 crude residual was then chromatographed on SiO₂ (3:1 hexanes: ethyl acetate,
Rₖ = 0.3), melting point: 136-138° C, yield: 80.3%. [α]²⁵D = +13° (c = 1.0, CHCl₃).
¹H NMR (300 MHz, CDCl₃): δ 7.4-7.15 (m, 4 H), 6.29 (bs, 1H), 5.15 (d, J = 3.9
Hz, 1H), 3.9 (m, 3H), 2.39 (s, 3H), 0.91 (s, 9H), 0.03 (s, 6H). ¹³C NMR (75.0

(4R,5R)-(+) -4-(4'-p-Toluenesulfonyloxymethyl)-5-(o-
Methyl)-phenyloxazolidin-2-one (7.5). Carbamate 7.4 (0.66 g, 2.04
mmol) was dissolved in acetonitrile (8 mL) at RT under
atmospheric conditions. To the solution, 49% aqueous
hydrofluoric acid (0.3 mL, 4 equiv.) was added to the solution.
The progress of the reaction was monitored by TLC. Upon completion, solvent
was removed under reduced pressure. Resulting solid was dissolved in freshly
distilled pyridine (5 mL) under argon at RT. Tosyl chloride (0.15 g, 0.80 mmol,
1.5 equiv.) was added in one portion. Once complete, the solvent was removed
in vacuo and the crude product was chromatographed with SiO₂. The pure product was isolated in 82.6% yield. (1:1 EtOAc: hexanes, Rᵣ = 0.4). [α]²⁵D = +50° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.8-7.1 (m, 8H), 6.65 (bs, 1H) 5.46 (d, J = 4.6 Hz, 1H), 4.18 (dd, J = 10.6, 5.1 Hz, 1H), 4.12 (dd, J = 10.5, 4.9 Hz, 1H), 3.85 (ddd, J = 4.9, 4.9, 5.1, 1H), 2.45 (s, 1H), 2.39 (s, 3H). ¹³C NMR (75.0 MHz, CDCl₃): δ 160, 146, 137, 131, 130, 129, 128, 127, 126, 124, 78, 70, 58, 21, 19.

(4R,5R)-(+)-[(N-Morpholino)methyl]-5-(o-methyl)-phenyloxazolidin-2-one (7.6). Tosylate 7.5 (0.20 g, 0.525 mmol.) was dissolved in dry THF (4 mL) under an argon atmosphere. Freshly distilled morpholine (130 μL, 1.6 mmol., 3 equiv.) was added to the solution and the resulting solution was heated to a gentle reflux. After 16 hr., solvent was removed under reduced pressure and the resultant was chromatographed on SiO₂ (EtOAc, Rᵣ = 0.3). The pure product was isolated in 82% yield. [α]²⁵D = +51° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.4-7.1 (m, 4H), 5.60 (bs, 1H), 5.40 (d, J = 4.9 Hz, 1H), 3.85 (q, J = 7.3, 5.5 Hz, 1H), 3.65 (t, J = 4.6 Hz, 4H), 2.65 (m, 6H), 2.39 (s, 3H). ¹³C NMR (75.0 MHz, CDCl₃): δ 159, 128, 126, 125, 79, 67, 63, 57, 54, 19.

(1R,2R)-(+)-1-o-Tolyl-2-decanoylamino-3-(N-morpholino)-1-propanol [D-threo-TDMP] (7.7a). Morpholino carbamate 7.6 (0.19 g, 0.67 mmol.) was dissolved in a 4:1 MeOH: H₂O solution and treated with 2 M KOH (5 mL). The resulting solution was heated to a gentle reflux. Progress of the reaction was monitored via TLC. After reaction deemed complete, solvent was removed under reduced pressure and the aqueous layer was extracted with CH₂Cl₂. The organic layer was washed with saturated
NaHCO₃ twice. The organic layer was then dried with K₂CO₃, filtered, and concentrated. The crude product was then dried over P₂O₅ overnight, then dissolved in freshly distilled pyridine (5 mL). To the solution, para-nitro phenyl decanoate (0.20 g, 0.67 mmol., 1 equiv.) and 1-hydroxybenzotriazole (6 mg, 0.04 mmol., 0.1 equiv.) were added to the reaction under argon. The reaction was allowed to agitate at RT until deemed complete via TLC. Once reaction was completed, solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂. The organic layer was washed with 1M NaOH three times, then chromatographed with SiO₂ (95: 5 Chloroform: Methanol, Rf = 0.5) 67.7% yield over two steps, oil product. [α]²⁵ D = +7° (c = 1.0, CHCl₃). HRMS (FAB+H) calculated: 405.3117 found: 405.3116. 

¹H NMR (300MHz, CDCl₃): δ 7.4-7.1 (m, 4H), 6.05 (d, J = 7.3 Hz, 1H), 5.22 (d, J = 2.9 Hz, 1H), 4.18 (m, 1H), 3.71 (t, J = 4.6 Hz, 4H), 2.70 (dd, J = 13.1, 6.2 Hz, 1 H), 2.65 (m, 5H), 2.33 (s, 3H), 2.05 (t, J = 7.8 Hz, 2 H), 1.50 (m, 2 H), 1.25 (s, 12 H), 0.90 (t, J = 5.8 Hz, 3 H). ¹³C NMR (75.0 MHz, CDCl₃): δ 173, 139, 134, 130, 128, 125.9, 125.7, 72, 66, 62, 60, 55, 50, 37, 32, 29.4, 29.3, 29.2, 29.1, 26, 23, 19, 14.

(1R,2R)-(−)-1-o-Tolyl-2-palmylamino-3-(N-morpholino)-1-propanol [D-threeo-TPMP] (7.7b).

Compound provided by same procedure utilized in generation of 7.7a. HRMS (FAB+H): calculated: 405.3117, found: 489.4 [α]²⁵ D = +7° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.4-7.1 (Ar-H, m, 4H), 6.05 (N-H, bd, J = 7.3 Hz, 1H), 5.22 (CH-OH, d, J = 2.9 Hz, 1H), 4.18 (αH, m, 1H), 3.71 (CH₂-Omorph, t, J = 4.6 Hz, 4H), 2.70 (βH, dd, J = 13.1, 6.2 Hz, 1 H), 2.65 (CH₂-Nmorph, m, 5H), 2.33 (Ar-CH₃, s, 3H), 2.05 (amide-CH₂-, t, 2 H), 1.50 (amide-CH₂-CH₂-, m, 2 H), 1.25 (alkyl-H, bs, 22 H), 0.90 (-CH₃, t, 3 H) ¹³C NMR (75.0 MHz, CDCl₃): δ 173, 139, 134, 130,
128, 125.9, 125.7, 72, 66, 62, 60, 55, 50, 37, 32, 29.4, 29.3, 29.2, 29.1, 26, 23, 19, 14.

\[(1R,2R)-(+)-o-Tolyl-2-(3,6-bis-oxadecanoyl)amino-3-(N-morpholino)-1-propanol \text{ [D-three-TbisoxaDMP] (7.7c).}\]

Compound provided by same procedure utilized in generation of 7.7a. HRMS (FAB+H): calculated: 405.3117, found: 405.3116 \([\alpha]^{25}_D = +7^\circ (c = 1.0, \text{CHCl}_3)\). \(^1\text{H NMR (300MHz, CDCl}_3\): \(\delta 7.4-7.1\) (Ar-H, m, 4H), 6.05 (N-H, bd, \(J = 7.3\) Hz, 1H), 5.22 (CH-OH, d, \(J = 2.9\) Hz, 1H), 4.18 (\(\alpha\)H, m, 1H), 3.71 (CH\(_2\)-O\_morph, t, \(J = 4.6\) Hz, 4H), 2.70 (\(\beta\)H, dd, \(J = 13.1, 6.2\) Hz, 1 H), 2.65 (CH\(_2\)-N\_morph, m, 5H), 2.33 (Ar-CH\(_3\), s, 3H), 2.05 (amide-CH\(_2\)_-, t, 2 H), 1.50 (amide-CH\(_2\)-CH\(_2\)_-, m, 2 H), 1.25 (alkyl-H, bs, 12 H), 0.90 (-CH\(_3\), t, 3 H) \(^{13}\text{C NMR (75.0 MHz, CDCl}_3\): \(\delta 173, 139, 134, 130, 128, 125.9, 125.7, 72, 66, 62, 60, 55, 50, 37, 32, 29.4, 29.3, 29.2, 29.1, 26, 23, 19, 14.}]}
Chapter 8 Experimentals

(1R,2R)-(−)-2-Amino-N-(diphenylmethylene)-3-O-(tert-butylidimethylsilyl)-1-(p-methoxy)phenylpropane-1,3-diol (8.3a). Protected-D-serine (2.0 g, 5 mmol) was dissolved in dry CH₂Cl₂ and chilled to -78°C. To this solution was added iBu₂AlH/iBu₃Al (5.25 mmol of each in 10.5 mL of hexanes) dropwise over one hr. The solution turned yellow upon addition of iBu₂AlH. After addition iBu₃AlH is complete, para-methoxy phenyl magnesium bromide (20 mL, 20 mmol) was added dropwise over 30 min. Reaction is then allowed to warm to RT. Reaction flask was placed in an ice bath and the excess Grignard reagent was quenched by the slow addition of saturated NaHCO₃. Solution was then diluted with CH₂Cl₂ and transferred to a separatory funnel. Organic layer was washed with saturated NaHCO₃ twice. Organic layer was then dried with K₂CO₃ and filtered. Solvent was removed under reduced pressure and chromatographed with SiO₂ (5% EtOAc: Hexanes, Rf = 0.4) yielded 0.95 g of pure threo product as a colorless oil in 40% yield, [α]²⁵D = -87° (c = 1.0, CHCl₃) ¹H NMR (300MHz, CDCl₃): δ 7.8-6.8 (Ar-H, m, 16 H), 4.8 (CH⁻OH, d, J = Hz, 1H), 3.9 (βH, dd, J = Hz, 1H), 3.8 (Ar-OCH₃, s, 3H) 3.6 (β'H, dd, J = Hz, 1H), 3.3 (αH, bs, 1H), 3.1 (N-H, bs, 1H), 0.91 [(CH₃)₃C-, s, 9H], 0.03 [(CH₃)₂Si-, s, 6H]. ¹³C NMR (75.0 MHz, CDCl₃): δ 160, 146, 132, 130, 128, 127, 126, 125, 113, 100, 82, 68, 59, 55, 25, 18, -6.

(1R,2R)-2-Amino-3-O-(tert-butylidimethylsilyl)-1-(p-methoxy)phenylpropane-1,3-diol (8.4a). Threo product 8.3a (0.97 g, 2.1 mmol) was dissolved in THF (10 mL) and H₂O (2 mL) at RT. Pyridinium p-toluenesulfonate (1.0 g, 4.2 mmol, 2 equiv) was added and reacted for 5 hr. THF was removed under reduced
pressure. The residue was dissolved in CH$_2$Cl$_2$ (30 mL). The organic solvent was washed with saturated NaHCO$_3$ (3 x 30mL), dried over K$_2$CO$_3$. The crude product was then chromatographed on silica gel (9:1 CHCl$_3$: MeOH, R$_f$ = 0.5), which yielded clear oil in 70 % yield. $[\alpha]^{25}_{D} = -11^\circ$ (c = 1.0, CHCl$_3$. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.3 (Ar-H, d, J = 8.8Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 4.6 (CH$_{2}$OH, d, J = 4.6 Hz, 1H), 3.9 (Ar-O-CH$_3$, s, 3H), 3.8 (pH, dd, J = 10.9, 4.4 Hz, 1H), 3.7 (p'H, dd, J = 10.8, 5.1 Hz, 1H), 2.9 (aH, ddd, J = 4.4, 5.1, 4.6, 1H), 0.91 [(CH$_3$)$_3$C-, s, 9H)], 0.03 [(CH$_3$)$_2$-Si-, s, 6H)]. $^1$C NMR (75.0 MHz, CDCl$_3$): $\delta$ 159, 134, 129, 114, 74, 65, 59, 55, 26, 19, -5.

(4R,5R)-(+)-4-(tert-Butyldimethylsilyloxymethyl)-5-(p-methoxy)-phenyloxazolidin-2-one (8.5a). Amino alcohol 8.4a (0.42 g, 1.3 mmol) was dissolved in dry THF (6 mL) under argon. 1,1'-diimidazole carbonyl (0.21 g, 1.3 mmol) was added in one portion. Once deemed complete via thin layer chromatography, solvent was removed under reduced pressure. The crude residual was then chromatographed on SiO$_2$ (3:1 hexanes: ethyl acetate, R$_f$ = 0.3), which resulted in a white solid with a melting point of 84°C in 64% yield. $[\alpha]^{25}_{D} = +49^\circ$ (c = 1.0, CHCl$_3$). $^1$H NMR (300MHz, CDCl$_3$): $\delta$ 7.3 (Ar-H, d, J = 8.8Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 6.9 (N-H, bs, 1H), 5.3 (CH$_{2}$OH, d, J = 5.1 Hz, 1H), 3.9 (pH, p'H, Ar-O-CH$_3$, aH, m, 6H), 0.91 [(CH$_3$)$_3$C-, s, 9H]), 0.03 [(CH$_3$)$_2$-Si-, s, 6H)]. $^1$C NMR (75.0 MHz, CDCl$_3$): $\delta$ 160, 131, 129, 115, 80, 65, 61, 55, 26, 19, -5.
(4R,5R)-(+) 4-(4'-Sulfonyloxymethyl)-5-(p-methoxy)-phenyloxazolidin-2-one (8.6a) Silyl carbamate 8.5a (0.29 g, 0.86 mmol) was dissolved in acetonitrile (6 mL) at RT. Aqueous HF (0.1 mL, 30 M, 4 equiv.) was added in one portion. Once reaction was deemed complete via TLC, solvent was removed under reduced pressure. Crude product was dried over vacuumed and carried-on without further purification. Dry, crude hydroxy carbamate (0.14 g, 0.65 mmol) was dissolved in freshly distilled pyridine (5 mL) under argon at RT. Mesyl chloride (0.19 g, 0.98 mmol, 1.5 equiv.) was added in one portion. Once complete, the solvent was removed in vacuo and the crude product was chromatographed with SiO₂. The pure product was isolated in 54% yield over two steps. (1:1 EtOAc: hexanes, Rₚ = 0.4). melting point: 94°C [α]⁺D = +55° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 6.5 (N-H, bs, 1H) 5.2 (CH-OH, d, J = Hz, 1H), 4.4 (pH, dd, J = 10.8, 4.3 Hz, 1H), 4.3 (p'H, dd, J = 10.7, 5.4 Hz, 1H), 4.1 (αH, d, J = 7.3 Hz, 1H), 3.8 (Ar-O-CH₃, s, 3H), 3.6 (CH₂-Omorph, t, J = 4.6 Hz, 4H).

8.6a

OMe

(4R,5R)-(+) 4-(4'-Sulfonyloxymethyl)-5-(p-methoxy)-phenyloxazolidin-2-one (8.7a). Mesylate 8.6a (0.15 g, 0.405 mmol.) was dissolved in dry THF (4 mL) under an argon atmosphere. Freshly distilled morpholine (170 μL, 2.0 mmol., 5 equiv.) was added to the solution and the resulting solution was heated to a gentle reflux. After 16 hr., solvent was removed under reduced pressure and the resultant was chromatographed on SiO₂ (EtOAc, Rf = 0.3). The pure product was isolated in 90% yield. [α]⁺25 = +57° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 5.8 (N-H, bs, 1H) 5.1 (CH-OH, d, J = Hz, 1H), 3.9 (αH, q, J = 7.3, 5.5 Hz, 1H), 3.8 (Ar-O-CH₃, s, 3H), 3.6 (CH₂-Omorph, t, J = 4.6 Hz, 4H),

Morpholino carbamate 8.7a (0.11 g, 0.36 mmol.) was dissolved in a 4:1 MeOH: H₂O solution and treated with 2 M KOH (5 mL). The resulting solution was heated to a gentle reflux. Progress of the reaction was monitored via TLC. After reaction deemed complete, solvent was removed under reduced pressure and the aqueous layer was extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃ twice. The organic layer was then dried with K₂CO₃, filtered, and concentrated. The crude product was dried over P₂O₅ overnight, then dissolved in freshly distilled pyridine (5 mL). To the solution, para-nitro phenyl decanoate (0.11 g, 0.37 mmol,) and 1-hydroxybenzotriazole (7 mg., 0.04 mmol., 0.1 equiv.) were added to the reaction under argon. The reaction was allowed to agitate at RT until deemed complete via TLC. Once reaction was completed, solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂. The organic layer was washed with 1M NaOH three times, chromatographed with SiO₂ (95: 5 Chloroform: Methanol, Rf = 0.5) 61% yield over two steps, oil product. [α]²⁵⁰D = +7° (c = 1.0, CHCl₃). HRMS (FAB+H) calculated: 421.3066, found: 421.3076. ¹H NMR (300MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 5.8 (N-H, bd, J = 7.3 Hz, 1H), 4.9 (CH-OH, d, J = 2.9 Hz, 1H), 4.2 (aH, m, 1H), 3.8 (Ar-O-CH₃, s, 3H), 3.7 (CH₂-Omorph, t, J = 4.6 Hz, 4H), 2.7 (βHs, CH₂-Nmorph, m, 6H), 2.05 (amide-CH₂-, t, J = 7.8 Hz, 2 H), 1.50 (amide-CH₂-CH₂, m, 2 H), 1.25 (alkyl-H, bs, 12 H), 0.90 (-CH₃, t, J = 5.8 Hz, 3 H). ¹³C NMR (75.0 MHz, CDCl₃): δ 174, 155, 136, 126, 124, 79, 75, 66, 60, 54, 50, 36, 32, 30, 29, 24, 22, 21, 15.
(1R,2R)-(-)-2-Amino-N-(diphenylmethylene)-3-O-(tert-butyldimethylsilyl)-1-(p-tbutoxyl)-phenylpropane-1,3-diol (8.3b). Protected-D-serine 8.2 (5.2 g, 13.07 mmol) was dissolved in dry CH$_2$Cl$_2$ (20 mL) and chilled to -78°C. To this solution was added iBu$_2$AlH/iBu$_3$Al (14.4 mmol of each in 30 mL of hexanes) dropwise over one hr. The solution turned yellow upon addition of iBu$_2$AlH/iBu$_3$Al. After addition iBu$_2$Al$_2$H is complete, para-tbutoxy phenyl magnesium bromide (39 mL, 39.4 mmol) was added dropwise over 30 min. Reaction is then allowed to warm to RT. Reaction flask was placed in an ice bath and the excess Grignard reagent was quenched by the slow addition of saturated NaHCO$_3$. Solution was then diluted with CH$_2$Cl$_2$ and transferred to a separatory funnel. Organic layer was washed with saturated NaHCO$_3$ twice. Organic layer was then dried with K$_2$CO$_3$ and filtered. Solvent was removed under reduced pressure and chromatographed with SiO$_2$ (5% EtOAc: Hexanes, R$_f$ = 0.4) yielded 3.05 g of pure threo product as a colorless oil in 45.1% yield. [α]$^D_{25}$ = -88° (c = 1.0, CHCl$_3$) $^1$H NMR (300MHz, CDCl$_3$): δ 7.8-7.1 (Ar-H, m, 16 H), 4.9 (CH-OH, d, J = 8.1 Hz, 1H), 3.9 (βH, dd, J = 10.7, 2.7 Hz, 1H), 3.6 (β'H, d, J = 10.7 Hz, 1H), 3.3 (αH, bs, 1H), 3.1 (N-H, bs, 1H), 1.3 (Ar-O-C(CH$_3$)$_3$-s, 9H), 0.91 [(CH$_3$)$_2$-Si-, s, 6H]). $^{13}$C NMR (75.0 MHz, CDCl$_3$): δ 155, 145, 136, 129, 128, 127, 126, 125, 124, 123, 122, 100, 81, 68, 60, 29, 24, -6.

(1R,2R)-(+)2-Amino-3-O-(tert-butyldimethylsilyl)-1-(p-tbutoxyl)-phenylpropane-1,3-diol (8.4b). Threo product 8.3b (0.58 g, 1.12 mmol) was dissolved in THF (10 mL) and H$_2$O (2 mL) at RT. Pyridinium p-toluenesulfonate (0.6 g, 2.2 mmol, 2 equiv) was added and reacted for 5 hr. THF was removed under reduced pressure. The residual was dissolved in CH$_2$Cl$_2$ (30 mL). The organic solvent was washed with saturated NaHCO$_3$ (3 x 30mL), dried over K$_2$CO$_3$. The
crude product was then chromatographed on silica gel (9:1 CHCl₃: MeOH, Rᵣ = 0.5), which yielded 0.26 g of clear oil in 70% yield. [α]²⁵D = +6.7° (c = 1.0, CHCl₃)

¹H NMR (300 MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8 Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2 H), 4.6 (CH-OH, d, J = 5.6 Hz, 1H), 3.6 (βH, dd, J = 9.9, 3.8 Hz, 1H), 3.5 (β'H, dd, J = 10.0, 5.1 Hz, 1H), 2.9 (αH, ddd, J = 3.8, 5.1, 5.6, 1H), 1.3 (Ar-O-C(CH₃)₃, s, 3H), 0.9 [(CH₃)₂C-, s, 9H]], 0.3 [(CH₃)₂-Si-, s, 6H]]. ¹³C NMR (75.0 MHz, CDCl₃): δ 155, 136, 126, 123, 78, 74, 66, 58, 29, 25, -6.

(4R,5R)-(+)−4-(tert-Butyldimethylsilyloxymethyl)-5-(p-tbutoxy)-phenyloxazolidin-2-one (8.5b). Amino alcohol 8.4b (0.90 g, 2.54 mmol) was dissolved in dry THF (6mL) under argon. 1,1′-diimidazole carbonyl (0.40 g, 2.54 mmol) was added in one portion. Once deemed complete via TLC, solvent was removed under reduced pressure. The crude residual was then chromatographed on SiO₂ (3:1 hexanes: ethyl acetate, Rᵣ = 0.3), which provided 0.18 g of clear oil in 55% yield. [α]²⁵D = +38° (c = 1.0, CHCl₃).

¹H NMR (300MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8 Hz, 2 H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 5.6 (N-H, bs, 1H), 5.1 (CH-OH, d, J = 5.4 Hz, 1H), 3.7 (βH, β'H, αH, m, 3H), 1.4 (Ar-O-C(CH₃)₃, s, 3H), 0.9 [(CH₃)₂C-, s, 9H]], 0.3 [(CH₃)₂-Si-, s, 6H]]. ¹³C NMR (75.0 MHz, CDCl₃): δ 158, 134, 126, 124, 79, 68, 65, 61, 28, 26, -6.

(4R,5R)-4-(4'-p-Toluenesulfonyloxymethyl)-5-(p-tbutoxy)-phenyloxazolidin-2-one (8.6b). Silyl carbamate 8.5b (0.29 g, 0.80 mmol) was dissolved in acetonitrile (5 mL) at RT. Aqueous HF (0.1 mL, 30M, 4 equiv.) was added in one portion. Once reaction was deemed complete via TLC, solvent was removed under reduced pressure. Crude product was dried over vacuumed and carried-on without further purification. Dry, crude hydroxy carbamate was dissolved in freshly distilled pyridine (5 mL) under argon at RT. Tosyl chloride (0.15 g, 0.80
mmol, 1.0 equiv.) was added in one portion. Once complete, the solvent was removed in vacuo and the crude product was chromatographed with SiO₂. The pure product was isolated in 83% yield over two steps. (1:1 EtOAc: hexanes, Rf = 0.4). ¹H NMR (300MHz, CDCl₃): δ 7.4 (Ar-H, m, 4H), 7.3 (Ar-H, d, J = 8.8 Hz, 2H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 5.8 (N-H, bs, 1H), 5.2 (CH-OH, d, J = 3.8 Hz, 1H), 3.9 (α-H, q, J = 7.3, 5.5 Hz, 2H), 3.5 (CH₃, t, J = 4.6 Hz, 4H), 2.65 (CH₂-N-morph, β-H, β'-H, m, 6H), 1.4 (Ar-OC(CH₃)₃, s, 3H). ¹³C NMR (75.0 MHz, CDCl₃): δ 160, 134, 130, 128, 126, 124, 79, 69, 59, 29, 22.

(4R,5R)-(+)−[(N-Morpholino)methyl]-5-(p-tbutoxyl)-phenyloxazolidin-2-one (8.7b). Tosylate 8.6b (0.54 g, 1.3 mmol.) was dissolved in dry THF (4 mL) under an argon atmosphere. Freshly distilled morpholine (320 µL, 3.9 mmol., 3 equiv.) was added to the solution and the resulting solution was heated to a gentle reflux. After 16 hr., solvent was removed under reduced pressure and the resultant was chromatographed on SiO₂ (EtOAc, Rf = 0.3) resulting in 0.40 g of clear oil product in 93% yield. [α]²⁵° = +55° (c = 1.0, CHCl₃). ¹H NMR (300MHz, CDCl₃): δ 7.3 (Ar-H, d, J = 8.8 Hz, 2H), 6.9 (Ar-H, d, J = 8.8 Hz, 2H), 5.6 (N-H, bs, 1H), 5.2 (CH₂-OH, d, J = 4.2 Hz, 1H), 3.9 (α-H, q, J = 7.3, 5.5 Hz, 2H), 3.7 (CH₂-O-morph, t, J = 4.6 Hz, 4H), 2.65 (CH₂-N-morph, β-H, β'-H, m, 6H), 1.4 (Ar-OC(CH₃)₃, s, 3H). ¹³C NMR (75.0 MHz, CDCl₃): δ 158, 134, 127, 124, 81, 67, 62, 58, 55, 29.

(1R,2R)-(+)−p-tButoxyl-2-decanoylamino-3-(N-morpholino)-1-propanol [D-threeo-BDMP] (8.1b). Morpholino carbamate 8.7b (0.40 g, 1.2 mmol.) was dissolved in a 4:1 MeOH: H₂O solution and treated with 2 M KOH (5 mL). The resulting solution was heated to a gentle reflux. Progress of the reaction was monitored via TLC. After reaction deemed complete, solvent was removed under reduced pressure
and the aqueous layer was extracted with CH$_2$Cl$_2$. The organic layer was washed with saturated NaHCO$_3$ twice. The organic layer was then dried with K$_2$CO$_3$, filtered, and concentrated. The crude product was dried over P$_2$O$_5$. Dry, crude product was dissolved in freshly distilled pyridine (5 mL). To the solution, para-nitro phenyl decanoate (0.35 g, 1.2 mmol, 1 equiv.) and 1-hydroxybenzotriazole (20 mg, 0.12 mmol, 0.1 equiv.) were added to the reaction under argon. The reaction was allowed to agitate at RT until deemed complete via TLC. Once reaction was completed, solvent was removed under reduced pressure and the residue was dissolved in CH$_2$Cl$_2$. The organic layer was washed with 1M NaOH three times, chromatographed with SiO$_2$ (95: 5 Chloroform: Methanol, Rf = 0.5) resulting in clear oil product in 68% yield over two steps. $[\alpha]^{25}_{D} = +4.5^\circ$ (c = 1.0, CHCl$_3$). HRMS (FAB+H) calculated: 463.3536 found: 463.3549. $^1$H NMR (300MHz, CDCl$_3$): $\delta$ 7.3 (Ar-H, d, $J = 8.8$ Hz, 2 H), 6.9 (Ar-H, d, $J = 8.8$ Hz, 2 H), 5.9 (N-H, bd, $J = 7.3$ Hz, 1H), 4.9 (C H-OH, d, $J = 2.9$ Hz, 1H), 4.2 ($\alpha$H, m, 1H), 3.7 (CH$_2$-O-morph, t, $J = 4.6$ Hz, 4H), 2.7 ($\beta$Hs, CH$_2$-N$_{\text{morph}}$, m, 6H), 2.1 (amide-$CH_2$, t, $J = 7.8$ Hz, 2 H), 1.5 (amide-CH$_2$-CH$_2$, m, 2 H), 1.3 (Ar-O-C(CH$_3$)$_3$, s, 3H), 1.25 (alkyl-H, bs, 12 H), 0.9 (-CH$_3$, t, $J = 5.8$ Hz, 3 H). $^{13}$C NMR (75.0 MHz, CDCl$_3$): $\delta$ 174, 155, 136, 127, 124, 79, 76, 67, 60, 54, 51, 37, 32, 29, 26, 23, 14.

(1R,2R)-2-Amino-N-(diphenylmethylene)-3-O-(tert-butylidimethylsilyl)-1-(3,4-methylenedioxy)-phenylpropane-1,3-diol (8.3c). Protected-D-serine 8.2 (9.5 g, 24.0 mmol) was dissolved in dry CH$_2$Cl$_2$ and chilled to -78°C. To this solution was added iBu$_2$AlH/iBu$_3$Al (26.0 mmol of each in 30 mL of hexanes) dropwise over one hr. The solution turned yellow upon addition of iBu$_2$AlH/iBu$_3$Al. After addition iBu$_5$Al$_2$H is complete, 3,4-methylene dioxy phenyl magnesium bromide (72 mL, 3 eq., 1M in THF/toluene, 72.0 mmol) was added dropwise over 30 min. Reaction is then allowed to warm to RT.
Reaction flask was placed in an ice bath and the excess Grignard reagent was quenched by the slow addition of saturated NaHCO₃. Solution was then diluted with CH₂Cl₂ and transferred to a separatory funnel. Organic layer was washed with saturated NaHCO₃ twice. Organic layer was then dried with K₂CO₃ and filtered. Solvent was removed under reduced pressure and chromatographed with SiO₂ (5% EtOAc: Hexanes, Rᵣ = 0.4) yielded 4.8 g of pure threo product as a colorless oil in 40% yield. NMR (300MHz, CDCl₃): δ 7.8-7.1 (Ar-H, m, 14 H), 5.9 (CH₂-(O)₂-Ar, s, 2H), 4.9 (CH-OH, d, J = 7.2 Hz, 1H), 3.9 (βH, dd, J = 10.4, 4.4 Hz, 1H), 3.6 (β'H, dd, J = 10.5, 4.8 Hz, 1H), 3.2 (αH, bs, 1H), 3.0 (N-H, bs, 1H), 0.9 [(CH₃)₃C-, s, 9H], 0.5 [(CH₃)₂Si-, s, 6H]. NMR (75.0 MHz, CDCl₃): δ 148, 146, 136, 135, 134, 131, 128, 127, 126, 125, 124, 107, 106, 100, 81, 68, 59, 25, 19, -5.

(1R,2R)-(−)-2-Amino-3-O-(tert-butyldimethylsilyl)-1-(3,4-methylenedioxy)-phenylpropane-1,3-diol (8.4c).

Threo product 8.3c (4.76 g, 9.7 mmol) was dissolved in THF (10 mL) and H₂O (2 mL) at RT. Pyridinium p-toluenesulphonate (4.7 g, 19.3 mmol, 2 equiv.) was added and reacted for 5 hr. THF was removed under reduced pressure. The residual was dissolved in CH₂Cl₂ (30 mL). The organic solvent was washed with saturated NaHCO₃ (3 x 30mL), dried over K₂CO₃. The crude product was then chromatographed on silica gel (9:1 CHCl₃: MeOH, Rᵣ = 0.5), which yielded clear oil in 40% yield (1.15 g). [α]₀²⁵ = -2.1° (c = 1.0, CHCl₃). NMR (300 MHz, CDCl₃): δ 6.9-6.7 (Ar-H, m, 3 H), 6.0 (CH₂-(O)₂-Ar, s, 2H), 4.5 (CH-OH, d, J = 5.6 Hz, 1H), 3.6 (βH, dd, J = 10.2, 4.0 Hz, 1H), 3.5 (β'H, dd, J = 10.0, 4.5 Hz, 1H), 2.9 (αH, ddd, J = 4.5, 5.6, 4.0, 1H), 0.9 [(CH₃)₃C-, s, 9H], 0.5 [(CH₃)₂Si-, s, 6H]. NMR (75.0 MHz, CDCl₃): δ 148, 146, 136, 120, 108, 106, 101, 75, 65, 58, 26, 18, -5.
(4R,5R)-(+)-4-(tert-Butylidimethylsilyloxymethyl)-5-(3,4-methylenedioxy)-phenyloxazolidin-2-one (8.5c). Amino alcohol 8.4c (1.15 g, 3.5 mmol) was dissolved in dry THF (6 mL) under argon. 1,1'-diimidazole carbonyl (0.66 g, 4.24 mmol, 1.2 equiv.) was added in one portion. Once deemed complete via thin layer chromatography, solvent was removed under reduced pressure. The crude residual was then chromatographed on SiO₂ (3:1 hexanes: ethyl acetate, Rₜ = 0.3) yielding 0.675 g of clear oil in 56% yield. [α]³⁰ = +17.4° (c = 1.0, CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 6.9-6.7 (Ar-H, m, 3 H), 6.3 (N-H, bs, 1H), 6.0 (CH₂-(O)₂-Ar, s, 2H), 5.2 (CH-OH, d, J = 4.9 Hz, 1H), 3.8 (βH, β'H, αH, m, 3H), 0.9 [(CH₃)₃C-, s, 9H), 0.5 [(CH₃)₂-Si-, s, 6H]). ¹³C NMR (75.0 MHz, CDCl₃): δ 160, 138, 134, 131, 129, 127, 126, 79, 65, 61, 26, 19, -5.

(4R,5R)-(+) -4-(4'-p-Toluenesulfonyloxymethyl)-5-(3,4-methylenedioxy)-phenyloxazolidin-2-one (8.6c) Silyl carbamate 8.5c (0.675 g, 1.9 mmol) was dissolved in acetonitrile (8 mL) at RT. Aqueous HF (0.26 mL, 30 M, 4 equiv.) was added in one portion. Once reaction was deemed complete via TLC, solvent was removed under reduced pressure. Crude product was dried in vacuo and carried on without further purification. Dry, crude hydroxy carbamate (0.35 g, 1.5 mmol) was dissolved in freshly distilled pyridine (5 mL) under argon at RT. Tosyl chloride (0.34 g, 1.8 mmol, 1.2 equiv.) was added in one portion. Once complete, the solvent was removed in vacuo and the crude product was chromatographed with SiO₂ (1:1 EtOAc: hexanes, Rₜ = 0.4). The solid product was isolated, 0.28 g, in 48% yield over two steps, melting pt.: 126 °C, [α]²⁵ = +26.9° (c = 1.0, CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 7.8-7.1 (Ar-H, m, 7H), 6.5 (N-H, bs, 1H) 6.0 (CH₂(O)₂-Ar, s, 2H), 5.1 (CH-OH, d, J = 4.6 Hz, 1H), 4.1 (βH, dd, J = 10.6, 5.1 Hz, 1H), 4.0 (β'H, dd, J = 10.5, 4.9 Hz, 1H), 3.85
(αH, ddd, J = 4.6, 4.9, 5.1, 1H), 2.45 (CH₃-TosO₂, s, 1H). ¹³C NMR (75.0 MHz, CDCl₃): δ 158, 148, 146, 132, 131, 130, 128, 120, 108, 106, 101, 80, 69, 59, 22.

(4R,5R)-(+)-[(N-Morpholino)methyl]-5-(3,4-methylenedioxy)-phenyloxazolidin-2-one (8.7c). Tosylate 8.6c (0.28 g, 0.72 mmol.) was dissolved in dry THF (4 mL) under an argon atmosphere. Freshly distilled morpholine (240 μL, 2.9 mmol., 4 equiv.) was added to the solution and the resulting solution was heated to a gentle reflux. After 16 hr., solvent was removed under reduced pressure and the resultant was chromatographed on SiO₂ (EtOAc, Rf = 0.3). The pure product, 0.25 g, was isolated in 92% yield. ¹H NMR (300MHz, CDCl₃): δ 7.4-7.1 (Ar-H, m, 3H), 6.1 (N-H, bs, 1H), 6.0 (CH₂(=O)₂Ar, s, 2H), 5.1 (CH-OH, d, J = 4.9 Hz, 1H), 3.8 (αH, q, J = 7.3, 5.5 Hz, 1H), 3.6 (CH₂-O-morph, t, J = 4.6 Hz, 4H), 2.6 (CH₂-N-morph, βH, βH', m, 6H). ¹³C NMR (75.0 MHz, CDCl₃): δ 159, 148, 132, 120, 108, 106, 102, 101, 82, 66, 62, 57, 54.

(1R,2R)-1-(3,4-Methylenedioxy)-phenyl-2-decanoylamino-3-(N-morpholino)-1-propanol [D-threo-PipDMP] (8.1c). Morpholino carbamate 8.7c (0.25 g, 0.79 mmol.) was dissolved in a 4:1 MeOH: H₂O solution and treated with 2 M KOH (5 mL). The resulting solution was heated to a gentle reflux. Progress of the reaction was monitored via TLC. After reaction deemed complete, solvent was removed under reduced pressure and the aqueous layer was extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃ twice. The organic layer was then dried with K₂CO₃, filtered, and concentrated. The crude product was dried over P₂O₅ overnight, then dissolved in freshly distilled pyridine (5 mL). To the solution, para-nitro phenyl decanoate (0.23 g, 0.79 mmol., 1 equiv.) and 1-
hydroxybenzotriazole (12 mg., 0.08 mmol., 0.1 equiv.) were added to the reaction under argon. The reaction was allowed to agitate at RT until deemed complete via TLC. Once reaction was completed, solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂. The organic layer was washed with 1M NaOH three times, then chromatographed with SiO₂ (95: 5 Chloroform: Methanol, Rf = 0.5) 66% yield over two steps, oil product. HRMS (FAB+H) calculated: 435.2859 found: 435.2844. ¹H NMR (300MHz, CDCl₃): δ 7.4-7.1 (Ar-H, m, 4H), 6.05 (N-H, bd, J = 7.3 Hz, 1H), 6.0 (CH₂−(O)₂−Ar, s, 2H), 5.22 (CH-OH, d, J = 2.9 Hz, 1H), 4.18 (αH, m, 1H), 3.71 (CH₂-Omorph, t, J = 4.6 Hz, 4H), 2.70 (βH, dd, J = 13.1, 6.2 Hz, 1 H), 2.65 (CH₂-Nmorph, m, 5H), 2.33 (Ar-CH₃, s, 3H), 2.05 (amide-CH₂−, t, J = 7.8 Hz, 2 H), 1.50 (amide-CH₂-CH₂−, m, 2 H), 1.25 (alkyl-H, bs, 12 H), 0.90 (-CH₃, t, J = 5.8 Hz, 3 H). ¹³C NMR (75.0 MHz, CDCl₃): δ 173, 139, 134, 130, 128, 125.9, 125.7, 72, 66, 62, 60, 55, 50, 37, 32, 29.4, 29.3, 29.2, 29.1, 26, 23, 19, 14.
Chapter 9 Experimentals

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-propane-1,3-diol (9.1'). Amino diol 9.1 (5.0 g, 30 mmol) was dissolved in MeOH at 0° C. To this solution, triethyl amine (5.3 mL, 37.5 mmol, 1.25 equiv.) and benzyl chloroformate (5.3 mL, 37.5 mmol, 1.25 equiv.) were added. After 12 h at RT, MeOH was removed in vacuo and EtOAc was added. The organic layer was washed with sat. NaHCO₃, brine, and dried over MgSO₄. Filtration, concentration, and recrystallization (from EtOAc) afforded a white to pale yellow powder in 88% yield. M.P. 104° C, ¹H NMR (300 MHz, CDCl₃): δ 7.2-7.4 (m, 10H), 5.6 (bd, J = 8.8 Hz, 1H), 4.9 (bs, 3H) 3.6 (m, 5H). NMR (75 MHz, CDCl₃): 157, 141, 136, 128, 127, 126, 73, 67, 63, 57.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-p-toluenesulfonyloxy-propane-1-ol (9.2). To a solution of amino-protected phenyl diol 9.1' (2.00 g, 6.8 mmol) in pyridine (2 mL), tosyl chloride (1.34 g, 7.0 mmol, 1.03 equiv.) was added at 0° C under Ar. The solution was allowed to warm to RT overnight. After 12 h, solvent was removed under reduced pressure. The resultant solid was dissolved in EtOAc and washed with brine twice. The organic layer was dried over MgSO₄, filtered, and concentrated to afford a white powder in 80% yield. Flash chromatography (3: 7 EtOAc: hexanes) could be performed, though product was usually carried-on without chromatograph purification. MP: 107° C. ¹H NMR (300 MHz, CDCl₃): δ 7.2-7.8 (m, 14H), 5.2 (d, J= 7.8 Hz, 1H), 5.0 (s, 2H), 4.9 (d, J = 3.9 Hz, 1H), 4.2 (dd, J = 6.4 Hz, 9.2Hz, 1H), 4.1 (m, 1H), 4.0 (dd, J= 4.9 Hz, 9.2 Hz, 1H), 2.8 (s, 1H), 2.4 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): 156, 145, 140, 136, 132, 130, 128, 72, 69, 67, 58, 22.
General Procedure for Displacement Products. Dried tosylate 9.2 was dissolved in freshly distilled cyclic amine (5-10 equivalents). Due to the viscosity of hydroxyethyl piperazine and N-methyl piperazine, DMF was employed as a solvent. In most instances, RT for 24 hours was sufficient for completion. Reactions that required DMF as solvent proceed faster if heated to ~50° C. Reactions were quenched by the addition of water. The aqueous layer was extracted three times with EtOAc. Organic layer was dried with MgSO₄, filtered, and concentrated in vacuo. Column chromatography provided displacement products in the yields provided in Table 9.1.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-morpholino-propane-1-ol (9.3a). Product: oil. ¹H NMR (300 MHz, CDCl₃): δ 2.6 (m, 6H), 3.7 (t, J= 6.6 Hz, 4H), 4.1 (m, 1H), 4.9 (d, J= 3.9 Hz, 1H), 5.0 (s, 2H), 5.2 (d, J= 7.8Hz, 1H), 7.2-7.6 (m, 10 H). ¹³C NMR (75 MHz, CDCl₃): 52, 54, 60, 67, 75, 126, 127, 128, 136, 141, 156.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-(1-methyl)-piperazine-propane-1-ol (9.3b). Product: oil. ¹H NMR (300 MHz, CDCl₃): δ 2.2 (s, 3H), 2.3-2.6 (m, 10H), 4.0 (m, 1H), 5.0 (d, J =3.9 Hz, 1H), 5.1 (s, 2H), 5.2 (bd, J = 7.8 Hz), 7.2-7.4 (m, 10 H). ¹³C NMR (75 MHz, CDCl₃): 46, 53, 54, 55, 60, 67, 74, 126, 127, 128, 129, 137, 142, 156.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-(1-acetylhydroxyethyl)-piperazine-propane-1-ol-acetate (9.3c). Product: oil. ¹H NMR (300 MHz, CDCl₃): δ 2.1 (s, 6H), 2.4 (dd, J=2.4, 7.2 Hz, 2H), 2.5 (s, 8H), 2.6 (t, J= 6.0 Hz, 2H), 4.2 (t, J= 6.2 Hz, 2H), 5.0 (bs, 3H), 6.0
(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-pyrrolidino-propane-1-ol (9.3d). Product: oil. \( ^1H \) NMR (300 MHz, CDCl\textsubscript{3}): \( \delta \) 1.7 (bs, 4H) 2.6 (m, 5H), 2.8 (m, 1H), 4.1 (m, 1H), 4.9 (m, 3H), 5.1 (d, J= 7.8 Hz, 1H), 7.2-7.4 (m, 10 H). \( ^{13}C \) NMR (75 MHz, CDCl\textsubscript{3}): 21, 54, 57, 59, 62, 70, 75, 127, 128, 129, 137, 138, 156, 170, 171.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-piperidino-propane-1-ol (9.3e). Product: oil. \( ^1H \) NMR (300 MHz, CDCl\textsubscript{3}): \( \delta \) 1.5 (m, 2H) 1.7 (m, 2H), 2.6 (m, 6H), 4.2 (bs, 1H), 4.9 (d, J= 7.8 Hz, 1H), 5.0 (s, 2H), 5.1 (d, J= 3.6 Hz, 1H), 7.2-7.5 (m, 10 H). \( ^{13}C \) NMR (75 MHz, CDCl\textsubscript{3}): 24, 26, 52, 56, 60, 67, 76, 126, 127, 128, 136, 141.

(1R,2R)-2-Carbobenzyloxyamino-1-phenyl-4-O-tert-butyldimethylsilyl propane-1-ol (9.5). To a solution of amino-protected phenyl diol 9.1' (8.0 g, 26.7 mmol) in DMF (20 mL), tert-butyldimethylsilyl chloride (4.43 g, 29.3 mmol, 1.1 equiv.) and imidazole (4.35 g, 64.0 mmol, 2.4 equiv.) was added at 0° C under Ar. The solution was allowed to warm to RT overnight. After 12 h, reaction was quenched by addition of water. Aqueous solution was extracted with EtOAc and washed with bicarb and brine twice. The organic layer was dried over MgSO\textsubscript{4}, filtered, and concentrated to afford a waxy, clear solid in 72% yield. Product was carried-on without chromatograph purification. MP: 82° C. \( ^1H \) NMR (300 MHz, CDCl\textsubscript{3}): \( \delta \) 0.5 (s, 6H), 0.9 (s, 9H), 3.8 (m, 4H), 5.0 (m, 3H), 5.2 (bd, J
166 Hz, 1H), 7.2–7.4 (m, 10H). $^{13}$C NMR (75 MHz, CDCl$_3$): 156, 141, 136, 129, 128, 127, 125, 76, 74, 66, 65, 57, 56, 18, 16.

(4R,5R)-4-(tert-Butyldimethylsilyloxyethyl)-5-phenyloxazolindin-2-one (9.6). To a chilled solution (~ 0°C) of mono-protected amino diol 9.5 (6.8 g, 16.3 mmol) in anhydrous THF (10 mL), nBuLi (12.2 mL, 19.6 mmol, 1.2 equiv.) was added dropwise. After required time, reaction was quenched via addition of distilled water. Aqueous solution was extracted with diethyl ether and washed with bicarb and brine. Organic layer was dried with MgSO$_4$, filtered, and concentrated. Crude product was recrystallized (hexanes) to yield white crystals in 75% yield. MP: 90°C. $^1$H NMR (300 MHz, CDCl$_3$): δ 0.5 (s, 6H), 0.9 (s, 9H), 3.8 (m, 3H), 5.3 (d, J = 4.6 Hz, 1H), 6.0 (bs, 1H), 7.2–7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 160, 140, 129, 126, 80, 64, 62, 28, 18, -5.

(4R,5R)-4-(4'-p-Toluenesulfonyloxymethyl)-5-phenyloxazolindin-2-one (9.7). To a solution of carbamate 9.6 (2.2 g, 7.3 mmol) at 0°C in ACN (5 mL), HF (370 µL, 11.0 mmol, 1.5 equiv.) was administered. Once deprotection was deemed complete, solution was removed under reduced pressure. Crude product was dissolved in distilled pyridine (5 mL) and chilled to 0°C. To that solution, tosyl chloride (2.5 g, 13.1 mmol, 1.8 equiv.) was added in one portion. Once complete, pyridine was removed in vacuo and resulting solid was dissolved in EtOAc and washed with 1M HCl, sat. bicarbonate, and brine. Organic layer was dried with MgSO$_4$, filtered and concentrated to provide crude tosylate in 88% yield. Crude tosylate was deemed stable to column chromatography; however, usually crude material was usually employed. MP: 117°C. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.8 (m, 9H), 6.3 (bs, 1H), 5.2 (d, J = 5.5 Hz, 1H), 4.2 (dd, J = 5.2, 5.4 Hz, 2H), 4.0 (dd, J = 5.3,
5.2 Hz, 1H), 2.5 (s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): 159, 145, 137, 132, 129, 128, 127, 126, 79, 69, 59, 22.

(1R,2R)-3-Diethanol-(diacetate)amino-5-phenyloxazolindin-2-one (9.8a). Crude tosylate 9.7 (1.8 g, 5.2 mmol) was dissolved in diethanol amine (2 mL) and DMF (2 mL) solution. Reaction was heated to 50° C and allowed to stir overnight. Once complete, quench with water, extract with CH$_2$Cl$_2$. Crude extract was treated with acetic anhydride (3 equiv.). Solvent was removed under reduced pressure and purified via column chromatography (9: 1 EtOAc: MeOH) to provide displaced product in 52% yield (oil). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.1 (s, 6H), 2.8 (m, 6H), 3.7 (dt, $J = 5.4$, 7.8 Hz, 1H), 4.1 (m, 4H), 5.1 (d, $J = 5.4$ Hz, 1H), 7.2 – 7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 171, 159, 139, 129, 128, 126, 81, 62, 63, 59, 21.

(1R,2R)-3-(N-methyl)-N-ethanol-(acetate) amino-5-phenyloxazolindin-2-one (9.8b). See above procedure. Product: oil, yield: 40%. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.9 (s, 3H), 2.2 (s, 3H), 2.8 (m, 4H), 3.7 (dd, $J = 6.4$, 12.2 Hz, 1H), 4.1 (m, 4H), 5.2 (d, $J = 5.4$ Hz, 1H), 7.2 – 7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 171, 158, 139, 129, 128, 126, 125, 82, 81, 64, 63, 62, 61, 60, 59, 54, 53, 22, 21, 20.

General Procedure for Saponification/N-acylation. To a solution of displacement product 9.3a-c and 9.8a-b in MeOH, KOH (10 equivalents) was added. Reaction was allowed to stir under refluxing conditions overnight. Once deemed complete, MeOH was removed in vacuo. Remaining solution was diluted with THF. To that solution, decanoyl chloride (1 equivalent) was added. Once complete, THF was removed under reduced pressure. Resulting solution was
extracted with CH$_2$Cl$_2$ twice, dried with MgSO$_4$, filtered and purified via column chromatography to provide D-threo-PDMP head-group analogues in yields reported in Table 9.3.

**OH (1R,2R)-1-Phenyl-2-Decanoyl amino 3-Morpholino Propan-1-ol (L-threo-PDMP) (9.9a).** Product: oil, yield: 74%. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.8 (t, J = 6.6 Hz, 3H), 1.2 (bs, 12H), 1.5 (p, J = 6.9 Hz, 2H), 2.1 (t, J = 7.5 Hz, 2H), 2.6 (m, 6H), 3.7 (t, J = 6.6 Hz, 4H), 4.1 (m, 1H), 4.9 (d, J = 3.4 Hz, 1H), 5.8 (bd, J = 7.3 Hz, 1H), 7.2 - 7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 173, 141, 128, 127, 126, 77, 74, 66, 59, 54, 51, 36, 31, 29, 28, 25, 22, 14. HRMS (FAB+H) calculated: 390.3327, found: 390.3347.

**OH (1R,2R)-1-Phenyl-2-Decanoyl amino 3-(1-Methyl)-Piperazine Propan-1-ol (L-threo-PDMPP) (9.9b)** Product: oil, yield: 45%. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.8 (t, J = 6.6 Hz, 3H), 1.2 (bs, 12H), 1.5 (p, J = 6.9 Hz, 2H), 2.1 (t, J = 7.5 Hz, 2H), 2.2 (s, 3H), 2.4 (m, 10H), 4.1 (m, 1H), 4.8 (d, J = 2.9 Hz, 1H), 5.9 (bd, J = 7.8 Hz, 1H), 6.1 (bs, 1H), 7.2 - 7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 173, 141, 128, 127, 126, 74, 58, 55, 53, 51, 46, 36, 32, 29, 28, 25, 22, 14. HRMS (FAB+H) calculated: 404.3277, found: 404.3268.

**OH (1R,2R)-1-Phenyl-2-Decanoyl amino 3-(1-HydroxyEthyl)-Piperazine Propan-1-ol (L-threo-PDHEPP) (9.9c).** Product: oil, yield: 45%. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.8 (t, J = 6.6 Hz, 3H), 1.2 (bs, 12H), 1.5 (p, J = 6.9 Hz, 2H), 2.1 (t, J = 7.5 Hz, 2H), 2.5 (m, 12H), 3.6 (t, J = 5.1 Hz, 2H), 4.3 (m, 2H), 4.9 (d, J = 3.4 Hz, 1H), 5.8 (bd, J = 7.3 Hz, 1H), 7.2 - 7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 173, 141, 128, 127, 126, 75, 59, 58, 54, 53,

(1R,2R)-1-Phenyl 2-Decanoyl amino 3-(N-Diethanol) amino Propan-1-ol (L-threo-PDDEAP) (9.9d). Product: oil, yield: 44%. $^1$H NMR (300 MHz, CDCl$_3$): δ 0.8 (t, J = 6.6 Hz, 3H), 1.2 (bs, 12H), 1.5 (p, J = 6.9 Hz, 2H), 2.1 (t, J = 7.5 Hz, 2H), 2.8 (m, 6H), 3.6 (m, 4H), 4.1 (m, 1H), 4.8 (bs, 2H), 5.1 (d, J = 2.0 Hz, 1H), 6.5 (bd, J = 8.3 Hz, 1H), 7.2–7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 174, 141, 128, 127, 126, 77, 72, 59, 57, 56, 54, 37, 32, 29, 26, 23, 14. HRMS (FAB+H) calculated: 409.3066, found: 409.3101.

(1R,2R)-1-Phenyl 2-Decanoyl amino 3-(N-Methyl-N-Ethanol) amino Propan-1-ol (L-threo-PDMEAP) (9.9e). Product: oil, yield: 64%. $^1$H NMR (300 MHz, CDCl$_3$): δ 0.8 (t, J = 6.6 Hz, 3H), 1.2 (bs, 12H), 1.5 (p, J = 6.9 Hz, 2H), 2.1 (t, J = 7.5 Hz, 2H), 2.4 (s, 3H), 2.8 (m, 4H), 2.9 (dd, J = 6.8, 12.7 Hz, 2H), 3.6 (t, J = 4.9 Hz, 4H), 4.2 (m, 1H), 4.9 (d, J = 2.9 Hz, 1H), 5.3 (bs, 1H), 6.4 (bd, J = 8.8 Hz, 1H), 7.2–7.4 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$): 174, 141, 128, 127, 126, 73, 59, 58, 57, 56, 52, 43, 36, 32, 29, 28, 26, 22, 14. HRMS (FAB+H) calculated: 379.2961, found: 379.2939.
Chapter 10 Experimentals

For the following experimentals: (1R,2R)-2-Carbobenzyloxyamino-1-phenyl-propane-1,3-diol (9.1'), (1R,2R)-2-Carbobenzyloxyamino-1-phenyl-3-p-toluenesulfonyloxy-propane-1-ol (9.2), (1R,2R)-2-Carbobenzyloxyamino-1-phenyl-4-O-ferf-butyldimethylsilyl propane-1-ol (9.5), (4R,5R)-4-(tert-Butyldimethylsilyloxymethyl)-5-phenyloxazolindin-2-one (9.6) and (4R,5R)-4-(4'-p-Toluenesulfonyloxymethyl)-5-phenyloxazolindin-2-one (9.7), see Chapter 9 Experimentals. Compounds 10.7-10.10 were synthesized using published procedures by H. Razavi, with contributions from Sames to complete the synthesis of compound 10.4.

(2S,3R,4R)-N-Diphenylmethyl-5-O-tert-butyldimethylsilyl-4-aminopentane-1,2,3,5-tetrol (10.11). To a solution of imine 10.10 (830 mg, 1.61 mmol) in dry THF (15 mL), lithium borohydride (142 mg, 6.45 mmol) was added. The solution was allowed to agitate at RT. After 6 h, MeOH (261 μL, 6.45 mmol) was added drop-wise and mixture was heated to reflux for 8 h, cooled to 0° C, and quenched with water. Aqueous layer was extracted with CH₂Cl₂ (10 mL). Organic layer was dried with MgSO₄, filtered, concentrated and purified via column chromatography (1: 1 EtOAc: hexanes) to provide 10.11 in 30% yield (oil). [α]²⁵ = +17.1° (c=1, CHCl₃) ¹H NMR (300 MHz, CDCl₃): δ 7.2-7.4 (m, 10H), 5.0 (s, 1H), 4.1 (dd, J = 4.15, 10.5 Hz, 1H), 3.80 (m, 2H), 3.72 (dd, J = 3.41, 10.5 Hz, 1H), 3.63 (m, 1H), 3.57 (m, 1H), 2.85 (m, 1H), 0.91 (s, 9H), 0.5 (s, 6H). ¹³C NMR (75MHz, CDCl₃): 140, 129, 128, 127, 126, 74, 64, 62, 56, 25, 16, -5.
5-O-tert-butyldimethylsilyl-\textit{N}-diphenylmethyl-1,4-dideoxy-1,4-imino-D-lyxitol (10.12). To a solution of tetrol 10.11 (0.201 g, 0.462 mmol) in dry DMF (5 mL) at RT under argon was added triphenylphosphine (0.243 g, 0.928 mmol), carbon tetrachloride (90 \mu L, 0.928 mmol), and triethylamine (130 \mu L, 0.928 mmol) in the dark. Once deemed complete, reaction quenched by addition of dry MeOH and diluted with CH₂Cl₂ (10 mL). Organic layer was washed with sat. NaHCO₃ twice. Organic layer was dried with K₂CO₃, filtered, concentrated, and purified via flash chromatography (3:1 hexanes: EtOAc) to provide cyclized product in 56% yield (solid). [\alpha]^{25}_D = -44.4 ^\circ (c=1, CHCl₃) (lit. [\alpha]^{25}_D = -44.4 ^\circ ), mp: 80-81\degree C. \textsuperscript{1}H NMR (300 MHz, CDCl₃): \delta 7.2-7.4 (m, 10H), 5.0 (s, 1H), 4.25 (dd, J = 4.9, 9.0 Hz, 1H), 3.96 (t, J = 3.7 Hz, 1H), 3.50 (dd, J = 2.0, 10.5 Hz, 1H), 3.3 (dd, J = 4.1, 10.5 Hz, 1H), 3.08 (dq, J = 2.0, 3.66, 9.23 Hz, 1H), 3.04 (dd, J = 1.2, 10.8 Hz, 1H), 2.47 (dd, J = 3.7, 10.8 Hz, 1H), 0.91 (s, 9H), 0.5 (s, 6H). \textsuperscript{13}C NMR (75 MHz, CDCl₃): 140, 129, 128, 127, 126, 74, 64, 57, 56, 25, 16, -5.

5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-D-lyxitol diacetate (10.4). To a solution of triol 10.12 (100 mg, 0.23 mmol) in pyridine (3 mL) was added acetic anhydride (60 \mu L, 0.57 mmol). Once complete, pyridine was removed under reduced pressure. Crude product was dissolved in MeOH (5 mL), treated with activated Pd (C) and purged with hydrogen. After 2 h, CH₂Cl₂ (10 mL) was added, then solution filtered through Celite\textsuperscript{®} and concentrated giving relatively pure amine in quantitative yield over two steps. \textsuperscript{1}H NMR (300 MHz, CDCl₃): \delta 5.41 (t, J = 4.88 Hz, 1H), 5.25 (q, J = 6.10 Hz, 1H), 3.72 (dd, J = 6.72, 9.88 Hz, 1H), 3.65 (dd, J = 6.48 Hz, 9.88 Hz, 1H), 3.37 (dd, J = 5.00, 6.41 Hz, 1H), 3.28 (dd, J = 7.16, 11.47 Hz, 1H), 3.10 (dd, J = 6.10, 11.48 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 0.9 (s, 9H), 0.5 (s, 6H). \textsuperscript{13}C NMR (75 MHz, CDCl₃): 180, 74, 64, 57, 56, 25, 19, 16, -5.
(1R,2R)-3-Azido-2-Carbobenzyloxyamino-1-phenyl-propane-1-ol (10.21) To a solution of tosylate 9.2 (5.65 g, 12.4 mmol) in DMF (8 mL), sodium azide (8.10 g, 124 mmol) was added. Reaction was heated to 60° C and allowed to agitate at this temp overnight. Once deemed complete via TLC, reaction was cooled to RT and quenched by addition of water. Resulting solution was extracted with EtOAc twice. Organic layer was dried with MgSO4, filtered, concentrated and purified via flash chromatography (3: 7 EtOAc: hexanes) to provide displaced product (white solid) in 43% yield, mp: 82-84° C, 1H NMR (300 MHz, CDCl3): δ 7.2-7.4 (m, 10 H), 5.2 (bd, J = 8.3 Hz, 1 H), 5.05 (s, 2 H), 4.95 (d, J = 4.8 Hz, 1H), 3.9 (ddd, J = 3.9, 4.9, 8.3 Hz, 1 H), 3.8 (dd, J = 5.4, 5.9, 12.0 Hz, 1H), 3.5 (dd, J = 3.9, 5.4, 12.0 Hz) 2.6 (bs, 1 H). 13C NMR (75 MHz, CDCl3): 156, 140, 137, 129, 128, 127, 126, 125, 72, 67, 56, 52. IR (CHCl3): 3423, 2100, 1704, 1511 cm⁻¹.

(4R,5R)-4-Azidomethyl-5-phenyloxazolidin-2-one (10.26). To a solution of tosylate (1.00 g, 3.26 mmol) in DMF (3 mL), sodium azide (1.27 g, 19.6 mmol) was added. Reaction was heated to 60° C and allowed to agitate at this temp overnight. Once deemed complete via TLC, reaction was cooled to RT and quenched by addition of water. Resulting solution was extracted with EtOAc twice. Organic layer was dried with MgSO4, filtered, concentrated and purified via flash chromatography (1:1 EtOAc: hexanes) to provide displaced product (clear oil) in 75% yield over three steps. 1H NMR (300 MHz, CDCl3): δ 7.6 (m, 5H), 6.8 (bs, 1H), 5.3 (d, J = 5.4 Hz, 1H), 3.9 (dd, J = 5.4, 4.9 Hz, 1H), 3.7 (dd, J = 5.2, 12.2 Hz, 1H), 3.5 (dd, J = 5.9, 12.2 Hz). 13C NMR (75 MHz, CDCl3): 159, 137, 129, 128, 125, 80, 59, 52. IR (CHCl3): 3278, 2097, 1744 cm⁻¹.
(4R,5R)-4-{P-Triphenyl-phospho-iminomethyl-5-phenyloxazolidin-2-one (10.27). To a solution of azide (2.8 g, 13.1 mmol) in anhydrous THF (15 mL) was treated with triphenylphosphine (3.1 g, 11.8 mmol, 0.9 equiv.) under Argon. Once complete, THF was removed under reduced pressure, leaving crude solid product. Crude product was recrystallized with hot EtOAc, yielding 4.5 g of recrystallized product in 76% yield. M.P.: 136° C. IR (KBr pellet): 1737, 1441, 1171 cm⁻¹.

5-[[acetyl-(2-oxo-5-phenyl-oxazolidin-4-ylmethyl)-amino]-methyl]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yl ester (10.29). To a solution of aldehyde 10.15 (0.07 g, 0.48 mmol) in dichloroethane (3 mL) under argon at RT, phospho-imine 10.27 (0.17 g, 0.48 mmol) was added. After one hour, sodium triacetoxyborohydride (0.21 g, 1.0 mmol) was added. After five hours, reaction was diluted with CH₂Cl₂ and washed with sat. bicarb and brine. Organic layer was dried with MgSO₄, filtered and concentrated. Crude product was treated with acetic anhydride (0.7 mL) in pyridine (2 mL). That crude product was purified via flash chromatography (EtOAc) to provide product in 25% yield (product also contained triphenylphosphine oxide). ¹H NMR (300 Mz, CDCl₃): δ 6.5 (bs, 1H), 5.8 (d, J = 3.9 Hz, 1H), 5.2 (d, J = 3.3 Hz, 1H), 5.1 (d, J = 4.7 Hz, 2H), 4.4 (m, 3H), 3.7 (m, 2H), 2.6 (m, 4H), 2.2 (s, 3H), 2.0 (s, 5H), 1.4 (s, 5H), 1.2 (s, 6H), ¹³C NMR (75Mz, CDCl₃): δ 170, 159, 138, 135, 132, 131, 130, 129, 128, 127, 125, 112, 105, 83, 81, 79, 78, 76, 62, 58, 56, 44, 26, 25, 21.

1,2: 5,6-Di-O-isopropylidene-D-mannitol (10.30). To a suspension of D-mannitol (20 g, 110 mmol) in distilled DMSO, 2,2-dimethoxypropane (29 mL, 240 mmol, 2.2 equiv.) and catalytic pTsOH (1 g) were added and resulting
mixture was allow to stir at RT overnight. Reaction was quenched with sat. NaHCO₃. Aqueous layer was extracted twice with EtOAc. Organic layer was dried with MgSO₄, filtered and concentrated in vacuo. Crude product was recrystallized from EtOAc: hexanes to provide pure bis-acetonide product in 66% yield. M.P.: 97° C. ¹H NMR (300 Mz, CDCl₃): δ 4.2 (q, J = 6.35 Hz, 2H), 4.1 (dd, J = 8.31, 6.35 Hz, 2H), 3.9 (dd, J = 8.31, 5.62 Hz, 2H), 3.7 (dd, J = 6.35, 5.62 Hz, 2H), 2.6 (d, J = 6.35 Hz, 1H), 1.4 (s, 3H), 1.3 (s, 3H). NMR (75Mz, CDCl₃): δ 109, 76, 71, 67, 27, 25.

1,2: 5,6-Di-O-isopropylidene-3,4-di-O-allyl-D-mannitol (10.31). To a suspension of sodium hydride (1.22 g, 51 mmol) in anhydrous THF (25 mL) at 0° C, bis-acetonide D-mannitol 10.30 (6.62 g, 25.5 mmol) was added and allowed to stir at that temperature for 1 h. After 1 h at 0° C, freshly distilled allyl bromide (4.4 mL, 51 mmol) was added. After deemed complete via TLC, solvent was removed under reduced pressure. Crude material was dissolved in Et₂O at 0° C and quenched with water. Organic layer was washed with sat. NaHCO₃ and sat. NaCl, dried with MgSO₄, filtered and concentrated. Product was purified via column chromatography (9: 1 Hexanes: EtOAc, Rf = 0.4) to provide fully protected D-mannitol as a clear oil in 85% yield. ¹H NMR (300 Mz, CDCl₃): δ 5.9 (ddd, J = 17.34, 10.25, 5.86 Hz, 2H), 5.2 (ddd 17.3, 10.25, 3.00 Hz, 4H), 4.2 (m, 10H), 4.1 (dd, J = 8.31, 5.86 Hz, 2H), 3.9 (dd, J = 8.31, 6.83 Hz, 2H), 3.7 (d, J = 6.35 Hz, 2H), 2.6 (d, J = 6.35 Hz, 1H), 1.4 (s, 6H), 1.3 (s, 6H). ¹³C NMR (75Mz, CDCl₃): δ 135, 117, 108, 80, 76, 74, 67, 27, 25.
1,2-O-isopropylidene-3,4-di-O-allyl-D-arabinose (10.33).

To a solution of fully protected mannitol 10.31 (1.02 g, 3.0 mmol) in cold methanol (3 mL) at 0°C under Ar, acetyl chloride (1.2 g, 15.0 mmol) was added. After approx. 15 minutes, reaction was quenched by sat. NaHCO₃ and extracted with CH₂Cl₂ twice. Organic layer was dried with MgSO₄, filter and concentrated. Crude diol was dissolved in a CH₂Cl₂: H₂O mixture and treated with silica (1.0 g) and sodium periodate (0.72 g, 3.3 mmol). After ten minutes, organic layer was separated from aqueous layer, followed by extraction of aqueous with additional CH₂Cl₂ (5 mL) twice. Organic layer was dried (MgSO₄), filtered and concentration. Product was purified using flash chromatography (9:1 Hexanes: EtOAc) to provide aldehyde in 81% yield (plus, 12% unreacted starting material).

**1H NMR (300 MHz, CDCl₃):** δ 9.8 (s, 1H), 5.9 (ddd, J = 17.34, 10.25, 5.86 Hz, 2H), 5.2 (ddd 17.3, 10.25, 3.00 Hz, 4H), 4.2 (m, 10H), 4.1 (dd, J = 8.31, 5.86 Hz, 2H), 3.9 (dd, J = 8.31, 6.83 Hz, 2H), 3.7 (d, J = 6.35 Hz, 2H), 1.4 (s, 6H).

**13C NMR (75MHz, CDCl₃):** δ 203, 136, 134, 128, 127, 126, 117, 116, 115, 109, 84, 80, 76, 75, 74, 72, 67, 27, 26, 25, IR (KBr pellet): 3074, 2100, 1728, 1451, 1367, 1217, 1073 cm⁻¹

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4-[[2,3-Bis-allyloxy-3-(2,2-dimethyl-[1,3]dioxolan-4-yl)-propylamino]-methyl]-5-phenyl-oxazolidin-2-one (10.34). To a solution of aldehyde 10.33 (0.29 g, 1.08 mmol) in dichloroethane (3 mL) under argon at RT, phospho-imine 10.27 (0.48 g, 1.08 mmol) was added. After one hour, sodium triacetoxyborohydride (0.57 g, 2.7 mmol) was added. After five hours, reaction was diluted with CH₂Cl₂ and washed with sat. bicarb and brine. Organic layer was dried with MgSO₄, filtered and concentrated. Crude product was purified via flash chromatography (EtOAc) to provide product in 25% yield (product also contained triphenylphosphine oxide). **1H NMR (300 MHz, CDCl₃):**...
Mz, CDCl₃): δ 5.9 (ddd, J = 17.34, 10.25, 5.86 Hz, 2H), 5.2 (ddd 17.3, 10.25, 3.00 Hz, 4H), 4.0 (m, 6H), 3.6 (m, 2H), 2.8 (m, 4H), 1.4 (s, 3H), 1.3 (s, 3H) ¹³C NMR (75Mz, CDCl₃): δ 159, 138, 135, 132, 131, 130, 129, 128, 127, 125, 116, 108, 81, 79, 78, 76, 73, 72, 66, 60, 52, 50, 26, 25.

(4R,5R)-4-Pyrrolidinomethyl-5-phenyloxazolidin-2-one (10.36). To a chilled (0°C) solution of azide 10.26 (0.5 g, 2.32 mmol) under atm pressure. Once decomposition and hydrolysis complete (water, 2 mL, added if phospho-imine 10.27 hydrolysis was incomplete), THF was removed under reduced pressure. Crude product was dissolved in 1,2-dichloroethane (3 mL). To this solution, aqueous 1,4-dial (prepared by acidic hydrolysis of 1,4-dimethoxytetrahydrofuran) was added (1.25 M, 1.9 mL, 2.32 mmol) at room temperature, followed by sodium triacetoxyborohydride (1.38 g, 6.5 mmol). Reaction was allowed to stir at RT for 24 hours. Once complete, reaction was diluted with CH₂Cl₂ and quenched by addition of sat. NaHCO₃. Organic layer was consecutively washed with brine and distilled water. Organic layer was dried with MgSO₄, filtered, concentrated and purified via flash chromatography (EtOAc) to provide pure pyrrolidine in a clear oil in 70% yield. ¹H NMR (300 MHz, CDCl₃): δ 7.2-7.4 (m, 5H), 7.0 (bs, 1H), 5.2 (d, J = 5.86 Hz, 1H), 3.6 (ddd, J = 5.37, 5.86, 8.30 Hz, 1H), 2.7 (dd, J = 8.30, 12.2 Hz, 1H), 2.6 (dd, J = 5.38, 12.2 Hz, 1H), 2.5 (dd, J = 6.35, 8.8 Hz, 4H), 1.9 (m, 4H) ¹³C NMR (75 MHz, CDCl₃): 159, 139, 129, 128, 125, 81, 60, 59, 54, 23.

(4R,5R)-1-Phenyl-2-Palmitoyl amino-3-Pyrrolidino-1-Propanol (L-threo-PPPP or P4) (10.37). To a solution of oxazolidinione 10.36 (0.382 g, 1.55 mmol) in MeOH (2 mL), aqueous KOH (6 mL, 2 M, 11.5 mmol) was added. The mixture was heated to reflux for 5-10 h. Once saponification was complete,
MeOH was removed _in vacuo_ and the crude amine was dissolved in THF (2 mL). Palmitoyl chloride (350 μL, 1.16 mmol) was added at 0° C. After two hours, THF was removed and crude product was dissolved in CH₂Cl₂ and consecutively washed with sat. NaHCO₃ and brine. Organic layer was dried with MgSO₄, filtered, concentrated and purified via flash chromatography (9: 1 EtOAc: MeOH) to provide pure P4, as a solid, in 56% yield, mp: 90-2° C (lit: oil)⁴⁰⁵, ¹H NMR (300 MHz, CDCl₃): δ 7.4-7.1 (m, 4H), 6.05 (d, J = 7.3 Hz, 1H), 5.22 (d, J = 2.9 Hz, 1H), 4.18 (m, 1H), 2.80 (dd, J = 13.1, 6.2 Hz, 1H), 2.65 (m, 4H), 2.05 (t, J = 7.8 Hz, 2H), 1.80 (bs, 4H), 1.60 (m, 2H), 1.20 (bs, 24H), 0.90 (t, J = 5.8 Hz, 3 H). ¹³C NMR (75.0 MHz, CDCl₃): δ 173, 141, 128, 127, 125, 58, 55, 52, 37, 32, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 26, 24, 23, 14.

1,3:4,6-di-O-benzylidene 2,5-dioxo-D-mannitol hydrate (10.38). To a solution of bis-benzylidene D-mannitol (1.67 g, 4.7 mmol) in CH₃CN: CH₂Cl₂ (12: 4 mL) mixture, PCC (5.1 g, 23.5 mmol) and NaOAc (2.3 g, 28.2 mmol) was added. Mixture was allowed to stir overnight. Organic solvent was removed under reduced pressure. Crude product was purified via flash chromatography (2: 8, EtOAc: hexanes) to provide 10.38 in 38% yield. ¹H NMR (300 MHz, CDCl₃): δ 7.4-7.1 (m, 10 H), 5.5 (s, 1H), 4.3 (s, 1H), 4.25 (d, J = 12.7, 2H), 4.15 (d, J = 12.7, 2H). ¹³C NMR (75.0 MHz, CDCl₃): δ 136, 129, 128, 126, 100, 99, 83, 70.
Experimentals for Chapter 11

(R)-Methyl- N-(diphenylmethylene)-D-alanate (11.4a).
Compound was prepared following the published procedure.406

(R)-Methyl-O-(tert-butyldimethylsilyl)-N-(diphenylmethylene)-D-serinate (11.4b). Compound was prepared following the published procedure.5

(R)-2-(Benzyldihydridene-amino)-N-methoxy-N-methyl
propionamide (11.5a). To a suspension of N, O-dimethyl
hydroxylamine hydrochloride (1.06 g, 10.8 mmol) in anhydrous
THF (5 mL) at 0° C, trimethyl aluminum (2M in toluene, 5.4
mL) was added drop wise and allowed to agitate and evolve methane for 1 h at
0° C. After 1 h, a solution of methyl alanate 11.4a (1.45 g, 5.4 mmol) in dry THF
(5 mL) was added in one portion. Resulting solution was allowed to warm to RT
and stir overnight. Solvent was removed under reduced pressure and resulting
oil was dissolved in EtOAc and carefully quenched with water. Organic layer was
washed with sat. NaHCO₃, brine and dried with MgSO₄. Filtration and
concentration under reduced pressure provide crude product. Recrystalization
(EtOAc: hexanes) provided transparent crystals in 59% yield. M.P.: 102-4° C. IR
(neat): 1662, 1446 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ 7.6-7.1 (m, 10H), 4.4 (q, J
= 6.35 Hz, 1H), 3.3 (s, 3H), 3.1 (s, 3H), 1.3 (d, J = 6.35 Hz, 3H). ¹³C NMR (75.0
MHz, CDCl₃): δ 169, 139, 136, 131, 129, 127, 126, 61, 58, 32, 18.

2-(Benzyldihydridene-amino)-3-(tert-butyl-dimethyl-
silyloxy)-N-methoxy-N-methyl-propionamide (11.5b).
Same procedure as above. Yield: 50%, M. P.: 116-8° C,
IR (neat): 1662, 1446 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ
7.7-7.1 (m, 10H), 4.6 (bs, 1H), 4.2 (dd, J = 9.3, 6.1 Hz), 3.9 (dd, J = 9.3, 6.8 Hz),
3.2 (s, 3H), 3.1 (s, 3H), 0.8 (s, 9H), -0.5 (s, 6H). 

**13C NMR (75.0 MHz, CDCl₃):**
171, 140, 137, 130, 129, 128, 127, 65, 64, 61, 32, 18, -5.

**O**

**Ph**

(R)-2-(Benzyhydrylidene-amino)-1-phenyl-propan-1-one (11.6a). To a solution of Weinreb amide 11.5a (0.63 g, 2.1 mmol) in anhydrous THF (3 mL) at 0°C, phenyl magnesium bromide (1.0M, 8.4 mL) was added drop-wise and allowed to warm to RT overnight. THF was removed in vacuo and residual was diluted in Et₂O and carefully quenched by the addition of ice. Wash organic with sat. NaHCO₃ and brine. Dry organic with MgSO₄, filter and concentrate. Crude product was purified via flash chromatography (5: 95: 0.1 EtOAc: Hexanes: Et₂N) to provide clear oil product in 95% yield. Chromatography-induced hydrolysis was observed. Author would advice avoiding column chromatography. [α]²³ = +9.5° (c = 2.6, CHCl₃). IR (CHCl₃): 1686, 1446 cm⁻¹. 

**1H NMR (300 MHz, CDCl₃):**
δ 7.9-7.1 (m, 15H), 5.0 (q, J = 6.84 Hz, 1H), 1.5 (d, J = 6.35 Hz, 3H). 

**13C NMR (75.0 MHz, CDCl₃):** δ 200, 169, 139, 136, 132, 131, 129, 128, 127, 126, 64, 20.

**O**

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**1H NMR (300 MHz, CDCl₃):** δ 7.7-7.1 (m, 15H), 5.1 (t, J = 6.35 Hz, 1H), 4.3 (dd, J = 9.27, 6.35 Hz, 1H), 4.1 (dd, J = 9.77, 6.35 Hz, 1H), 0.8 (s, 9H), -0.5 (s, 6H). 

**13C NMR (75.0 MHz, CDCl₃):** 199, 171, 140, 138, 136, 132, 131, 130, 129, 128, 78, 71, 66, 18, -5.

(R)-2-(amino)-1-phenyl-propan-1-one hydrochloride (11.7). To a solution of ketone 11.6a (.353 g, 1.11 mmol) in EtOAc (2 mL), 3M HCl (2 mL, aqueous) was added. Reaction was allowed to stir at RT until
hydrolysis was complete. Removal of solvents under reduced pressure, followed by recrystallization (EtOH; Et₂O) provided cathinone HCl in quantitative yield. M. P.: 171° C (lit.: 180° C), ¹H NMR (300 MHz, D₂O): δ 7.8 (d, J = 7.8 Hz, 2H), 7.6 (t, J = 7.3 Hz, 1H), 7.4 (m, 2H), 5.0 (q, J = 7.3 Hz, 1H), 1.4 (d, J = 7.3 Hz, 3H). ¹³C NMR (75 MHz, D₂O): 198, 135, 132, 129, 128, 52, 17.

(R)-2-(decanoyl amino)-1-phenyl-propan-1-one (11.8). To a ice-bath-chilled solution of crude amine 11.7 in CH₂Cl₂, decanoyl chloride (.256 g, 1.11 mmol) and triethylamine (.42 mL, 2.9 mmol) were added. Once reaction deemed complete via TLC (4: 1 Hexanes: EtOAc), reaction was quenched with water. Separation, followed by organic extraction of aqueous layer, drying with MgSO₄, filtration, concentration and chromatography provide amide as a clear oil in 57% yield (over two steps). IR (neat): 3296, 1686, 1632, 1217 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ 7.8 (d, J = 7.8 Hz, 2H), 7.6 (t, J = 7.3 Hz, 1H), 7.4 (m, 2H), 6.8 (d, J = 7.32 Hz, 1H) 5.6 (dd, J = 7.32, 6.84 Hz, 1H), 2.1 (t, J = 7.8 Hz, 2 H), 1.5 (m, 2 H), 1.25 (bs, 12 H), 0.9 (t, J = 5.8 Hz, 3 H). ¹³C NMR (75 MHz, CDCl₃): 199, 173, 134, 129, 128, 50, 36, 32, 29, 26, 22, 19, 14.

MRRL-CH1 cell line procedure: The cell line, MRRL-CH1, which was derived originally from embryonic Manduca sexta by Dwight Lynn (USDA), is maintained in a 10-ml volume in 50-ml Falcon flasks in a 27° incubator in room air. The cells grow attached to the bottom of the flask, and are passaged approximately every 3-4 weeks by using the outflow of a medium-filled pipette to dislodge cells that have grown to confluence, then seeding a new flask containing 10 ml fresh DL medium with approximately 300 µl of the cell suspension. For the current experiments, 1.2 ml DL medium and 120 µl of cell suspension from the 50-ml flask were added to each well of a sterile12-well plate. Analogues were added 2-4 days after initiation of the well cultures. 48 hrs after
the drugs were added, 0.9 ml of medium were removed from each well and replaced with 0.9 ml fresh medium, and the step was repeated to decrease the concentration of the remaining analogue by 16-fold. Digital photographs were taken just before adding the analogue, at 24 and 48 hrs after analogue administration, and at 24, 48, and 144 hrs after analogue removal.

Because the PDMP analogues were lipophilic, each analogue was massed using a microgram scale found in the Mass Spectroscopy Facility (Old Chem., first floor). In one trial, the following masses were used in testing: L-threo-PDMP (2.736 mg, 6.42 µmol), L-threo-PDDEAP (4.038 mg, 8.45 µmol), L-threo-PDHEPP (2.445 mg, 5.21 µmol) and D-threo-PDMP (2.288 mg, 5.37 µmol).

Because the PDMP analogues were lipophilic, each drug was dissolved in DMSO in an amount appropriate to yield a 100 mM stock solution, and then diluted in 1 ml DL medium to yield a 100 µM solution. One analogue, L-threo-PDDEAP, could be administered using water as the vehicle. In that case, the same dilution scheme used in the DMSO case was employed. Warming, vortexing, and sonication were necessary for solubility and the analogue-containing solutions were used immediately after preparation. Further dilutions from the 100 µM solution were made by removing from each well the volume of medium-containing analogue to be added. All doses were tested in duplicate. Vehicle control wells were given an amount of DMSO equal to the amount of DMSO that the cells exposed to the highest analogue dose tested received.
APPENDIX. Selected $^1$H and $^{13}$C NMR Spectra
**d-threo tolyl SB**

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>ppm</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.95</td>
<td>0.29</td>
</tr>
<tr>
<td>1.00</td>
<td>0.92</td>
</tr>
<tr>
<td>1.27</td>
<td>1.78</td>
</tr>
<tr>
<td>2.46</td>
<td>8.80</td>
</tr>
<tr>
<td>8.60</td>
<td>6.21</td>
</tr>
</tbody>
</table>
Tolyl amino alcohol
AMINO ALCOHOL

\[
\text{\begin{center}
\includegraphics[width=0.2\textwidth]{amino_alcohol.png}
\end{center}}
\]
tolyl silyl carbamate
$^{13}$C tolyl silyl carbamate

![Chemical Structure](image)

**NMR Spectrum**

- ppm scale from 160 to 20 ppm
d-threo-tolyl tosylate
tolyl tosylate

[Chemical structure image]

[Graph with peaks at ppm values]
Tolyl Morpholino carbamate

![Chemical structure of Tolyl Morpholino carbamate]
L3C morpholine tolyl oxazolidinone
Pulse Sequence: $2pul$
$^{13}C$ d-threo-TPHP

Diagram of a 13C NMR spectrum with chemical shift values in ppm.
anisole amino alcohol

\[
\text{TBSC-} \quad \text{NH}_2 \quad \text{OH} \\
\]

![Chemical Structure of Anisole Amino Alcohol](image)

**NMR Spectrogram**

- **ppm:** 2.27, 2.04, 1.00, 2.26, 5.27, 1.08, 6.08, 5.34
$^1$H anisole silyl carbamate

Pulse Sequence: sipul

![Chemical Structure Image]
$^{13}C$ anisole silyl carbamate

Pulse Sequence: s2pul

\[ OM e \]

TBSO

\[ HO \]

\[ 160 \quad 140 \quad 120 \quad 100 \quad 80 \quad 60 \quad 40 \quad 20 \quad \mu m \]
anisole
methylene-dioxy-phenyl mesylate

\[
\text{Structure Image}
\]
$^{13}$C anisole mesylate
anisole morpholino carbamate

\[ \text{OMe} \]

\[ \text{HN} \]

The diagram shows a spectrum with peaks labeled at various ppm values:
- 2.05 ppm
- 1.97 ppm
- 1.05 ppm
- 1.00 ppm
- 4.06 ppm
- 4.07 ppm
- 6.21 ppm
IH butoxy phenyl SB
Pulse Sequence: sızpul
threo-tButoxyphenyl-5B

\[
\begin{align*}
\text{TBSO} & \quad \text{OH} \\
\text{Ph} & \quad \text{Ph} \\
\end{align*}
\]
threo tbutoxyphenyl amino alcohol

\[ \text{Structure Image} \]

\[ \text{NMR Spectrum Image} \]
three tbutoxy phenyl amino alcohol
tbutoxyphenyl carbamate
touful phenyl ether tosylate

![Chemical Structure of Toluyl Phenyl Ester Tosylate](image)

**NMR Spectrum**

- Chemical Shifts:
  - 1.00 ppm
  - 2.30 ppm
  - 4.30 ppm
  - 9.82 ppm

**Touful phenyl ether tosylate**

- Description of the chemical structure and NMR spectrum for the compound.
13C t-butyl phenyl ether tosylate
tert-butyl phenyl ether morpholino carbamate
t-butyl phenyl ether morpholino carbamate
13C OBSERVE

Pulse Sequence: s2pul
$^{13}$C NMR of amino alcohol
methylene dioxy silyl carbamate
$^{13}$C silyl methylene dioxy phenyl carbamate
methylene dioxy phenyl tosyl carbamate

Pulse Sequence: 2paul

![Molecule Structure]

1.80 3.40 2.33
1.90 0.99

ppm
methylene dioxy phenyl tosylate

Pulse Sequence: s2pul
methylene dioxy phenyl morpholino carbamate

Pulse Sequence: s2pul

![Chemical Structure]
13C methylene dioxy morpholino carbamate

Pulse Sequence: s2pul
$^{13}$C D-threo-PipDMP

Pulse Sequence: s2DJi
2-M-Chr 1-phenyl propane-1,3-diol
Pulse Sequence: s2pul
1-phenyl 2-alkyl-Cbz 3-tosyl propa-1-ol

Pulse Sequence: s2pul

1H NMR (CDCl3)
13C l-phenyl 3-aminocbz 3-tosyl propan-1-ol
Pulse Sequence: t2pu1
l-phenyl 2-fl-Cbz 3-pyrroloidin l-propano

Pufse $equencse Zpul

O

\[ \text{Diagram} \]

\[ \text{Chemical Structure} \]
13C l-phenyl 2-N-Cbz 3-pyrrolidine 1-propenoic
Pulse Sequence: 52pul
1-phenyl 2-N-Cbz 3-piperidine 1-propanol
Pulse Sequence: s2pu1
$^{13}$C 1-phenyl 2-aminocbz 3-(1'-methyl)piperazine propyl-d$_3$

Pulse Sequence: f2pu1
peracetylated HEP displacement product
Pulse Sequence: 62p1p1

Chemical Shifts:
- 1.87
- 1.88
- 3.05
- 3.15
- 3.30
- 3.34
- 3.75
- 5.94

ppm
13C peracetylated hydroxy ethyl piperazine product
Pulse Sequence: s2pu1
1-0-tertbutyl-dimethylsilyl 2-H-C82 1-phenyl propen-1-ol
Pulse Sequence: spsp

\[
\begin{align*}
\text{TBSO} & \quad \text{Ph} \\
\text{OH} & \quad \text{Ph} \\
\text{N} & \quad \text{N}
\end{align*}
\]
3-tert-butyldimethylsilyl oxy 2-Cbz-amino 1-phenyl propan-1-ol

Pulse Sequence: 1H-13C
phenyl silyl carbamate

Pulse Sequence: $s^{pu}$

![Chemical Structure Image]
$^{13}$C phenyl silyl carbamate

Pulse Sequence: s2pul
1H 3 methyl ethanol amine (GNE) phenyl oxazolidinone
Pulse Sequence: 90° 90°
pyrrolidine polyol w/ d2O
trial protected pyrrolidine

\[
\text{TBSP}^+ 
\begin{array}{c}
\text{TBSP}^+
\end{array}
\text{OAc}
\text{OAc}
\]
1-Azido 2-N-Cbz 3-phenyl propanol

Pulse Sequence: n2pul

10.49

0.86 1.21 1.21 1.17 1.17 2.15 0.94 1.14

ppm
arson oxazolidinone
Pulse Sequence: 62px1
1H NMR 3,4-diethyl 1,1-1,4-bis acetonide 3-mannitol
Pulse Sequence: zlipol
Crude 5,8-diol oxidative cleavage
Pulse Sequence: sspp1
13C oxidation of 1,2-acetonide 3,4-diallyl 5,6-diol mannitol

Pulse sequence: 2D NMR
Reductive amination with C5 aldehyde.

Pulse Sequence: 5ppl
$^{13}$C 1-phenyl 3-pyrrolidino 1,2-thiazolidinone

Pulse Sequence: 1pul
D-serine D3 Valerol amide
Pulse Sequence: 90°/90°
$^{13}$C<sub>salbutamol HCl</sub>

Pulse Sequence: stpul
$^{13}$C NMR spectrum of an amide compound.

Pulse sequence: edpul

[Chemical structure of the amide compound]
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