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 known, 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.

Signature:

_____	_____
Abdul M. Kouanda	04/20/2010

Synthesis of Novel Anti-Cancer Compounds Based on Sphingolipid Analogs

by

Abdul M. Kouanda

Adviser: Frank E. McDonald

Department of Chemistry

_____
Frank E. McDonald
Adviser

_____
Brenda Harmon
Committee Member

_____
Darrel Stokes
Committee Member

_____
04/20/2010

Synthesis of Novel Anti-Cancer Compounds Based on Sphingolipid Analogs

By

Abdul M. Kouanda

Adviser: Frank E. McDonald

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 Science with Honors

Department of Chemistry

2010

Abstract

Synthesis of Novel Anti-Cancer Compounds Based on Sphingolipid Analogs

By Abdul M. Kouanda

Sphingosine is a compound that induces apoptosis in cancer cells, including androgen independent prostate cancer. It fails in that its actions are reversed upon phosphorylation by sphingosine kinase. A collaboration with Dr. Dennis C. Liotta of Emory University and Dr. Alfred H. Merril of Georgia Tech have developed a 1-deoxysphingolipid derivative, enigmol. Enigmol is more potent against tumor cells than sphingosine because it is not metabolized as quickly. In this thesis, a proposed synthesis of novel 1-deoxysphingolipid compounds as potential anti-cancer compounds, is explored. The building blocks of these novel analogs, including their direct precursor, have been successfully synthesized and fully characterized by ^{1}H NMR, ^{13}C NMR, FT IR, and HRMS. Our proposed synthesis approach has been a difficult, but successful method and we are at the dawn of the synthesis of the first of these analogs.

Synthesis of Novel Anti-Cancer Compounds Based on Sphingolipid Analogs

By

Abdul M. Kouanda

Adviser: Frank E. McDonald

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 Science with Honors

Department of Chemistry

2010

Acknowledgements

Before I began working in McDonald lab, my practical knowledge of the Chemistry lab was essentially zero. My thought process was that research experience gives you an advantage in the medical school application process, I am Chemistry major, so I might as well do research in Chemistry. After almost two years in the lab, I have become so fond of the discipline, and I am now even considering pursuing the MD-PhD in Chemistry. I have learned and gained so much experience in research with the help of my fellow lab mates.

First and foremost I would like to thank Dr. McDonald for giving me the opportunity to work in the lab; I am most grateful. I would also like to thank Bradley Balthaser and Claney Pereira for teaching and advising me time and time again. It is through these individuals' efforts that my work was possible. Last but not least, my partner on this project, Alex Wein, for his advice as well.

Thank you Dr. Stokes, and Ms. Harmon for taking the time to be on my committee; I appreciate this very much and I hope you enjoy my thesis.

Table of Contents

Figures

"The process of *scientific discovery* is, in effect, a continual flight from *wonder*."

Albert Einstein.

1. Introduction

According to the American Cancer Society, prostate cancer is the second leading cause of cancer death in men in the United States, and 1 man in 6 will be diagnosed during his lifetime.[1] There are currently a number of therapeutic options for treating prostate cancer when detected early; including radiation therapy, chemotherapy, and hormone therapy. The results of early detection have given victims of prostate cancer a prognosis of 100% survival within five years and 91% survival within 10 years. [1] However, if the cancer is not detected early, it may proceed into an advanced form known as metastic androgen independent prostate cancer. This form of prostate cancer is very resilient and chemotherapy resistant, leading to a survival rate of approximately 33% after five years.[2] Certainly this is a significant health issue and thus we have taken steps to address it by developing a family of anti-cancer agents based on analogs of cell membrane sphingolipids. I will begin this introductory discussion with the basic biochemistry of sphingolipids, followed by an analysis of current sphingolipid analogs explored as anticancer agents, and finally our proposed synthesis approach for developing novel analogs.

The cell membrane is comprised of three classes of lipids: glycerol-lipids, sterols, and sphingolipids. Sphingolipids are amphipathic molecules, containing both

[1] *American Cancer Society*. www.cancer.gov
[2] Kent, K. D.; Clubbs, E. A.; Harper, W. J.; Bomser, J. A. *Lipids* **2008**, *43*, 143.

hydrophobic and hydrophilic properties. They generally consists a sphingoid base, usually sphingosine (**Figure 1A**), attached by an amide bond on carbon 2 to a fatty acid, as in the simplest case of the molecule ceramide (**Figure 1B**). [3] There is a great deal of structural variety among sphingolipids: five sphingoid bases are known in mammalian cells, with over 20 different fatty acid chains that can be attached. [3]

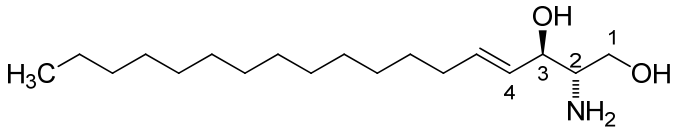


Figure 1A Sphingosine

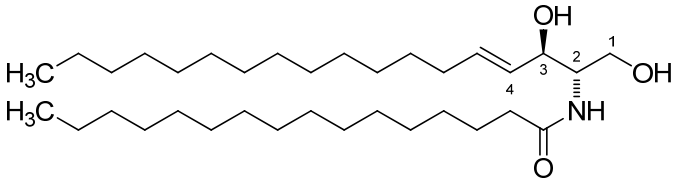


Figure 1B Ceramide

Sphingolipid synthesis in the cell begins with the condensation of palmitoyl-CoA and L-serine in the endoplasmic reticulum to form 3-ketosphinganine; which is then reduced to sphinganine. A 14-26 carbon fatty acid chain is then linked via an amide bond to the amino group on carbon 2 to form dihydroceramide. A head group (e.g. carbohydrate) is then added to the primary hydroxyl to form the native sphingolipid structure. In eukaryotes a 4, 5-trans alkene is introduced to form ceramide, the

[3] Humpf, H.-U.; Schmelz, E.-M.; Meredith, F. I.; Vesper, H.; Vales, T. R.; Wang, E.; Menaldino, D. S.; Liotta, D. C.; Merrill, A. H. *Journal of Biological Chemistry* **1998**, *273*, 19060.

precursor to more complex sphingolipids. Sphingosine is formed from the hydrolysis of ceramide.[4] The biosynthetic pathway of sphingolipids is summarized in **Figure 2.**[5]

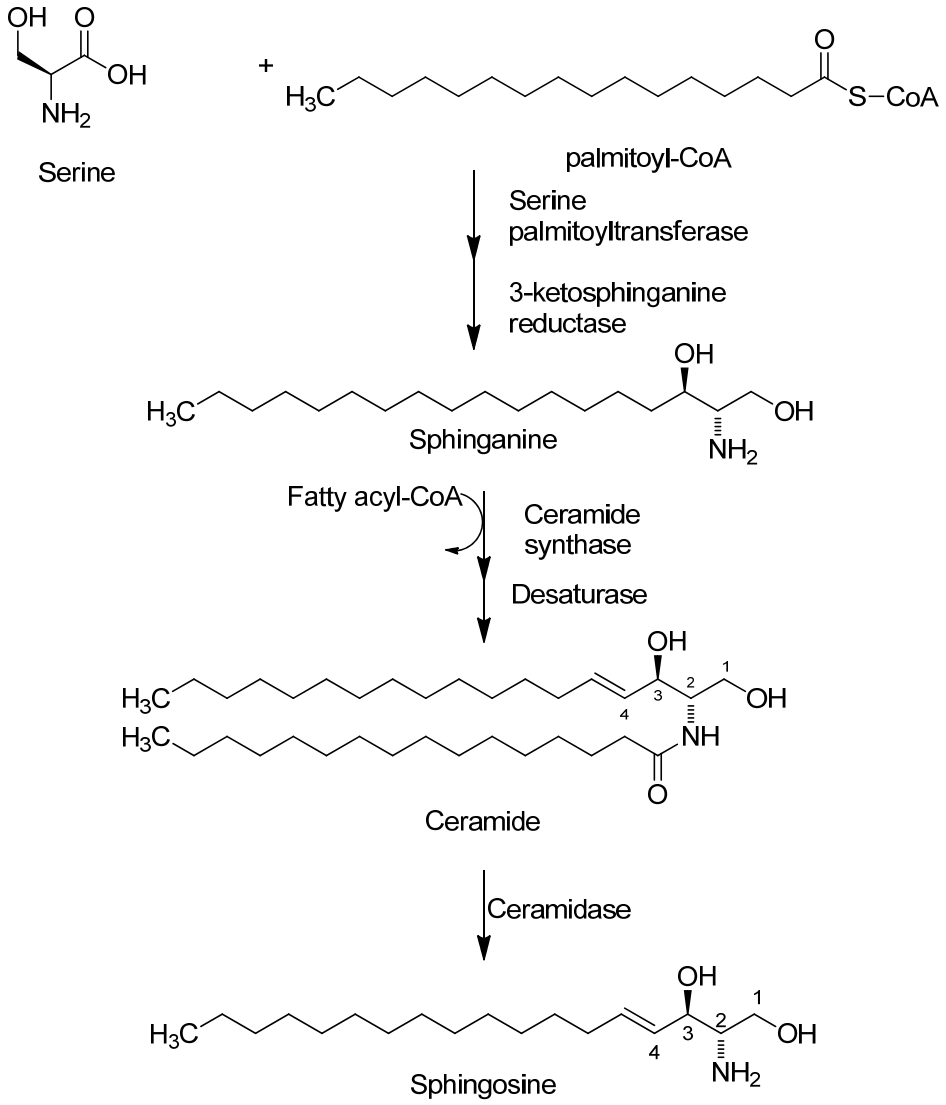


Figure 2 Sphingolipid Biosynthetic Pathway

[4] Zeidan, Y. H.; Hannun, Y. A. *Trends in Molecular Medicine* **2007**, *13*, 327.
[5] Menaldino, D. S.; Bushnev, A.; Sun, A.; Liotta, D. C.; Symolon, H.; Desai, K.; Dillehay, D. L.; Peng, Q.; Wang, E.; Allegood, J.; Trotman-Pruett, S.; Sullards, M. C.; Merrill, A. H. *Pharmacological Research* **2003**, *47*, 373.

In addition to maintaining cell membrane integrity, sphingolipids are now considered significant bioactive molecules that are responsible for cell differentiation, proliferation, cell-cell interactions that lead to inflammations, and transformations such as angiogenesis.[4] The key molecules central to these developments are ceramide, sphingosine, sphingosine-1-phosphate, and ceramide-1-phosphate.[6] Sphingosine and ceramide have been found to function as regulators of stress and as tumor suppressers, mediating apoptosis, growth, and differentiation.[6] They accomplish these affects by altering cell membrane fluidity and by altering signal transduction cascades leading to cell cycle arrest and apoptosis.[2] *In vivo,* sphingosine is phosphorylated on its primary hydroxyl by sphingosine kinase to form sphingosine-1-phosphate (**Figure 3)**.[6]

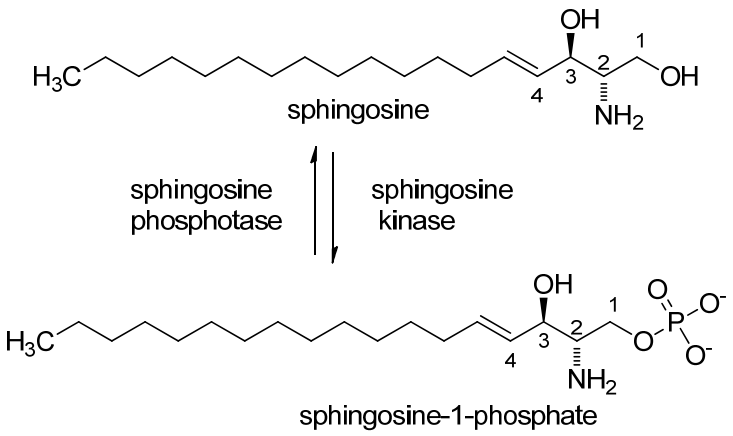


Figure 3 Addition of a phosphate group to sphingosine is the difference between tumor suppression and cell proliferation

Sphingosine-1-phosphate has been shown to be a tumor-promoting lipid, inhibiting apoptosis; and to be involved in cell proliferation, migration, angiogenesis, and

[6] Pchejetski, D.; Golzio, M.; Bonhoure, E.; Calvet, C.; Doumerc, N.; Garcia, V.; Mazerolles, C.; Rischmann, P.; Teissie, J.; Malavaud, B.; Cuvillier, O. *Cancer Research* **2005**, *65*, 11667.

mitogenesis.[6] Thus it is evident that the actions of the phosphorylated and unphosphorylated forms of these signal molecules are antagonistic and the role sphingosine kinase plays in maintaining balance is critical to the suppression of tumors.

In light of these findings, developments of synthetic sphingolipid analogs that may not be subject to the effects of sphingosine kinase are being explored. Safingol[7] and fingolimod[8] (**Figure 4)** are two synthetic sphingolipid analogs currently in clinical trials.

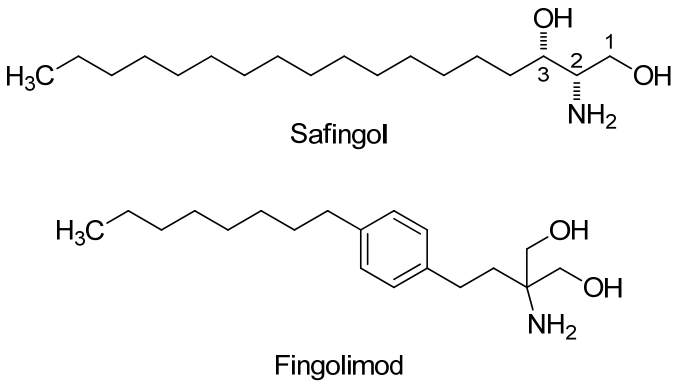


Figure 4 Sphingolipid analogs currently in clinical trials

Safingol is identical to sphingosine in chain length (18 Carbon). These compounds differ in that safingol has S stereochemistry on carbon 3 whereas sphingosine is R. Also, safingol is saturated and sphingosine contains a *trans* alkene. From these similarities, we would expect these two molecules to share similar biological activity, and this is the case. Also, safingol is a protein kinase C (PKC) inhibitor. PKC is a phospholipid dependent enzyme which plays a role in signal transduction pathways leading to the formation of tumors. When used in conjunction with the

---

[7]Maurer, B. J.; Melton, L.; Billups, C.; Cabot, M. C.; Reynolds, C. P. *Journal of the National Cancer Institute*. **2000**, *92*, 1897.

[8] Schmid, G.; Guba, M.; Papyan, A.; Ischenko, I.; Brückel, M.; Bruns, C. J.; Jauch, K. W.; Graeb, C. *Transplantation Proceedings* **2000,** *37*, 110.

chemotherapeutic agent doxorubicin (DOX) and mitomycin C, safingol enhanced the onset of apoptosis on cancer cell lines. However, when used independently, it did not show significant anti-cancer activity.[7] Safingol is currently in phase 1 clinical trials in conjunction with cisplatin (platinum based chemotherapy drug)[9] for the treatment of locally advanced or metastatic solid tumors.

Unlike sphingosine and safingol, fingolimod is achiral, is a 1-2 diol, and contains a benzene ring which conformationally constrains the center of this molecule. Fingolimod is an immunosuppressant which also induces apoptosis and prevents metastasis and tumor growth.[8] In a study of mice injected with Lewis Lung Cancer Carcinoma cells, fingolimod was shown to be an antiangiogenic dose dependent inhibitor of subcutaneous tumors.[8] The tumors of the experimental mice were less than half the size of the control mice at a dosage of 10 mg/kg.[8] Fingolimod is currently in a number of phase III trials for treatment of multiple sclerosis.

In addition to these sphingolipid analogs, a number of 1-deoxysphingolipid naturally occurring molecules have been discovered (**Figure 5)**. Among their physical differences are the absolute stereochemistries of the aminoalcohol, chain lengths, degree of unsaturation, alkyl substituents, and amphipathic nature. Clavaminol A[10] (**Figure 5)** differs from spisulosine[11] (**Figure 5)** in both chain length and stereochemistry. Rhizochalin[12] (**Figure 5)** is a bicephalic sphingolipid and halaminol C[13] (**Figure 5)** shows

---

[9] Rosenberg, B.; Vancamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, 222, 385.
[10] Aiello, A.; Fattorusso, E.; Giordano, A.; Menna, M.; Navarrete, C.; Munoz, E. *ChemInform* **2007**, *38*.
[11] Sánchez, A. M.; Malagarie-Cazenave, S.; Olea, N.; Vara, D.; Cuevas, C.; Díaz-Laviada, I. *European Journal of Pharmacology* **2008**, *584*, 237.
[12] Molinski, T. F.; Makarieva, T. N.; Stonik, V. A. *Angewandte Chemie* **2000**, 4076.

cis-unsaturation as opposed to the *trans*-unsaturation of sphingosine. Spisulosine has efficacy against androgen-independent prostatic carcinoma and lymphocyte androgen-dependent carcinoma.[11]

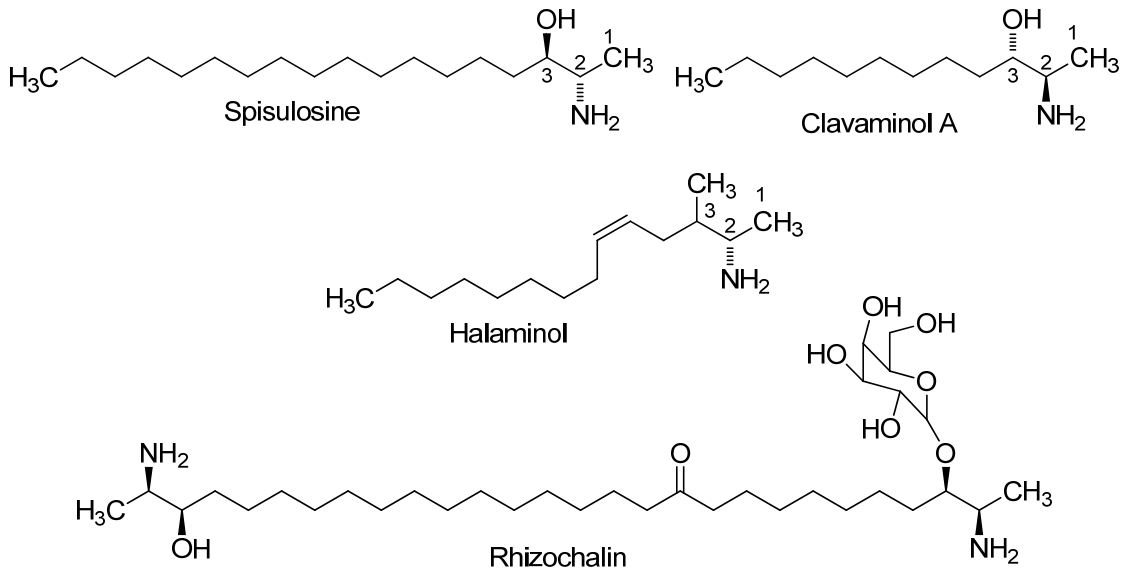


Figure 5 Naturally occurring 1-deoxysphingolipid products

These promising compounds inspired the collaboration of the laboratories of Professors Alfred H. Merrill of Georgia Institute of Technology and Dennis C. Liotta of Emory University, to develop 1-deoxysphingolipids as anti-cancer compounds. The first generation lead is Enigmol (**Figure 6),** which has the hydroxyl group switched from carbon 1 to carbon 5.[5]

[13] Clark, R. J.; Garson, M. J.; Hooper, J. N. A. *Journal of Natural Products* **2001**, *64*, 1568.

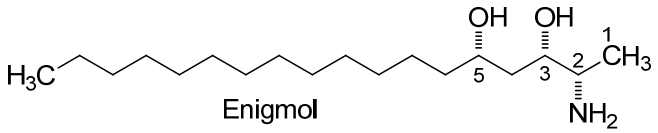


Figure 6 Structure of Enigmol

Enigmol has an identical 2,3-stereochemistry to safingol (**Figure 4)** and the same chain length as sphingosine. Enigmol is a very promising lead towards the synthesis of more efficient anti-cancer sphingolipid analogs for several reasons. The actions of sphingosine kinase are impeded due to the switch of the hydroxyl group from carbon 1 to carbon 5. Enigmol is thus a more potent agent because it is not metabolized as quickly, while still sharing many of the same biological roles of the naturally occurring sphingolipids. Enigmol was shown to be 2 to 5-fold more potent than sphingosine on androgen-independent prostate cancer cell cultures.[14] Also, *in vivo* studies of mice containing prostate cancer xenografts showed that enigmol significantly inhibited tumor growth at 10 mg/kg as opposed to the control mice, and that enigmol is an orally deliverable drug.[15]

[14] Humpf, H.-U.; Schmelz, E.-M.; Meredith, F. I.; Vesper, H.; Vales, T. R.; Wang, E.; Menaldino, D. S.; Liotta, D. C.; Merrill, A. H. *Journal of Biological Chemistry* **1998**, *273*, 19060.
[15] Ramaraju, H.; Bushnev, A.; Sun, C. Q.; Liotta, D. C.; Merrill, A. H.; Petros, J. A. *The Journal of Urology* **2008**, *179*, 46.

2. RESEARCH METHODS TO BE APPLIED

Given the success of enigmol, we aspire to develop 1-deoxysphingolipid analogs as chemotherapeutic agents with greater *in vivo* efficacy, while limiting cytotoxicity. Our generalized approach to synthesizing these compounds is summarized in (**Figure 7**).

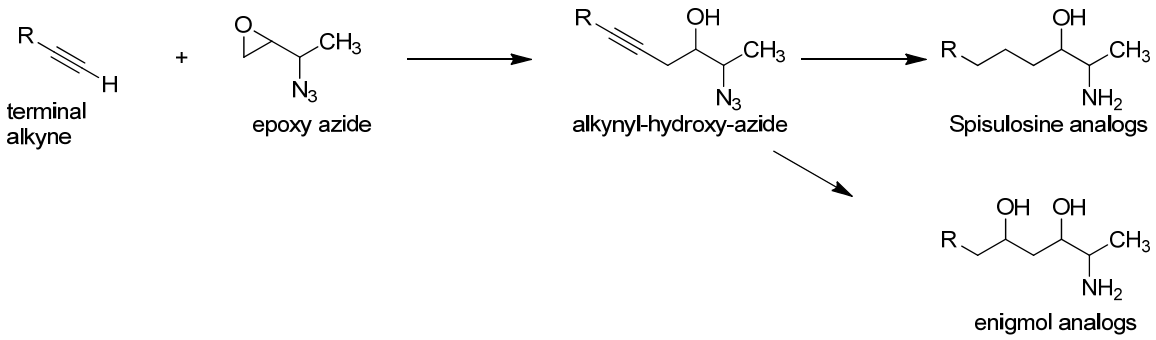


Figure 7 Generalized synthesis approach

The lipid sections of these analogs will be formed using commercially available terminal alkynes ranging from 2 to 17 carbons (**Figure 8A)**. These can be further lengthened or modified by coupling with monosubstituted diynes (**Figure 8B)** or alkynyl-substitued aryl halides (**Figure 7C)**. The epoxyazide head group of these analogs will be formed from Sharpless enantioselective epoxidation[16] followed by the Sharpless regioselective and stereospecific introduction of azide[17] to *cis* or *trans* crotyl alcohol. The resulting azidodiol will then be dehydrated to form the epoxy azide via tosylimidazole mediation.[18] The coupling of various lipid sections and head groups will lead to a very

[16] Gao, Y.; Klunder, J. M.; Hanson, R. M.; Masamune, H.; Ko, S. Y.; Sharpless, K. B. *Journal of the American Chemical Society* **1987**, *109*, 5765.
[17] Caron, M.; Carlier, P. R.; Sharpless, K. B. *The Journal of Organic Chemistry* **1988**, *53*, 5185.
[18] Cink, R. D.; Forsyth, C. J. *The Journal of Organic Chemistry* **1995**, *60*, 8122.

diverse library of alkynyl-hydroxy-azides (**Figure 7)** which can undergo further modifications to form spisulosine, fingolimod, and enigmol analogs.

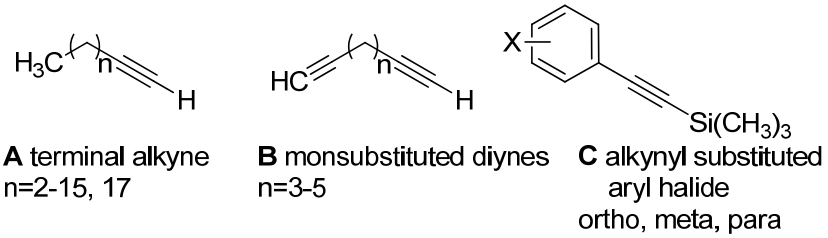
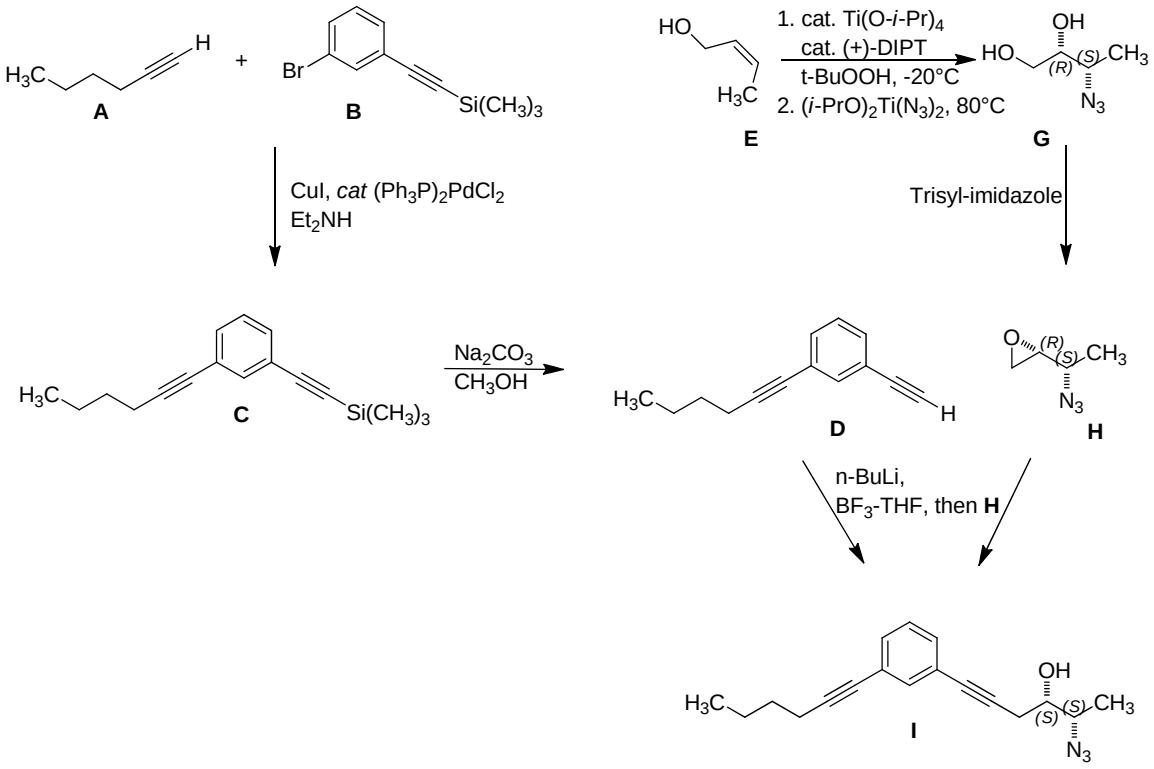


Figure 8 Lipid section constituents. Combinations of these will lead to a wide variety of respective lipid sections.

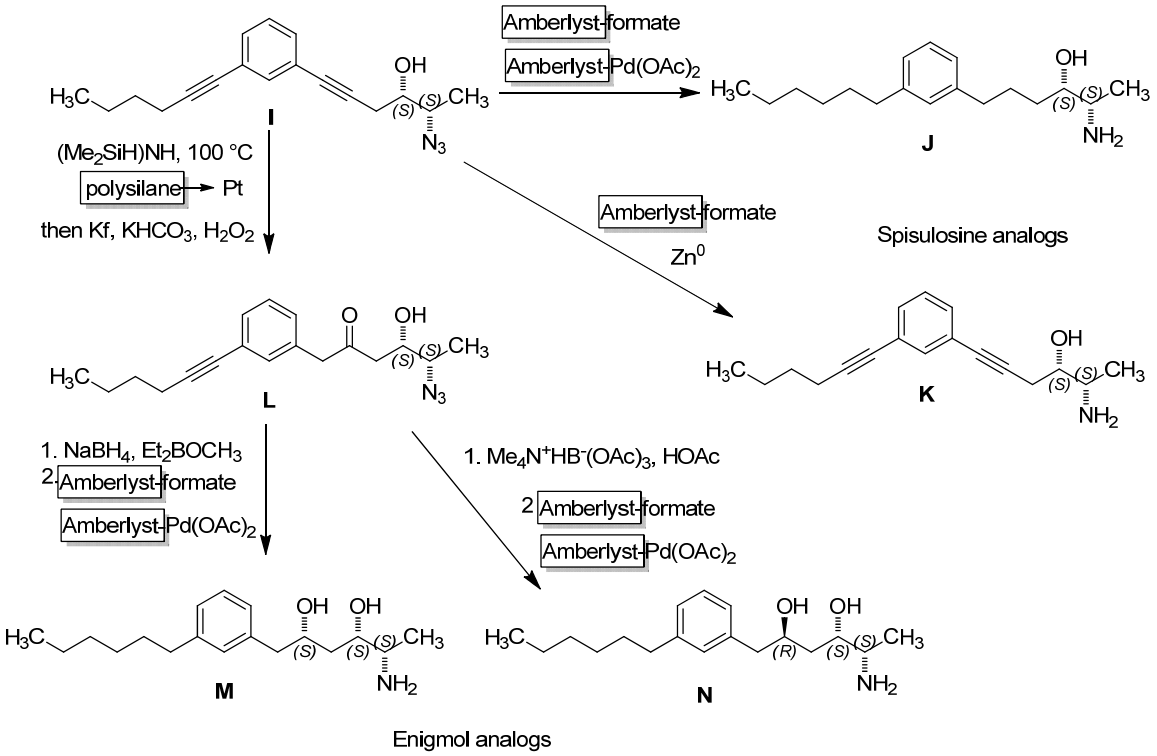
Dr. Frank McDonald of Emory University has proposed a synthesis plan for a sphingolipid analog with characteristics of both enigmol and fingolimod (**Scheme 1)**. The azidodiol **G** will be prepared by performing a Sharpless enantioselective epoxidation[16] on commercially available *trans*-crotyl alcohol **E**, followed by a Sharpless stereospecific and regioselective introduction of azide[17]. Using the (-)-DIPT in the epoxidation step will result in the enantiomer of **G** and using *trans*-crotyl alcohol will result in the diastereomer; allowing us a total of four stereoisomeric head groups.



Scheme 1 Synthesis of *meta*-aryl alkynyl-hydroxy-azide intermediate

The lipid section **D** will be prepared from the Sonogoshira-cross coupling[19] of 1-hexyne **A** and (3-bromophenylethynyl)-trimethylsilane **B,** followed by basic methanolysis to desilylate the alkyne. Perhaps the most challenging part of this scheme, the epoxyazide **H** will be formed *in situ* via trisylimidazole-mediated dehydrative epoxidation.[17] Simultaneously, the timely *n*-butyllithium deprotonation and addition of the alkyne **D** to the already formed epoxide will form **I** as a key intermediate in the synthesis of 1-deoxysphingolipid analogs.

[19]Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Letters* **1975**, *16*, 4467.



Scheme 2

From key intermediate **I**, we have a number of options to form 1-deoxysphingolipid analogs (**Scheme 2)**. We can hydrogenate the azide and both alkynes to form **J**, a spisulosine analog. Alternately, we can also selectively reduce the azide alone using zinc metal and formic acid[20] to form an alkyne containing spisulosine analog **K**. The Enigmol derivatives can also be formed through hydroxyl-directed intramolecular hydrosilylation[21] followed by oxidation to form the keto-hydroxy-azide **L**. Based on the method of reduction[22,23], the enigmol derivatives **M** and **N** can then be synthesized.

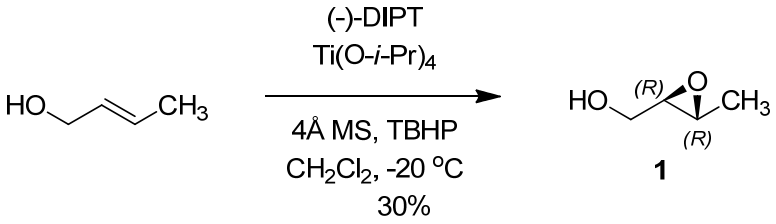
[20] Prakasha, K. C.; Sindhumol, M.; Baba, A. R.; Gowda, D. C. *ChemInform* **2007**, *38*.
[21] Oyamada, H.; Akiyama, R.; Hagio, H.; Naito, T.; Kobayashi, S. *Chemical Communication.* **2006,** 4297
[22] Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Letter* **1987**, 28, 155.
[23] Evans, D. A.; Chapman, K. T.; Carreira, E. M. *Journal of the American Chemical Society* **1988**, 110, 3560.

3. EXPERIMENTAL

3.1 General Methods: All solvents and starting materials were obtained from commercial suppliers and were used without further purification, unless otherwise noted. These include, but are not limited to, acetone, ethyl ether, methylene chloride, ethyl acetate, and hexane. All reactions were carried out in anhydrous solvents which were dried over 4 Å molecular sieves purchased from Sigma Aldrich. The solvents were tested for trace amount of water using a Coulometric KF titrator from Denver instruments. All reactions were carried out under flame dried glassware and inert argon atmosphere. Thin Layer Chromatography (TLC) was performed using precoated glass backed silica gel $60F_{254}$ plates purchased from Whatman. Flash column chromatography was carried out using silica gel 60 (230-400 mesh) from EM Science.

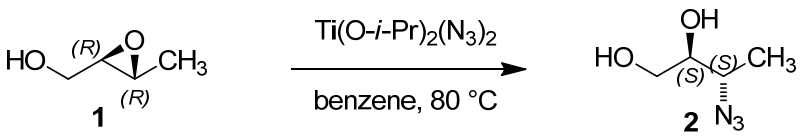
^{1}H NMR and ^{13}C NMR spectra were obtained using INOVA 400 spectrometer. Deuterated chloroform ($CDCl_3$) was used as the NMR solvent and an internal standard of 7.26 ppm was set for ^{1}H NMR and 77.23 ppm was set for ^{13}C NMR. Abbreviations for the splitting patterns are reported as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), qd (quartet of doublets), p (pentet), bs (broad singlet), and m (multiplet). IR spectra were obtained from Mattson Genesis II FT-IR spectrometer as neat films on sodium chloride discs. Mass spectra were obtained from Finnigan LTQ HRMS Mass spectrometer. Optical rotations were measured using Perkin-Elmer 341 polarimeter.

3.2 Synthesis of epoxyalchohol



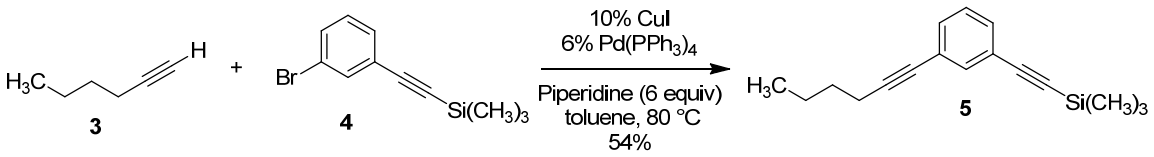
((2R,3R)-3-methyloxiran-2-yl)methanol (1): To a suspension of 4Å molecular sieves (10 g) in CH_2Cl_2 (130 mL) at -20 °C were added D-(-)-diisopropyl tartrate (10.9 mL, 52 mmol) and titanium tetraisopropoxide (11.1 mL, 38 mmol). The mixture was stirred for 15 minutes after which *tert*-butylhydroperoxide (15 mL, 5.5 M in decane) was added dropwise. The resulting solution was stirred for 30 minutes at -20 °C before the *trans*-crotyl alcohol (5.9 mL, 69 mmol) was added. The reaction mixture was placed in a -20 °C freezer for 45 hours and then quenched with a 1:1:1 solution of citric acid, acetone, and ether. The solution was then stirred for 15 minutes, filtered through celite, and concentrated under reduced pressure. The crude product was purified via flash chromatography using 30% ethyl ether in hexanes as the eluent, to provide compound **1** (1.45 g, 30%) as a clear oil. **^{1}H NMR** δ 3.876 (dd, 1H, *J* = 2.4, 12.4 Hz), 3.583 (dd, 1H, *J* = 4.4, 12.4 Hz), 3.018 (qd, 1H, *J* = 2.4, 5.2 Hz), 2.871 (p, 1H, *J* = 2.4 Hz), 2.350 (s, 1H), 1.308 (d, 3H, *J* = 5.2 Hz); **^{13}C NMR** δ 61.8, 59.7, 52.0, 17.3; **HRMS** (APCI): *m/z* calcd. for $C_4H_9O_2$ ($M+H^+$) 89.06025 found 89.05960; **FT-IR**: 3398, 2973, 2850, 1743, 1454, 1365 cm^{-1}; **[α]**$_D^{25}$ = - 23.0 (c = 0.16, $CHCl_3$).

3.3 Synthesis of epoxy azide



(2R,3S)-3-azidobutane-1,2-diol (**2**): Trimethylsilylazide (4.37 mL, 32.9 mmol) and titanium tetraisopropoxide (4.81 mL, 16.45 mmol) were refluxed in benzene (120 mL) for 5 hours at 80 °C to generate $Ti(O\text{-}i\text{-}Pr)_2(N_3)_2$. The epoxyalcohol **1** (1.45 g, 16.45 mmol) in a solution of benzene (10 mL) was then added, and the reaction was stirred for 15 minutes and then cooled to room temperature. The benzene was removed in vacuo and the crude product was diluted with ether (100 mL), and diluted with 5% H_2SO_4 (50 mL). The resulting solution was then stirred for 1 hour until two clear phases formed. The aqueous layer was extracted with ether and the combined organic phases were purified on a silica gel column and concentrated to give **2** as an oil (0.97 g, 45%)**.** **^{1}H NMR** δ 3.748-3.618 (m, 4H), 2.646 (bs, 1H), 2.172 (bs, 1H), 1.319 (d, 1H, *J* = 6.4 Hz); **^{13}C NMR** δ 74.0, 63.0, 59.0, 14.9; **HRMS** (APCI): *m/z* calcd. for $C_4H_{11}N_3O_2$ ($M+2H^+$) 133.08512, found 133.06464; **FT IR**: 3398, 2978, 2939, 2503, 2117, 1643 cm^{-1}.

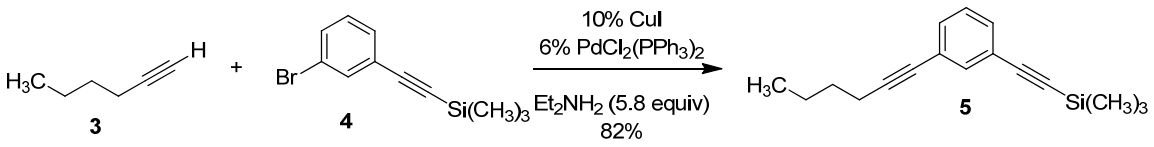
3.4 Synthesis of Lipid section[24]



((3-(hex-1-yn-1-yl)phenyl)ethynyl)trimethylsilane (**5**): A 50 mL roundbottom flask was charged with tetrakis(triphenylphosphine)palladium(0) (0.27 g, 0.24 mmol), Copper(I) iodide (76 mg, 0.39 mmol), and ((3-bromophenyl)ethynyl)trimethylsilane **4** (0.84 mL, 3.95 mmol) . While stirring, dry toluene (20 mL, starting material halide is 0.2 M in toluene) was added followed by piperidine (2.34 mL, 23.6 mmol). 1-Hexyne **3** (0.53 mL, 4.74 mmol) was then added and the reaction vessel was heated to 80 °C by a sand bath and left stirring overnight. Workup involved partitioning the reaction with ethyl ether and water (50 mL each). The organic layer was washed with 1 M HCL (3X 50 mL), saturated NaCl (1X 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by chromatography with 100% hexane to give **5** (0.54 g, 54 %) as a clear oil. **^{1}H NMR** δ 7.516 (s, 1H), 7.361-7.318 (m, 2H), 7.206 (t, 1H, *J* = 7.6 Hz), 2.398 (t, 2H, *J* = 6.8 Hz), 1.602-1.531 (m, 4H), 0.953 (t, 3H, *J* = 6.8 Hz), 0.251 (s, 9H); **^{13}C NMR** δ 135.2, 131.7, 131.0, 128.3, 104.5, 94.7, 91.3, 79.9, 30.9, 22.2, 19.2, 13.8, 0.1; **HRMS** (APCI): *m/z* calcd. for $C_{17}H_{23}Si$ ($M+H^+$) 255.15689, found 255.15634; **FT IR**: 3062, 2958, 2931, 2360, 2337, 2229, 2159 cm^{-1}.

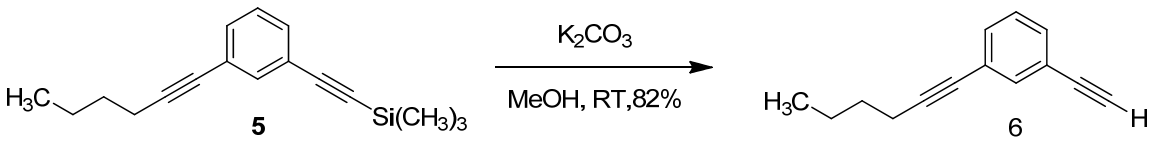
[24] Mio, M. J.; Kopel, L. C.; Braun, J. B.; Gadzikwa, T. L.; Hull, K. L.; Brisbois, R. G.; Markworth, C. J.; Grieco, P. A. *Organic Letters* **2002**, *4*, 3199.

3.5 Alternate synthesis of lipid section[19]



((3-(hex-1-yn-1-yl)phenyl)ethynyl)trimethylsilane (**5**): Copper(I) iodide (0.76 mg, 0.39 mmol) was added to a diethylamine (2.4 mL, 23.6 mmol) solution of bis(triphenylphosphine)palladium(II) dichloride (0.17 g, 0.24 mmol) and ((3-bromophenyl)ethynyl)trimethylsilane **4** (0.85 mL, 4.0 mmol) at room temperature. 1-Hexyne **3** (0.54 mL, 4.8 mmol) was then added dropwise and the reaction was left stirring for 24 hours. The diethylamine was then removed under reduced pressure and the residue was diluted with water. The solution was then extracted with ether, passed over a bed of celite, and dried with Na_2SO_4. The crude was purified by column chromatography with 100% hexane to give **5** (0.84 g, 82%) as a clear oil. **^{1}H NMR** δ 7.516 (s, 1H), 7.361-7.318 (m, 2H), 7.206 (t, 1H, *J* = 7.6 Hz), 2.398 (t, 2H, *J* = 6.8 Hz), 1.602-1.531 (m, 4H), 0.953 (t, 3H, *J* = 6.8 Hz), 0.251 (s, 9H); **^{13}C NMR** δ 135.2, 131.7, 131.0, 128.3, 104.5, 94.7, 91.3, 79.9, 30.9, 22.2, 19.2, 13.8, 0.1; **HRMS** (APCI): *m/z* calcd. for $C_{17}H_{23}Si$ ($M+H^+$) 255.15689, found 255.15634; **FT IR**: 3062, 2958, 2931, 2360, 2337, 2229, 2159 cm^{-1}.

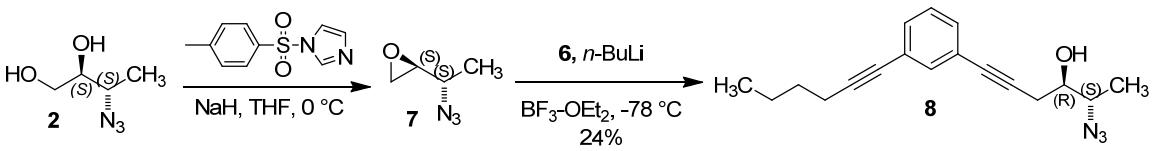
3.6 Removal of TMS group[25]



1-ethynyl-3-(hex-1-yn-1-yl)benzene (**6**): A mixture of anhydrous potassium carbonate (12.5 mg, 0.1 mmol), anhydrous methanol (3.0 mL, 74 mmol), and **5** (0.25 g, 1.0 mmol) was stirred for 3 hours at room temperature. The solvent was removed under reduced pressure and the diluted with aqueous sodium bicarbonate. The aqueous phase was then extracted with diethyl ether and the combined organic fractions were dried over Na_2SO_4. The crude material was purified by column chromatography with hexane as the eluent to afford **6** (0.15 g, 82%) as a colorless oil. **^{1}H NMR** δ 7.530 (s, 1H), 7.400-7.354 (m, 2H), 7.248 (t, 1H, *J* = 10 Hz), 3.065 (s, 1H), 2.408 (t, 2H, *J* = 8.8 Hz), 1.617-1.443 (m, 4H), 0.954 (t, 3H, *J* = 8 Hz); **^{13}C NMR** δ 135.3, 132.1, 131.2, 128.5, 91.5, 79.8, 77.7, 77.6, 30.9, 22.2, 19.2, 13.8; **HRMS** (APCI): *m/z* calcd. for $C_{14}H_{15}$ ($M+H^+$) 183.11737, found 183.11674 ; **FT IR**: 3297, 2958, 2931, 2865, 2360, 2337, 22292 cm^{-1}.

[25] Xu, Y. P.; Hu, R. H.; Cai, M. Z. *ChemInform* **2008**, *39*.

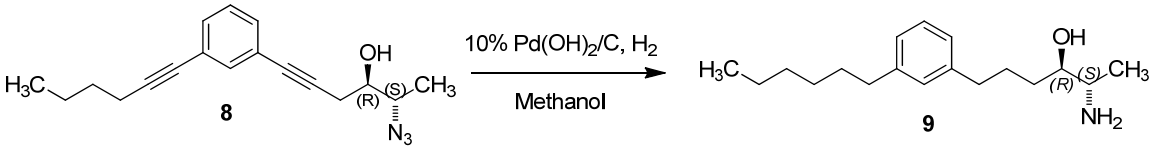
3.7 Synthesis of alkynylhydroxyazide intermediate



(2S,3R)-2-azido-6-(3-(hex-1-yn-1-yl)phenyl)hex-5-yn-3-ol (**8**): Sodium hydride (46.0 mg, 1.14 mmol, 60% in mineral oil) was added to a 0 °C solution of the azidodiol **2** (60.0 mg, 0.46 mmol) in THF (4.6 mL). The solution was allowed to warm to room temperature over 1 hour and then cooled back to 0 °C. 1-(p-Toluenesulfonyl)imidazole (0.102 g, 0.46 mmol) was added in 3 portions over 20 minutes. The resulting solution was stirred and allowed to warm to room temperature over 40 minutes to generate a solution of the epoxide intermediate **7**, then cooled to -78 °C. In a second flask, *n*-BuLi (0.328 mL, 2.5 M in hexane, 0.82 mmol) was added dropwise to a -78 °C solution of **6** (0.15 g, 0.82 mmol) in THF (2 mL). The solution was stirred for 15 minutes then added to the *in situ* formed epoxide **7** via cannula. BF_3-OEt_2 (0.234 mL, 1.90 mmol) was then added dropwise and the solution was stirred for an additional 30 minutes at -78 °C. Workup consisted of addition of aqueous ammonium chloride (10 mL) followed by dilution with ether (50 mL). The organic phase was washed with water (25 mL) and brine (25 mL). The combined aqueous phase was extracted with ether (50 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography (Hexane-ether, 4:1) to afford **8** (32.8 mg, 24%). **^{1}H NMR** δ 7.440 (s, 1H), 7.332-7.259 (m, 2H), 7.231 (t, 1H, *J* = 10 Hz), 3.798-3.656 (m, 2H), 2.699 (d, 2H, *J* = 7.2 Hz), 2.399 (t, 2H, *J* = 8.8 Hz), 2.198 (d, 1H, *J* = 5.6 Hz), 1.606-1.396

(m, 4H), 1.339 (d, 3H, *J* = 3 Hz), 0.9447 (t, 3H, *J* = 9.2 Hz); **^{13}C NMR** δ 134.9, 131.4, 130.8, 128.4, 91.4, 79.9, 77.5, 72.6, 60.4, 30.9, 24.5, 22.2, 19.2, 14.4, 13.8; **HRMS** (APCI): *m/z* calcd. for $C_{18}H_{22}N_3O$ ($M+H^+$) 296.17627, found 296.17567; **FT IR**: 3421, 2958, 2931, 2869, 23560, 2233, 2113 cm^{-1}.

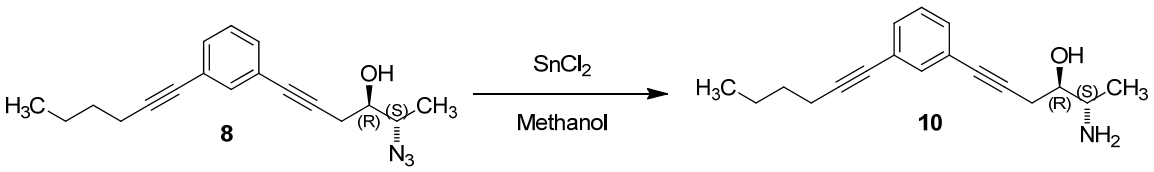
Synthesis of spisulosine analog[26]



(2S,3R)-2-amino-6-(3-hexylphenyl)hexan-3-ol (**9**): Compound **8** was dissolved in methanol (2 mL). Pearlman's catalyst ($Pd(OH)_2/C$) was then added and the reaction mixture was evacuated under vacuum for 5 minutes. The reaction was then backfilled with a balloon of hydrogen gas and stirred for 24 hours. The reaction mixture was then filtered through a bed of Celite and the solvent was removed under reduced pressure. The crude was loaded on to a silica gel column and eluted with 1 : 9 CH_2Cl_2 : MeOH. The expected product was not validated by NMR.

[26] Pereira, C. L.; Chen, Y.-H.; McDonald, F. E. *Journal of the American Chemical Society* **2009**, *131*, 6066.

Synthesis of alternate spisulosine analog[27]



(2S,3R)-2-amino-6-(3-(hex-1-yn-1-yl)phenyl)hex-5-yn-3-ol (10): Compound 8 was dissolved in a solution of methanol (2 mL) and $SnCl_2$ (38.0 mg, 0.13 mmol), and this mixture was stirred for 2 hours. Methanol was removed under reduced pressure and diethylamine (0.5 mL) was added to make the solution alkaline. The product was purified on a silica gel column with 1 : 9 CH_2Cl_2 : MeOH. ^{1}H NMR, ^{13}C NMR, and FT IR were unattainable due to limited amount of sample. **HRMS** (APCI): *m/z* calcd. for $C_{18}H_{24}O_1N_1$ ($M+H^+$) 270.18577, found 270.18519.

[27] Maiti, S. N.; Singh, M. P.; Micetich, R. G. *Tetrahedron Letters* **1986**, *27*, 1423.

4. DISCUSSION

Synthesis of the epoxy alcohol resulted in a low yield (30%) for several reasons. Possessing a relatively low boiling point (18 mmHg, 81- 82 °C),[16] a significant amount of product may have been lost on the rotary evaporator. In addition, the use of acetic acid instead of citric acid to quench the reaction may have also resulted in significant loss of the epoxyalcohol in the aqueous phase. It is recommended to use both a non-aqueous workup (the epoxide is soluble in water) and a distillation to purify this compound.[16] Fortunately, this reaction was performed on a large scale and enough product was synthesized to move forward (1.45 g). Optimizations of this reaction are in progress. In a recent experiment, full consumption of the starting material alcohol and appearance of the epoxide was observed by TLC. After the epoxide was purified by column chromatography, only a 27% yield was observed. This loss may be due to opening of the epoxide on the column. Low yields from the Sharpless epoxidation result from loss of product during the extraction and purification; therefore distillations will performed in future experiments. The reaction was only observed by TLC to completion when kept stirring at -20 °C, as opposed to being placed in the -20 °C freezer without stirring. To improve the yield of the azide insertion, use of a slight excess of the titanium azide reagent as well as increased temperature conditions will be explored.

Synthesis of the lipid section was carried out by both the original Sonogoshira coupling[18] method and by a modified Sonogoshira coupling method.[20] Not only did the original method retain a greater yield (82% as opposed to 54%) it is a much milder and easier

reaction to set up. The modified method requires elevated temperature conditions as well as additional reagents not needed in the original method. The original Sonogoshira procedure calls for the starting material halide to be 0.17 M in diethylamine, but the reaction was run in a 1.17 M solution. Thus decreasing the reaction concentration may lead to better yields. A reoccurring issue with the purification of this compound is an impurity with a very similar Rf (retention factor). Although hexane is used as eluent for the purification of this compound, heptane may yield a better separation since it is more polar. This will be investigated in future experiments.

The one pot synthesis of the alkynyl-hydroxy-azide intermediate proved a challenge. The first time this reaction was carried out it failed. It was later determined that both the sodium hydride and tosylimidazole used were expired. Two more trials of this reaction were performed, resulting in 19% and 24% yields respectively. These are well below the yields reported in the literature.[17] Possible reasons for this include incomplete *in situ* formation of the epoxide. This is caused by either use of insufficient amount of sodium hydride, or insufficient time allotted. After more starting material azide and alkyne have been synthesized, optimization of this reaction will be explored.

The first time the hydrogenation was performed, the starting material was impure. This resulted in a large number of reduced materials as observed from TLC; making the desired product unattainable. On the second try, what was believed to be the product on TLC was disproved by NMR. On failing to completely reduce the intermediate, specific reduction of the azide was performed via tin(II) chloride in methanol. Upon

detecting the product by TLC, extraction was carried out and the product was unfortunately lost during transfer. A small amount of starting material was recovered and upon running the reaction again, NMR of the isolated product did not seem accurate. However, a mass spectrometer sample gave an m/z of 270.18524 for the M+1, consistent with the product with a calculated M+1 of 270.17795. Elemental composition also matched that of the product compound. Since it was such a small sample (approximately 3 mg), NMR would have needed a much greater number of scans to develop an accurate spectrum. From this it seems as though reduction of the azide portion prior to alkyne reduction should be investigated. Since the product is a hydroxy amine, it is water soluble and a non aqueous quench and purification must be developed.

5. Conclusion and Future Endeavors

The synthesis of these potential 1-deoxysphingolipid anti-cancer compounds is within reach. The foundations for the synthesis of these compounds have been laid and more efficient experimental procedures are being explored. Once we have synthesized libraries of these 1-deoxysphingolipids, they will be evaluated on a number of dimensions. First, their cytotoxicity against prostate cancer cell lines in culture will be determined. The most effective compounds will then be reevaluated for rate of metabolism and their inhibition of sphingosine-1-phosphate. The most promising leads will then be tested on mouse xenografts of prostate cancer. We will then either send the most effective compounds to clinical trials or establish enigmol as the most effective of these analogs.