Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter know, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Type name here	Type date here
Allison R. Beckett	12/2/2010

“Progress Toward a Common Intermediate for C1 Modified Enigmols”

by

Allison Rankin Beckett

Dr. Dennis Liotta
Adviser

Department of Chemistry

Dr. Dennis Liotta

Adviser

Dr. Matthew Weinschenk

Committee Member

Dr. Pat Marsteller

Committee Member

12/2/2010

"Progress Toward a Common Intermediate for C1 Modified Enigmols"

By

Allison Rankin Beckett

Dr. Dennis Liotta

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Chemistry

2010

Abstract

"Progress Toward a Common Intermediate for C1 Modified Enigmols"

By Allison Rankin Beckett

Recent discoveries recognize the sphingolipid pathway to be involved in cell proliferation, apoptosis, and signaling. The balance of sphingoid bases and metabolites present govern these actions, rendering the pathway an effective target for modulation via therapeutic agents. Currently compounds modulating the sphingolipid pathway are in clinical trials, and have shown to be effective in treating cancer and inflammatory diseases. Though very little is known about the mechanistic actions of these agents, the successful results warrant further investigation. The Liotta group has efficiently synthesized Enigmol, a sphingosine analogue efficacious in a number cancer cell lines and malaria, and is continually working toward the development of novel analogues which display improved potency, physiochemical and pharmocodynamic properties. In an attempt to modify the C1 position of Enigmol, a synthetic strategy has been developed to produce a common intermediate from which various substitutions can be made. The synthesis begins with a Liebeskind thioester-boronic acid coupling to yield an enone in multi-gram quantities. Attempts to stereoselectively reduce the enone (3) have yielded at best a 60% de of the desired syn diastereomer. Attempts to increase the diastereoselectivity or synthesize the desired intermediate (6) are underway but have thus far been unsuccessful.

“Progress Toward a Common Intermediate for C1 Modified Enigmols”

By

Allison Rankin Beckett

Dr. Dennis Liotta

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Chemistry

2010

Table of Contents

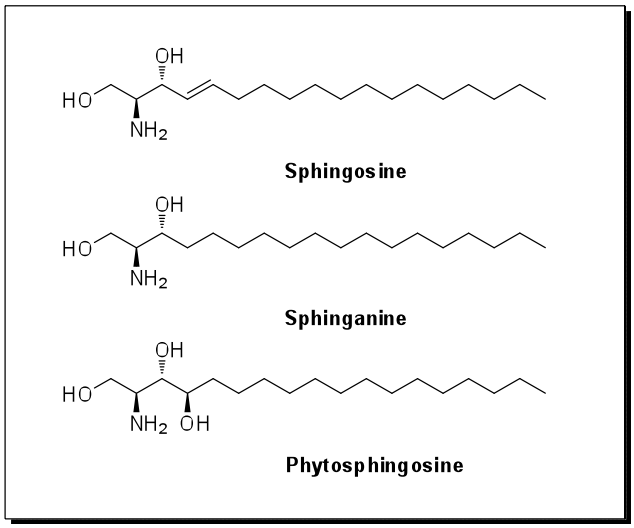
List of Figures

List of Schemes

Introduction

Discovered by L.W. Thudichum in 1884 and later synthesized by David Shapiro in 1954, sphingolipids were aptly named, as their structure and function prove as enigmatic as the Sphinx.[1,2] Sphingolipids are a member of phospholipids, one of six classes of lipids, and are sub-categorized by their sphingoid bases. Sphingolipids are found in all eukaryotes and in some viruses and prokaryotes, where they participate in cell structure, and intracellular communication and trafficking.[3] Common sphingoid bases include sphinganine, sphingosine, and phytosphingosine, as shown in Figure 1.

Figure 1: Common Sphingoid Bases

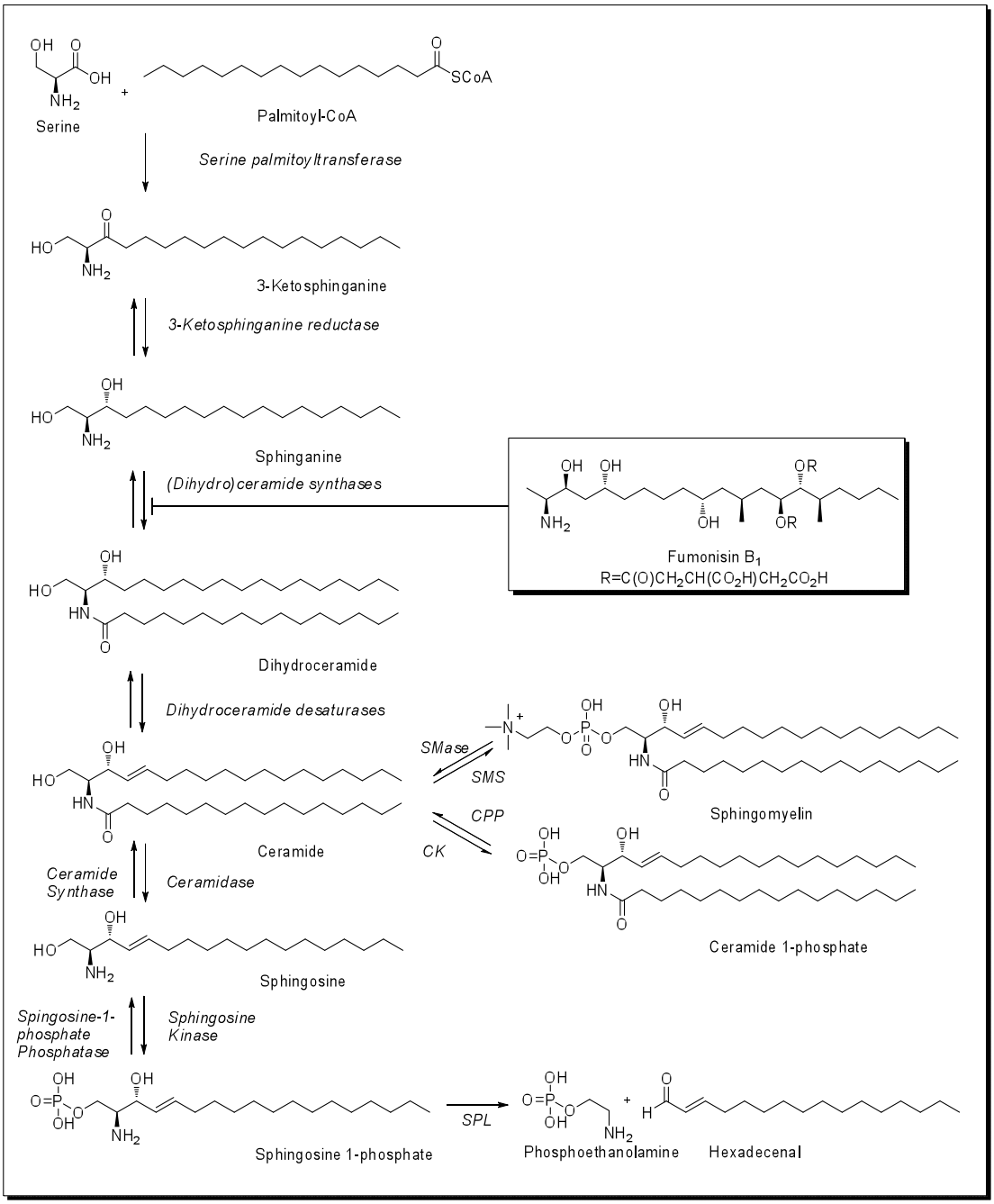


Until recently, sphingolipids were thought to be relegated to the plasma membrane but new research suggest they are active in intracellular and nuclear functions.[4,5] In 1986 Merrill and Hannun discovered that sphingoid bases inhibit protein kinase C (PKC), initiating research into the sphingolipid metabolic pathway.[6] De novo biosynthesis and turnover of complex sphingolipids produces bioactive species, including ceramindes and sphingoid bases, which are involved in cell signaling.[3] The sphingolipid biosynthetic pathway (shown in Scheme 1) occurs primarily in the

endoplasmic reticulum.[7] It begins with the condensation of Serine and Palmitoyl-CoA to yield 3-ketosphinganine, which is readily reduced to sphinganine by the enzyme *3-Ketosphinganine reductase*. The amine of sphinganine is then acetylated with fatty acyl-CoA by the action of the enzyme *(Dihydro)ceramide synthase*, to yield dihydroceramide. Mediated by the *Dihydroceramide desaturase enzyme*, ceramide is formed as result of unsaturation between C4 and C5. Ceramide has anti-mitotic and anti-proliferative effects.[8] It also serves as a basis for several more complex sphingolipds, including sphingomyelin, sphingosine, galactosylceramide, glucosylceramide, and ceramide-1-phosphate. Sphingomyelin is formed by the addition of phosphocholine to the primary hydroxyl end of ceramide in the presence of *Sphingomyelin synthase* (SMS). Sphingomyelin can then be converted back to ceramide by the action of *sphingomyelinase* (SMase). Due to quantities in excess of the signaling requirements, sphingomyelin is suggested to have important roles in maintaining membrane homeostasis and may also inhibit inflammation.[9] The primary hydroxyl of ceramide can also be phosphorylated and dephosphorylated by the enzymes *Ceramide kinase* (CK) and *Ceramide-1-phosphate phosphatase* (CPP), respectively. Ceramide-1-phosphate is currently thought to have two roles in the cell. It is pro-mitotic, having the opposite effect of ceramide, and stimulates macrophage chemotaxis, proving to be important mediator in the inflammatory response.[10] Sphingosine is formed upon *Ceramidase* hydrolysis of the fatty acid chain of Ceramide and the hydrolysis of sphingomyelin and glycolipids, and is involved in both cell proliferation and apoptosis. Sphingosine is an effector molecule in apoptosis by inhibiting the synthesis of the DNA primase and also cell growth by inducing early dephosphorylation of the Retinoblastoma (Rb) protein (a nuclear protein which functions as a tumor suppressor) and protein kinase C, as mentioned previously.[11] Conversely, sphingosine also induces cell proliferation. Subsequent phorphorylation at the C1 of sphingosine produces sphingosine-1-phosphate, which can be further decomposed by *sphingosine-1-phosphate lyase* into hexadecanal

and phosphorylethanolamine. Sphingosine-1-phosphate plays an integral role in several processes including angiogenesis regulation, and lymphocyte trafficking. The interconnected sphingolipid metabolic and cell signaling pathways are indicative of the pleiotropic nature of the sphingolipid metabolites.

Scheme 1: Sphingoid Base and Ceramide Biosynthesis[8]

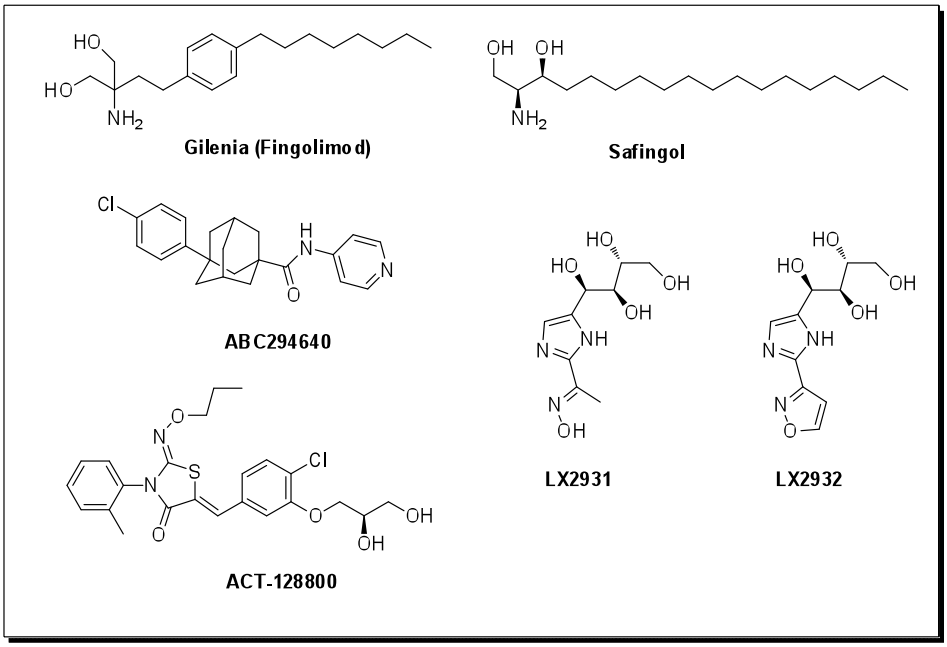


The term "sphingolipid rheostat" describes the interconversion and balance between sphinganine, ceramide, sphingosine-1-phosphate, and sphingomyelin. The relative amounts of these species control whether a cell lives or dies.[12] High amounts of ceramide and its catabolite sphingosine inhibits cell growth and promotes apoptosis, while, in contrast, high levels of sphingosine-1-phosphate stimulates cell growth and inhibits apoptosis.[13,14,15] Naturally this delicate balance has implications in the development of cancer therapies, and currently attempts are being made to develop compounds that can modulate one or more targets in the sphingolipid pathway.[16,17]

Several drugs which act on the sphingolipid pathway are in the pre-clinical and clinical stages or have recently been approved, as shown in Figure 2. In 2010, the FDA approved the drug Gilenya (FTY720), previously Fingolimod, to treat relapsing remitting multiple sclerosis. It is a sphingosine-1-phosphate receptor modulator, which blocks the ability of lymphocytes to exit lymph nodes, thereby reducing the number of lymphocytes in the blood.[18] Safignol, the synthetic L-threo-stereoisomer of sphinganine, is another drug which targets the sphingolipid pathway by inhibiting protein kinase C and sphingosine kinase. After three clinical trials its therapeutic effects were proven unsuccessful in humans, but it provides a rational for alteration of sphingoid bases to produce potential drug candidates. Apogee has been approved to begin clinical trials in 2011 to test the efficacy of ABC294640 against solid tumors and advanced pancreatic cancer. In laboratory tests it has been shown to selectively inhibit *Sphingosine Kinase 2* with an IC_{50} in the 10 μM range, inhibit tumor growth in a mouse mammary adenocarcinmoma model, and is active against several cancers *in vitro* (IC_{50}'s: 6-48μM).[19] Lexicon's LX2931 is currently in phase 2 clinical trials for Rheumatoid Arthritis and other immune diseases. LX2931 regulates lymphocytes by inhibiting *Sphingosine-1-phosphate lyase*, thereby increasing concentrations of sphingosine-1-phosphate, resulting in a reduced immune response.[20] Actelion's ACT-128800 is also in advanced clinical development for the autoimmune

diseases Multiple Sclerosis and Psoriasis, and works by similar mechanism of action as fumonisin.[21] Although the direct mechanisms of actions are not known in all cases, these drugs demonstrate the validity of the sphingolipid biosynthetic pathway as well as the importance of further development of drugs targeting it.

Figure 2: Drugs Acting on the Sphingolipid Rheostat

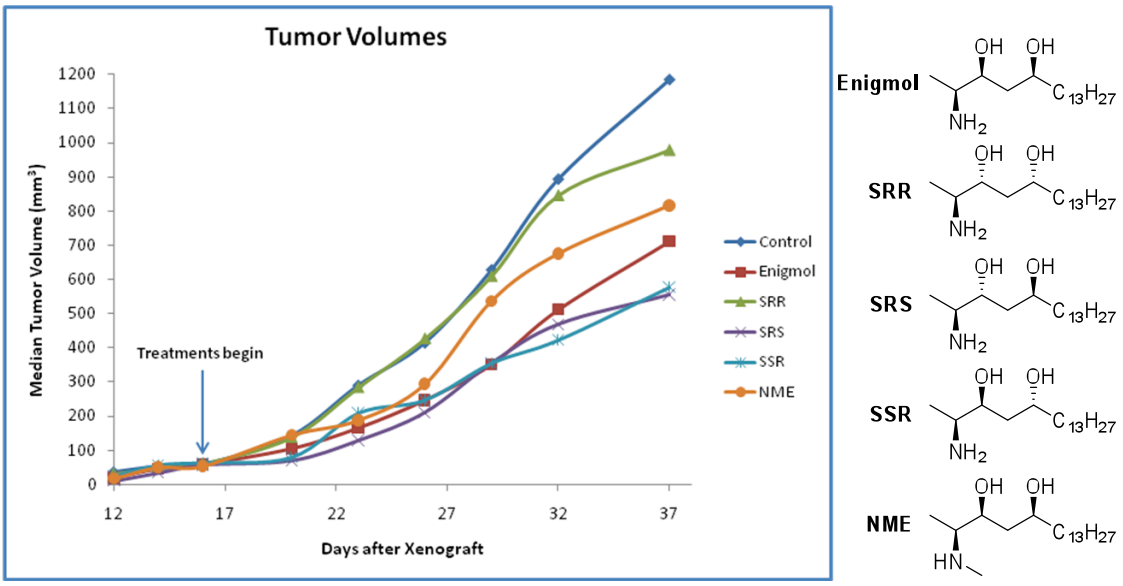


Produced by the fungus *Fusarium moniliforme*, fumonisins are mycotoxins which inhibit *ceramide synthase*, thus blocking conversion of sphinganine to dihydroceramide.[22] As a result, the substrate sphinganine accumulates and is then converted to sphinganine1-phosphate, while the product ceramide decreases. This disruption of the sphingolipid rheostat is likely responsible for the reported toxicity of fumonisin.[23] In order to develop modulators, agonists, and antagonists of the sphingolipid pathway it was theorized that 'unnatural' structural modifications of the sphingoid bases may result in compounds with better pharmacological properties and therapeutic effects.[19] Several independent studies have shown the structure and stereochemistry of sphingosine to be most effective as an 18-membered, saturated carbon chain, with N-methyl substitution, in the L-threo (2*S*,3*S*) stereoisomer

form.[24,25,26] In an effort by the Liotta group to create novel sphingosine analogues, which can survive the metabolic enzymes of the sphingosine pathway, it was deemed necessary to remove the C1 hydroxyl group to prevent phosphorylation by *sphingosine kinase* or metabolism by the addition of headgroups that could increase the rate of excretion.[27] Also, it seems phorporylation could be the major difference between the pro-mitotic activity and mitotic activity of the sphingosine metabolites.[28] It was thought that upon removing the hydroxyl group the hydrophobicity would be increased and the solubility altered in an undesirable direction. It was therefore postulated that moving the C1 hydroxyl to the C5 position, akin to the head group structure of fumonisin, would eliminate the primary alcohol yet retain similar hydrophobicity to that of sphingosine. A series of compounds with the 1-deoxy-2S-amino-3,5-dihydroxyoctadecane skeletal structure, and various stereochemical combinations and functional groups were produced to test for potency.

Of these analogues Enigmol, 2*S*-amino-3*S*,5*S*-dihydroxyoctadecane was discovered to be the most effective. The 4 diastereomers of Enigmol (shown in Figure 3) were examined in an in vivo prostate cancer mouse model. 4 week old nude mice were injected with PC-3 cells and dosed daily with 10mg/kg Enigmol diastereomers for 37 days. Enigmol (2S, 3S, 5S) and SSR and SRS showed similar efficacy, with only SRR showing significantly decreased potency.

Figure 3: Effects of Enigmol Diastereomers on PC-3 (unpublished)

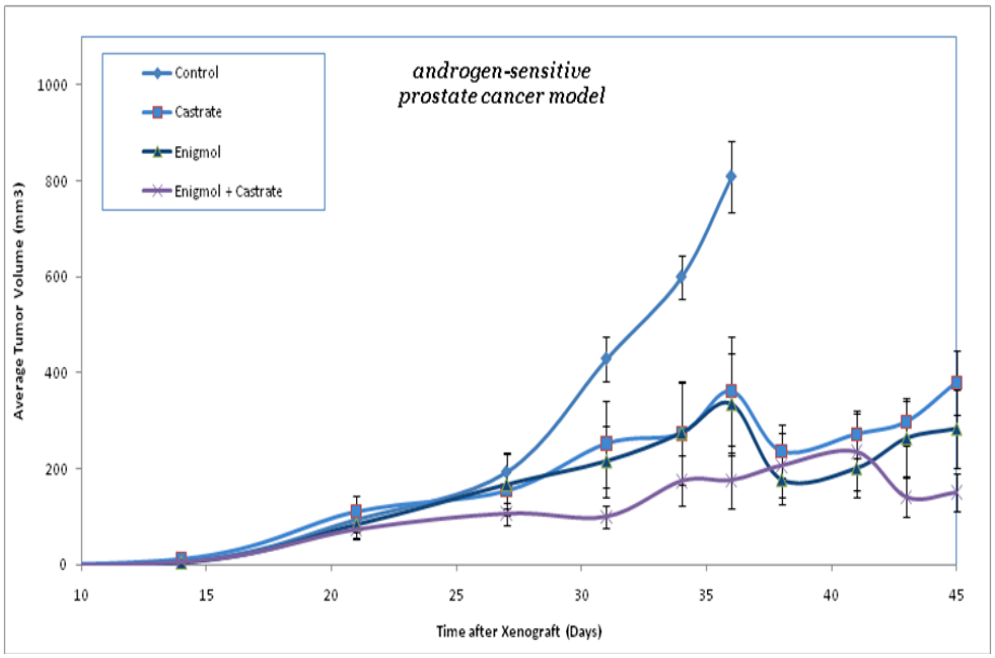


Enigmol was tested for efficacy against cancer by the NCI in 57 cell lines, resulting in IC_{50} values between 0.4 and 14 µM. [28] When analyzed against colon cancer in HT29 cells, Enigmol exhibited an IC_{50} value of 8 µM, effectively limiting tumor growth, with a three-fold lower dose than required of sphingosine and sphinganine. Enigmol showed greater cytotoxicity and a longer biological lifetime than sphingosine and sphinganine. To test anti-tumor activity in vivo, Enigmol was administered to mice induced with colon cancer which resulted in reduced tumor size. Mice were fed a sphingolipid-free diet, and those fed 0.025% and 0.1% Enigmol diets had 52% and 37% fewer tumors, respectively.

Efficacy and toxicity of Enigmol was also tested in mouse xenografts for prostate cancer. Mice were injected with Enigmol at 8mg/kg of body weight for 5 days and showed significant suppression of growth in DU145 cells. There were no signs of toxicity at this dosage. Oral administration also proved successful. Mice implanted with PC3 cells and were dosed with 10mg/kg Enigmol in 200µl of

olive oil. A 50% reduction in tumor size was seen in both cell lines, as compared to control (as shown in Figure 4). Upon evaluating the pharmacokinetics, enigmol was present in both the tumors and the bloodstream indicating good absorption. Enigmol has also shown in vitro efficacy in glioblastoma cell lines LN18, LN229, and U87MG.

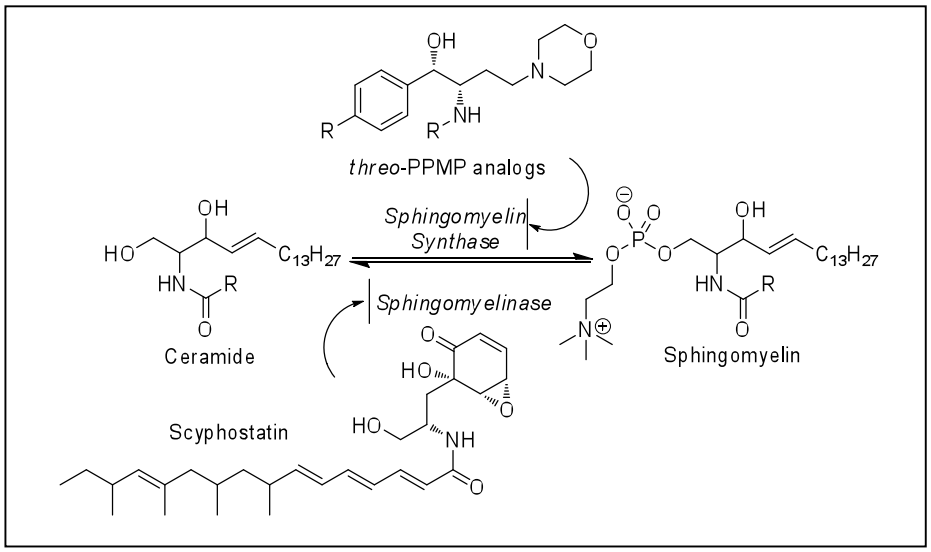
Figure 4: Androgen-Sensitive Prostate Cancer Model (unpublished)



Enigmol analogues are also being tested for efficacy against *P. falciparum*, the parasite that causes malaria. The interest in testing sphingosine like analogues originated from the knowledge that d,1-threo-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (threo-PPMP) analogues have shown efficacy against malaria infected red blood cells by inhibiting *Sphingomyelin Synthase,* which modulates conversion of ceramide to sphingomyelin.[30] This effectively prevented the formation of the tubovesicular network which delivers nutrients to the parasite. Scyphostatin also inhibits intraerythrocytic parasite replication by inhibiting sphingomyelinase, which modulates conversion of sphingomyelin to ceramide, as shown in Figure 6. [31] Thus far Enigmol and five novel compounds have

shown low micromolar inhibition of Plasmodium, the most potent analogue being N-methylenigmol. IC_{50}'s are comparable or better in the chloroquine resistant strain (W2) vs. the chloroquine sensitive strain (D6). Similar efficacy has also been shown against P. knowlesi suggesting further development is warranted.

Figure 5: Sphingolipids as Anti-Malarials

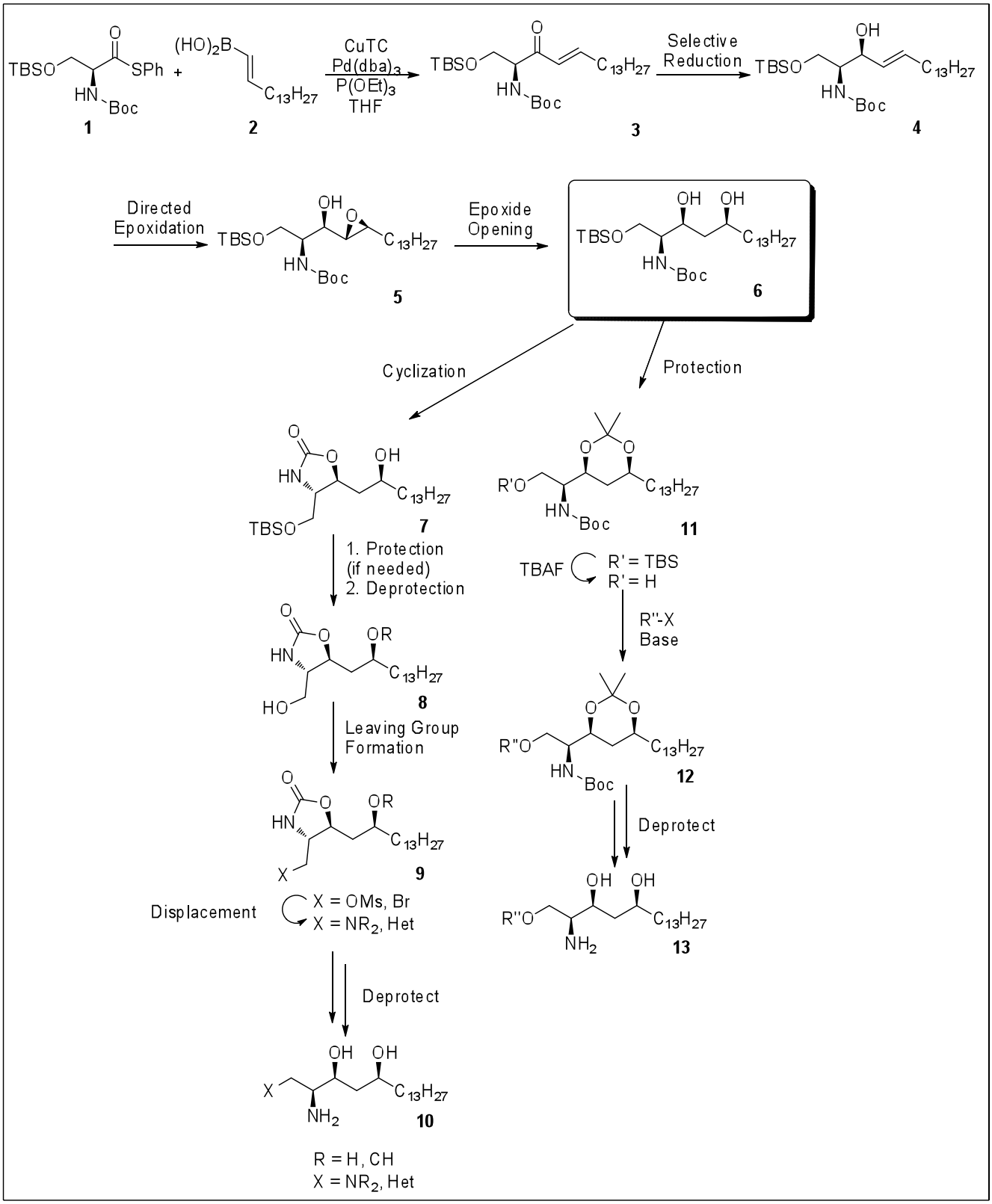


Though specific mechanisms of action are unknown, Enigmol and other sphingosine analogues have shown efficacy, low toxicity, and good metabolism and absorbance in a number of cancer cell lines in vitro and in vivo and malaria in vitro warranting further investigation into the discovery of additional analogues. Recently, Leibeskind and Yang reported an effective synthesis of sphingosine using a novel peptidyl thio ester-boronic acid cross coupling reaction.[32] We wish to use a modified version of Liebeskind's method to produce a common intermediate (X), from which analogues of enigmol can be generated through modification at C1, as shown in Scheme 2.

The synthesis of the common intermediate (6) could be realized via Liebeskind-Srogl coupling to give enone (3), selective reduction to give allylic alchohol (4), hydroxyl directed epoxidation to give

epoxide (5), and finally epoxide opening to give the 1,3-diol (6). This intermediate could then be elaborated further to generate analogs by alkylation or replacement of the oxygen. In the case where oxygen would need to be converted to a leaving group the NHBoc group would be converted to the cyclic carbamate (7) to avoid aziridine formation. Described herein is the progress toward the synthesis of the common intermediate (6).

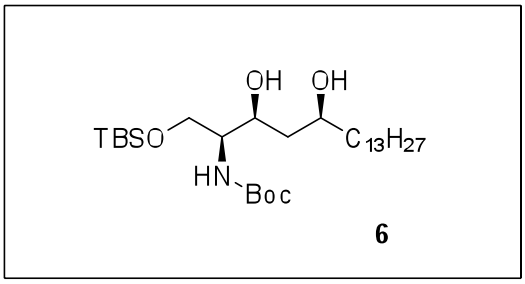
Scheme 2: Proposed Modification of the C1 Position



Results and Discussion

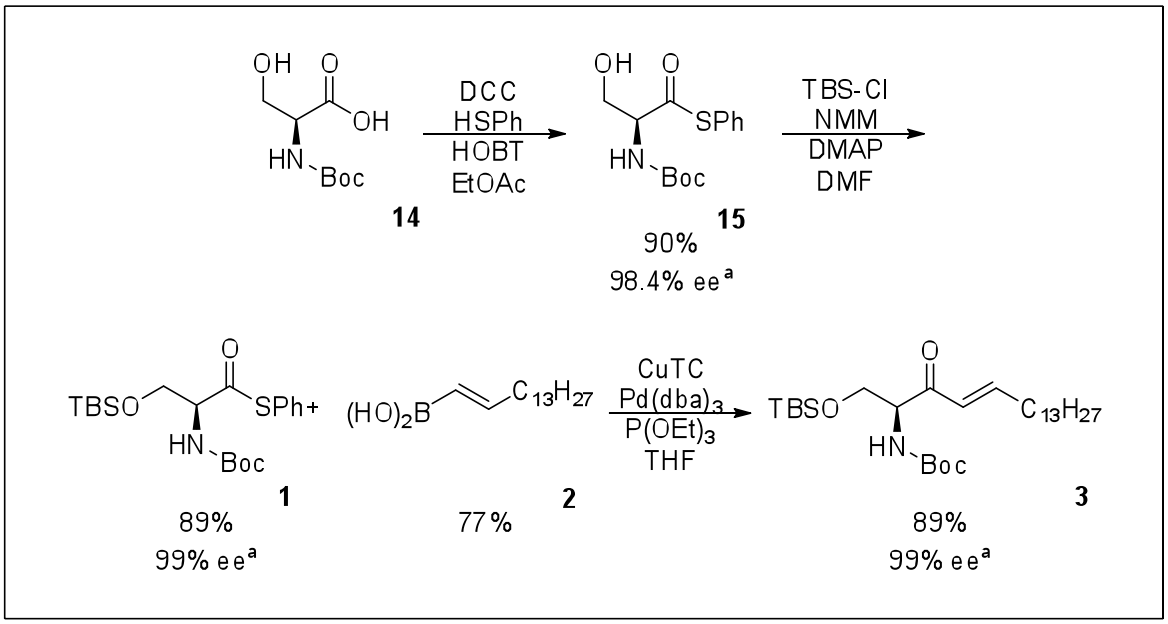
The efforts to synthesize a common intermediate (6) were initiated using chemistry previously published by Liebeskind and Yang in the synthesis of sphingosine.[32]

Figure 6: A Common Intermediate



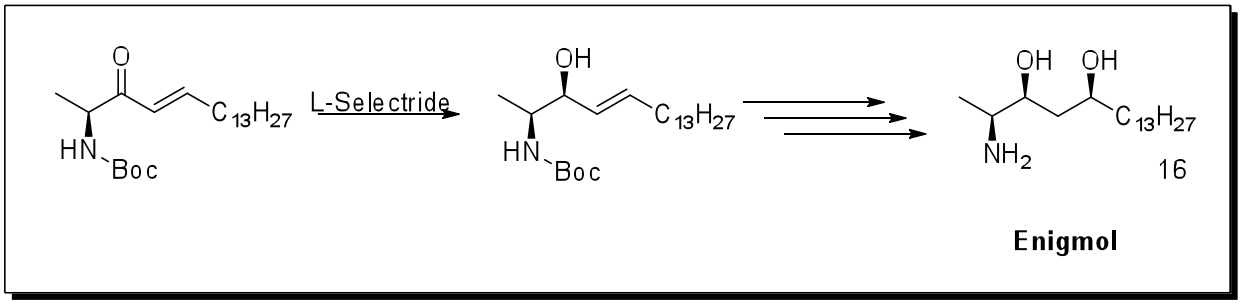
The synthesis began with the synthesis of enone (3) via Liebeskinds literature procedure.[32] Commercial available Boc-L-serine, which resembles sphingosine in that it has the C-1 hydroxyl group and the C-2 chiral center. A DCC coupling reaction between Boc-L-serine and thiophenol gave **15** in 90% yield with 98.4% ee. The primary alcohol was then protected with a TBS group to give **1** in 89% yield with 99% ee . The *R* enantiomer of each intermediate was also carried through for use as an HPLC reference. The *S* thioester intermediate was set-up to undergo Liebeskind-Srogl cross-coupling with boronic acid (2), synthesized via hydroboration of 1-pentadecyne,[33] to give enone 3 in 91% yield with 99% ee. The above sequence was used to produce multigram quantities of **3** (Scheme 3).

Scheme 3: Synthesis of the Enone Intermediate



In the synthesis of sphingosine by Liebeskind and Yang, **3** was stereoselectively reduced with lithium tri-tert-butoxy-aluminohydride to give the anti diastereomer. Conversely, in the Liebeskind group's synthesis of Enigmol (unpublished) L-Selectride was used to attain the desired syn diastereomer (16) (Scheme 4).

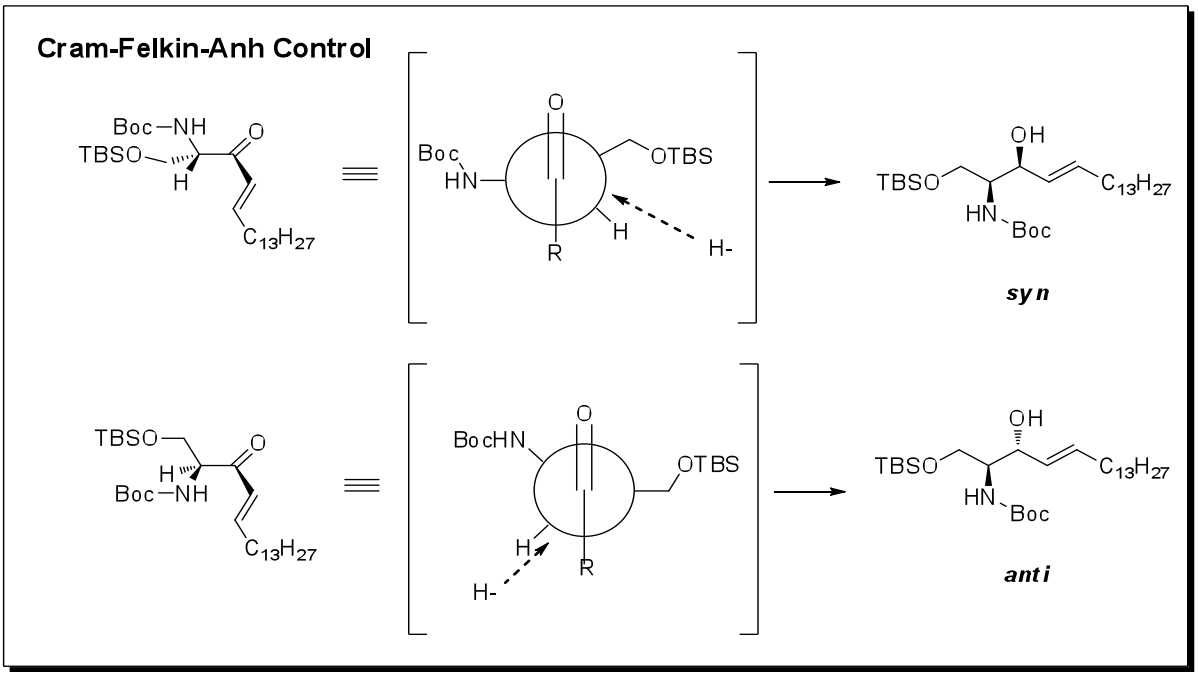
Scheme 4: Liebeskind and Yang Synthesis of Enigmol (unpublished)



Attempts to reduce **3** to yield the syn diastereomer using L-Selectride produced diastereomers in a 1:1 ratio. The stereocontrol of each reducing agent can be explained using three models. Cram-

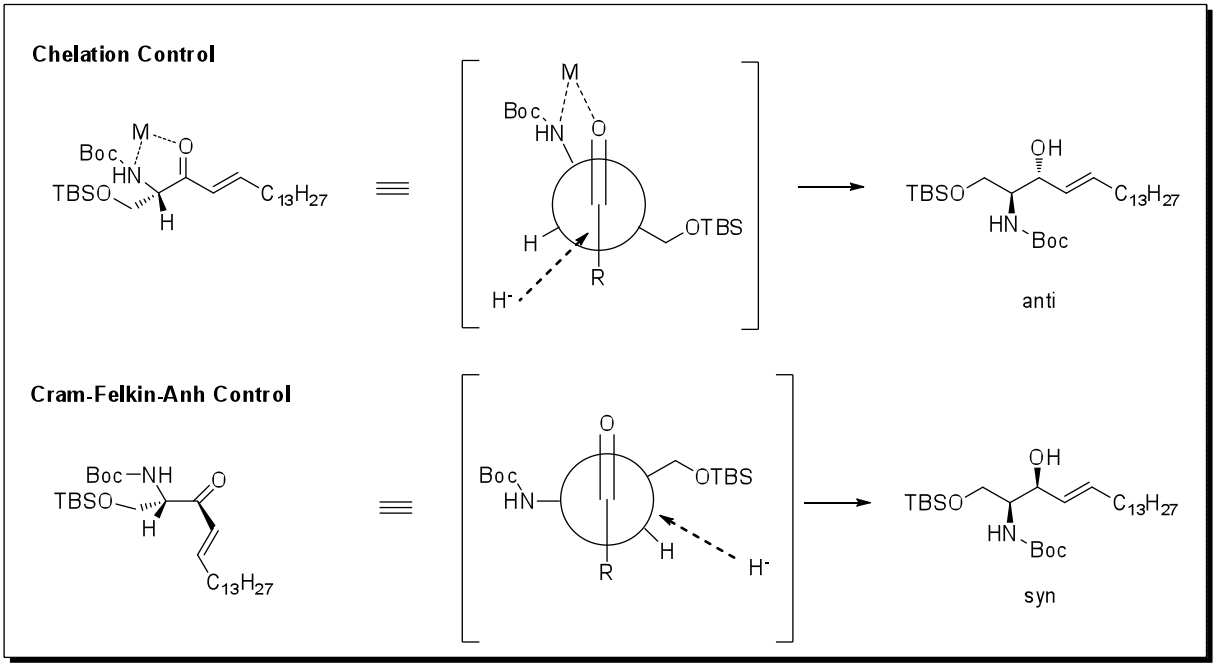
Felkin-Anh control results in a dihedral 90º angle between the ketone and the largest group, in this case the amine, thereby providing maximum electronic interactions which leads to syn diastereomer formation.[34] The similarity in size of the OTBS and NHBoc groups suggests that differentiation between the two groups is what leads to a lack of stereoselectivity using L-Selectride.

Figure 7: Lack of Selectivity Due to Similarities in Size of NHBoc and OTBS Groups



It is also possible that a cyclic-Cram model, in which the oxygen of the carbonyl group forms a hydrogen bond with the NHBoc group, directs a hydride attack to the less hindered *si* face, similar to the transition state shown in the chelation model in figure 8, except where M is a hydrogen. Alternatively, when using lithium tri-tert-butoxy-aluminohydride, chelation control (Figure 8) resulting from metal ion coordination with the amine nitrogen and carbonyl oxygen, creates a syn-periplanar structure allowing for formation of the anti diastereomer.[35] The inability to achieve complete syn reduction of the ketone using L-selectride prompted screening of various other reduction agents in an effort to gain better selectivity.

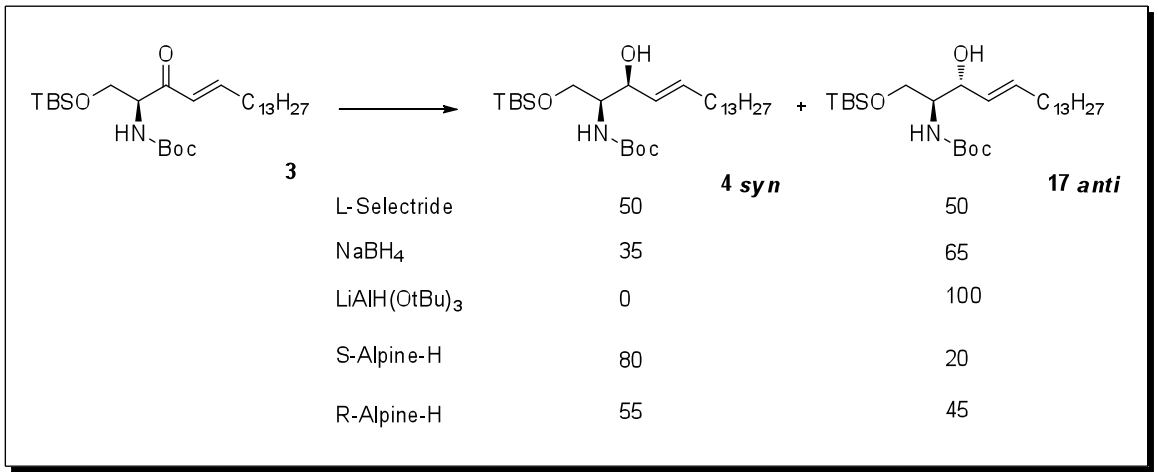
Figure 8: Stereocontrol



Hoffman, R. V.; Maslouh, N.; Cervantes-Lee, F. J. Org. Chem. 2002, 67, 1045-1056.34
Våbenø, J.; Brisander, M.; Lejon, T.; Luthman, K. J. Org. Chem. 2002, 67, 9186-9191.35

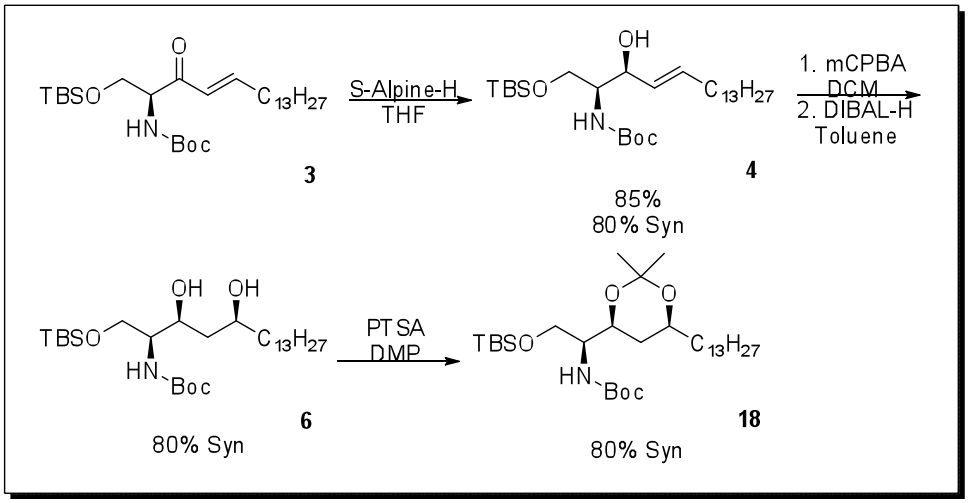
In order to reduce the ketone to yield the desired, syn diastereomer, various reducing agents were tested. Sodium borohydride reduction yielded diastereomers in a 65:35 anti/syn ratio. Corey-Bakshi-Shibata reagent (CBS) reduction produced a complex mixture of products which were unable to be isolated, thus the yields were not determined. As mentioned before, lithium tri-tert-butoxy-aluminohydride produced the undesired, anti diastereomer in 96% yield and 100% de. Literature from Våbenø suggested diastereoselective reduction of a chiral N-Boc-protected δ-Amino-α,β-unsaturated ketones could be achieved using aluminum and boron-based reducing agents, with S-Alpine Hydride yielding good results.[34] S-Alpine-Hydride yielded the best diastereoselective reduction with an 80:20 syn/anti ratio of diastereomers. R-Alpine-Hydride reduction of **3** yielded diastereomers in a 55:45 anti/syn ratio. Chromatographic separation of the 80:20 mixture of diastereomers was attempted but was not possible.

Scheme 5: Ketone Reduction Routes



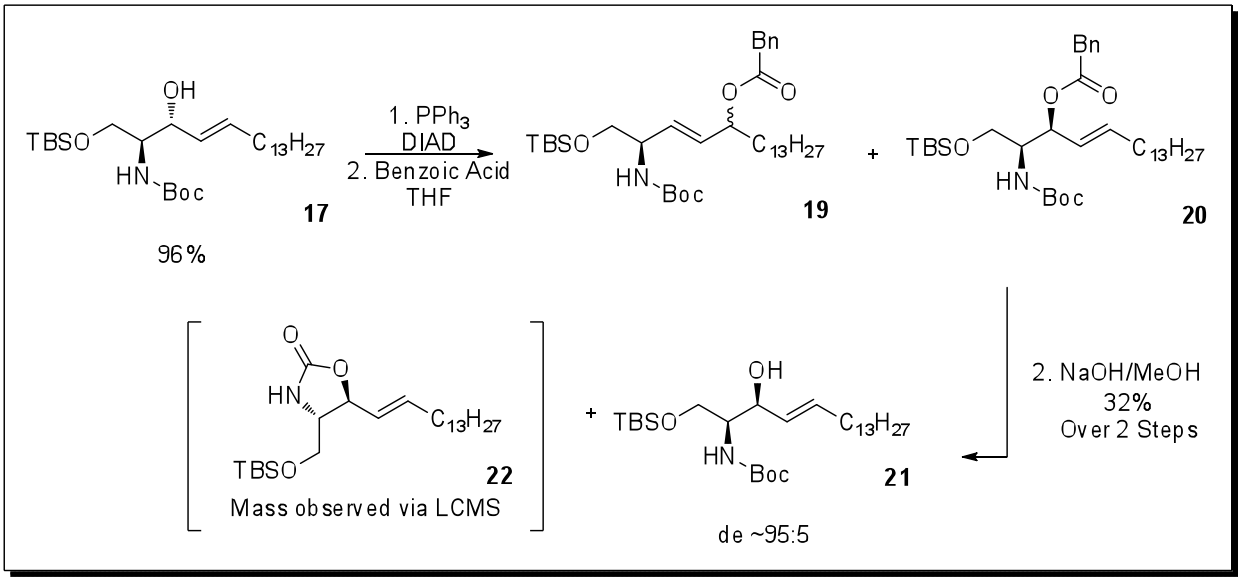
The 80:20 mixture was carried through subsequent steps with attempts to separate the mixture made at each step. The mixture underwent an epoxidation with mCPBA, followed by an epoxide ring opening with DIBAL-H to give **6**, and then acetonide formation using paratoluene sulfonic acid monohydrate and 2,2'-dimethoxypropane to give **18** but separation could never be achieved. In light of the difficulties attaining complete diastereoselectivity during reaction and the failure to separate the diastereomers, other solutions were explored in order to attain the allylic alcohol (4) in a pure form.

Scheme 6: Attempt to Separate Diastereomers



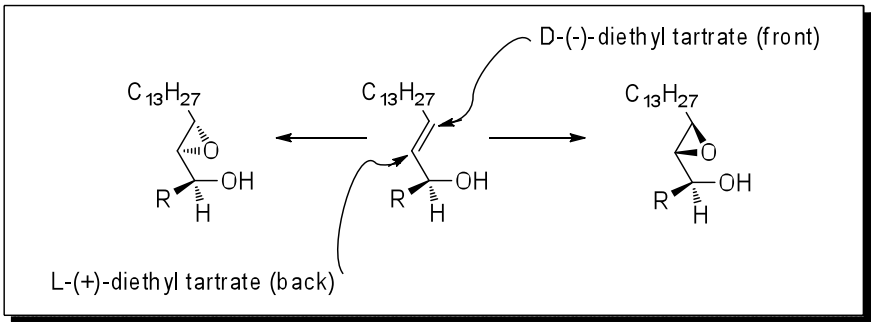
The first attempted solution was to perform a Mitsunobu inversion of the C3 hydroxyl group after stereoselective anti reduction by lithium tri-tert-butoxy-aluminohydride . Although it is known that a Mitsunobu inversion of an allylic alcohol might be problematic due to the possibility of rearrangements there are examples in the literature.[36] Mitsunobu inversion was performed with triphenylphosphine, diisopropyldiazodicarboxylate, and benzoic acid in THF. Although the ester (20) was formed and isolated there was also an appreciable amount of the rearranged product (19**)**. Upon separation and saponification of **20** to give **21**, it was shown that 4%-6% of the anti diastereomer still remained, which suggests that the rearrangement proceeds through a carbo cation intermediate. In addition, a byproduct was observed after saponification, which by ^{1}H NMR and LCMS showed loss of the tert-butyl portion of the Boc group and were consistent with cyclic carbamate formation (22). It is hypothesized that the addition of the water during the work-up raised the pH, thereby facilitating carbamate formation. If the residue resulting from the saponification is extracted with organic prior to the addition of water cyclic carbamate formation is significantly reduced. However, due to low yields (<20 %) and at best a 95:5 mixture of diasteromers this route was not further pursued.

Scheme 7: Mitsunobu Inversion



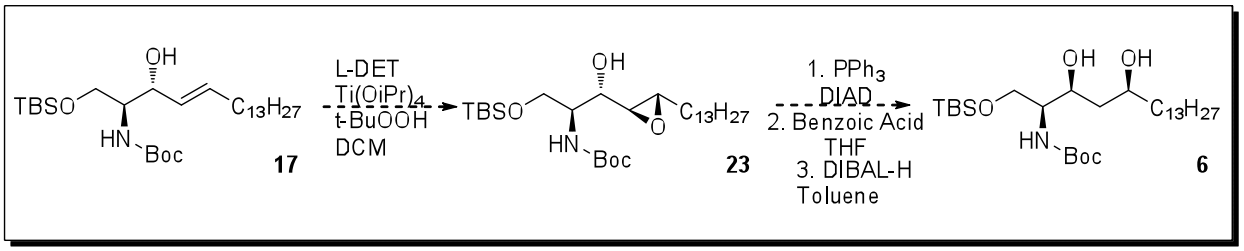
In a second effort to utilize the pure anti diastereomer (17) we reasoned that a Sharpless epoxidation of allylic alcohol (17) before Mitsunobu inversion could lead to the desired product (6), circumventing the issue of racimization. Diethyl-L-tartrate provided for stereoselective epoxidation from the backside of **17**.

Figure 9: Diastereoselective Facial Preference of Substrate



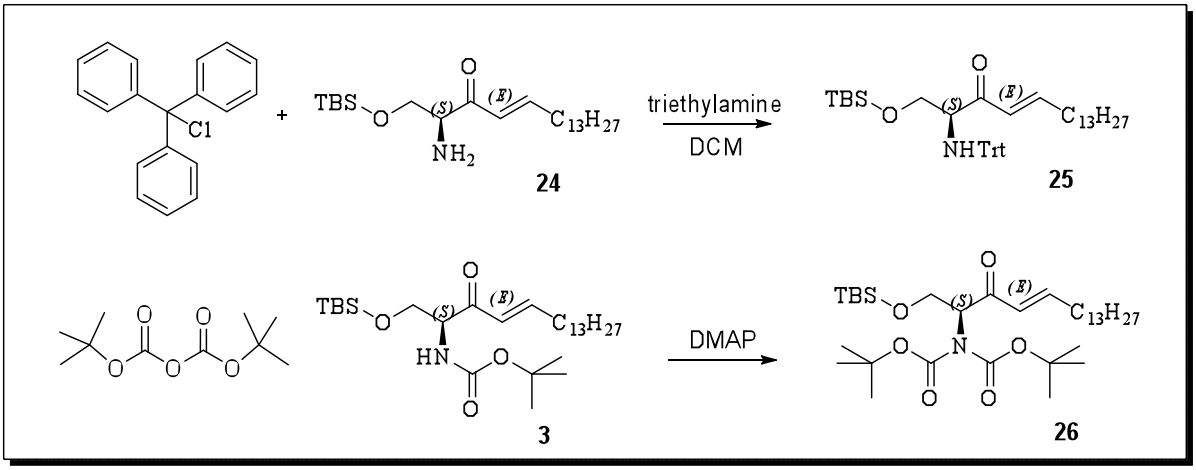
The Sharpless Epoxidation was attempted three times and each time it proceeded extremely slowly. The small amount of product which was observed after reaction times > 1 week appeared to be a 50:50 mixture of diastereomers. In light of the exceedingly long reaction times as well as the apparent lack of selectivity, this route was abandoned. A Shi epoxidation was also attempted and produced no product. It is possible that the difficulties encountered during epoxidation were due to the steric bulk of the substrate.

Scheme 8: Sharpless Epoxidation Followed by Mitsunobu Inversion



Based on the initial hypothesis that lack of selectivity of the reduction of **3** with L-selectride was due to the size similarity of the NHBoc and OTBS groups, it was postulated that increasing the size of the amine protecting group may increase stereoselectivity. Two attempts were made to increase the steric bulk of the amine. First, the Boc group was successfully removed from enone **3** with (trimethylsilyl) trifluoromethanesulfonate (TMSOTf) and **24** was subjected to trityl chloride and triethylamine. Product formation could not be monitered via LCMS due to the polarity of the molecule as well as the instability of the trityl protecting group. TLC did show a high Rf spot, and this spot was isolated. Spectral data was inconclusive and the yield was poor. This reaction pathway was abandoned in favor of a second strategy, in which we attempted to add an additional Boc group to increase the size of the nitrogen substituent. In an initial attempt 3 equivalents of Boc-Anhydride and 0.25 eq of DMAP produced approximately 50% conversion by TLC to the desired product (26) (confirmed by LCMS). In an attempt to increase the percent conversion the reaction was heated at 60 degrees Celsius for 10 minutes. Unfortunately, heating resulted in decomposition of the reaction mixture with loss of the TBS group observed as the major produce. A second attempt was allowed to run for 7 days with 5 equivalents of Boc-Anhydride and again the conversion by TLC yield did not exceed 50%, the product was not isolated due to the very small scale of the reaction. Optimization, isolation of the product, and subsequent reduction is in progress.

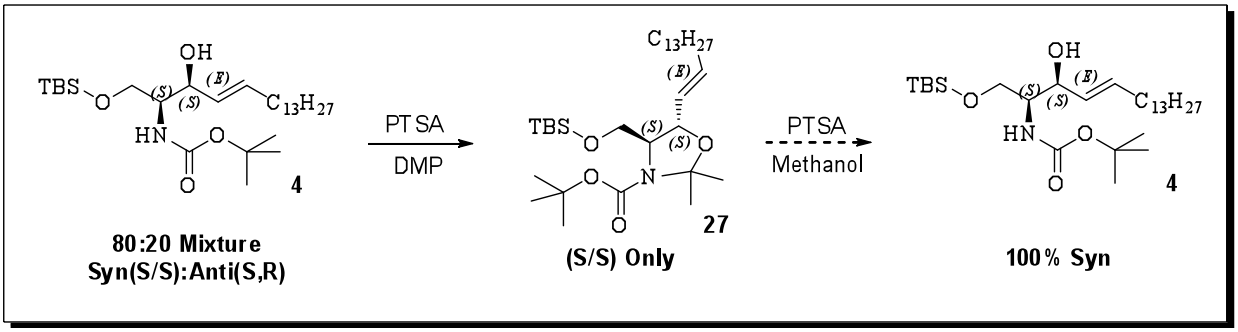
Scheme 9: Attempts to Increase Steric Bulk of Amine



Due to the inability to isolate the pure syn diastereomer, kinetic resolution of the 80:20 syn/anti mixture was pursued. In his 1993 Dissertation David Menaldino separates a chromatographically inseparable mixture of similar diastereomers by formation of an N-O acetonide where formation of the trans 5 membered N,O-acetonide was significantly faster than the cis.[37] Using p-toluenesulfonic acid (PTSA), the 80:20 mixture of **4** was reacted with dimethoxypropane (DMP) at ambient temperature to yield acetonide **27**. This first acetonide reaction proceeded too quickly, as almost all of the starting material was consumed in thirty minutes. Complicating things further, the product mass was not able to be observed by LCMS due to its very low polarity. Isolation of the highest spot (by TLC) yielded a compound that was consistent with product by ^{1}HNMR, but the diasteromeric ratio was inconclusive. We decided to remove the acetonide and test the product to see if the syn/anti ratio of **4** had increased. Unfortunately, during removal of the acetonide with PTSA and methanol (4 hrs at RT then heating at 40 degrees Celsius) the TBS group fell off. The product mass was observed via LCMS less the TBS group, possibly due to heat. In order to better observe the disappearance of the syn diastereomer during N,O-acetonide formation, enone X was reduced to give a more equal concentration of diastereomers with sodium borohydride. Reduction yielded a 35:65 syn/anti mixture of the reduced product (X). The

hemiaminal ether formation reaction was cooled to 0 in order to slow the rate of the reaction. The reaction of the product was monitored every thirty minutes by LCMS. The mass of the syn diastereomer disappeared while the mass of the anti diastereomer remained, though we were not able to monitor the conversion of starting material to product directly as the product was not observable by LCMS. ^{1}HNMR confirms the product was formed and appears to be one isomer, while the remaining starting material appears to be only the undesired anti diastereomer. Removal of the aminal and analysis of the diasteromeric ratio is in progress.

Scheme 10: Kinetic Resolution of Syn Diastereomer

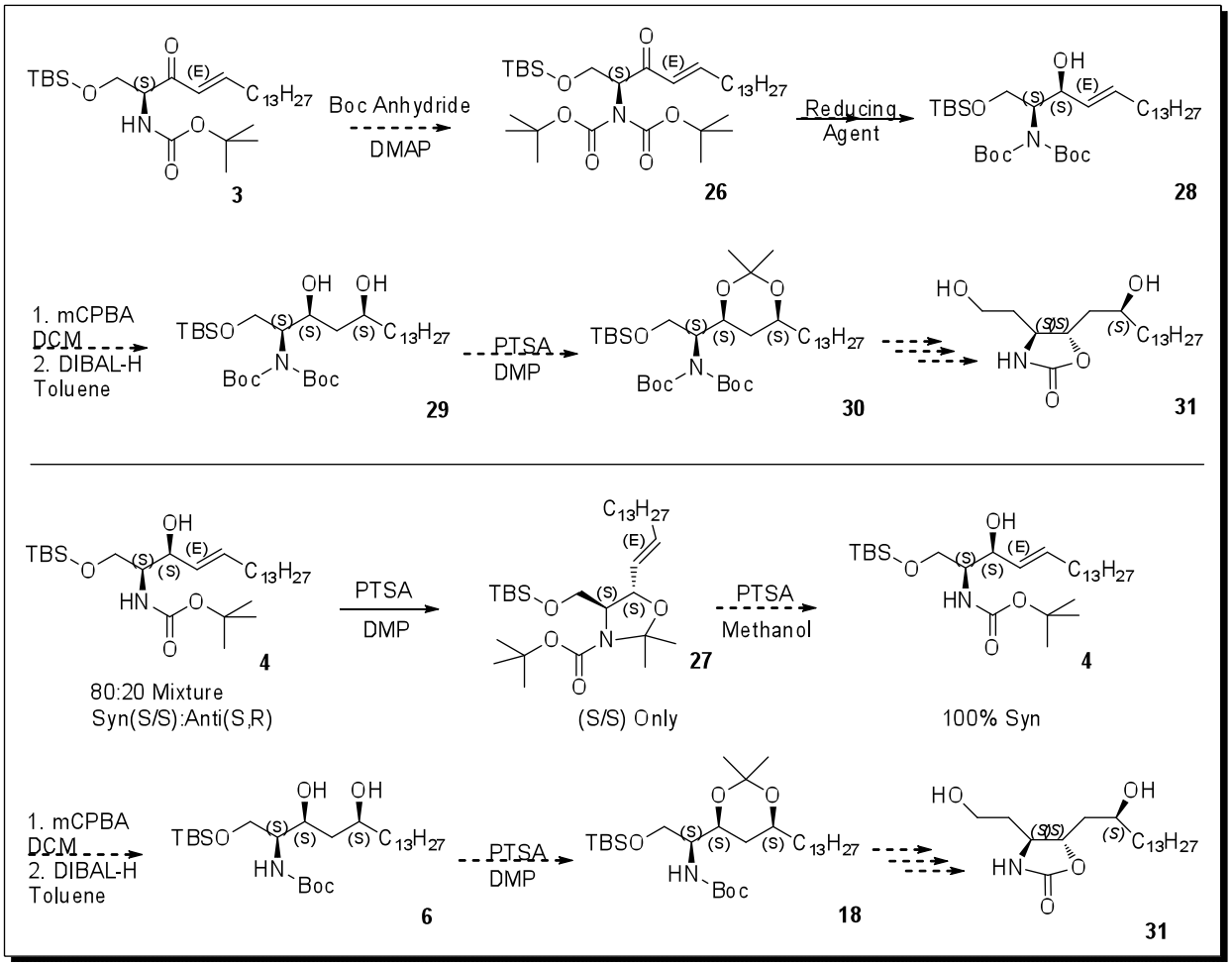


Future Work

Optimization of the diBoc protected enone (26) reaction is thought to be the most promising route. This allows us to continue to use the TBS protecting group on the primary alcohol and add the second boc group after DCC coupling, maximizing yields and theoretically achieving stereoselective reduction of the enone (26). The second promising route would be to devise an effective procedure for removing the aminal without also removing the TBS group. Depending upon which route will allow for a stereoselective, scalable synthesis of the reduced product (4), appropriate steps will be take to synthesize the desired intermediate (6), as shown in Scheme X. In the event that neither solution works or allows the synthesis of large quantities of material, replacement of the serine oxygen protecting

group will be attempted. Possible less bulky substitutes that are compatible. are the benzyl, *p*-methoxy benzyl and, Methoxymethyl ether (MOM) group.

Scheme 11: Proposed Synthetic Methods for Intermediate (6)



Conclusion

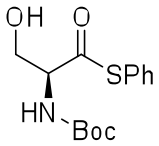
Imbalance in the sphingolipid rheostat governs cell proliferation, apoptosis, and cell signaling, providing an exciting drug discovery opportunity. Recently, compounds targeting the sphingolipid pathway have been synthesized and are in clinical trials, signifying the importance of this class of lipids to medicinal chemistry. The Liotta group has effectively synthesized Enigmol, a sphingosine

analogue efficacious against several cancers and malaria. Attempts to discover novel analogues encourages exploration of the head group of Enigmol. Efforts to synthesize a common intermediate with a primary oxygen, to which various functional groups can be added, have proved unsuccessful thus far. Using Leibeskind chemistry, the enone (3) was successfully synthesized in multi-gram quantities. Reduction of the enone proved problematic due to lack of stereoselectivity and the subsequent inability to separate the similar diastereomers. Attempts to utilize the pure anti diastereomer reduced product (17) and the 80:20 syn/anti mixture of reduced products (4) resulted in poor yields and product mixtures. Future optimization of the diboc and acetonide reactions should result in pure reduced product (4), which can then be taken forward to yield the desired intermediate (6) in substantial yields.

Experimental Details

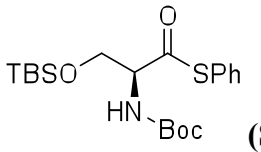
General Experimental: All reactions were run under inert atmosphere unless otherwise stated. All chemicals used were purchased from commercial sources and used as received, unless otherwise stated. All solvents termed "dry" were dried via a Glass Contour solvent purification system (SG Water, USA LLC), or purchased as anhydrous. ^{1}H NMR spectra were obtained on a 400 or 600 MHz Varian Spectrometer, referenced to residual solvent or for chloroform to TMS. ^{13}C NMR spectra were taken on a 100 or 150 MHz Varian Spectrometer referenced to the solvent carbon signal. High resolution mass spectrometry was performed by the Emory University Mass Spectrometry Center (Dr. Fred Strobel). Optical rotation was measured on a Perkin Elmer 341 Polarimeter. Microwave reactions were performed in a Biotage Initiator microwave synthesizer. Chiral HPLC was performed on a Varian ProStar using a Chiral Technologies Inc ChiralPak® AD-H (0.46cm X 25cm) column, at a flow rate of 1 mL/min.

Note: Enantiomeric excess was determined by chiral HPLC by comparision of the other enantiomer which for each compound was synthesized in parallel.

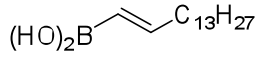


(S)-S-phenyl 2-(tert-butoxycarbonylamino)-3-hydroxypropanethioate (15): Thiophenol (6.18 ml, 60.2 mmol) was added to hydroxybenzotriazole hydrate (4.61 g, 30.1 mmol) and (S)-2-(tert-butoxycarbonylamino)-3-hydroxypropanoic acid (6.179 g, 30.1mmol) in dry ethyl acetate (300 ml) at 0 °C. Dicyclohexylcarbodiimide (6.52 g, 31.6 mmol) was added slowly, in portions, and solution was stirred for 20 hours allowing to warm to ambient temperature. 3 ml of 50% acetic acid in ethyl acetate was added and then the reaction mixture was cooled to 0 °C, filtered through a plug of celite and the solvent removed in vacuo. The product was tritrated in hexanes to remove the excess

thiophenol and the resulting solid was filtered, then purified by chromatography (silica, 120g, 0-40% EtOAc/Hex. over 36 minutes) to yield 8.06 g (90 %) of a white solid. 1H NMR, 13C NMR, and LCMS were consistent with the literature[32] with very slight dicyclohexylurea contamination. Optical rotation $[a]^{20}_{D}$= -80.5 deg (c = 1.68, $CHCl_3$) Lit. $[a]^{20}_{D}$= -85.2 (c = 0.99, $CHCl_3$). Chiral HPLC: 98.4% ee (AD-H, 10% iPrOH:Hex). Product was used without further purification.

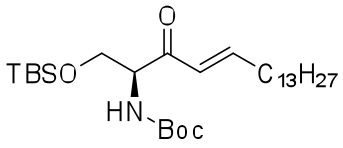


(S)-S-phenyl 2-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsilyloxy)propanethioate (1): 4-methylmorpholine (3.03 ml, 27.6 mmol) was added to a solution of tert-butylchlorodimethylsilane (19.12 ml, 110 mmol), N,N-dimethylpyridin-4-amine (0.674 g, 5.52 mmol), (S)-S-phenyl 2-(tert-butoxycarbonylamino)-3-hydroxypropanethioate (X) (8.2038 g, 27.6 mmol), and DMF (138 ml) at 0 °C. The reaction mixture was stirred warming to room temperature over 1 hour. TLC shows complete reaction. Diluted with 600 mL of EtOAc and washed with 0.1 M HCl, sat. $NaHCO_3$, and brine, dried over magnesium sulfate and concentrated to give an oil. Purified via chromatography 0-5% EtoAc/Hex. To give 10.08 g (89%) of a clear/slightly yellow oil. . 1H NMR, 13C NMR, and LCMS were consistent with the literature.[32] Chiral HPLC: 99.0 % ee (AD-H, 10% iPrOH:Hex).

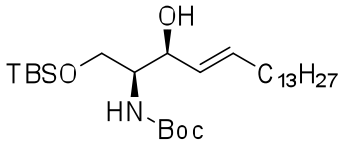


(E)-pentadec-1-enylboronic acid (2): To pentadec-1-yne (20 ml, 76 mmol) and dichloromethane (83 mL) was added dibromo(dimethylsulfonio)hydroborate (13.27 ml, 80 mmol) dissolved in 20 ml DCM, at 0 °C. The solution was stirred, allowing to warm to room temperature, for 7 hrs. The solution was cooled to 0 °C, 40 mL of ether was added and then 40 mL of cold water was

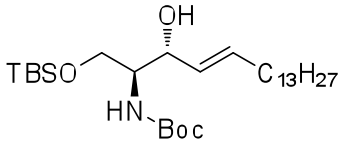
added carefully over ~10 min. The resulting solution was stirred for 1 hr then cooled to 0 °C. The resulting precipitate was filtered off and dissoved in EtOAc (600 mL) and washed with sat. sodium bicarbonate (600 mL). The aqueous phase was extracted with EtOAc (400 mL) and the organics were combined, dried over magnesium sulfate and concentrated to give 4.30 g of a white solid (77%). 1H NMR, 13C NMR, and LCMS were consistent with the literature.[32]



(S,E)-tert-butyl 1-(tert-butyldimethylsilyloxy)-3-oxooctadec-4-en-2-ylcarbamate (3): To an argon flushed flask was added: (S)-S-phenyl 2-(tert-butoxy carbonylamino)-3-(tert-butyldimethylsilyloxy)propanethioate (X) (4.53 g, 11.01 mmol), (E)-pentadec-1-enylboronic acid (X) (4.76 g, 18.71 mmol), (thiophene-2-carbonyloxy)copper (3.57 g, 18.71 mmol), Tris(dibenzylideneacetone)dipalladium(0) (0.252 g, 0.275 mmol) and THF (110 ml). Triethyl phosphite (0.383 ml, 2.201 mmol) was then added and the reaction mixture was stirred for 6 hrs. The solvent was removed under reduced pressure, and the resulting residue was taken up in EtOAc (500 mL), filtered through a pad of celite to remove excess copper and then washed with 2% wt ammonium hydroxide (2 x 200 mL), 0.1 M HCl (2 x 200 mL), sat. sodium bicarbonate (1 x 250 mL), and brine (1 x 150 mL). The organic was dried over magnesium sulfate and concentrated to give a brown residue. The residue was purified via chromatography (dry load, 120 g, 0-10% over 25 min) to give 5.04 g (89%) of a light yellow, oily liquid. 1H NMR, 13C NMR, and LCMS were consistent with the literature. Chiral HPLC shows 99% ee (AD-H, 4% iPrOH:Hex).

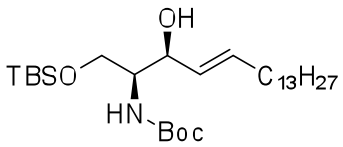


tert-Butyl (2S,3S,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxyocta dec-4-en-2-ylcarbamate (4): (S,E)-tert-butyl 1-(tert-butyldimethylsilyloxy)-3-oxooctadec-4-en-2-ylcarbamate (X) (1.0 g, 1.95 mmol) was dissolved in tetrahydrofuran (72.4 mL) and cooled to -78 °C. (S)-Alpine Hydride 0.5 M in THF (4.30 ml, 2.15 mmol) was added slowly to the reaction mixture over 1/2 hour via syringe pump and the solution was stirred at -78 °C for 1 hr. Quenched with 10% citric acid (10 mL) and partitioned between ether (250 mL) and brine (250 mL). The organics were then separated, dried over magnesium sulfate and concentrated to give a clear oil. Purified via chromatography (silica, 80 g, 0-10% EtOAc:Hex over 15 min) to give 851 mg (85%) of a clear oil. LCMS shows 80/20 inseparable mix of diastereomers. 1H NMR is consistent with the product and shows both diastereomers present (80:20).



tert-Butyl (2S,3R,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxyocta dec-4-en-2-ylcarbamate (17): A solution of (S,E)-tert-butyl 1-(tert-butyldimethylsilyloxy)-3-oxooctadec-4-en-2-ylcarbamate (X) (1.0 g, 1.95 mmol) in Ethanol (32.6 ml) was cooled to -78 °C. Lithium tri-tert-butoxy-aluminohydride (3.91 ml, 3.91 mmol) was added and solution was stirred for 4 hrs at -78 °C. Quenched with 10 mL of 0.1 M HCl, diluted into EtOAc (150 mL) and washed with sat. sodium carbonate. Washed with brine, dried over magnesium sulfate and concentrated to give a yellow oil. Purified via chromatography (silica, 40g, 0-50% EtOAc: Hex over 16 min) to give 964 mg (96%) of a clear oil. 1H NMR, 13C NMR, and LCMS were consistent with the literature.[32]

Mistunobu Inversion



tert-Butyl (2S,3S,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxyocta dec-4-en-2-ylcarbamate (4): tert-Butyl (2S,3R,E)-1-(tert-butyldi methylsilyloxy)-3-hydroxyoctadec-4-en-2-ylcarbamate (X) (964 mg, 1.876 mmol) and triphenylphosphine (590 mg, 2.251 mmol) were dissolved in dry THF (6 mL) and added to a solution of diisopropyl azodicarboxylate (0.443 ml, 2.251 mmol) and benzoic acid (275 mg, 2.251 mmol) in THF (2 mL) at 0 °C. The mixture was stirred for 1 hr at 0 °C. The solvent was removed under reduced pressure and the resulting oil was purified by chromatography (silica, dry load, 4 g, 0-10% EtOAc:Hex over 16 min) to yield 653 mg as a yellow oil (Rf= 0.5 ,10% EtOAc:Hex), 1H NMR and LCMS are consistent with the benzoate ester. Subjected the material to a solution of 5% NaOH in MeOH (10 mL) and let stir for 3 hrs. Concentrated and partitioned between water and ether, dried the organic fraction over magnesium sulfate, and concentrated to give a white residue. Purified via chromatography (silica, solid load, 40 g, 0-40% EtOAc:Hex over 16 min) to give a clear oil. 1H NMR shows 5% of the undesired diastereomer.

References

1. Thudichum, J. L. W. *A Treatise on the Chemical Constitution of Brain*. Bailliere, Tindall and Cox: London, **1884.**

2. Shapiro, D.; Segal, K., *Journal of the American Chemical Society* **1954,** 76, 5894.

3. Merrill Jr., A; Wang, M; Park, M; Sullards, M. (glyco)sphingolipidology: an amazing challenge and opportunity for systems biology. *TRENDS in Biochemical Sciences* **2007,** *32* (10), 457-68.

4. Degroote, S. The cell biology of glycosphingolipids. Semin. *Cell Dev. Biol.* **2004,** 15, 375–387.

5. Ledeen, R.; Wu, G. Sphingolipids of the nucleus and their role in nuclear signaling. *Biochim. Biophys. Acta.* **2006,** 1761, 588–598.

6. Hannun, Y.; Loomis, C.; Merrill, Jr., A; Bell, R. Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. *J Biol Chem*. **1986,** 261(27):12604-9.

7. Lodish, H.; Berk, A.; Kaiser, C.; Krieger, M.; Scott, M.; Zipursky, S.; Darnell, J. Molecular Cell Biology, 5th ed.; **2004**. *W. H. Freeman and Company*: New York. 8 Merrill AH Jr. & Sweeley CC (1996).

8. Jayadev, S.; Liu, B.; Bielawaka, A.; Lee, J.; Nazaire, F.; Pushkarevs, M.; Obeid, L.; Hannun, Y.; Role for ceramide in cell cycle arrest. *J. Biol. Chem.* **1995,** 270, 2047-2052.

9. Subbaiah, P.; Sargis, R. Sphingomyelin: a natural modulator of membrane homeostasis and inflammation. Medical Hypotheses. **2001,** (57) 2:135-8.

10. Arana, L.; Gangoiti, P.; Ouro, A.; Trueba, M.; Gómez-Muñoz, A. Ceramide and ceramide 1-phosphate in health and disease. *Lipids Health Dis*. **2010,** 9:15.

11. Simbulan, M.; Tamiya-Koizuma, K.; Suzuki, M.; Shoji, M.; Taki, T.; Shonen, Y. Sphingosine Inhibits the Synthesis of RNA Primers by Primase in vitro. *Biochemistry* **1994,** *33* (30), 9007-9012.

12. Cuvillier, O.; Pirianov, G. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature* **1996,** 381: 800-803.

13. Huwiler, A.; Pfeilschifter, J. Altering the Sphingosine-1-Phosphate/Ceramide Balance: A Promising Approach for Tumor Therapy. *Curr. Pharm. Design*. **2006**, *12*, 4625 – 4635.

14. Fyrst, H.; Saba, J. An update on sphingosine-1-phosphate and other sphingolipid mediators. *Nat. Chem Biol.* **2010**, *17*, 489 – 497.

15. Cuvillier, O. Sphingosine in apoptosis signaling. Biochim. Biophys. Acta. **2002,** 1585 (2-3): 153-162.

16. Padrón, J. Sphingolipids in Anticancer Therapy. *Curr. Med. Chem*. **2006,** *13*, 755 – 770.

17. Curfman, C.; Kirkland, K.; Merrill, A. Recent anticancer agents targeting sphingolipid pathways. *Exp. Opin. Therap. Patents.* **2006**, *16*, 1129 – 1147.

18. Gilenya. (2010, September). *Highlights of prescribing information*. http://www.pharma.us.novartis.com/product/pi/pdf/gilenya.pdf

19. French, K. Pharmacology and Antitumor Activity of ABC294640, a Selective Inhibitor of Sphingosine Kinase-2. J. Pharm. Exp. Ther. **2010,** 333, 129 – 139.

20. Lexicon (2010). *Lx2931*. http://www.lexicon-genetics.com/pipeline/clinical-trials/lx2931.html

21. Actelion (2009, Decebmer 8). Actelion's selective s1p1 receptor agonist moves into advanced clinical development in psoriasis - phase iib dose-finding study in multiple sclerosis already ongoing. http://www1.actelion.com/en/journalists/news-archive.page?newsId=1359631

22. Desai, K; Sullards, M.; Allegood, J.; Wang, E.; Schmelz, E.; Hartl, M.; Humpf, H.; Liotta, D.; Peng, Q.; Merrill Jr., A. Fumonisins and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. *Biochim. Biophys. Acta.* **2002,** 1585: 188-92.

23. Zitomer, N.; Mitchell, T.; Voss, K.; Bondy, G.; Pruett, S.; Garnier-Amblard, E.; Liebeskind, L.; Park, H.; Wang, E.; Sullards, M.; Merrill Jr., A.; Riley, R. Ceramide Synthase Inhibition By Fumonisin B_1 Causes Accumulation Of 1-Deoxysphinganine: A Novel Category Of Bioactive 1-Deoxysphingoid Bases And 1-Deoxydihydroceramides Biosynthesized By Mammalian Cell Lines And Animals *J. Biol. Chem.* **2009,** 284: 4786-4795.

24. Nimkar, S.; Menaldino, D.; Merrill Jr., A.; Liotta, D. A stereoselective synthesis of sphingosine, a protein kinase c inhibitor. *Tetrahedron Lett.* **1988**, *29(25*). 3037-3040.

25. Schmelz, E.; Bushnev, A.; Dillehay, D.; Liotta, D.; Merrill Jr., A. Suppression of aberrant colonic crypt foci by synthetic sphingomyelins with saturated or unsaturated sphingoid base backbones. *Nutr. Cancer*. **1997**, *28(1*). 81-85.

26. Birt, D.; Merrill Jr., A.; Barnett, T.; Enkvetchakul, B.; Pour, P.; Liotta, D.; Geisler, V.; Menaldino, D.; Schwartzbauer, J. Inhibition of skin carcinomas but not papillomas by sphingosine, N-methylsphingosine, and N-acetylsphingosine. *Nutr. Cancer.* **1998**, *31(2*). 119-126.

27. Menaldino, D.; Bushnev, A.; Sun, A.; Liotta, D.; Symolon, H.; Desai, K.; Dillehay, D. L.; Peng, Q.; Wang, E.; Allegood, J.; Pruett, S.; Sullards, M.; Merrill Jr., A. Sphingoid bases and de novo

ceramide synthesis: enzymes involved, pharmacology and mechanisms of action. *Pharmacol. Res.* **2003**, *47*. 373-381.

28. Bushnev, A.; Baillie, M.; Holt, J.; Menaldino, D.; Merrill Jr., A.; Liotta, D. An efficient asymmetric synthesis of Enigmols (1-deoxy-5-hydroxysphingoid bases), an important class of bioactive modulators. *ARKIVOC*. **2010**, *viii*. 263-277.

29. Symolon, H.; Bushnev, A.; Peng, Q.; Ramaraju, H.; Mays, S.; Allegood, J.; Pruett, S. T.; Sullards, M.; Petros, J.; Dillehay, D.; Liotta, D.; Merrill Jr., A. Enigmol: a novel sphingolipid analog with anti-cancer activity against cancer cell lines and *in vivo* models for intestinal and prostate cancer. *Unpublished*.

30. Labaied, M. *Malar. J.* **2004**, *3*, 49-58.

31. Hanada, K. *J. Exp. Med.* **2002**, *195*, 23-34.

32. Liebeskind, L.; Yang, H. Concise and Scalable Synthesis of High Enantiopurity (−)-d-erythro-Sphingosine Using Peptidyl Thiol Ester−Boronic Acid Cross-Coupling. *Organic Letters* **2007,** *9* (16), 2993-2995.

33. Brown, H.; Basavaiah, D.; Bhat, N.; A convenient synthesis of alkyldibromoboranes and dialkylbromoboranes via hydroboration-redistribution. *Organometallics* **1983,** 2 (10), 1311-1316.

34. Våbenø, J.; Brisander, M.; Lejon, T.; Luthman, K. Diastereoselective Reduction of a Chiral N-Boc-Protected δ-Amino-α,β-unsaturated γ-Keto Ester Phe-Gly Dipeptidomimetic. *The Journal of Organic Chemistry* **2002,** *67* (26), 9186-9191.

35. Hoffman, R.; Maslouh, N.; Cervantes-Lee, F. Highly Stereoselective Syntheses of syn- and anti-1,2-Amino Alcohols. *The Journal of Organic Chemistry* **2002,** *67* (4), 1045-1056.

36. Evans, P.; Cui, J.; Gharpure, S.; Polosukhin, A.; Zhang, H. Enantioselective Total Synthesis of the Potent Antitumor Agent (—)-Mucocin Using a Temporary Silicon-Tethered Ring-Closing Metathesis Cross-Coupling Reaction. *J Am Chem Soc* **2003,** 125(48):14702-3.

37. Menaldine, D. Asymmetric syntheses of amino alcohols as bioactive compounds: Chapter One. Syntheses of sphingolipid analogues as inhibitors of the protein kinase C enzyme system. Chapter Two. Syntheses of fumonisin analogues as non-metabolizable protein kinase C inhibitors and as proposed ceramide synthase inhibitors. *Dissertation (1993): 196.*