Novel Syntheses of Sphingolipids and Their Analogues

by

Xuequan Lu

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2005

This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Sept. 7, 2005 — Prof. Robert Bittman
Date — Chair of Examining Committee

Sept. 7, 2005 — Prof. Gerald W. Koeppl
Date — Executive Officer

Prof. David R. Mootoo
Prof. Alexander Greer
Supervisory Committee

The City University of New York

Abstract

Novel Syntheses Sphingolipids and Their Analogues

by

Xuequan Lu

Research Adviser: Professor Robert Bittman

This dissertation presents the asymmetric total syntheses of naturally occurring sphingoid bases and novel sphingolipid analogues, including (a) photoactivatable sphingosine 1-phosphate (S1P), (b) photoactivatable sphingosylphosphorylcholine (SPC), (c) phytosphingosine 1-phosphonate, (d) a ceramide bearing a C4-methylene group, (e) chiral phosphates and phosphonates of FTY720, and (f) an α-*C*-glycoside of the KRN7000. Also included in this dissertation is a novel synthesis of a β-*C*-glycoside analogue of the ether lipid ET-18-OCH_3.

Chapter 1 presents the first synthesis of two [^{32}P]-labeled photoaffinity probes of sphingosine 1-phosphate (S1P): (2*S*,3*R*)-14-(*O*)-(4'-benzoyl)phenyl- and 14-(*O*)-(4'-trifluoromethyldiazirinyl)phenyl-4(*E*)-tetradecen-2-amino-3-hydroxy-1-phosphate.

Chapter 2 presents the synthesis of the first photoaffinity analogue of SPC. This probe, which contains a ^{14}C-isotopic label in the choline methyl groups and a benzophenone in the long-chain base, may be a useful tool in the identification of receptors that have been postulated to interact directly and specifically with SPC.

Chapter 3 presents the first synthesis of an isosteric phosphonate analogue of the aminotriol lipid (2*S*,3*S*,4*S*)-phytosphingosine, together with an improved synthesis of (2*S*,3*S*,4*S*) and (2*S*,3*S*,4*R*)-phytosphingosine. A key intermediate is a 3-pentylidene acetal.

Chapter 4 presents the synthesis of a new analogue of (2*S*,3*R*)-ceramide with a methylene group at C4. Tebbe methylenation was the key step in the synthesis.

Chapter 5 presents efficient routes to the synthesis of (2*S*,3*R*)-2-azido-3-*O*-benzylsphingosine and (2*S*,3*R*)-sphingosine from (2*R*,3*S*)-2-*O*-benzyl-3,4-*O*-(3'-pentylidene)-2,3,4-trihydroxybutanal, which was prepared from D-tartaric acid. .

Chapter 6 presents a concise synthesis of FTY720 and the first syntheses of its chiral phosphonate and regioisomeric analogues. A 2,3-epoxy alcohol served as a common precursor of these targets. An intramolecular reaction of a 2,3-epoxytrichloroacetimidate mediated by Et_2AlCl gave a five-membered oxazoline ring, which created the quaternary center.

Chapter 7 presents a short synthesis of a new *β-C*-gaactosyl analogue of ET-18-OCH_3 via the addition of a chiral propargylic alcohol to 2,3,4,6-tetra-*O*-benzylgalacto-δ-lactone.

Chapter 8 presents the first synthesis of a α-*C*-galactosylceramide analogue that is tentatively assigned to bear an D-*arabino*-phytosphingosine backbone. The method has only 11 steps (or 13 steps with the final acylation and deacylation) in the longest pathway and an overall yield of 8.8%.

Dedicated to those who made this thesis possible:

My parents, my wife

Acknowledgments

I am sincerely grateful to my research mentor, Distinguished Professor Robert Bittman, for his professional guidance. He not only helped me in project design and development in my research work, but also took much time and energy to review all of my reports and papers and made valuable comments as well as corrections.

I thank Professor David R. Mootoo, Professor Alexander Greer, Professor A. David Baker, Professor William H. Hersh, Dr. Hoe-Sup Byun, and Professor Gopal Subramaniam, who gave me many valuable suggestions.

I am very thankful to my colleagues and to all those people who offered me a great deal of help.

Special thanks are also given to my parents and my wife for their consistent encouragement during my Ph.D. research work.

Finally, I acknowledge the financial support from the National Institutes of Health.

Portions of this Dissertation have been published in the following journals.

A. "Reprinted with permission from *The Journal of Organic Chemistry*. Copyrights 2003, 2004, and 2005 American Chemical Society."

1. Lu, X.; Cseh, S.; Byun, H.-S.; Tigyi, G.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 7046-7050. (Chapter 1)

2. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438. (Chapter 3)

3. Lu, X.; Bittman, R. *J. Org. Chem.* **2005**, *70*, 4746-4750. (Chapter 2)

B. "Reprinted with permission from *Organic Letters*. Copyright 2005 American Chemical Society."

1. Lu, X.; Arthur, G.; Bittman, R. *Org. Lett.* **2005**, *7*, 1645-1648. (Chapter 4)

C. "Reprinted with permission from *Tetrahedron Letters*. Copyright 2005 Elsevier Science Ltd."

1. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 1873-1875. (Chapter 5)

2. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 3165-3168. (Chapter 3)

Table of Contents

List of Schemes

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of Figures, Charts, & Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Abbreviations

Ac	acetyl
AEL	antitumor ether lipid
ATP	adenosine 5’-triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BSA	bovine serum albumin
Bz	benzoyl
CSA	(+)-10-camphorsulfonic acid
DBU	1,5-diazabicyclo[5.4.0]undec-5-ene
DCC	*N*,*N’*-dicyclohexylcarbodiimide
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIPT	diisopropyl tartrate
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylene diaminetetraacetic acid
FAB	fast atom bombardment
HMPA	hexamethylphosphoramide
HR-MS	high-resolution mass spectrum

HWE	Horner-Wadsworth-Emmons
MOM	methoxymethyl
MS	mass spectrum
NCS	*N*-chlorosuccinimide
NKT cells	natural killer T cells
NMR	nuclear magnetic resonance
PBS	phosphate buffer saline
PCC	pyridinium chlorochromate
PHS	phytosphingosine
PMB	*p*-methoxybenzyl
Py	pyridine
S1P	sphingosine 1-phosphate
SPC	sphingosylphosphorylcholine
TBAB	tetra-*n*-butylammonium bromide
TBAF	tetra-*n*-butylammonium fluoride
THF	tetrahydrofuran
TMS	trimethylsilyl
TBS	*tert*-butyldimethylsilyl
TLC	thin-layer chromatography
Ts	*p*-toluenesulfonyl
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
UV	ultraviolet

Chapter 1

Total Synthesis of Two Photoactivatable Analogues of the Growth Factor-like Mediator Sphingosine 1-Phosphate: Differential Interaction with Protein Targets

Abstract

The first synthesis of two photoreactive analogues of the lipid mediator and second messenger sphingosine 1-phosphate (S1P), [^{32}P]-labeled (2*S*,3*R*)-14-*O*-(4'-benzoylphenyl)- and (2*S*,3*R*)-14-*O*-((4'-trifluoromethyldiazirinyl)phenyl)-(4*E*)-tetradecenyl-2-amino-3-hydroxy-1-phosphate, is described. The interactions of these probes with the S1P type-1 receptor ($S1P_1$) transfected into membranes of rat hepatoma cells and with plasma proteins were analyzed. The $S1P_1$ receptor interacted in a specific manner with the benzophenone-containing ligand (K_D = 84 ± 10 nM vs K_D for S1P = 36 ± 2 nM); in contrast, no saturable specific binding was found with the diazirine-containing ligand. However, the same pattern was found for labeling of plasma proteins by both probes, indicating that different parts of the S1P pharmacophore underlie the interaction of S1P with its receptor and plasma carrier proteins.

Introduction

Sphingosine 1-phosphate (S1P) is a member of the lysophospholipid growth factor family with diverse actions in almost every cell type.[1] S1P can act as an extracellular mediator through the activation of any of five G-protein-coupled plasma membrane receptors encoded by some of the endothelial differentiation gene (EDG)

family, which are named S1P(1-5).[1c, 2] Intracellularly, when generated from sphingosine by sphingosine kinases (SKs), S1P can also act as a second messenger through a set of targets that are not yet fully characterized.[3] Because of the many extra- and intracellular targets that bind to S1P, photoactivatable analogues of S1P would be of great practical importance provided that the photoreactive moiety does not compromise the specificity and often high-affinity interaction of the ligand with its binding protein. Ligand recognition of S1P by the $S1P_1$ receptor has been partially mapped to a cluster of three charged amino acids, but the position of the hydrophobic tail remains unknown.[4] Although residues involved in the catalytic activity of SKs have been identified, the binding of the substrate and the precise catalytic mechanism remain to be elucidated.[5] Elucidation of the S1P-mediated receptor activation or of the enzymatic mechanisms underlying S1P production are just two examples of important molecular events that may benefit from the availability of photoreactive S1P analogues. Binding of S1P to serum proteins has been shown to attenuate its biological activity.[6] Identification of S1P binding proteins in biological fluids and their binding domain could lead to the development of synthetic peptides that offer therapeutic applicability to block S1P-induced tumor angiogenesis.

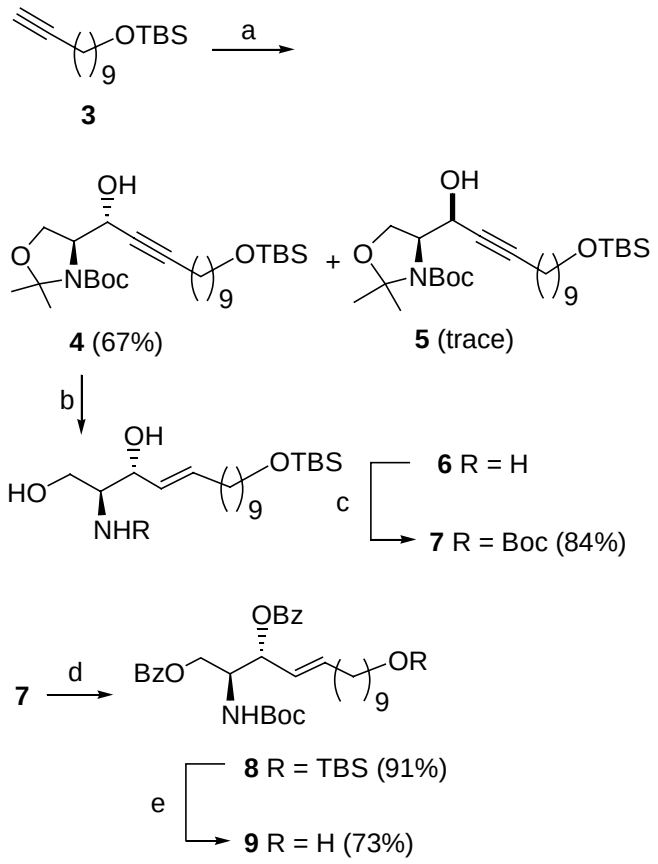
The benzophenone and trifluoromethylphenyldiazirine chromophores have many useful features in common, including high chemical stability in subdued ambient light, photoactivation using near-UV light ($\lambda > 350$ nm), and the ability to insert randomly into accessible C-H bonds of amino acid residues to form photoadducts.[7] This chapter describes the total synthesis of two new S1P derivatives that bear a benzophenone or a (4-trifluoromethyl)phenoxydiazirine moiety in the sphingoid chain of S1P (compounds **1**

and **2**, respectively) and the characterization of their interaction with the $S1P_1$ receptor and S1P-binding proteins present in rat plasma. These two new S1P analogues may facilitate the dissection of the molecular mechanisms involved in the specific lipid-protein interaction taking place between S1P and its protein targets.

Results and Discussion

Preparation of ω-Hydroxysphingosine Derivative 9 (Scheme 1). The hydroxy group of 10-undecyn-1-ol was protected as its TBS ether **3** with TBSCl (1.5 equiv) and imidazole (3 equiv) in CH_2Cl_2. The acetylide anion derived from **3** was coupled diastereoselectively with (*S*)-Garner aldehyde[8] in the presence of HMPA,[9] giving *erythro*-isomer **4** in 67% yield and, in some reactions, a trace amount of *threo*-isomer **5**. Birch reduction of *erythro*-**4** afforded crude 2-amino-1,3-diol **6**. To introduce a benzophenone (Scheme 2) or (4-trifluoromethyl)phenoxydiazirinyl (Scheme 3) moiety at the terminal position of the long-chain base, the hydroxy and amino groups must be protected. As shown in Scheme 1, the amino group was protected as an *N*-BOC carbamate to afford **7** in 84% yield (for two steps from **4**). Then, the hydroxy groups of **7** were protected as benzoyl esters, affording **8** in 91% yield. The TBS group of **8** was cleaved (TBAF, THF) to give the ω-hydroxysphingosine intermediate **9** in 73% yield.

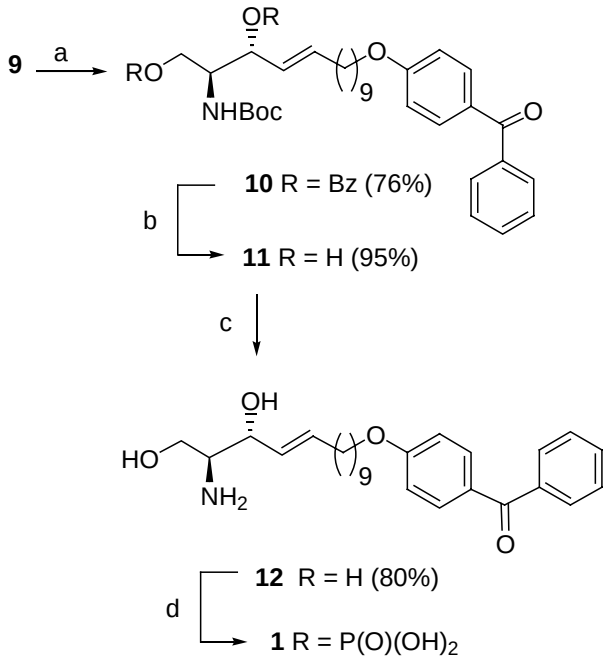
Scheme 1: Preparation of ω-hydroxysphingosine 9



Reagents and conditions: (a) *n*-BuLi, THF, HMPA, (*S*)-Garner aldehyde, -78 °C; (b) Li, $EtNH_2$, THF, -78 °C; (c) Boc_2O, Et_3N, dioxane; (d) Bz_2O, DMAP, Py, rt; (e) TBAF, THF, rt. See reference 15.

Synthesis of the Benzophenone S1P Analogue, Compound 1. Compound **1** was synthesized as shown in Scheme 2. The key step is the Mitsunobu reaction (DIAD/Ph_3P)[10] of 4-hydroxybenzophenone with intermediate **9**, which afforded coupling product **10** in 76% yield. After the benzoyl esters of **10** were hydrolyzed (1 M NaOH in MeOH) and the *N*-BOC group of **11** was removed by treatment with 4 M HCl in THF, sphingosine analogue **12** was obtained in 80% yield.

Scheme 2: Synthesis of analogue 1

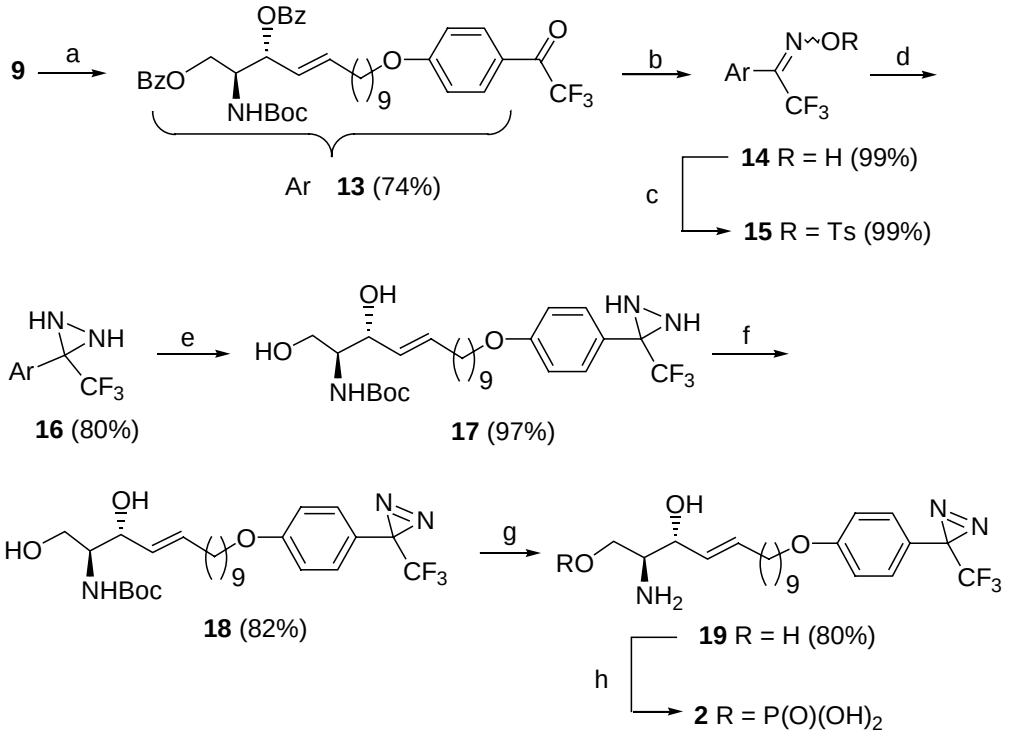


Reagents and conditions: (a) DIAD, PPh_3, 4-hydroxybenzophenone, CH_2Cl_2, 0 °C - rt; (b) 1 M NaOH, MeOH, rt; (c) 4 M HCl, THF, rt; (d) sphingosine kinase, ATP. See reference 15.

Synthesis of the (4-Trifluoromethyldiazirinyl)phenoxy S1P Analogue, Compound 2. Compound **2** was prepared as shown in Scheme 3. Mitsunobu reaction (DIAD/Ph_3P) of 4-hydroxytrifluoroacetophenone (**22**) (prepared by trifluoroacetylation of the Grignard reagent of 4-bromoanisole with *N*-(trifluoroacetyl)piperidine (**20**), followed by *O*-demethylation of **21** with LiCl in DMF) with **9** gave trifluoroacetophenone ether **13** in 74% yield. The trifluoroacetyl group of **13** was converted to the (4-trifluoromethyl)phenoxydiazirinyl moiety by using standard procedures.[11] Briefly, a mixture of (*E*/*Z*)-oximes **14** derived from **13** was treated with *p*-TsCl and triethylamine in the presence of a catalytic amount of DMAP in CH_2Cl_2 to afford tosylates **15**. Tosylates **15** were converted to diaziridine **16** with liquid ammonia,

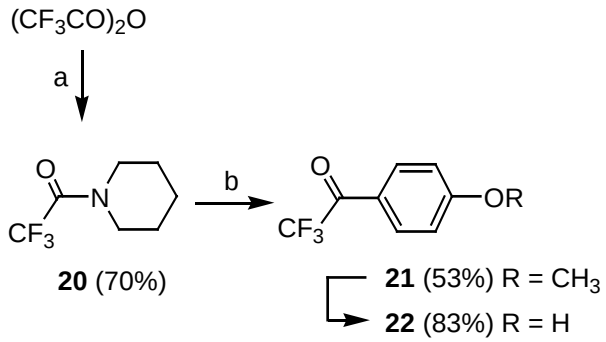
and base-catalyzed hydrolysis of benzoyl ester **16** with 10% methanolic NaOH gave diol **17**. Oxidation of **17** (I_2, Et_3N, MeOH) gave the desired diazirine **18**. Finally, the *N*-BOC group of **18** was removed to give sphingosine analogue **19**.

Scheme 3: Synthesis of analogue 2



Reagents and conditions: (a) DIAD, PPh_3, CH_2Cl_2, 4-$CF_3C(O)C_6H_4OH$ (**22**), rt; (b) NH_2OH, Py, EtOH, 50-60 °C; (c) *p*-TsCl, NEt_3, DMAP, CH_2Cl_2, 0 °C - rt; (d) liquid NH_3; (e) 1 N NaOH, MeOH, rt; (f) I_2, Et_3N, MeOH, rt; (g) 4 M HCl, THF, rt; (h) sphingosine kinase, [γ-^{32}P]ATP. See reference 15.

Scheme 4: Synthesis of 4-hydroxytrifluoroacetophenone 22



Reagents and conditions: (a) piperidine, Et_3N, Et_2O; (b) Mg, 4-bromoanisole, THF, 0 °C – rt; (c) LiCl, DMF, reflux, 2 h.

Synthesis of 4-hydroxytrifluoroaacetophenone 22. As shown in Scheme 1, *N*-(trifluoroacetyl)piperidine **20** was prepared from trifluoroacetic anhydride and piperidine in the presence of triethylamine in Et_2O. Trifluoroacetylation of the Grignard reagent derived from anisole with **20** provided trifluoroacetophenone analogue **21** in 53% yield. *O*-Demethylation of anisole **21** with lithium chloride in DMF at reflux provided phenol derivative **22** in 83% yield.

Radiolabeling of S1P and the Sphingosine Photoactivatable Analogues. The following experiment was carried out by Dr. Sandor Cseh in the laboratory of Professor Gabor Tigyi (University of Tennessee Health Science Center, Memphis, TN). The reaction buffer contained 20 mM Tris, pH 7.5, 12 mM $MgCl_2$, 2 mM DTT, 0.25 mM EDTA, 5 mM NaF, 12 mM β-glycerophosphate, 1 mM sodium pyrophosphate, 5% glycerol, and 1% protease inhibitor cocktail. A 65 nmol sample of sphingosine or its analogue was incubated in 1 mL of reaction buffer containing 100 μg of protein from the cell lysate of RH7777 cells that had been transfected with SK and 250 μCi of [γ-^{32}P]ATP (30 Ci/mmol) at 37 °C for 1 h. The product was extracted with a mixture of 1.6 mL of

$CHCl_3$/MeOH/HCl (100:200:1), 1 mL of 2 M KCl, and 1 mL of $CHCl_3$. The organic phase was extracted again with 2 mL of MeOH, 1 mL of $CHCl_3$, 2 mL of 2 M KCl, and 100 μ L of NH_4OH. The aqueous phase was extracted with 3 mL of $CHCl_3$ and 200 μL of 14 N HCl. The organic extract was dried under a stream of N_2, and the residue was redissolved in 0.5 mL of PBS containing 1 mM BSA.

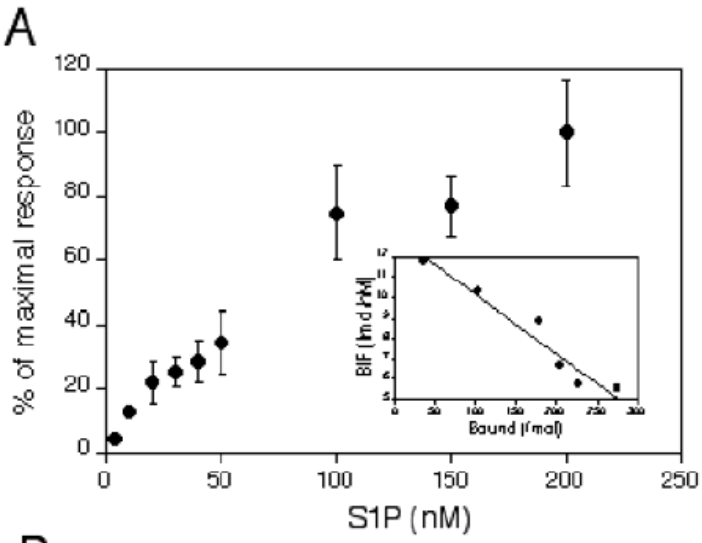
Radioligand Binding Assay. The following experiment was carried out by Sandor Cseh. The rat hepatoma RH7777 cell line was obtained from ATCC and was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). This cell line does not express endogenous $S1P_1$ and is nonresponsive to S1P.[4] Binding of ^{32}P-labeled S1P and the photoactivatable analogues to intact cells was carried out in subdued light after transient transfection of the $S1P_1$ receptor into RH7777 cells as described earlier.[12] Scatchard analysis of the binding curves was carried out using the Kaleidograph software. Each binding experiment was carried out on triplicate samples and was repeated at least three times using a new transfection of cells.

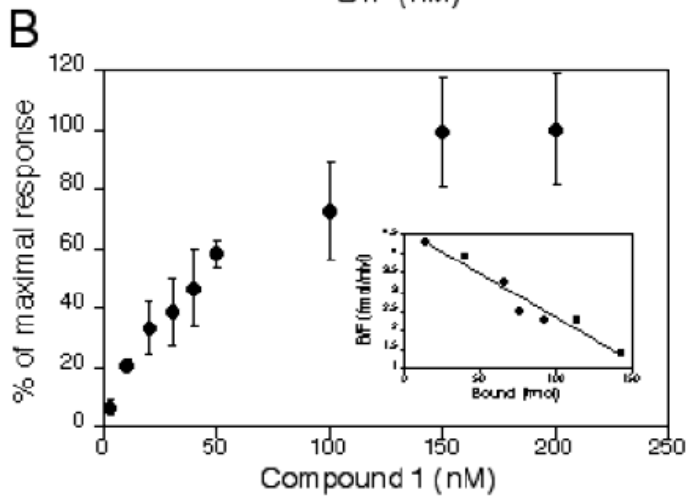
Photoaffinity Labeling of Plasma. The following experiment was carried out by Sandor Cseh. Rat plasma diluted to 5% (v/v) in PBS was mixed with 5 nM photoaffinity analogue on ice. The sample was irradiated at 365 nm on ice for 10 min using a UV lamp. Aliquots of 10 μL were loaded on reducing 12.5% SDS-PAGE gels. The gels were silver stained using the SilverQuest kit and exposed to a phosphorimager screen for 24 h.

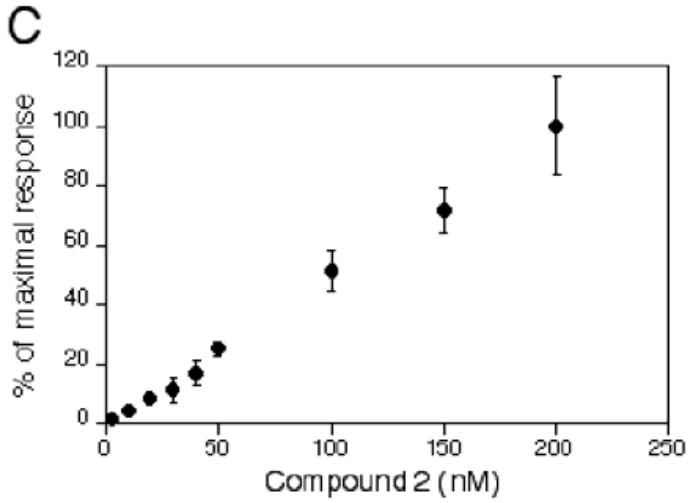
Ligand Binding Properties of Compounds 1 and 2 on $S1P_1$ Receptors. The following experiment was carried out by Sandor Cseh. The RH7777 cell line does not express endogenous S1P receptors and has been used for the heterologous expression of

the $S1P_1$ receptor.[4] Binding of the S1P photoactivatable analogues to $S1P_1$ was tested in the dark after transient transfection of the receptor into RH7777 cells. The dose-response curves of S1P (Figure 1A) and compound **1** (Figure 1B) showed saturable binding, whereas that of compound **2** did not (Figure 1C), even though both probes were tethered to the same position of the sphingoid base. Scatchard analysis yielded K_D values of 36 ± 2 and 84 ± 10 nM for S1P and compound **1**, respectively, indicating that compound **1** closely mimics the binding properties of the endogenous ligand for $S1P_1$. Because of the lack of saturable binding, the K_D value could not be determined for compound **2**. These results indicate that compound **2** does not interact specifically with the high-affinity binding pocket of the $S1P_1$ receptor. These binding studies suggest a highly restricted interaction between $S1P_1$ and its ligand that has been compromised for compound **2** but was relatively unaffected for compound **1**.

Figure 1 (see page 10). Binding of S1P and photoactivatable analogues to RH7777 cells transfected with $S1P_1$ receptor: (A) concentration dependence of specific binding of S1P (the inset shows the Scatchard plot); (B) concentration dependence of specific binding of compound **1** (the inset is the Scatchard plot); (C) concentration dependence of specific binding of compound **2**. Note that the binding of S1P and compound **1** shows saturation, whereas the binding of compound **2** does not.


A
% of maximal response
S1P (nM)
B/F (fmol/nM)
Bound (fmol)


B
% of maximal response
Compound 1 (nM)
B/F (fmol/nM)
Bound (fmol)


C
% of maximal response
Compound 2 (nM)

Photoaffinity Labeling of Rat Plasma with Compounds 1 and 2. The following experiment was carried out by Sandor Cseh. To map the S1P interactions with plasma proteins, the photoactivatable analogues were used to label normal and analbuminemic rat plasma samples (Figure 2). The analbuminemic Nagase rats, which were derived from the Sprague-Dawley strain, contain reduced concentrations of albumin (19.4 mg/mL vs 0.4 mg/mL) because of a defect in albumin synthesis by the liver.[13] Both compounds gave an identical footprint of labeling of plasma proteins. Besides albumin, another protein with an apparent molecular weight of 25000 was labeled. The addition of S1P at 20 μM competed for the labeling of this protein, indicative of the specific nature of the interaction. Labeling of albumin was not affected by 20 μM S1P, possibly because of the high-affinity binding and the high concentration of albumin (~14.5 μM) in the plasma samples.

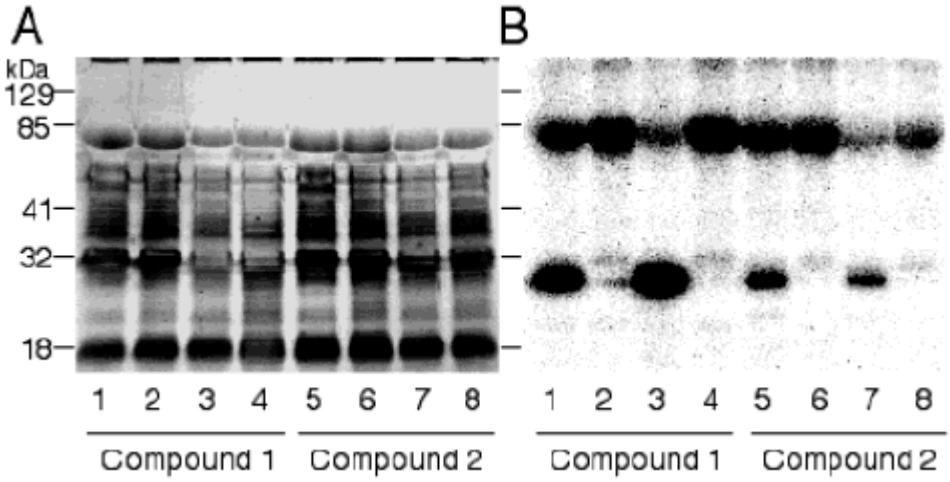


Figure 2. Photoaffinity labeling of normal Sprague-Dawley and analbuminemic Nagase rat plasma with compounds **1** and **2**. The labeled plasma was separated on a 12.5% SDS-PAGE gel, silver-stained (A), and exposed to a phosphorimager screen (B). Lanes 1 and 5: normal rat plasma. Lanes 2 and 6: normal rat plasma labeled in the

presence of 20 μM S1P. Lanes 3 and 7: analbuminemic rat plasma. Lanes 4 and 8: analbuminemic rat plasma labeled in the presence of 20 μM S1P competitor. See reference 15.

Conclusions

A convenient method has been demonstrated for the first synthesis of two photoactivatable S1P analogues. This method can be readily modified to prepare a range of compounds carrying linkers with different chain lengths to the photoreactive moiety at the terminal position of the long-chain base. The application of these photoactivatable S1P agents in the present study suggests that their interactions with the $S1P_1$ receptor, albumin, and an unidentified molecular weight 25000 novel plasma protein are affected by different parts of the S1P pharmacophore. With respect to $S1P_1$ recognition, we found that a benzophenone-containing derivative of S1P but not a diazirine analogue showed competitive displacement of S1P with nanomolar affinity from the ligand-binding site. In contrast, similar patterns of labeled proteins were obtained for both analogues in rat plasma, where albumin was the predominant carrier of the S1P analogues. The availability of these probes is likely to fuel future studies aimed at the identification of the binding sites of the biological targets of S1P at the molecular level, which will also provide important information for the synthesis of drugs selectively targeting proteins that bind S1P and regulate its biological activity.

Experimental Section

General Information. Flash chromatography was carried out with 230-400 mesh silica gel. TLC was carried out on 0.25-mm thick silica gel 60 F254 aluminum sheets. Visualization was with 10% sulfuric acid solution in EtOH. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$. Optical rotations were measured on a Jasco digital polarimeter at room temperature. The solvents were dried with the following reagents and distilled before use: CH_2Cl_2 (CaH_2); THF (sodium/benzophenone), toluene (CaH_2). All manipulations were carried out in a partially darkened room, and aluminum foil was used to exclude light from the reaction flasks containing the diazirine group. (*S*)-Garner aldehyde was prepared from L-(*N*-Boc)-serine methyl ester.

11-*tert*-Butyldimethylsiloxy-1-undecyne (3). To a solution of *tert*-butyldimethylsilyl chloride (4.52 g, 30 mmol) in dry CH_2Cl_2 (20 mL) was added imidazole (4.08 g, 60 mmol) at rt under N_2 atmosphere. The mixture was stirred for 1 h before adding a solution of 10-undecyn-1-ol (3.36 g, 20 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred overnight, and then quenched with water (10 mL). After the mixture was extracted with CH_2Cl_2 (3 x 25 mL), the combined organic phases were washed with brine (40 mL) and dried ($MgSO_4$). The residue was purified by flash chromatography to afford **3** (5.53 g, 98%) as a colorless oil; R_f 0.95 (EtOAc:hexane 1:3); ^{1}H NMR δ 0.01 (s, 6H), 0.85 (s, 9H), 1.23-1.53 (m, 14H), 1.87 (s, 1H), 2.12 (m, 2H), 3.55 (m, 2H); ^{13}C NMR δ –5.25, 18.2, 18.3, 25.6, 25.7, 28.4, 28.5, 28.7, 29.0, 29.1, 29.3, 29.4, 29.5, 32.8, 63.1, 68.0.

***N*-*tert*-Butoxycarbonyl (*4S,1'R*)-2,2-Dimethyl-4-(1'-hydroxy-12'-*tert*-butyl-dimethylsiloxy-2'-dodecynyl)oxazolidine (4).** To a solution of alkyne **3** (5.08 g, 18.0

mmol) in dry THF (150 mL) was added *n*-BuLi (2.5 M in hexane, 6.6 mL, 1.65 mmol) at –78 °C under N_2 atmosphere. The mixture was stirred for 2 h before adding HMPA (116 mg, 114 μL, 0.65 mmol). After the mixture was stirred for another 30 min, a solution of (*S*)-Garner aldehyde (3.45 g, 15.0 mmol) in 15 mL of dry THF was added slowly. The solution was stirred at –78 °C for 2.5 h before quenching with aqueous saturated NH_4Cl solution (40 mL). The mixture was extracted with Et_2O (3 x 50 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography to afford **4** (4.34 g, 67%) as a colorless oil; $[\alpha]^{25}_D$ – 33.2° (*c* 5.0, $CHCl_3$); R_f 0.61 (EtOAc/hexane 1:3); ^{1}H NMR δ 0.01 (s, 6H), 0.85 (s, 9H), 1.23-1.53 (m, 29H), 2.15 (m, 2H), 3.54 (t, 2H, *J* = 6.4 Hz), 3.89 (s, 1H), 4.08 (m, 2H), 4.51 (s, 1H); ^{13}C NMR δ –5.25, 18.3, 18.8, 25.8, 28.3, 28.4, 28.5, 29.1, 29.4, 29.5, 32.9, 62.9, 63.3, 64.2, 65.1, 81.2, 86.6, 94.9, 99.4, 154.1; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{28}H_{53}NO_5SiNa$ 534.3591, found 534.3561.

(*2S,3R*)-2-(*N-tert*-Butoxycarbonyl)amido-14–(*tert*-butyldimethylsilyloxy)-4(*E*)-tetradecene-1,3,14-triol (7). To a blue solution prepared from lithium (1.40 g, 200 mmol) and dry $EtNH_2$ (50 mL) was added a solution of **4** (5.12 g, 10 mmol) in THF (10 mL) at –78 °C. The reaction mixture was stirred for 4 h at –78 °C and then quenched with solid NH_4Cl. After $EtNH_2$ was removed under reduced pressure, the aqueous solution was extracted with CH_2Cl_2 (3 x 10 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated. To the residue were added dioxane (60 mL), H_2O (20 mL), Et_3N (5 mL), and Boc_2O (2.62 g, 12 mmol). The solution was stirred overnight at rt before quenching with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with Et_2O (3 x 50 mL), and the combined organic phases were

washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane 1:1), providing **7** (3.97 g, 84%) as a colorless wax; $[\alpha]^{25}_D$ –1.33° (*c* 11.7, $CHCl_3$); R_f 0.43 (EtOAc/hexane 1:1); ^{1}H NMR δ 0.00 (s, 6H), 0.85 (s, 9H), 1.12-1.47 (m, 23H), 1.98 (q, 2H, *J* = 7.2 Hz), 3.55 (t, 2H, *J* = 6.4 Hz), 3.56–3.62 (m, 2H), 3.73 (m, 1H), 3.83 (m, 2H), 4.19 (m, 1H), 5.43 (t, 1H, *J* = 6.4 Hz), 5.47 (d, 1H, *J* = 6.4 Hz), 5.75 (m, 1H); ^{13}C NMR δ –5.26, 18.3, 25.8, 26.0, 28.4, 29.1, 29.4, 29.6, 32.3, 32.8, 55.5, 62.3, 63.3, 74.1, 79.6, 129.2, 133.7, 156.3. MS (ESI) *m/z* 474.3 (MH^+).

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N*-*tert*-butoxycarbonylamido)-14-(*tert*-butyl-dimethylsilyloxy)-4(*E*)-tetradecene-1,3,14-triol (8). Dimethylaminopyridine (137 mg, 0.55 mmol) was added to a solution of **7** (1.37 g, 2.89 mmol) and benzoic anhydride (2.61 g, 11.6 mmol) in pyridine (30 mL), and the mixture was stirred at rt overnight. Volatiles were evaporated under vacuum, and the residue was purified by chromatography (elution with EtOAc/hexane 1:6), providing **8** (1.80 g, 91%) as a colorless oil; $[\alpha]^{25}_D$ –4.14° (*c* 5.6, $CHCl_3$); R_f 0.61 (EtOAc/hexane 1:3); ^{1}H NMR δ 0.00 (s, 6H), 0.84 (s, 9H), 1.20-1.37 (m, 21H), 1.43 (m, 2H), 2.01 (q, 2H, *J* = 7.2 Hz), 3.54 (t, 2H, *J* = 6.8 Hz), 4.46 (m, 3H), 4.94 (d, 1H, *J* = 9.6 Hz), 5.54 (m, 1H), 5.63 (m, 1H), 5.88 (m, 1H), 7.37 (m, 4H), 7.50 (m, 2H), 7.99 (m, 4H); ^{13}C NMR δ –5.26, 18.4, 25.8, 26.0, 28.3, 28.7, 29.2, 29.4, 29.5, 32.3, 32.9, 52.3, 63.3, 63.6, 75.1, 79.8, 124.0, 128.3, 128.4, 129.5, 129.7, 130.0, 132.8, 133.1, 137.2, 155.3, 165.4, 166.4. MS (ESI) *m/z* 699.3 (MNH_4^+).

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N*-*tert*-butoxycarbonylamido)-4(*E*)-tetradecene-1,3,14-triol (9). A solution of TBAF (1 M, 7.2 mL) in THF (1.88 g, 7.20 mmol) was

added to a solution of **8** (1.65 mg, 2.42 mmol) in THF (80 mL). After the mixture was stirred at rt overnight, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane 1:6), providing **9** (1.00 g, 73%) as a colorless oil; $[\alpha]^{25}_{D}$ –5.85° (*c* 4.80, $CHCl_3$); R_f 0.39 (EtOAc/hexane 1:1); ^{1}H NMR δ 1.20-1.58 (m, 23H), 2.04 (q, 2H, *J* = 7.2 Hz), 3.62 (t, 2H, *J* = 6.8 Hz), 4.46 (m, 3H), 4.90 (d, 1H, *J* = 9.2 Hz), 5.54 (dd, 1H, *J* = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (td, 1H, *J* = 6.4, 15.6 Hz), 7.41 (m, 4H), 7.57 (m, 2H), 8.03 (m, 4H); ^{13}C NMR δ 25.7, 28.3, 28.7, 29.0, 29.2, 29.3, 29.4, 32.3, 32.8, 52.4, 63.0, 63.6, 75.1, 79.9, 124.0, 128.4, 129.7, 129.8, 130.0, 133.1, 137.2, 155.3, 165.4, 166.5; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{33}H_{45}NO_7Na$ 590.3094, found 590.3069.

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N-tert*-butoxycarbonylamido)-14-(*O)*-(4'-benz-oylphenyl)-4(*E*)-tetradecene-1,3,14-triol (10). To a solution of DIAD (50 mg, 0.25 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added a solution of Ph_3P (70 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred for 10 min, a solution of 4-hydroxybenzophenone (56 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL) was added over a period of 20 min. The reaction mixture was stirred for another 10 min, and a solution of alcohol **9** (94 mg, 0.17 mmol) in dry CH_2Cl_2 (10 mL) was added over a period of 20 min. After the mixture was stirred for 10 min at 0 °C, the ice bath was removed, and the mixture was stirred at rt overnight. TLC indicated that **9** had disappeared. The organic solution was concentrated, and the resulting residue was purified by chromatography (elution with EtOAc/hexane 1:6) to afford **10** (93 mg, 76%) as a white wax; $[\alpha]^{25}_{D}$ –2.71° (*c* 2.8, $CHCl_3$); R_f 0.42 (EtOAc/hexane 1:3); ^{1}H NMR δ 1.27-1.58 (m, 21H), 1.77 (m, 2H), 2.04 (q, 2H, *J* = 7.2 Hz), 4.02 (t, 2H, *J* = 6.4 Hz), 4.42 (m, 1H), 4.50 (m, 2H), 4.87

(d, 1H, *J* = 9.2 Hz), 5.54 (dd, 1H, *J* = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (td, 1H, *J* = 6.4, 15.6 Hz), 6.93 (m, 2H), 7.45 (m, 6H), 7.57 (m, 3H), 7.75 (d, 2H, *J* = 7.2 Hz), 7.80 (d, 4H, *J* = 8.8 Hz), 8.04 (m, 4H); ^{13}C NMR δ 22.0, 26.0, 28.3, 28.7, 29.1, 29.3, 29.4, 30.9, 32.3, 40.4, 52.3, 63.6, 75.1, 79.9, 114.0, 124.1, 128.2, 128.4, 129.7, 129.9, 130.0, 131.8, 132.6, 133.2, 137.2, 138.4, 155.3, 162.9, 165.4, 166.5, 195.6.

(*2S,3R*)-2-(*N-tert*-Butoxycarbonylamido)-14-(*O*)-(4'-benzoylphenyl)-4(*E*)-tetradecene-1,3,14-triol (11). To a solution of 1 M NaOH (10 mL) in MeOH was added **10** (57 mg, 0.076 mmol). After the solution was stirred overnight at rt, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane 1:6), providing diol **11** (38.7 mg, 70%) as a white wax; $[\alpha]^{25}_{D}$ -1.21° (*c* 2.4, $CHCl_3$); R_f 0.38 (EtOAc/hexane 1:1); ^{1}H NMR δ 1.26-1.50 (m, 21H), 1.79 (m, 2H), 2.04 (q, 2H, *J* = 7.2 Hz), 3.60 (m, 1H), 3.71 (dd, 1H, *J* = 3.6, 11.6 Hz), 3.92 (dd, 1H, *J* = 3.6, 11.6 Hz), 4.04 (t, 2H, *J* = 6.4 Hz), 4.31 (m, 1H), 5.34 (m, 1H), 5.51 (dd, 1H, *J* = 6.4, 15.6 Hz), 5.75 (dt, 1H, *J* = 8.0, 15.6 Hz), 6.93 (d, 2H, *J* = 7.2 Hz), 7.46 (t, 2H, *J* = 8.0 Hz), 7.57 (t, 1H, *J* = 7.2 Hz), 7.74 (d, 2H, *J* = 6.8 Hz), 7.80 (d, 2H, *J* = 7.6 Hz); ^{13}C NMR δ 24.0, 28.4, 29.0, 29.2, 29.3, 29.4, 29.5, 32.3, 55.4, 62.6, 68.3, 74.6, 79.7, 114.0, 124.0, 128.1, 129.0, 129.5, 129.7, 129.8, 131.9, 132.6, 133.8, 138.3, 156.3, 162.9, 195.7. MS (ESI) *m/z* 540.3 (MH^+).

(*2S,3R*)-2-Amido-14-(*O*)-(4'-benzoylphenyl)-4(*E*)-tetradecene-1,3,14-triol (12). To a solution of 4 M HCl (10 mL) and THF (10 mL) was added **11** (42 mg, 0.080 mmol). The solution was stirred at rt overnight and then neutralized with 4 M NaOH (10 mL). The product was extracted with EtOAc (3 x 15 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). The volatiles were evaporated under

vacuum, and the residue was purified by chromatography (elution with $CHCl_3$/MeOH/conc. NH_4OH 130:25:4), providing **12** (28 mg, 80%) as a white solid; mp 79.5-80.8 °C; $[\alpha]^{25}_D$ -6.24° (*c* 2.43, $CHCl_3$); R_f 0.30 ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4); ^{1}H NMR δ 1.26-1.50 (m, 12H), 1.79 (m, 2H), 2.04 (q, 2H, *J* = 7.2 Hz), 3.02 (s, 1H), 3.45 (s, 4H), 3.71 (m, 2H), 4.02 (t, 2H, *J* = 6.4 Hz), 4.20 (m, 1H), 5.47 (dd, 1H, *J* = 6.4, 15.6 Hz), 5.78 (dt, 1H, *J* = 8.0, 15.6 Hz), 6.93 (d, 2H, *J* = 7.2 Hz), 7.46 (t, 2H, *J* = 8.0 Hz), 7.57 (t, 1H, *J* = 7.2 Hz), 7.74 (d, 2H, *J* = 6.8 Hz), 7.80 (d, 2H, *J* = 7.6 Hz); ^{13}C NMR δ 26.0, 29.1, 29.2, 29.3, 29.4, 29.5, 32.3, 56.4, 62.4, 68.3, 73.8, 114.0, 124.0, 128.1, 128.3, 129.7, 129.8, 131.9, 132.6, 134.7, 138.3, 162.9, 195.7; HR-MS [DCI, MH^+] *m/z* calcd for $C_{27}H_{38}NO_4$ 440.2801, found 440.2795 .

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N*-*tert*-butoxycarbonyl)amido-14-((4'-trifluoro-acetyl)phenoxy)-4(*E*)-tetradecene-1,3,14-triol (13). To a solution of DIAD (277 mg, 1.37 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added a solution of Ph_3P (395 mg, 1.51 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred for 10 min, a solution of 4-hydroxytrifluoroacetophenone (**22**) (260 mg, 1.37 mmol) in dry CH_2Cl_2 (5 mL) was added over a 20-min period. The reaction mixture was stirred for another 10 min, and a solution of **9** (521 mg, 0.92 mmol) in dry CH_2Cl_2 (10 mL) was added over a 20-min period. After the mixture was stirred for 10 min at 0 °C, the ice bath was removed, and the mixture was stirred at rt overnight. TLC (EtOAc/hexane 1:6) indicated that **9** had disappeared. The organic solution was concentrated and the residue was purified by chromatography (elution with EtOAc/hexane 1:6), giving **13** (491 mg, 74%) as a colorless wax; $[\alpha]^{25}_D$ –2.82° (*c* 5.0, $CHCl_3$); R_f 0.41 (EtOAc/hexane 1:3); ^{1}H NMR δ 1.27-1.58 (m, 21H), 1.79 (m, 2H), 2.04 (q, 2H, *J* = 7.2 Hz), 4.02 (t, 2H, *J* = 6.4 Hz), 4.42

(m, 1H), 4.50 (m, 2H), 4.87 (d, 1H, J = 9.2 Hz), 5.54 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (dt, 1H, J = 6.4, 15.6 Hz), 6.97 (d, 2H, J = 9.2 Hz), 7.43 (m, 4H), 7.57 (m, 2H), 8.02 (m, 6H); ^{13}C NMR δ 25.2, 27.5, 28.0, 28.2, 28.3, 28.6, 28.7, 31.6, 31.9, 38.9, 51.7, 62.9, 67.9, 74.5, 79.2, 114.2, 114.8 (q, J_{CF} = 290 Hz), 121.8, 123.4, 127.7, 129.0, 129.2, 132.0, 133.4, 136.5, 154.6, 164.4, 164.7, 165.7, 178.3 (q, J_{CF} = 34 Hz). MS (ESI) *m/z* 757.2 (MNH_4^+).

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N*-*tert*-butoxycarbonyl)amido-14-(*O*)-(4'-trifluoroacetylphenyl)-4(*E*)-tetradecene-1,3,14-triol Oxime (14). A solution of **13** (393 mg, 0.53 mmol) and hydroxylamine hydrochloride (38 mg, 0.55 mmol) in absolute EtOH (5 mL) and dry pyridine (2 mL) was heated at 50-60 °C for 12 h. The solvents were evaporated and the residue was partitioned between water and Et_2O. The organic layer was dried ($MgSO_4$) and concentrated. The crude oxime was purified by chromatography (elution with CH_2Cl_2/MeOH 20:1) to provide **14** (396 mg, 99%) as a colorless oil; $[\alpha]^{25}_D$ –2.24° (*c* 5.8, $CHCl_3$); R_f 0.39 (EtOAc/hexane 1:3); ^{1}H NMR δ 1.26-1.44 (m, 21H), 1.75 (m, 2H), 2.04 (m, 2H), 3.97 (t, 2H, J = 6.8 Hz), 4.47 (m, 3H), 4.94 (d, 1H, J = 9.2 Hz), 5.56 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.92 (dt, 1H, J = 6.4, 15.6 Hz), 6.94 (d, 2H, J = 8.8 Hz), 7.42 (m, 4H), 7.53 (m, 4H), 8.02 (m, 4H), 9.76 (s, 1H); ^{13}C NMR δ 25.1, 27.4, 27.9, 28.2, 28.3, 28.4, 28.5, 31.5, 51.5, 62.7, 67.1, 74.3, 79.3, 113.4, 117.2, 118.7 (q, J_{CF} = 273 Hz), 123.1, 127.6, 128.8, 128.9, 129.1, 129.6, 132.4, 136.4, 145.6 (q, J_{CF} = 32 Hz), 154.6, 159.7, 164.7, 165.7. MS (ESI) *m/z* 755.2 (MH^+), 772.2 (MNH_4^+).

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N*-*tert*-butoxycarbonylamido)-14-(*O*)-((4'-trifluoroacetyl)phenyl)-4(*E*)-tetradecene-1,3,14-triol *O*-Tosyl Oxime (15). To a solution of oxime **14** (352 mg, 0.47 mmol), triethylamine (142 mg, 1.4 mmol), and DMAP (4.0

mg, 0.030 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added *p*-tosyl chloride (106 mg, 0.56 mmol) portionwise with stirring. The reaction mixture was stirred for 45 min at rt. The mixture was washed with water, and the organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane 1:3), providing **15** (420 mg, 99%) as a light yellow oil; $[\alpha]^{25}_D$ –2.13° (*c* 9.2, $CHCl_3$); R_f 0.38 (EtOAc/hexane 1:3); ^{1}H NMR δ 1.26-1.56 (m, 21H), 1.77 (m, 2H), 2.05 (m, 2H), 2.46 (m, 3H), 3.97 (t, 2H, *J* = 6.8 Hz), 4.47 (m, 3H), 4.90 (d, 1H, *J* = 9.2 Hz), 5.56 (dd, 1H, *J* = 7.2, 15.2 Hz), 5.68 (m, 1H), 5.92 (dt, 1H, *J* = 6.4, 15.6 Hz), 6.92 (m, 2H), 7.43 (m, 8H), 7.53 (m, 2H), 7.88 (m, 2H), 8.03 (m, 4H); ^{13}C NMR δ 21.8, 26.0, 28.3, 28.7, 29.0, 29.1, 29.3, 29.4, 30.3, 31.3, 32.3, 52.3, 63.6, 68.2, 75.1, 79.9, 114.6, 114.7, 119.5, 119.7 (q, J_{CF} = 273 Hz), 122.9, 124.1, 128.4, 129.1, 129.3, 129.7, 129.8, 129.9, 130.3, 130.6, 130.7, 131.3, 131.6, 133.2, 137.2, 145.9, 146.0, 154.0 (q, J_{CF} = 32 Hz), 155.3, 161.7, 162.1, 165.4, 166.4. MS (ESI) *m/z* 926.2 (MNH_4^+).

(*2S,3R*)-1,3-(*O*-Dibenzoyl)-2-(*N-tert*-butoxycarbonylamido)-14-(*O*)-((4'-trifluoromethyldiaziridinyl)phenyl)-4(*E*)-tetradecene-1,3,14-triol (16). To a solution of **15** (378 mg, 0.42 mmol) in 10 mL of dry CH_2Cl_2 in a thick-wall, three-neck flask at –78 °C was added liquid ammonia (5 mL). The flask was sealed, and the mixture was stirred at rt for 18 h. The excess ammonia was allowed to evaporate at rt. The residue was partitioned between water and CH_2Cl_2, and the organic layer was dried ($MgSO_4$). After evaporation of the solvent, the residual oil was purified by chromatography (elution with Et_2O) to give **16** (282 mg, 80%) as a colorless wax; $[\alpha]^{25}_D$ –4.78° (*c* 8.3, $CHCl_3$); R_f 0.35 (EtOAc/hexane 1:3); ^{1}H NMR δ 1.24-1.41 (m, 21H), 1.75 (m, 2H), 2.04 (m, 2H), 2.18 (d, 1H, *J* = 8.4 Hz), 2.74 (d, 1H, *J* = 8.4 Hz), 3.97 (t, 2H, *J* = 6.8 Hz), 4.47 (m, 3H), 4.91 (d,

1H, J = 9.2 Hz), 5.56 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.92 (dt, 1H, J = 6.4, 15.6 Hz), 6.91 (d, 2H, J = 8.8 Hz), 7.43 (m, 4H), 7.54 (m, 4H), 8.03 (m, 4H); ^{13}C NMR δ 26.0, 28.3, 28.7, 29.1, 29.2, 29.4, 32.3, 52.3, 57.5 (q, J_{CF} = 32 Hz), 63.6, 68.1, 75.1, 80.0, 114.6, 121.3 (q, J_{CF} = 273 Hz), 123.4, 124.1, 128.4, 129.4, 129.7, 129.8, 130.0, 132.2, 137.2, 155.3, 160.4, 165.4, 166.4. MS (ESI) *m/z* 754.2 (MH^+).

(*2S,3R*)-2-(*N-tert*-Butoxycarbonylamido)-14-(*O*)-((4'-trifluoromethyldiaziridinyl)phenyl)-4(*E*)-tetradecene-1,3,14-triol (17). To a solution of NaOH (1 M, 10 mL) in MeOH was added **16** (155 mg, 0.21 mmol). After the solution was stirred at rt overnight, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane 2:1), providing **17** (110 mg, 97%) as a colorless oil; $[\alpha]^{25}_D$ –1.24° (*c* 5.0, $CHCl_3$); R_f 0.56 (EtOAc/hexane 2:1); ^{1}H NMR δ 1.24-1.47 (m, 21H), 1.75 (m, 2H), 2.04 (m, 2H), 2.27 (d, 1H, J = 8.4 Hz), 2.82 (d, 1H, J = 8.4 Hz), 3.43 (s, 2H), 3.57 (s, 1H), 3.67 (m, 1H), 3.86 (m, 1H), 3.95 (t, 2H, J = 6.8 Hz), 4.25 (m, 1H), 5.41 (d, 1H, J = 8.0 Hz), 5.51 (dd, 1H, J = 7.2, 15.2 Hz), 5.74 (dt, 1H, J = 6.4, 15.6 Hz), 6.91 (d, 2H, J = 8.8 Hz), 7.49 (d, 2H, J = 8.4 Hz); ^{13}C NMR δ 26.0, 28.3, 29.1, 29.2, 29.3, 29.4, 29.5, 32.3, 55.5, 57.5 (q, J_{CF} = 32 Hz), 62.5, 68.1, 74.4, 79.8, 114.6, 121.3 (q, J_{CF} = 273 Hz), 123.4, 129.1, 129.5, 134.0, 156.3, 160.4. MS [ESI] *m/z* 546.3 (MH^+).

(*2S,3R*)-2-(*N-tert*-Butoxycarbonylamido)-14-(*O*)-((4'-trifluoromethyldiazirinyl)phenyl)-4(*E*)-tetradecene-1,3,14-triol (18). A mixture of **17** (30 mg, 0.056 mmol), I_2 (8.5 mg, 0.067 mmol), and Et_3N (16 mg, 0.17 mmol) in MeOH (10 mL) was stirred at rt for 1 h. After 20 mL of Et_2O was added, the mixture was washed with brine and dried ($MgSO_4$). The product (25 mg, 82%) was obtained by chromatography (elution

with CH_2Cl_2); R_f 0.46 (EtOAc/hexane 1:1); ^{1}H NMR δ 1.24-1.47 (m, 21H), 1.75 (m, 2H), 2.04 (m, 2H), 3.60 (s, 1H), 3.71 (m, 1H), 3.95 (t, 2H, J = 6.8 Hz), 4.30 (m, 1H), 5.30 (m, 1H), 5.53 (dd, 1H, J = 7.2, 15.2 Hz), 5.77 (dt, 1H, J = 6.4, 15.6 Hz), 6.90 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.4 Hz); ^{13}C NMR δ 26.0, 28.0 (q, J_{CF} = 32 Hz), 28.3, 29.1, 29.3, 29.4, 29.5, 32.2, 55.3, 62.6, 68.1, 74.8, 79.8, 114.8, 120.6, 121.0 (q, J_{CF} = 273 Hz), 128.0, 129.0, 156.3, 160.2; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{27}H_{40}F_3N_3O_5Na$ 566.2818, found 566.2841.

(*2S,3R*)-2-Amido-14-(*O*)-((4'-trifluoromethyldiazirinyl)phenyl)-4(*E*)-tetradecene-1,3,14-triol (19). To a solution of 4 M HCl (10 mL) and THF (10 mL) was added **18** (42 mg, 0.080 mmol), and the solution was stirred at rt overnight before neutralizing with 4 M NaOH (10 mL). The product was extracted with EtOAc (3 x 15 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). The volatiles were evaporated under vacuum, and the residue was purified by chromatography (elution with $CHCl_3$/MeOH/conc. NH_4OH 130:25:4), providing **19** (28 mg, 80%) as a white solid; R_f 0.30 ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4); ^{1}H NMR δ 1.26-1.50 (m, 12H), 1.76 (m, 2H), 2.04 (q, 2H, J = 6.8 Hz), 2.77 (s, 4H), 2.93 (s, 1H), 3.68 (m, 2H), 3.93 (t, 2H, J = 6.4 Hz), 4.07 (m, 1H), 5.47 (dd, 1H, J = 6.4, 15.6 Hz), 5.78 (td, 1H, J = 8.0, 15.6 Hz), 6.89 (d, 2H, J = 6.8 Hz), 7.13 (t, 2H, J = 8.8 Hz); ^{13}C NMR δ 25.9, 28.0 (q, J_{CF} = 32 Hz), 28.9, 29.1, 29.2, 29.3, 29.4, 29.5, 32.3, 56.4, 62.4, 68.1, 73.8, 114.8, 120.6, 121.9 (q, J_{CF} = 273 Hz), 128.0, 128.8, 134.7, 160.2; HR-MS [DCI, MH^+] *m/z* calcd for $C_{22}H_{33}F_3N_3O_3$ 444.2474, found 444.2478.

***N*-Trifluoroacetylpiperidine (20).** To a 100-mL, 3-neck flask were added piperidine (3.8 mL, 0.04 mol), triethylamine (4.6 mL, 0.032 mol), and 15 mL of Et_2O.

The reaction mixture was cooled in an ice bath, and a solution of $(CF_3CO)_2O$ (4.6 mL, 0.032 mol) in 10 mL of Et_2O was added dropwise with stirring. After the reaction mixture was stirred overnight at rt, the mixture was diluted with Et_2O (25 mL) and washed with 0.2 M HCl until neutral, then with brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane 1:6) to give 4.03 g (70%) of **20** as a colorless oil; R_f 0.48 (EtOAc/hexane 1:6); ^{1}H NMR δ 1.70 (m, 6H), 3.60 (m, 4H); ^{13}C NMR δ 24.3, 25.5, 26.5, 44.7, 47.0, 115.4 (q, J_{CF} = 286 Hz), 155.2 (q, J_{CF} = 35 Hz).

4-Methoxytrifluoroacetophenone (21). Mg turnings (0.241 g, 10 mmol), 4-bromoanisole (1.87 g, 10.0 mmol), and dry THF (10 mL) were placed in a 100-mL round-bottom flask. The mixture was cautiously heated until a vigorous reaction took place. The exothermic reaction was allowed to proceed until almost all of the Mg turnings had dissolved. The reaction mixture was then cooled in an ice bath. A solution of **20** (1.46 g, 8.0 mmol) in dry THF (2 mL) was added dropwise to the Grignard reagent over a 10-min period with stirring at 0 °C. After the mixture had stirred for 2 h at rt, the reaction was quenched with saturated aqueous NH_4Cl solution (5 mL), and the precipitate was removed by filtration. The filtrate was dried ($MgSO_4$) and evaporated, and the residue was distilled to give 4.81 g (53%) of **21** as a colorless oil; bp 84-85 °C (3.5 torr); R_f 0.46 (EtOAc/hexane 1:6); ^{1}H NMR δ 3.88 (s, 3H), 6.96 (d, 2H, J = 10.0 Hz), 8.00 (d, 2H, J = 9.2 Hz); ^{13}C NMR δ 55.8, 114.7, 115.8 (q, J_{CF} = 320.3 Hz), 122.9, 132.9, 165.7, 178.8 (q, J_{CF} = 35 Hz).

4-Hydroxytrifluoroacetophenone (22). A mixture of **21** (1.085 g, 5.34 mmol) and LiCl (690 mg, 16 mmol) in 20 mL of DMF was heated at reflux under N_2 for 2 h.

The mixture was cooled to rt, poured into water (50 mL), and acidified with 10% HCl. The product was extracted with Et_2O (2 x 50 mL). The ethereal layer was washed with brine twice, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane 1:3), providing 888 mg (88%) of **22** as white crystals; R_f 0.10 (EtOAc/hexane 1:6); ^{1}H NMR δ 6.09 (s, 1H), 6.97 (d, 2H, J = 9.6 Hz), 8.03 (d, 2H, J = 8.8 Hz); ^{13}C NMR δ 118.3, 119.1 (q, J_{CF} = 291.8 Hz), 125.1, 135.4, 164.4, 181.4 (q, J_{CF} = 35 Hz).

References

1. (a) Pyne, S.; Pyne, N. J. *Biochem. J.* **2000**, *349*, 385-402. (b) Spiegel, S.; Milstien, S. *J. Biol. Chem.* **2002**, *277*, 25851-25854. (c) Hla, T. *Pharmacol. Res.* **2003**, *47*, 401-407.

2. (a) Spiegel, S.; Milstien, S. *Biochim. Biophys. Acta* **2000**, *1484*, 107-116. (b) Spiegel, S.; English, D.; Milstien, S. *Trends Cell Biol.* **2002**, *12*, 236-242.

3. (a) Maceyka, M.; Payne, S. G.; Milstien, S.; Spiegel, S. *Biochim. Biophys. Acta* **2002**, *1585*, 193-201. (b) Liu, H.; Chakravarty, D.; Maceyka, M.; Milstien, S.; Spiegel, S. *Prog. Nucleic Acid Res. Mol. Biol.* **2002**, *71*, 493-511.

4. Parrill, A. L.; Wang, D.; Bautista, D. L.; Van Brocklyn, J. R.; Lorincz, Z.; Fischer, D. J.; Baker, D. L.; Liliom, K.; Spiegel, S.; Tigyi, G. *J. Biol. Chem.* **2000**, *275*, 39379-39384.

5. (a) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Xia, P.; Gamble, J. R.; Vadas, M. A.; D'Andrea, R. J.; Wattenberg, B. W. *J. Biol. Chem.* **2000**, *275*, 33945-33950. (b) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Vadas, M. A.; D'Andrea, R. J.; Wattenberg, B.

W. *FEBS Lett.* **2001**, *509*, 169-173. (c) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Zareie, R.; Derian, C. K.; Darrow, A. L.; Qi, J.; D'Andrea, R. J.; Bagley, C. J.; Vadas, M. A.; Wattenberg, B. W. *J. Biol. Chem.* **2002**, *277*, 49545-49553.

6. Murata, N.; Sato, K.; Kon, J.; Tomura, H.; Yanagita, M.; Kuwabara, A.; Ui, M.; Okajima, F. *Biochem. J.* **2000**, *352*, 809-815.

7. (a) Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687. (b) Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.

8. (a) Garner, P.; Park, J. M. *Org. Synth*. **1991**, *70*, 18-27. (b) Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136-2157.

9. Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362.

10. For a review of the Mitsunobu reaction, see: Hughes, D. L. *Org. React*. **1992**, *42*, 335-656.

11. Li, G.; Samadder, P.; Arthur, G.; Bittman, R. *Tetrahedron* **2001**, *57*, 8925-8932.

12. Wang, D. A.; Lorincz, Z.; Bautista, D. L.; Liliom, K.; Tigyi, G.; Parrill, A. L. *J. Biol. Chem.* **2001**, *276*, 49213-49220.

13. Nagase, S.; Shimamune, K.; Shumiya, S. *Science* **1979**, *205*, 590-591.

14. Kalivretenos, A.; Stille, J. K.; Hegedus, L. S. *J. Org. Chem.* **1991**, *56*, 2883-2894.

15. Lu, X.; Cseh, S.; Byun, H.-S.; Tigyi; G.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 7046-7050.

Chapter 2

Synthesis of a Photoactivatable (2*S*,3*R*)-Sphingosylphosphorylcholine Analogue

Abstract

The receptor for the lipid mediator sphingosylphosphorylcholine (SPC) has not yet been identified. This chapter describes the synthesis of the first photoaffinity analogue of SPC. This probe, which contains a ^{14}C-isotopic label in the choline methyl groups and a photoreactive benzophenone in the long-chain base, may be a useful tool in the identification of the G protein-coupled receptors that have been postulated to interact directly and specifically with SPC and in the definition of the ligand-binding sites. The key steps in the synthesis are selective reduction of the triple bond in enyne **6** to install the 4*E* double bond, Suzuki coupling to incorporate the benzophenone photophore at the end of the sphingoid chain, and reduction of the 2-azidoethyl phosphate head group of **13** followed by *N,N,N*-trimethylation to introduce the radiolabel into the choline moiety. The synthesis was completed by the release of the amino group at C2 of the sphingoid base of SPC analogue **2**.

Introduction

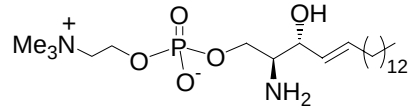
Sphingosylphosphorylcholine (SPC or lysosphingomyelin, **1**) is formed by *N*-deacylation of sphingomyelin (SPM).[1] SPC is a natural component of blood plasma[2] and high-density lipoproteins.[3] It accumulates in the brain of patients with Niemann-Pick

type A disease[4] and in the malignant ascites of patients with ovarian cancer.[5] SPC participates in the regulation of many cellular functions, including proliferation, cell migration, smooth muscle contraction, and wound healing.[6] SPC also has potential pathophysiological roles in angiogenesis (the growth of new capillary blood vessels)[7] and in enhancement of the elasticity of human epithelial tumor cells.[8] Several putative receptors for SPC have been suggested.[6,9] Several years ago it was concluded that the multiple cellular functions of SPC were directly transduced via a G protein-coupled receptor called GPR4.[10] However, this conclusion was retracted recently.[11] Nevertheless, very recently it was proposed that SPC-induced angiogenesis in endothelial cells is mediated by GPR4.[7] Thus the molecular identity of SPC receptor(s) is unclear at present. The availability of a photoreactive SPC analogue would provide the means for identifying the SPC receptor and help elucidate the molecular mechanisms underlying the normal and pathophysiological functions of SPC.

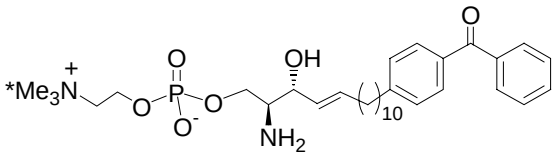
Photolabeling techniques employ molecules that are targeted to a biological system and, upon photolysis with UV light, generate short-lived, highly reactive intermediates that form covalent bonds with adjacent molecules. Photoactivatable analogues of phospholipids have been used to identify lipid-binding membrane proteins.[12] Chapter 1 describes a study of photoactivatable analogues of the lipid mediator sphingosine 1-phosphate (S1P);[13] a probe containing benzophenone was bound more tightly to a S1P receptor than a similar S1P analogue bearing a 3-trifluoromethyl-3-aryldiazirine probe, perhaps because of an electrostatic effect.[14] Therefore, the benzophenone photophore was selected for the study presented in this chapter. Among its many useful features are (a) a high degree of hydrophobicity, thus inserting

spontaneously into membrane bilayers; (b) chemical stability with respect to many solvents and reaction conditions, and stability in the absence of light; (c) photoactivatability to a triplet state with long wavelength UV light ($\lambda > 350$ nm), thus minimizing damage to proteins; and (d) the ability to undergo photochemical reactions by random insertion into accessible Cα-H bonds of amino acid residues. A radiolabel is generally incorporated into photoprobes to allow sensitive detection and identification of proteins covalently coupled to the probe. This chapter describes an efficient synthesis of a photoactivatable analogue **2** that bears a benzophenone moiety in the sphingoid chain and *N*-[^{14}C]-methyl groups in the polar head group.

Chart 1. Structures of SPC (1) and its analogue 2



SPC (**1**)



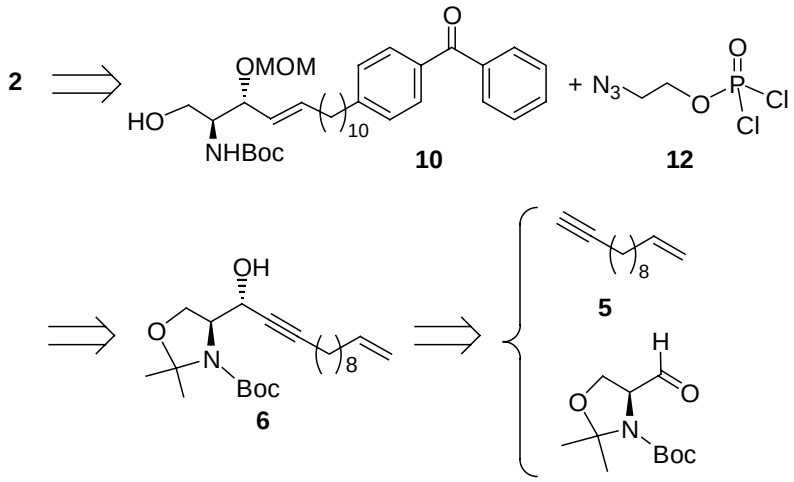
[^{14}C]-labeled photoprobe **2** (* = ^{14}C)

Results and Discussion

Synthetic Plan. As illustrated in the retrosynthetic analysis (Scheme 1), the synthesis of radiolabeled photoactivatable analogue **2** would start with the addition of the acetylide ion derived from enyne **5** to *N*-Boc-*N*,*O*-isopropylidene-L-serinal ((*S*)-Garner aldehyde).[15] Use of non-chelation controlled conditions would afford the requisite 2*S*,3*R* stereochemistry of the sphingoid backbone of **6**. After Red-Al reduction of progarylic

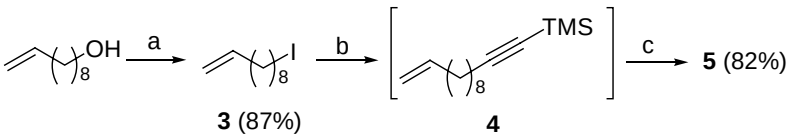
alcohol **6** and protection of the secondary alcohol, the benzophenone group would be installed by Suzuki coupling. The oxazolidine ring would be opened to provide alcohol **10**, with retention of the *N*-Boc group. Reaction with 2-azidoethyl phosphorochloridate **12**[16] and introduction of radioactivity into the choline moiety, followed by deprotection of the *O*-MOM and *N*-Boc groups in a one-pot reaction, would complete the synthesis of **2**.

Scheme 1. Retrosynthetic plan



Synthesis of Enyne 5. The synthesis of 1-dodecen-11-yne (**5**) began with 9-decen-1-ol, which was converted to iodide **3** as described previously[17] (Scheme 2). Alkynylation with lithium trimethylsilylacetylene afforded intermediate **4**, which was treated with 1 N NaOH to remove the TMS group, providing alkyne **5** (82% yield for two steps).

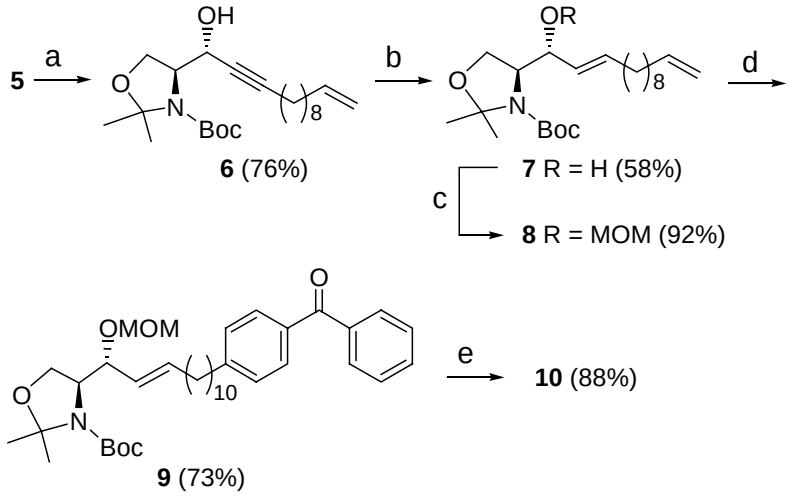
Scheme 2. Synthesis of 1-dodecen-11-yne (5)



Reagents and conditions: (a) PPh_3, I_2, imidazole, Et_2O/CH_3CN (3:1), 0-5 °C to rt; (b) TMS-acetylene, *n*-BuLi, HMPA, THF, -78 °C; (c) 1 N NaOH, Et_2O, rt. See reference 21.

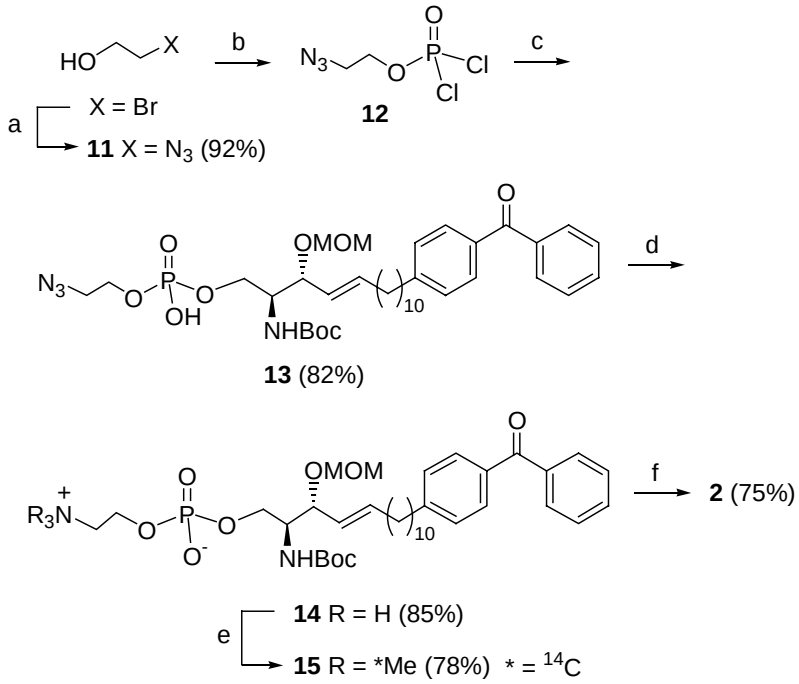
Synthesis of Alcohol 10. As outlined in Scheme 3, the acetylide anion derived from **5** was coupled diastereoselectively with (*S*)-Garner aldehyde in the presence of HMPA, giving *erythro* isomer **6** in 76% yield.[15] Reduction of propargylic alcohol **6** with 2 equiv of Red-Al afforded (4*E*)-diene **7** in moderate yield; 30% of starting material **6** was recovered. Use of longer reaction times or additional Red-Al resulted in oxazolidine ring opening. Protection of the hydroxy group of **7** as a MOM ether furnished **8**, which was subjected to hydroboration with 9-BBN (0 °C, overnight). After unreacted 9-BBN was destroyed, Suzuki coupling[18] with 4-bromobenzophenone afforded benzophenone-labeled sphingosine analogue **9** in 73% yield. Selective deprotection of **9** with 80% HOAc at 80 °C yielded primary alcohol **10** with retention of the *N*-Boc group.[19]

Scheme 3. Synthesis of alcohol 10



Reagents and conditions: (a) (*S*)-Garner aldehyde, *n*-BuLi, HMPA, THF, -78 °C; (b) Red-Al, Et_2O, rt, 4 h; (c) MOMCl, EtN(Pr-*i*)$_2$, CH_2Cl_2, 0 °C - rt; (d) (i) 9-BBN, THF, 0 °C–rt, (ii) $Pd(PPh_3)_4$, K_3PO_4, 4-bromobenzophenone, H_2O, dioxane, 85 °C; (e) 80% HOAc, 80 °C, 5 h. See reference 21.

Scheme 4. Synthesis of SPC photoprobe 2



Reagents and conditions: (a) NaN_3, *n*-Bu_4NBr, 15 h, 110 °C; (b) $POCl_3$, 0 °C; (c) (i) **10**, pyridine, Et_2O, rt (30 min), reflux (3 h), (ii) H_2O; (d) $HS(CH_2)_3SH$, Et_3N, MeOH, rt; (e) (i) [^{14}C]MeI, $NaHCO_3$, MeOH, 50 °C, pressure tube, 3 h, (ii) MeI (excess), 50 °C, pressure tube, 3 h; (f) 3 M HCl/THF (1:1), 70 °C, 3 h. See reference 21.

Synthesis of SPC Analogue 2. To prepare 2-azidoethyl chlorophosphate (**12**),[16] 2-bromoethanol was reacted with sodium azide, and the resulting 2-azidoethanol (**11**) was added to phosphorus oxychloride (Scheme 4). Phosphorylation was carried out by adding a solution of alcohol **10** in Et_2O to **12** in the presence of pyridine, providing 2-azidoethanol phosphate ester **13** in good yield. Reduction of the azido group to an amino group with PPh_3 or polymer-supported PPh_3 failed but reduction with 1,3-propanedithiol[20] in the presence of dry triethylamine afforded amine **14** in 85% yield. The radiolabel was introduced into the polar head group of **2** by *N*,*N*-dimethylation of 2-aminoethyl phosphate ester **14** with ~2.5 equiv of [^{14}C]methyl iodide in the presence of $NaHCO_3$ in MeOH at 50 °C in a pressure tube for 3 h. After complete *N*-methylation with a large excess (20 equiv) of unlabeled methyl iodide, the solvent and excess methyl iodide were evaporated, and the residue was purified on a short silica gel column to provide [^{14}C]-phosphocholine **15** in 78% yield. Deprotection of the *N*-Boc and *O*-MOM groups at the same time with 3 N HCl at 70 °C gave final product **2** in 75% yield.

In summary, a photoactivatable analogue of SPC bearing a benzophenone in the sphingoid base and radioactivity in the polar head group was prepared in 12 steps and 7.6% overall yield starting from Garner aldehyde. Suzuki coupling was used to install the photophore, and [^{14}C]-*N*-methyl groups were introduced in the penultimate step. The

specific activity of **2** was 3.2 mCi/mmol. It is anticipated that the availability of [^{14}C]-**2** will help unravel the identity of the SPC receptor(s), the nature of which has been elusive until now.[11]

Experimental Section

1-Dodecen-11-yne (5). *n*-BuLi (2.89 M in hexanes, 2.6 mL, 7.5 mmol) was added over 5 min to a stirred and cooled (-78 °C) solution of trimethylsilylacetylene (0.75 g, 7.5 mmol) in dry THF (20 mL). After 30 min, HMPA (5 mL) was added. A solution of iodide **3** (1.33 g, 5.0 mmol) in dry THF (5 mL) was added dropwise, the cold bath was removed, and stirring was continued overnight at rt The reaction was quenched with saturated aqueous NH_4Cl solution (20 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to give crude TMS-enyne **4**. To a solution of crude **4** in 15 mL of Et_2O was added 15 mL of aqueous 1 N NaOH solution. The mixture was stirred at rt for 1 h, then neutralized with 15 mL of 1 M HCl. The organic layer was isolated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (elution with hexane) gave alkyne **5** (0.67 g, 82%) as a colorless oil: R_f 0.80 (hexane); ^{1}H NMR ($CDCl_3$) δ 1.24-1.56 (m, 12H), 1.93 (t, 1H, J = 2.8 Hz), 2.04 (m, 2H), 2.17 (m, 2H), 4.94 (m, 2H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 18.4, 28.5, 28.7, 28.9, 29.1, 29.3, 29.7, 33.8, 68.4, 84.8, 114.1, 139.2.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*)-2,2-Dimethyl-4-(1'-hydroxy-2'-dodecyn-11'-enyl)oxazolidine (6).** To a solution of alkyne **5** (0.51 g, 3.1 mmol) in dry THF (50 mL)

was added *n*-BuLi (2.5 M in hexane, 1.24 mL, 3.1 mmol) at -78 °C under N_2. The mixture was stirred for 2 h before HMPA (45 mg, 44 μL, 0.25 mmol) was added. After the mixture was stirred for 30 min, a solution of (*S*)-Garner aldehyde (0.57 g, 2.5 mmol) in 5 mL of dry THF was added slowly. The solution was stirred at -78 °C for 2.5 h and then quenched with aqueous saturated NH_4Cl solution (40 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography (EtOAc/hexane 1:3) to afford **7** (0.77 g, 76%) as a colorless oil: R_f 0.59 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –34.1° (*c* 1.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24-1.65 (m, 27H), 2.04 (m, 2H), 2.20 (m, 2H), 3.91 (m, 1H), 4.11 (m, 2H), 4.52 (m, 1H), 4.94 (m, 2H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 18.8, 25.8, 28.4, 28.6, 29.1, 29.4, 33.8, 60.4, 62.8, 64.2, 77.9, 81.2, 86.6, 94.9, 114.2, 139.2, 154.1; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{23}H_{39}NO_4Na^+$ 416.2771, found 416.2777.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*)-2,2-Dimethyl-4-(1'-hydroxy-2'-dodecen-11'-enyl)oxazolidine (7).** To a solution of **6** (0.68 g, 1.67 mmol) in anhydrous Et_2O (20 mL) at 0 °C was added a solution of Red-Al (0.96 mL, 70% in toluene, 3.34 mmol) dropwise. After 10 min, the cooling bath was removed, and the reaction mixture was stirred at rt for 4 h. An aqueous saturated solution of NH_4Cl (2 mL) was slowly added (Caution! very exothermic). The resulting white slurry was diluted with Et_2O (10 mL), 1 N NaOH (5 mL), and water (5 mL), and the layers were separated. The aqueous phase was re-extracted with Et_2O (3 × 5 mL), and the combined organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc/hexanes 1:3) to afford 396 mg (58%) of diene **7** as a colorless oil: R_f 0.54 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –

22.3° (*c* 1.51, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.24-1.57 (m, 27H), 2.04 (m, 4H), 3.91 (m, 1H), 4.02 (m, 1H), 4.11 (m, 1H), 4.20 (m, 1H), 4.94 (m, 2H), 5.48 (m, 1H), 5.80 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.7, 26.2, 28.6, 28.9, 29.1, 29.2, 29.4, 29.7, 32.4, 33.8, 60.4, 62.3, 64.9, 74.0, 81.0, 94.4, 114.1, 128.2, 133.3, 139.2, 154.1; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{23}H_{41}NO_4Na^+$ 418.2928, found 418.2926.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*)-2,2-Dimethyl-4-(1'-methoxymethoxy-2'-dodecen-11'-enyl)oxazolidine (8).** To a solution of 409 mg (1.0 mmol) of alcohol **7** in 15 mL of anhydrous CH_2Cl_2 were added 269 mL (1.55 mmol) of $(i\text{-Pr})_2NEt$ and 118 mL (1.55 mmol) of MOMCl at 0 °C. After 10 min, the cooling bath was removed, and the reaction mixture was stirred overnight at rt. The reaction mixture was poured into H_2O (70 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexanes/EtOAc 6:1) gave 417 mg (92%) of ether **8** as a colorless oil: R_f 0.82 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –73.8° (*c* 1.50, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.24-1.60 (m, 27H), 2.04 (m, 4H), 3.36 (s, 3H), 3.92 (m, 2H), 4.07 (m, 1H), 4.28 (m, 1H), 4.51 (d, 1H, *J* = 6.4 Hz), 4.73 (d, 1H, *J* = 6.4 Hz), 4.94 (m, 2H), 5.32 (m, 1H), 5.69 (m, 1H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 22.7, 24.9, 26.2, 28.4, 29.0, 29.4, 32.4, 33.8, 55.8, 60.3, 64.6, 76.3, 79.9, 93.7, 94.3, 114.1, 126.7, 136.8, 139.2, 152.4; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{25}H_{45}NO_5Na^+$ 462.3190, found 462.3202.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*)-2,2-Dimethyl-4-(1'-methoxymethoxy-2'-dodecene-13'-benzoylphenyl)oxazolidine (9).** To a solution of 363 mg (0.80 mmol) of **8** in 5 mL of dry THF was added 1.8 mL (0.90 mmol) of a 0.5 M solution of 9-BBN in THF. The solution was stirred overnight until **8** had completely disappeared (TLC,

EtOAc/hexane 1:6). Unreacted 9-BBN was destroyed by adding 2 drops of H_2O with stirring for 10 min. To a solution of 209 mg (0.80 mmol) of 4-bromobenzophenone in 4 mL of dioxane were added $Pd(PPh_3)_4$ (28 mg, 0.024 mmol) and K_3PO_4 (0.92 g, 40 mmol). The reaction mixture was heated overnight at reflux (85 °C). After the solvents were removed, the residue was purified by chromatography (EtOAc/hexane 1:6), providing **9** (363 mg, 73%) as a colorless oil: R_f 0.51 (EtOAc/hexane 1:6); $[\alpha]^{25}_D$ –40.6° (*c* 0.83, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27-1.65 (m, 31H), 2.13 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 3.36 (s, 3H), 3.92 (m, 2H), 4.07 (m, 1H), 4.28 (m, 1H), 4.51 (d, 1H, *J* = 6.4 Hz), 4.73 (d, 1H, *J* = 6.4 Hz), 5.30 (m, 1H), 5.69 (m, 1H), 7.29 (d, 2H, *J* = 6.4 Hz), 7.47 (t, 2H, *J* = 7.2 Hz), 7.56 (m, 1H), 7.73 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 25.6, 28.4, 29.1, 29.4, 29.5, 31.2, 36.0, 55.8, 60.3, 64.6, 76.3, 79.9, 93.7, 94.3, 126.7, 128.2, 128.3, 130.0, 132.1, 135.0, 136.8, 137.9, 148.2, 152.4, 196.4; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{38}H_{55}NO_6Na^+$ 644.3922, found 644.3951.

(2*S*,3*R*)-2-*N*-(*tert*-Butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4'-benzoyl-phenyl)-(4*E*)-pentadecene-1,3-diol (10). Oxazolidine **9** (311 mg, 0.50 mmol) was dissolved in acetic acid (0.8 mL) and water (0.2 mL), and the mixture was stirred at 80 °C for 5 h. The mixture was concentrated and co-evaporated with heptane (2 × 1 mL) to provide a residue that was purified by chromatography (hexane/EtOAc 1:1), affording *N*-Boc alcohol **10** (256 mg, 88%) as a colorless oil: R_f 0.18 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ – 38.4° (*c* 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27-1.65 (m, 25H), 2.04 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 2.80 (br s, 1H), 3.36 (s, 3H), 3.68 (m, 2H), 3.93 (m, 1H), 4.24 (m, 1H), 4.51 (d, 1H, *J* = 6.4 Hz), 4.66 (d, 1H, *J* = 6.4 Hz), 5.26 (m, 1H), 5.36 (dd, 1H, *J* = 8.0, 15.6 Hz), 5.73 (m, 1H), 7.29 (d, 2H, *J* = 8.0 Hz), 7.47 (t, 2H, *J* = 7.2 Hz), 7.57 (m, 1H), 7.73

(d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 26.2, 26.4, 28.4, 29.0, 29.3, 29.6, 31.2, 32.3, 36.0, 55.7, 61.6, 62.4, 78.5, 79.5, 93.9, 126.0, 128.2, 128.3, 130.0, 132.1, 135.0, 137.0, 138.0, 148.2, 156.0, 196.5; HR-MS (FAB, MNa^+) m/z calcd for $C_{35}H_{51}NO_6Na^+$ 604.3609, found 604.3586.

2-Azidoethanol (11). To a 50-mL flask were added 2-bromoethanol (7.51 g, 60.5 mmol), NaN_3 (5.13 g, 122 mmol), and *n*-Bu_4NBr (500 mg, 1.5 mmol), with stirring for 15 h at 110 °C. After the mixture had cooled, the product was taken up with Et_2O (20 mL), and the precipitate (consisting of NaBr, unreacted NaN_3, and phase-transfer catalyst) was removed by filtration. The salt was washed with Et_2O (~20 mL). Evaporation of the solvents gave a yellow residue that was purified by distillation (bp 35 °C/1 Torr) to yield 5.0 g (95%) of **11** as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 2.06 (m, 1H), 3.45 (m, 2H), 3.78 (m, 2H); ^{13}C NMR δ 53.5, 61.5.

2-Azidoethyl Phosphorochloridate (12). To a 50-mL flask containing 8.86 g (58 mmol) of $POCl_3$ was added 2.5 g (28.7 mmol) of **11** dropwise at 0 °C. The mixture was heated at 70 °C for 20 h, and the remaining $POCl_3$ was evaporated at room temperature (1 Torr, two days) to give crude **12**, which was used without further purification.

(2*S*,3*R*)-1-*O*-[2'-Azidoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxycarbonyl-amido)-3-*O*-methoxymethyl-15-(4'-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (13). To a well-dried 50-mL flask containing 202 mg (1.0 mmol) of crude **12** in 15 mL of anhydrous Et_2O was added 0.16 mL (2.0 mmol) of anhydrous pyridine. After 30 min of stirring, a solution of 200 mg (0.34 mmol) of alcohol **10** in 2 mL of Et_2O was added dropwise. The reaction mixture was stirred at rt for 30 min, and then was heated at reflux

for about 3 h until the starting material (alcohol **10**) disappeared. Water (2 mL) was added at 0 °C, and stirring was continued at rt overnight. The solvent was removed and the residue was purified by chromatography ($CHCl_3$/MeOH 9:1, then 9:2) to give 206 mg (82%) of **13** as a wax: R_f 0.48 ($CHCl_3$/MeOH 9:2); $[\alpha]^{25}_D$ –24.4° (*c* 6.40, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26-1.65 (m, 25H), 2.03 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 3.36 (s, 3H), 3.48 (m, 2H), 3.85 (m, 1H), 4.09 (m, 5H), 4.53 (m, 1H), 4.67 (m, 1H), 5.33 (m, 1H), 5.71 (m, 1H), 7.28 (d, 2H, *J* = 7.2 Hz), 7.47 (t, 2H, *J* = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, *J* = 7.6 Hz), 7.78 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 28.4, 29.1, 29.3, 29.4, 29.5, 29.6, 31.2, 32.4, 36.0, 51.3, 54.0, 55.7, 64.8, 65.3, 79.4, 93.9, 125.9, 127.9, 128.2, 128.3, 130.0, 130.3, 132.1, 135.0, 137.4, 138.0, 148.2, 155.9, 196.5; ^{31}P NMR ($CDCl_3$) δ –0.28; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{37}H_{55}N_4O_9PNa^+$ 753.3599, found 753.3567.

(2*S*,3*R*)-1-*O*-[2'-Aminoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxycarbonyl-amido)-3-*O*-methoxymethyl-15-(4'-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (14). To a solution of azide **13** (197 mg, 0.27 mmol) in dry MeOH (5 mL) were added dry Et_3N (0.14 mL, 1.0 mmol) and 1,3-propanedithiol (0.10 mL, 1.0 mmol). The solution was stirred overnight at rt. A white precipitate formed, which was removed by filtration, and the filtrate was concentrated. The residue was purified by chromatography ($CHCl_3$/MeOH 2:1) to give 162 mg (85%) of **14** as a wax: R_f 0.47 ($CHCl_3$/MeOH 2:1); $[\alpha]^{25}_D$ –36.4° (*c* 0.78, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26-1.65 (m, 25H), 2.03 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 3.17 (m, 2H), 3.36 (s, 3H), 3.77 (m, 1H), 3.97-4.10 (m, 5H), 4.50 (m, 1H), 4.67 (m, 1H), 5.33 (m, 1H), 5.71 (m, 1H), 7.27 (d, 2H, *J* = 7.2 Hz), 7.47 (t, 2H, *J* = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 2H, *J* = 6.8 Hz), 8.51 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 28.5, 29.2, 29.4, 29.5, 29.6, 31.2, 32.4, 36.0, 40.3,

54.2, 55.6, 62.1, 64.7, 79.0, 93.7, 126.4, 127.9, 128.2, 128.3, 130.0, 130.1, 130.3, 132.1, 135.1, 137.2, 138.0, 148.2, 155.8, 196.5; ^{31}P NMR ($CDCl_3$) δ 0.61; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{37}H_{57}N_2O_9PNa^+$ 727.3694, found 727.3690.

(2*S*,3*R*)-1-*O*-[2'-[^{14}C]Trimethylaminoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4'-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (15). To a solution of 12 mg (0.017 mmol) of **14** in 2 mL of dry MeOH in a pressure tube with a stirring bar were added 6 mg (0.043 mmol, ~2.5 equiv) of [^{14}C]MeI (2.0 mCi, specific activity, 47.0 mCi/mmol) and 72 mg (0.85 mmol) of anhydrous $NaHCO_3$. After the tip of the tube containing [^{14}C]MeI was broken, the contents were transferred to the pressure tube and the vial was washed with MeOH (3 x 0.5 mL). The pressure tube was sealed and the contents were heated to 50 °C (no higher than 65 °C) in an oil bath for 3 h, then cooled to 0 °C in an ice bath, and 50 mg (0.35 mmol) of unlabeled MeI was added. The reaction mixture was again heated to 50 °C in an oil bath for 3 h. The reaction mixture was cooled to rt, and the contents of the tube were transferred to a 25-mL round-bottom flask. The tube was washed with MeOH (3 x 1 mL), and the washings were transferred to the flask. The solvent and excess MeI were removed by evaporation. The residue was dissolved in 10 mL of CH_2Cl_2/ H_2O (1:1), and the solution was transferred to a separatory funnel. The organic layer was collected, and the aqueous layer was washed with CH_2Cl_2 (3 x 3 mL). The combined CH_2Cl_2 layers were washed with brine, water, dried (Na_2SO_4), and concentrated. The residue was purified on a short silica gel column (3 cm); elution was first with 20 mL of $CHCl_3$/MeOH 9:1 (to remove an impurity), and then with 50 mL of $CHCl_3$/MeOH/H_2O 65:25:4 (to collect the product), affording compound **15** (10 mg, 78%) as a white wax.

To ensure that the product had been eluted completely, the fraction that was UV active and had R_f 0.23 (developed with $CHCl_3$/MeOH/H_2O 65:25:4) was monitored by TLC. For unlabeled **15**: R_f 0.23 ($CHCl_3$/MeOH/H_2O 65:25:4); $[\alpha]^{25}_D$ –23.6° (*c* 0.75, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26-1.39 (m, 23H), 1.63 (m, 2H), 2.03 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 3.34 (s, 3H), 3.39 (s, 9H), 3.76 (m, 1H), 3.86 (m, 2H), 3.99 (m, 1H), 4.10 (m, 2H), 4.36 (m, 2H), 4.50 (d, 1H, *J* = 6.4 Hz), 4.67 (d, 1H, *J* = 6.4 Hz), 5.33 (dd, 1H, *J* = 8.0, 15.6 Hz), 5.56 (m, 1H), 5.70 (m, 1H), 7.27 (d, 2H, *J* = 7.2 Hz), 7.47 (t, 2H, *J* = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 2H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 28.5, 29.1, 29.3, 29.5, 29.6, 31.2, 32.4, 36.0, 54.5, 55.7, 59.4, 64.2, 66.4, 78.9, 93.8, 126.2, 128.2, 128.3, 130.0, 130.1, 130.3, 132.2, 135.1, 137.2, 138.0, 148.2, 155.8, 196.6; ^{31}P NMR ($CDCl_3$) δ 0.61; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{40}H_{63}N_2O_9PNa^+$ 769.4163, found 769.4159.

(2*S*,3*R*)-1-*O*-[2'-[^{14}C]Trimethylaminoethyl(hydroxy)phosphoryl]-2-amino-15-(4'-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (2). (Unlabeled compound **2** is needed for competitive binding studies with putative membrane proteins.) For unlabeled probe **2**: A solution of **15** (10 mg, 0.013 mmol) in 5 mL of THF and 5 mL of 3 M HCl in a 25-mL round-bottom flask was heated at 70 °C in an oil bath for 5 h. After the solution was cooled to rt, the solvents were removed by vacuum evaporation. The residue was dried, and a drop of concentrated NH_4OH was added to neutralize the residue. After about 5 min, NH_4OH was removed and the residue was dried under vacuum. Dry MeOH (5 mL) was added with stirring, which dissolved the product, leaving some NH_4Cl remaining as a solid. The mixture was filtered through filter paper, which was washed with 3 mL of dry MeOH. The combined MeOH solutions were collected, and the solvent was removed by

vacuum evaporation. The residue was dissolved in $CHCl_3$/MeOH/H_2O (65:25:4) and loaded onto a TLC plate, which was developed with $CHCl_3$/MeOH/H_2O (65:25:4). The UV-active band (R_f 0.2) was scraped from the plate and extracted with $CHCl_3$/MeOH/H_2O (65:25:4). The solution was passed through an Osmonics Cameo syringe filter (elution with $CHCl_3$/MeOH 65:25) to remove traces of suspended silica gel. The solvents were removed and the residue was dried under vacuum to give product **2** as a white solid (6.0 mg, 75%): R_f 0.20 ($CHCl_3$/MeOH/H_2O 65:25:4); mp 178.5 °C-182.3 °C; $[\alpha]^{25}_D$ –5.4° (*c* 0.36, $CHCl_3$/MeOH 1:1); ^{1}H NMR (CD_3OD) δ 1.26-1.39 (m, 14H), 1.63 (m, 2H), 2.13 (m, 2H), 2.74 (t, 2H, *J* = 7.6 Hz), 3.26 (s, 9H), 3.33 (m, 1H), 3.69 (m, 2H), 4.11 (m, 2H), 4.31 (m, 3H), 5.50 (dd, 1H, *J* = 8.0, 15.6 Hz), 5.88 (m, 1H), 7.37 (d, 2H, *J* = 8.0 Hz), 7.55 (t, 2H, *J* = 8.0 Hz), 7.66 (m, 1H), 7.74 (d, 2H, *J* = 8.0 Hz), 7.77 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 30.2, 30.3, 30.5, 30.6, 30.7, 32.4, 33.4, 36.9, 54.7, 57.5, 60.7, 62.8, 63.6, 67.7, 70.7, 128.3, 129.5, 129.6, 130.9, 131.4, 133.7, 136.3, 137.2, 139.2, 149.9, 198.5; ^{31}P NMR (CD_3OD) δ –0.38; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{33}H_{51}N_2O_6PNa^+$ 625.3377, found 625.3403. Radioactivity was determined on a liquid-scintillation counter. The specific activity of [^{14}C]-labeled probe **2** was determined to be 3.2 mCi/mmol.

References

1. Ito, M.; Kita, K.; Kurita, T.; Sueyoshi, N.; Izu, H. *Methods Enzymol.* **2000**, *311*, 297-303.

2. Liliom, K.; Sun, G.; Bunemann, M.; Virag, T.; Nusser, N.; Baker, D. L.; Wang, D. A.; Fabian, M. J.; Brandts, B.; Bender, K.; Eickel, A.; Malik, K. U.; Miller, D. D.; Desiderio, D. M.; Tigyi, G.; Pott, L. *Biochem. J.* **2001**, *355*, 189-197.

3. Nofer, J. R.; Geigenmuller, S.; Gopfert, C.; Assmann, G.; Buddecke, E.; Schmidt, A. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 98-103.

4. Rodriguez-Lafrasse, C.; Vanier, M. T. *Neurochem. Res.* **1999**, *24*, 199-205.

5. Xu, Y.; Casey, G.; Mills, G. B. *J. Cell Physiol.* **1995**, *163*, 441-450.

6. (a) Xu, Y. *Biochim. Biophys. Acta* **2002**, *1582*, 81-88. (b) Meyer zu Heringdorf, D. M.; Himmel, H. M.; Jakobs, K. H. *Biochim. Biophys. Acta* **2002**, *1582*, 178-189. (c) Kye, K. C.; Chae, E. K.; Piao, Y. J.; Park, S.; Park, J. K.; Kim, C. D.; Lee, J. H.; Suhr, K. B. *J. Invest. Dermatol.* **2004**, *122*, 1365-1371.

7. Kim, K.-S.; Ren, J.; Jiang, Y.; Ebrahem, Q.; Tipps, R.; Cristina, K.; Xiao, Y.-J.; Qiao, J.; Taylor, K. L.; Lum, H.; Anand-Apte, B.; Xu, Y. *FASEB J.* **2005**, *19*, 819-821.

8. Beil, M.; Micoulet, A.; von Wichert, G.; Paschke, S.; Walther, P.; Omary, M. B.; Van Veldhoven, P. P.; Gern, U.; Wolff-Hieber, E.; Eggermann, J.; Waltenberger, J.; Adler, G.; Spatz, J.; Seufferlein, T. *Nat. Cell Biol.* **2003**, *5*, 803-811.

9. (a) Xu, Y.; Zhu, K.; Hong, G.; Wu, W.; Baudhuin, L. M.; Xiao, Y.; Damron, D. S. *Nat. Cell Biol.* **2000**, *2*, 261-267. (b) Ignatov, A.; Lintzel, J.; Hermans-Borgmeyer, I.; Kreienkamp, H. J.; Joost, P.; Thomsen, S.; Methner, A.; Schaller, H. C. *J. Neurosci.* **2003**, *23*, 907-914.

10. Zhu, K.; Baudhuin, L. M.; Hong, G.; Williams, F. S.; Cristina, K. L.; Kabarowski, J. H.; Witte, O. N.; Xu, Y. *J. Biol. Chem.* **2001**, *276*, 41325-41335.

11. Witte, O. N.; Kabarowski, J. H.; Xu, Y.; Le, L. Q.; Zhu, K. *Science* **2005**, *307*, 206.

12. (a) Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687. (b) Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.

13. Lu, X.; Cseh, S.; Byun, H.-S.; Tigyi, G.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 7046-7050.

14. Parrill, A. L.; Sardar, V. M.; Yuan, H. *Seminars Cell Develop. Biol.* **2004**, *15*, 467-476.

15. (a) Garner, P.; Park, J. M. *Org. Synth.* **1991**, *70*, 18-27. (b) Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136-2157.

16. Pfaendler, H. R.; Weimar, V. *Synthesis* **1996**, 1345-1349.

17. Wang, Y.; Dong, X.; Larock, R. C. *J. Org. Chem.* **2003**, *68*, 3090-3098.

18. For reviews of the Suzuki reaction, see: (a) Chemler, S. R.; Trauner, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 4544-4568. (b) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633-9695. (c) Bellina, F.; Carpita, A.; Rossi, R. *Synthesis* **2004**, 2419-2440.

19. Khiar, N.; Singh, K.; Garcia, M.; Martin-Lomas, M. *Tetrahedron Lett.* **1999**, *40*, 5779-5782.

20. Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, *19*, 3633-3634.

21. Lu, X.; Bittman, R. *J. Org. Chem.* **2005**, *70*, 4746-4750.

Chapter 3

Syntheses of L-*lyxo*-Phytosphingosine, Its 1-Phosphonate Analogue, and D-*ribo*-Phytosphingosine Using a Threitol Acetal Synthon

Abstract

The first synthesis of an isosteric phosphonate analogue of the aminotriol lipid (2*S*,3*S*,4*S*)-phytosphingosine (**3**), together with an improved synthesis of (2*S*,3*S*,4*S*)-phytosphingosine (**2**), (2*S*,3*S*,4*R*)-phytosphingosine (**1**), are described. A key intermediate is 3-pentylidene acetal **9**, which was prepared in two steps from dimethyl 2,3-*O*-benzylidene-D-tartrate (**7**).

Introduction

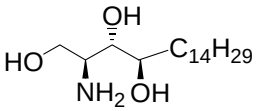
The trihydroxy sphingoid base phytosphingosine (4D-hydroxysphinganine, PHS) consists of an aliphatic chain (predominantly octadecyl) bearing a 2-amino-1,3,4-triol head group. PHS differs from sphingosine in that it possesses an additional hydroxy group (at C4) but does not possess a 4,5-trans double bond. PHS is the major sphingoid base in fungi, plants, and several mammalian tissues.[1] In addition to its structural role in membranes, PHS has a number of physiological roles, many of which remain to be elucidated. PHS is involved in the heat stress response of yeast cells[2] and induces apoptosis in cancer cells.[3] Amide-linked derivatives of PHS, which constitute ~30% of the total ceramide content of the stratum corneum (the water permeability barrier of

human skin),[4] self-associate extensively via hydrogen bonds even in the hydrated state and have different chain packing properties than ceramides with a sphingosine backbone.[4d] PHS is also the backbone of (a) KRN7000, an α-galactosylsphingolipid that stimulates natural killer cells to produce cytokines and strongly inhibits tumor metastasis in mice,[5] and (b) the glycosylphosphatidylinositol (GPI) of the membrane-anchored proteins in yeast.[6]

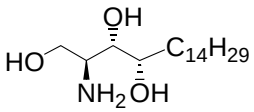
The construction of the aminotriol moiety of the phytosphingosines represents a synthetic challenge. A number of routes to naturally occurring (2*S*,3*S*,4*R* or D-*ribo*)-PHS (Chart 1, **1**) and some of its diastereomers have been reported.[7] The chiral centers of the PHS molecule have generally been derived from carbohydrate[8] and serine precursors.[9] In addition, chiral induction into PHS has been achieved by the Sharpless asymmetric epoxidation[10] and asymmetric dihydroxylation reactions,[11] asymmetric aldol reactions,[12] and other stereoselective methods.[13] This chapter describes an efficient method for the preparation of D-*ribo*-PHS (**1**) and L-*lyxo*-PHS (**2**)[11,14] that utilizes a pentylidene group to protect the 1,2-diol moiety of 3-*O*-protected-D-threitol **8** as an acetal. To further illustrate the utility if this method, an isosteric phosphonate derivative of **2**, L-*lyxo*-phytosphingosine 1-phosphonate (**3**), was synthesized. Our interest in the latter compound arises from the recent finding that a phosphate ester of PHS, D-*ribo*-phytosphingosine 1-phosphate (**4**), possesses a higher affinity than S1P for a widely distributed cell-surface G protein-coupled receptor.[15] Since the carbon-phosphorus bond is resistant to the action of lipid phosphohydrolases, a phosphonate derivative[16] of a bioactive lysosphingolipid such as **4**, in which the phosphate oxygen at C1 of **4** is replaced with a methylene group, is expected to have a long half-life in cells and thus be

a valuable pharmacological tool to probe receptor-specific interactions. In previous work from Prof. Bittman's laboratory, it was found that isosteric phosphonate analogs of glycerophospholipids retain the biological activity of the parent phosphate esters.[17] It should also be noted that nonhydrolyzable phosphonolipids with a 2-amino-3,4-dihydroxy head group are found in some bacteria[18] and mollusks.[19]

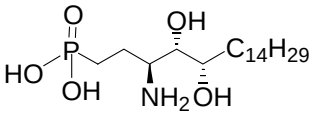
Chart 1



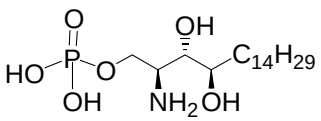
D-*ribo*-phytosphingosine **1**



L-*lyxo*-phytosphingosine **2**



L-*lyxo*-phytosphingosine 1-phosphonate **3**



D-*ribo*-phytosphingosine 1-phosphate **4**

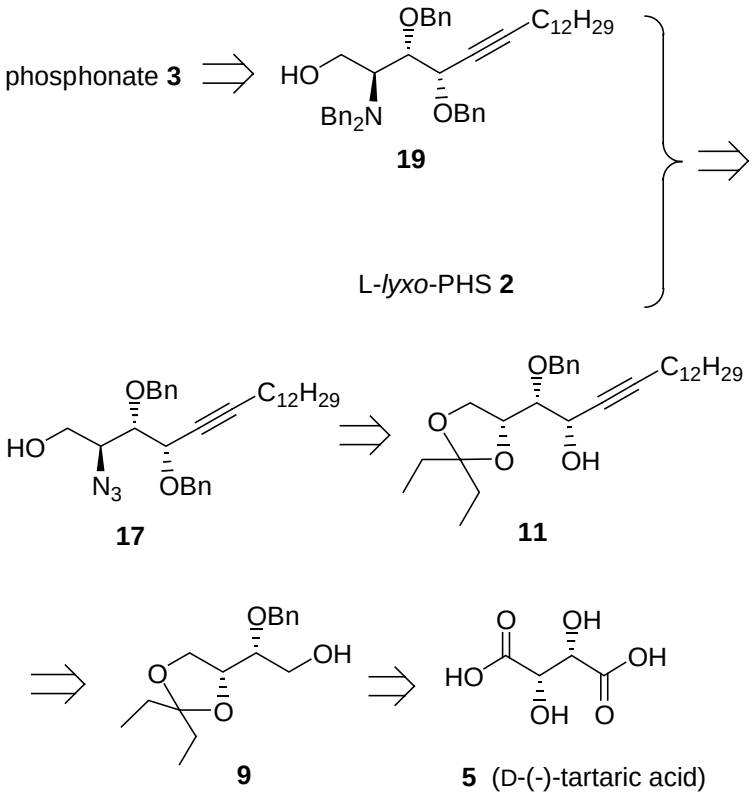
Structures of D-*ribo*-PHS (**1**) and its phosphate ester (**4**), and of L-*lyxo*-PHS (**2**) and its isosteric 1-phosphonate derivative (**3**).

Results and Discussion

As illustrated in the retrosynthetic analysis (Scheme 1), the syntheses of L-*lyxo*-phytosphingolipids **2** and **3** start with readily available D-(-)-tartaric acid (**5**), which would be converted to pentylidene-protected D-threitol derivative **9** (see Scheme 2). After Dess-Martin oxidation,[20] the aliphatic chain would be installed with concomitant construction of the third chiral center, the acetal of tetrol **11** will be released, and regioselective azidation of the secondary hydroxy group of the resultant 1,2-diol with inversion of configuration would be accomplished, affording azido alcohol **17**. For the

synthesis of L-lyxo-PHS (**2**), the azido group and triple bond of **17** will would reduced and the protecting groups at C3 and C4 would be removed in a one-pot reaction. For the synthesis of the isosteric phosphonate analogue **3**, azide **17** would be converted to protected 2-amino alcohol **19**. Condensation of the aldehyde derived from **19** with tetramethyl methylenediphosphonate, followed by ester hydrolysis, reduction of the unsaturation, and hydrogenolysis of the protecting groups, would afford PHS-phosphonate **3**.

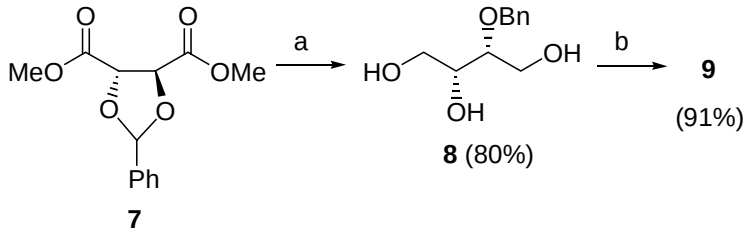
Scheme 1. Retrosynthetic plan



Synthesis of Threitol Derivative 9. D-(-)-Tartaric acid (**5**) has been used extensively as a chiral precursor of many natural products.[21] Scheme 2 shows a short synthesis of the protected threitol derivative **9**. Benzylidene acetal **7** is commercially available or can be readily prepared by esterification of **5** ($SOCl_2$, MeOH) to give

dimethyl ester **6**, followed by acetal protection of the hydroxy groups (PhCHO, *p*-TsOH, cyclohexane, reflux); alternatively, **7** is accessible from **5** in a one-pot reaction.[22] Both the ester and acetal functionalities of **7** were reduced with $LiAlH_4$ in the presence of $AlCl_3$,[23] affording triol **8** in 80% yield. 1,2-Isopropylidene-protected derivative of **8**, which is a widely used building block,[24] was unstable on storage, especially as a solution in chloroform. Therefore, triol **8** was converted to pentylidene acetal **9**, which is less acid sensitive and thus more stable than the isopropylidene acetal. The most suitable conditions for the formation of this new acetal derivative entail the brief heating of **8** with 3,3'-dimethoxypentane[25] (1.2 equiv) with a catalytic amount of 10-camphorsulfonic acid (CSA) in THF (reflux, 30 min; overall yield for the four steps >50%).

Scheme 2. Preparation of threitol derivative 9

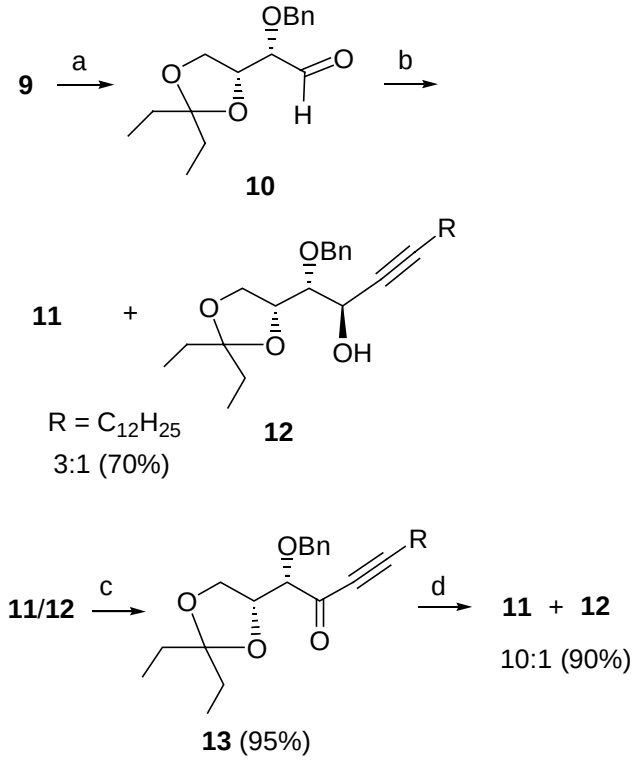


Reagents and conditions: (a) $LiAlH_4$, $AlCl_3$, Et_2O, reflux; (b) pentanone, $HClO_4$, molecular sieves (method A), or 3,3'-dimethoxypentane, 10% CSA, THF, reflux, 30 min, then K_2CO_3 (method B). See reference 41.

Synthesis of Propargyl Alcohol 11. Alcohol **9** was oxidized to the corresponding aldehyde **10** with PCC in the presence of NaOAc and molecular sieves (Scheme 3).[26] A chelation-controlled addition of tetradecynyllithium to aldehyde **10** in the presence of anhydrous $ZnBr_2$ afforded a mixture of **11** and **12** in a ratio of only 3:1.

The mild selectivity may be a result of 2,3-chelation of Zn^{2+} to give the undesired diastereomer **12**.[27] In an attempt to increase the yield of **11**, the mixture of **11** and **12** was oxidized to the propargylic ketone and then reduced diastereoselectively.[28] When the mixture of propargyl alcohols **11** and **12** was oxidized with the Dess-Martin periodinane reagent, ketone **13** was obtained exclusively. Unfortunately, during the purification of crude **13** by silica gel column chromatography, some loss of configuration at C3 took place. Therefore, the crude ketone **13** was used directly in the next step. Reduction of **13** with L-Selectride (–78 °C, THF) afforded propargyl alcohol **11** as the major product (C3,C4 syn). The ratio of **11**:**12** was 10:1, and the overall yield of the isolated products was 90%. Pure propargyl alcohol **11** was obtained by column chromatography (elution with hexane/EtOAc 6:1).[29]

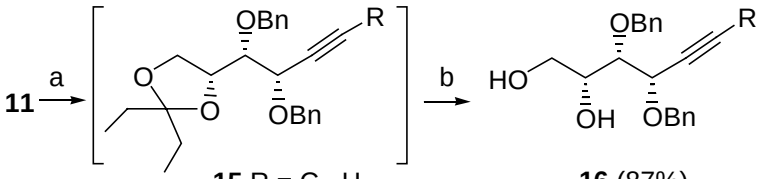
Scheme 3. Preparation of propargyl alcohol 11



Reagents and conditions: (a) PCC, NaOAc, CH_2Cl_2, MS; (b) *n*-BuLi, 1-tetradecyne, THF, $ZnBr_2$; (c) Dess-Martin periodinane, CH_2Cl_2, 1 h, rt; (d) L-Selectride, THF, -78 to 0 °C. See reference 41.

Synthesis of L-*lyxo*-PHS (2). Scheme 4 outlines the synthesis of product **2** from alcohol **11**. After the hydroxy group of **11** was protected as a benzyl ether (BnBr, NaH, catalytic *n*-Bu_4NBr (TBAB)), selective deprotection with 5% H_2SO_4 provided 1,2-diol **16** in 87% yield for the two steps. Diol **16** was converted to azido alcohol **17** in a one-pot reaction.[30a] This was accomplished by adding the diol to a mixture of diisopropyl azodicarboxylate (DIAD) and Ph_3P at 0 °C. After 3 h, $TMSN_3$ was added to accomplish the azide substitution reaction.[30b] Hydrolysis of the silyl ether of the primary hydroxy group and purification by column chromatography provided azido alcohol **17** in 61% yield. Simultaneous reduction of the azido group and the triple bond, together with hydrogenolysis of the benzyl groups in the presence of Pearlman's catalyst ($Pd(OH)_2/C$), gave L-*lyxo*-PHS **2** in 82% yield. The NMR spectra and rotation data were in full accord with previously reported data.[11]

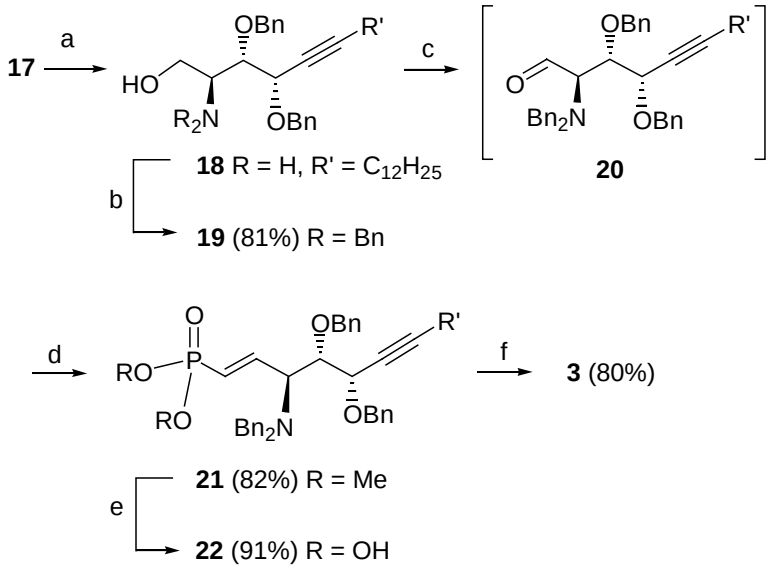
Scheme 4. Conversion of 11 to L-*lyxo*-PHS (2)



c → **17** (61%) d → **2** (82%)

Reagents and conditions: (a) BnBr, NaH, THF, reflux; (b) 5% H_2SO_4, MeOH, rt; (c) (i) PPh_3, DIAD, CH_2Cl_2, 0 °C, (ii) $TMSN_3$, 0 °C – rt, (iii) TBAF, THF; (d) $Pd(OH)_2/C$, H_2, THF. See reference 41.

Scheme 5. Conversion of 17 to L-*lyxo*-PHS 1-phosphonate (3)



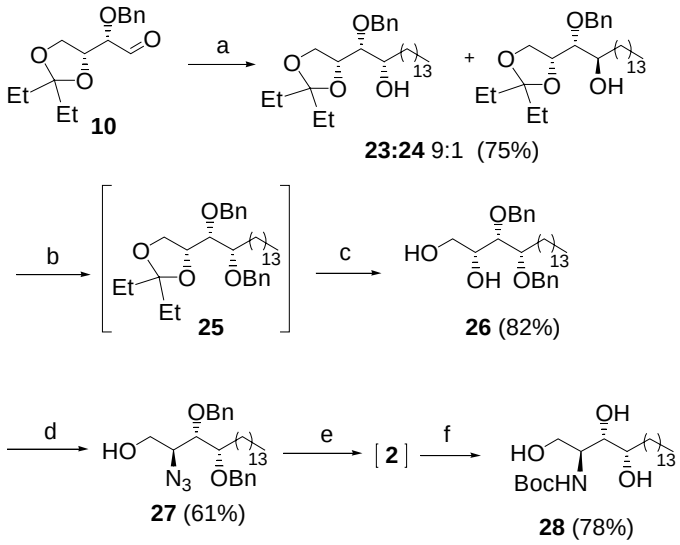
Reagents and conditions: (a) PPh_3, THF/H_2O 9:1; (b) K_2CO_3, BnBr, H_2O, NaOH; (c) Dess-Martin periodinane, CH_2Cl_2; (d) tetramethyl methylenediphosphonate, THF, NaH; (e) TMSBr, CH_2Cl_2; (f) $Pd(OH)_2/C$, H_2, MeOH. See reference 41.

Synthesis of L-*lyxo*-PHS 1-phosphonate (3). A Horner-Wadsworth-Emmons reaction was used to introduce an (*E*)-enephosphonate moiety (Scheme 5). A low yield was obtained in the reaction of tetramethyl methylenediphosphonate with the aldehyde derived by oxidation of azido alcohol **17**. Therefore, the azido group was first reduced to an amino group with PPh_3 in THF/H_2O (9:1) to afford 2-amino alcohol **18**. Reaction of the amino group with excess benzyl bromide in K_2CO_3/NaOH (1:1) in water gave *N*,*N*-dibenzyl amino alcohol **19** (81% yield for the two steps). Oxidation of **19** with the Dess-

Martin reagent afforded aldehyde 20,[31] which reacted with the anion derived from tetramethyl methylenediphosphonate to provide unsaturated phosphonate ester **21** (82% for the two steps). Treatment of **21** with trimethylsilyl bromide, followed by 5% aqueous MeOH, afforded phosphonic acid **22** in 91% yield. Finally, reduction of the double and triple bonds and deprotection of the *N*,*N*-benzyl groups all at the same time gave the desired (2*S*,3*S*,4*S*)-PHS-phosphonate analogue **3** in 80% yield.

More efficient syntheses of L-*lyxo*-phytosphingosine and D-*ribo*-phytosphingosine were established using a Grinard reagent to install the lipophilic chain instead of a lithated alkyne.

Scheme 6: Synthesis of *N*-Boc-L-*lyxo*-PHS (28)

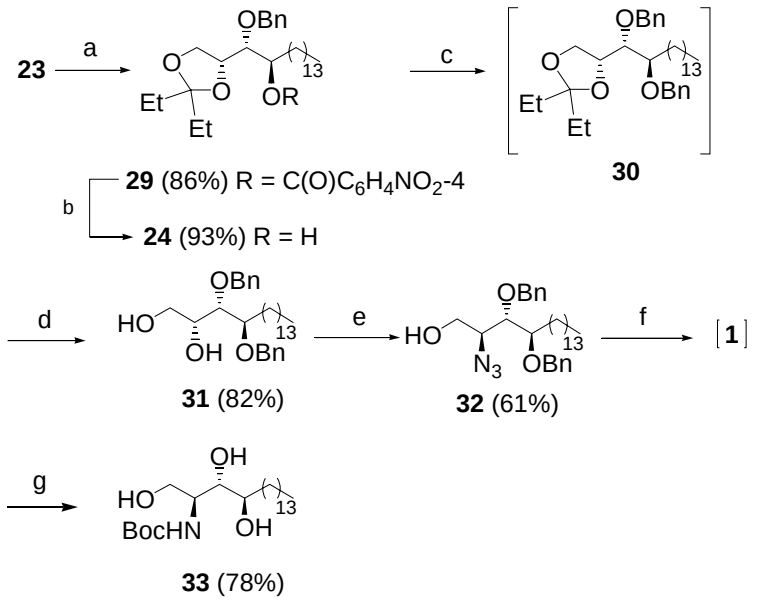


Reagents and conditions: (a) $C_{14}H_{29}Br$, Mg, $BrCH_2CH_2Br$, Et_2O; (b) BnBr, NaH, THF; (c) 5% H_2SO_4, MeOH; (d) (i) PPh_3, DIAD, CH_2Cl_2, 0 ^oC, (ii) $TMSN_3$, 0 ^oC – rt, (iii) TBAF, THF; (e) $Pd(OH)_2/C$, H_2, MeOH; (f) Boc_2O, Et_3N, dioxane/H_2O. See reference 42.

Scheme 6 outlines the synthesis of **2** from aldehyde **10**. Reaction of aldehyde **10** with tetradecylmagnesium bromide in Et_2O at 0 ˚C gave a mixture of compounds **23** and

24 in 9:1 ratio.[32] The diastereoselectivity of the Grignard addition is markedly higher than that of the reaction between aldehyde **10** and tetradecynyllithium in Et_2O at -20 ˚C in the presence of $ZnBr_2$, which furnished the 2*R*,3*R*,4*S* and 2*R*,3*R*,4*R* diastereomers in a 3:1 ratio. Thus Grignard addition afforded the chelation-controlled product **23**,[33] which was obtained in 68% yield after purification by column chromatography (elution with hexane/EtOAc 3:1). The configuration at C4 was confirmed when **23** was finally converted to L-*lyxo*-PHS (**2**). After the hydroxy group of **23** was protected as a benzyl ether (BnBr, NaH, catalytic *n*-Bu_4NBr (TBAB)), selective deprotection of **25** with 5% H_2SO_4 provided 1,2-diol **26**[34] in 82% yield for the two steps. Diol **26** was converted to azido alcohol **27**[35] in a one-pot reaction. This was accomplished by adding the diol to a mixture of diisopropyl azodicarboxylate (DIAD) and Ph_3P at 0 ^oC. After 3 h, $TMSN_3$ was added to accomplish the azide substitution reaction. Hydrolysis of the silyl ether of the primary hydroxy group with *n*-Bu_4NF (TBAF) provided azido alcohol **27** in 61% yield. Simultaneous reduction of the azido group and hydrogenolysis of the benzyl groups in the presence of Pearlman's catalyst ($Pd(OH)_2/C$) gave **2**, whose amino group was protected with a Boc group for ease of isolation to give compound **28**[36] (78% yield for the two steps).

Scheme 7. Synthesis of *N*-Boc-D-*ribo*-PHS (33)



Reagents and conditions: (a) DIAD, PPh_3, *p*-nitrobenzoic acid, CH_2Cl_2; (b) NaOMe, MeOH; (c) BnBr, NaH, THF; (d) 5% H_2SO_4, MeOH; (e) (i) PPh_3, DIAD, CH_2Cl_2, 0 ^oC, (ii) $TMSN_3$, 0 ^oC – rt, (iii) TBAF, THF; (f) $Pd(OH)_2/C$, H_2, MeOH; (g) Boc_2O, Et_3N, dioxane/H_2O. See reference 42.

Scheme 7 outlines the synthesis of **1** from alcohol **23**. The configuration at C4 of compound **23** was inverted by Mitsunobu reaction (*p*-nitrobenzoic acid, DIAD, PPh_3). Hydrolysis of benzoate ester **29**[37] with NaOMe in methanol gave alcohol **24** (80% overall yield for the two steps). As in the preparation of **2** (Scheme 8), the C4 hydroxy group was protected as a benzyl ether and the acetonide was opened by treatment with H_2SO_4. After the secondary hydroxy group of diol **31**[38] was converted to an azido group,[39] the azido group was reduced and the *O*-benzyl groups were deprotected to give product **1**. The amino group of **1** was protected as a carbamate to give compound **32**[40] (78% yield for two steps).

In summary, a novel synthesis of L-*lyxo*-PHS (**2**), D-*ribo*-PHS, and the first synthesis of L-*lyxo*-PHS 1-phosphonate (**3**) via the new D-threitol acetal derivative **9** have been reported here. Coupling of 1-tetradecyne with aldehyde **10** gave a mixture of alcohols **11** and **12**, which were oxidized and then reduced with L-Selectride to install the third chiral center. After protection of the 4-hydroxy group and deprotection of the 1,2-hydroxy groups, the 2-hydroxy group was converted to an azido group with inversion of configuration. Hydrogenolysis gave L-*lyxo*-PHS (**2**). An unsaturated phosphonate moiety was introduced by reaction of tetramethyl methylenediphosphonate with aldehyde **20**. Ester hydrolysis, followed by deprotection and reduction of the double and triple bonds, gave L-*lyxo*-PHS 1-phosphonate **3** in high yield. A shorter routes to L-*lyxo*-PHS (**2**) and D-*ribo*-PHS (**1**) via D-threitol acetal derivative **10** are also reported in this chapter, when tetradecylmagnesium bromide instead of lithiated tetradecyne was used as coupling reagent. If L-tartaric acid is used as the starting material, the methods shown here can be used to prepare the enantiomers of these phytosphingolipids. The availability of 1-phosphonate derivatives of PHS diastereomers is expected to spur analysis of the bioactivities of these lysolipids.

Experimental Section

(*2R,3R*)-2-*O*-Benzyl-3,4-*O*-(3'-pentylidene)-1-butanol [(+)-9]. Two methods were used to prepare acetal **9**. In method A, triol **8** underwent reaction with 3-pentanone whereas in method B it reacted with 3,3'-dimethoxypentane. *Method A.* A slurry of 8.4 g (39.6 mmol) of triol **8**, 25.2 g of 4Å molecular sieves, and 42 mL (396 mmol) of 3-pentanone in 300 mL of dry THF was treated with 4.1 mL (41 mmol) of concentrated $HClO_4$. The mixture was stirred overnight and then quenched by adding 40 g (290

mmol) of anhydrous K_2CO_3. The solid residue was removed by filtration and washed with Et_2O (2 x 100 mL). The solvent was evaporated and the residue was purified by column chromatography (elution with EtOAc/hexane 1:3) to give **9** (9.8 g, 88%) as a colorless liquid. *Method B.* To a solution of 8.4 g (39.6 mmol) of triol **8** and 930 mg (4.0 mmol) of CSA in 300 mL of dry THF was added 6.3 g (47.5 mmol) of 3,3'-dimethoxypentane. The solution was stirred at reflux for 0.5 h and then quenched by adding 4.0 g (29 mmol) of anhydrous K_2CO_3. The solid residue was removed by filtration and washed with Et_2O (2 x 100 mL). The solvent was evaporated and the residue was purified by chromatography (EtOAc/hexane 1:3) to give 10.2 g (91%) of **9** as a colorless liquid: $[\alpha]^{25}_D$ +15.4° (*c* 3.75, $CHCl_3$); R_f 0.30 (EtOAc/hexane 1:3); ^{1}H NMR (C_6D_6) δ 1.00-1.07 (m, 6H), 1.68-1.79 (m, 4H), 1.90 (s, 1H), 3.36 (m,1H), 3.43 (m, 1H), 3.57 (m, 2H), 3.84 (t, 1H, *J* = 6.4 Hz), 4.24 (m, 1H), 4.65 (d, 1H, *J* = 12.0 Hz), 4.74 (d, 1H, *J* = 12.0 Hz), 7.18 (m, 1H), 7.27 (m, 2H), 7.39 (m, 2H); ^{13}C NMR (C_6D_6) δ 8.36, 8.42, 29.7, 30.1, 62.3, 66.4, 72.8, 78.1, 80.0, 113.2, 128.0, 139.2; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{16}H_{24}O_4Na^+$ 303.1568, found 303.1574.

(*2R*,*3S*)-2-*O*-Benzyl-3,4-*O*-(3'-pentylidene)-2,3,4-trihydroxybutanal (10). A mixture of 5.6 g (20.0 mmol) of triol **8** and 5 g of 3Å molecular sieves in 200 mL of dry CH_2Cl_2 was stirred at rt for 2 h (mixture A). A slurry of 11.5 g (140 mmol) of NaOAc, 15.8 g (73.3 mmol) of PCC, and 5 g of 3Å molecular sieves in 200 mL of CH_2Cl_2 was stirred at rt for 2 h (mixture B). Mixture B was added to mixture A, with stirring at rt for 4 h. After 400 mL of dry Et_2O was added, stirring was continued for 30 min. The resulting precipitate was removed by filtration over a short pad of silica gel and thoroughly washed with Et_2O. The solvent was evaporated and the residue was dried to

give 5.0 g (91%) of **10** as a pale yellow liquid, which was used in the next reaction without further purification. A pure sample of aldehyde **10** was obtained by flash chromatography (3:1 hexane/EtOAc); R_f 0.59 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.84-0.92 (m, 6H), 1.58-1.72 (m, 4H), 3.85 (m, 2H), 4.05 (t, 1H, *J* = 6.8 Hz), 4.34 (m, 1H), 4.68 (d, 1H, *J* = 12.0 Hz), 4.78 (d, 1H, *J* = 12.0 Hz), 7.25-7.40 (m, 5H), 9.70 (d, 1H, *J* = 4.8 Hz); ^{13}C NMR ($CDCl_3$) δ 8.09, 8.14, 28.7, 39.3, 65.8, 73.4, 75.6, 83.1, 113.7, 128.1, 128.2, 128.4, 128.5, 137.1, 202.0.

(2*R*,3*R*,4*S*)- and (2*S*,3*R*,4*R*)-3-*O*-Benzyl-1,2-*O*-(3'-pentylidene)-5-octadecyn-1,2,3,4-tetrol (11) and (12). To a solution of 1.6 g (8.2 mmol) of 1-tetradecyne in 100 mL of dry Et_2O at –20 °C was added dropwise 3.3 mL (8.2 mmol) of *n*-BuLi (a 2.89 M solution in hexane) under nitrogen. The white suspension was stirred at –20 °C for 1 h, and then 2.15 g (9.5 mmol) of anhydrous $ZnBr_2$ was added at 0 °C. After 1 h at 0 °C and 1 h at rt, a solution of 1.9 g (6.8 mmol) of aldehyde **10** in 20 mL of dry Et_2O was added dropwise at –20 °C. The mixture was allowed to warm to rt overnight and then quenched by the addition of 20 mL of saturated aqueous NH_4Cl solution at –20 °C. After the mixture was diluted with 80 mL of water, the aqueous layer was extracted with Et_2O (2 x 100 mL). The combined extracts were washed with saturated aqueous NaCl solution, dried, and evaporated. The residue was purified by chromatography (EtOAc/hexane 1:3) to give a 3:1 mixture of **11**/**12** (2.2 g, 70%) as a colorless liquid.

(2*R*,3*R*)-3-*O*-Benzyl-1,2-*O*-(3'-pentylidene)-4-oxo-5-octadecyn-1,2,3-triol [(-)-13]. A solution of 2.00 g (5.0 mmol) of the 3:1 mixture of **11**/**12** in 100 mL of dry CH_2Cl_2 at rt was treated with 2.00 g (5.5 mmol) of Dess-Martin periodinane. After 1 h, TLC analysis indicated the complete consumption of starting material. An aqueous

solution of 10% $Na_2S_2O_3$ (50 mL, 20.2 mmol) was added. The mixture was stirred until both layers became clear. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution, water, and brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was dried to afford 1.89 g (95%) of ketone **13** as a colorless liquid. A pure sample of **13** was obtained by flash chromatography (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –28.4° (*c* 13.0, $CHCl_3$); R_f 0.87 (hexane/EtOAc 3:1); ^{1}H NMR (C_6D_6) δ 1.01 (m, 6H), 1.27 (t, 3H, *J* = 6.0 Hz) 1.26-1.40 (m, 20H), 1.68 (q, 2H, *J* = 7.2 Hz), 1.82 (q, 2H, *J* = 7.2 Hz), 2.02 (t, 2H, *J* = 6.8 Hz), 4.05 (m, 3H), 4.35 (d, 1H, *J* = 12.0 Hz), 4.66 (m, 2H), 7.17-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.56, 14.3, 19.0, 22.2, 27.8, 29.1, 29.4, 29.5, 29.8, 30.0, 30.1, 33.8, 66.9, 72.1, 73.4, 76.8, 85.9, 97.2, 114.0, 128.0, 128.1, 128.5, 137.8, 186.3; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{30}H_{46}O_4Na^+$ 493.3288, found 493.3288.

(2*R*,3*S*)-3-*O*-Benzyl-1,2-*O*-(3'-pentylidene)-4-oxo-5-octadecyn-1,2,3-triol [(-)-14]. PCC oxidation of a mixture of **11**/**12** gave a 1:1 mixture of **13**/**14** in 92% total yield. A pure sample of **14** was obtained by flash chromatography (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –14.4° (*c* 2.5, $CHCl_3$); R_f 0.93 (hexane/EtOAc 3:1); ^{1}H NMR (C_6D_6) δ 1.03-1.18 (m, 9H), 1.26-1.43 (m, 20H), 1.70 (q, 2H, *J* = 7.2 Hz), 1.85 (q, 2H, *J* = 7.2 Hz), 2.02 (t, 2H, *J* = 6.8 Hz), 3.91 (d, 1H, *J* = 5.2 Hz), 4.05 (m, 2H), 4.48 (d, 1H, *J* = 12.0 Hz), 4.64 (m, 1H), 4.85 (d, 1H, *J* = 12.0 Hz), 7.25-7.29 (m, 3H), 7.45 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 8.31, 8.36, 8.44, 14.4, 19.1, 23.1, 27.8, 29.1, 29.4, 29.5, 29.8, 30.0, 30.1, 32.3, 66.2, 73.2, 76.9, 81.0, 85.5, 97.4, 113.8, 128.0, 128.1, 128.5, 138.2, 186.6; MS (ESI) *m*/*z* 488.3 (MNH_4^+).

(2*R*,3*R*,4*S*)-3-*O*-Benzyl-1,2-*O*-(3'-pentylidene)-5-octadecyn-1,2,3,4-tetrol [(+)-11]. To a solution of 543 mg (1.19 mmol) of ketone **13** in 50 mL of dry THF was added

2.38 mL (2.4 mmol) of L-Selectride (a 1 M solution in THF) dropwise at –78 °C under nitrogen. The reaction mixture was stirred for 0.5 h at –78 °C and then allowed to warm to rt for 0.5 h. The mixture was diluted with 100 mL of EtOAc and filtered through a pad of silica gel, which was rinsed with 100 mL of EtOAc. The filtrate was concentrated and the residue was purified by chromatography (hexane/EtOAc 6:1) to give 489 mg (82%) of propargyl alcohol **11** as a colorless oil; $[\alpha]^{25}_{D}$ +11.2° (*c* 6.3, $CHCl_3$); R_f 0.75 (hexane/EtOAc 3:1); ^{1}H NMR (C_6D_6) δ 1.03-1.10 (m, 9H), 1.35-1.55 (m, 20H), 1.72-1.82 (m, 4H), 2.18 (m, 2H), 2.37 (d, 1H, *J* = 6.8 Hz), 3.58 (m, 1H), 3.77 (d, 1H, *J* = 8.4 Hz), 4.01 (m, 1H), 4.55 (m, 2H), 4.94 (m, 2H), 7.20-7.32 (m, 3H), 7.52 (d, 2H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 8.43, 14.4, 19.0, 23.1, 28.9, 29.3, 29.5, 29.8, 29.9, 30.0, 30.1, 32.3, 63.8, 66.8, 74.8, 78.0, 80.0, 83.0, 86.4, 113.0, 127.4, 127.7, 127.9, 128.0, 128.1, 128.5, 139.1; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{30}H_{48}O_4Na^+$ 495.3445, found 495.3437.

(2*R*,3*R*,4*S*)-3,4-Benzyloxy-5-octadecyn-1,2,3,4-tetrol [(+)-16]. To a mixture of 272 mg (6.78 mmol) of NaH (60%) and 1.60 g (3.39 mmol) of **11** in 100 mL of THF were added 870 mg (5.09 mmol) of benzyl bromide and 32 mg (0.10 mmol) of TBAB. The mixture was heated at reflux for 3 h, and then quenched by the addition of 50 mL of water. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution, water, and then dried ($MgSO_4$) and concentrated. To the dry residue of **15** in 100 mL of MeOH was added 5 mL of 5% aqueous H_2SO_4. The mixture was stirred at rt overnight. After 5.0 g (36 mmol) of solid K_2CO_3 was added, the mixture was filtered. The filtrate was evaporated to provide a residue that was purified by chromatography (hexane/EtOAc 3:1), affording 1.46 g of **16** (87% for two steps) as a colorless oil; $[\alpha]^{25}_{D}$ +8.9° (*c* 2.7, $CHCl_3$); R_f 0.24 (hexane/EtOAc 3:1); ^{1}H NMR (C_6D_6) δ 1.03 (t, 3H, *J* = 6.8

Hz), 1.33-1.53 (m, 20H), 2.14 (m, 2H), 2.82 (s, 1H), 3.54 (d, 1H, *J* = 4.4 Hz), 3.98 (m, 3H), 4.44 (m, 1H), 4.58 (d, 1H, *J* = 11.6 Hz), 4.65 (m, 1H), 4.70 (d, 1H, *J* = 11.6 Hz), 4.89 (d, 1H, *J* = 11.6 Hz), 4.95 (d, 1H, *J* = 11.6 Hz), 7.17-7.22 (m, 6H), 7.44 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.4, 19.0, 23.1, 28.9, 29.0, 29.3, 29.5, 29.8, 29.9, 30.0, 30.1, 32.3, 63.5, 70.8, 71.0, 72.1, 74.2, 77.1, 81.4, 89.2, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.5, 128.6, 128.7, 138.2, 138.9; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{32}H_{46}O_4Na^+$ 517.3288, found 517.3303..

(2*S*,3*S*,4*S*)-2-Azido-3,4-benzyloxy-5-octadecyn-1,3,4-triol [(+)-17]. To a solution of 1.63 g (3.4 mmol) of **16** and 1.34 g (5.1 mmol) of Ph_3P (both thoroughly dried overnight at 0.7 Torr) in 150 mL of dry CH_2Cl_2 was added 1.06 mL (5.1 mmol) of DIAD at 0 °C. After the yellow reaction mixture was stirred at 0 °C for 3 h under nitrogen, 592 μL (4.4 mmol) of Me_3SiN_3 was added. The reaction mixture was stirred at the same temperature for 3 h and then allowed to warm to rt overnight. After removal of the solvent, the residue was dissolved in 10 mL of THF and treated with 8.5 mL (8.5 mmol) of *n*-Bu_4NF (TBAF) (a 1 M solution in THF containing 5 wt % H_2O). The brown reaction mixture was stirred at rt until the silyloxy azides were consumed completely (TLC). Concentration gave a slurry that was dissolved in CH_2Cl_2 and passed through a pad of silica gel in a sintered glass funnel to remove $Ph_3P(O)$ and salts. The pad was washed with a mixture of hexanes/EtOAc (6:1). The crude products were purified by silica gel chromatography (hexanes/EtOAc 3:1) to give **17** (1.04 g, 61%) as a colorless oil; $[\alpha]^{25}_D$ +28.9° (*c* 1.75, $CHCl_3$); R_f 0.70 (hexane/EtOAc 3:1); ^{1}H NMR (C_6D_6) δ 1.03 (t, 3H, *J* = 6.8 Hz), 1.33-1.53 (m, 20H), 1.73 (t, 1H, *J* = 4.4 Hz), 2.14 (m, 2H), 3.83 (m, 2H), 3.91 (m, 1H), 3.96 (m, 1H), 4.52 (m, 1H), 4.60 (d, 1H, *J* = 11.6 Hz), 4.68 (d, 1H, *J* =

11.6 Hz), 4.95 (d, 1H, *J* = 11.6 Hz), 5.01 (d, 1H, *J* = 11.6 Hz), 7.17-7.32 (m, 6H), 7.47 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.4, 19.0, 23.1, 28.9, 29.2, 29.5, 29.8, 30.0, 30.1, 32.3, 62.4, 63.8, 70.4, 71.1, 75.1, 76.9, 81.8, 89.4, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 138.3, 138.6; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{32}H_{45}N_3O_3Na^+$ 542.3353, found 542.3328.

(2*S*,3*S*,4*S*)-Phytosphingosine [(-)-2]. To a solution of 63 mg (0.12 mmol) of **17** in 90 mL of MeOH was added 12.7 mg (0.024 mmol) of 20% $Pd(OH)_2$/C. The resulting suspension was purged with H_2 for approximately 10 min and then stirred with a balloon filled with H_2 overnight, when TLC indicated the complete consumption of the starting material. The crude reaction mixture was filtered through a short pad of Celite, and the solvent was evaporated to provide 32 mg (82%) of **2** as a white solid; mp 104.2-105.5 °C [lit.[11] mp 104.8-106.0 °C]; $[\alpha]^{25}_D$ –7.5° (*c* 1.00, C_5H_5N) [lit.[11] $[\alpha]^{25}_D$ –7.4° (*c* 0.9, C_5H_5N)]; R_f 0.56 ($CHCl_3$/MeOH/NH_4OH 130:25:4); ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.27-1.53 (m, 24H), 2.98 (m, 1H), 3.39 (m, 1H), 3.40 (m, 1H), 3.59 (m, 1H), 3.67 (m, 1H), 3.76 (m, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 23.9, 27.2, 30.6, 30.9, 31.0, 33.2, 34.5, 34.7, 55.9, 64.4, 72.6, 75.1; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{18}H_{39}NO_3Na^+$ 340.2822, found 340.2828.

(2*S*,3*S*,4*S*)-2-*N*,*N*-Dibenzylamino-3,4-benzyloxy-5-octadecyn-1,3,4-triol [(-)-19]. To a solution of 0.94 g (1.86 mmol) of **17** in 30 mL of THF/H_2O 9:1 was added 0.63 g (2.3 mmol) of Ph_3P. The reaction mixture was stirred at rt under nitrogen for 48 h. After the solvents were removed, a solution of 520 mg (3.72 mmol) of K_2CO_3 and 150 mg (3.72 mmol) of NaOH in 10 mL of water was added. The solution was heated at reflux with stirring for 30 min. To the refluxing mixture was added 0.96 g (5.58 mmol)

of benzyl bromide. The mixture was heated for an additional 2 h and then cooled to rt. The organic phase was separated, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography (hexane/EtOAc 3:1) to give 0.99 g (81% for two steps) of **19** as a colorless oil; $[\alpha]^{25}_D$ –25.5° (*c* 2.15, $CHCl_3$); R_f 0.74 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.22-1.47 (m, 20H), 2.12 (m, 2H), 2.72 (s, 1H), 3.30 (m, 1H), 3.44 (d, 2H, *J* = 13.6 Hz), 3.71 (m, 1H), 3.86 (d, 1H, *J* = 8.8 Hz), 3.90 (d, 2H, *J* = 13.6 Hz), 4.04 (m, 1H), 4.35 (m, 1H), 4.39 (d, 1H, *J* = 11.6 Hz), 4.64 (d, 1H, *J* = 11.2 Hz), 4.79 (d, 1H, *J* = 11.6 Hz), 5.01 (d, 1H, *J* = 11.2 Hz), 7.17-7.35 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.9, 22.7, 28.5, 29.0, 29.1, 29.4, 29.6, 29.7, 30.3, 31.9, 54.0, 58.4, 59.9, 71.0, 71.1, 72.1, 73.2, 76.4, 78.9, 89.4, 127.0, 127.6, 127.7, 127.8, 128.0, 128.2, 128.3, 128.4, 129.0, 130.1, 132.4, 137.9, 138.3, 139.6; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{46}H59NO_3Na^+$ 696.4387, found 696.4414.

Dimethyl (3*S*,4*S*,4*S*)-3-*N*,*N*-Dibenzylamino-4,5-dibenzyloxy-6-octadecyn-(1*E*)-enephosphonate [(+)-21]. A solution of 336 mg (0.50 mmol) of alcohol **19** in 50 mL of dry CH_2Cl_2 at rt was treated with 252 mg (0.60 mmol) of Dess-Martin periodinane. After 1 h, TLC analysis indicated the complete consumption of starting material. An aqueous solution of 10% $Na_2S_2O_3$ (50 mL) was added. The mixture was stirred until both layers became clear. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution, water, and dried ($MgSO_4$). The solvent was evaporated and the residue was dried to afford crude aldehyde **20** as a colorless liquid. To a mixture of 36 mg (1.5 mmol) of NaH and 50 mL of THF was added 348 mg (1.5 mmol) of tetramethyl methylenediphosphonate in 10 mL of THF at 0 °C. After the mixture was stirred for 10 min, a solution of aldehyde **20** in 10 mL of THF was added.

The reaction mixture was stirred for 2 h, diluted with Et_2O (20 mL), and extracted in successive order with 1 M NaOH/MeOH (7:3) (2 x 30 mL) to remove the excess diphosphonate, H_2O/brine (1:1) (1 x 30 mL), and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (hexane/EtOAc 1:1) to give 318 mg (82% for the two steps) of **21** as a colorless oil; $[\alpha]^{25}_D$ +22.8° (*c* 1.3, $CHCl_3$); R_f 0.73 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.22-1.35 (m, 20H), 1.99 (m, 2H), 3.56-3.68 (m, 8H), 3.86 (m, 3H), 4.08 (m, 1H), 4.30 (d, 1H, *J* = 6.4 Hz), 4.37 (d, 1H, *J* = 11.2 Hz), 4.67 (d, 1H, *J* = 10.8 Hz), 4.73 (d, 1H, *J* = 11.2 Hz), 5.03 (d, 1H, *J* = 10.8 Hz), 5.85 (dd, 1H, *J* = 17.2, 22.0 Hz), 6.98 (m, 1H), 7.17-7.35 (m, 20H); ^{13}C NMR ($CDCl_3$) 14.1, 18.6, 22.7, 28.4, 29.0, 29.2, 29.4, 29.6, 29.7, 31.9, 52.2, 54.9, 61.6 (d, J_{CP} = 22.0 Hz), 70.9, 74.2, 76.2, 89.4, 126.4, 126.9, 127.1, 127.3, 127.5, 127.7, 127.9, 128.0, 128.2, 128.4, 128.5, 128.6, 128.9, 129.0, 137.9, 138.3; HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{49}H_{64}NO_5PNa^+$ 800.4414, found 800.4408.

(3*S*,4*S*,4*S*)-3-*N*,*N*-Dibenzylamino-4,5-benzyloxy-6-octadecyn-(1*E*)-ene-phosphonic Acid [(+)-22]. To a solution of 200 mg (0.22 mmol) of dimethyl phosphonate **21** in 10 mL of dry CH_2Cl_2 at rt was added 0.29 mL (2.2 mmol) of bromotrimethylsilane. The reaction mixture was stirred for 4 h, at which time TLC indicated that all of the reactant had been consumed. The solvent was removed and the residue was dried. A solution of the residue in 1 mL of 95% MeOH was stirred for 1 h, and the solvent was removed to afford 174 mg (91%) of **22** as a white solid, mp 65.1-65.7 °C; $[\alpha]^{25}_D$ +40.9° (*c* 1.6, $CHCl_3$/MeOH 1:1); R_f 0.76 ($CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.22-1.35 (m, 20H), 1.84 (m, 2H), 4.30 (m, 3H), 4.37 (d, 1H, *J* = 11.2 Hz), 4.48 (m, 2H), 4.65 (d, 1H, *J* = 11.2 Hz), 4.75 (d, 1H, *J* = 11.6 Hz),

4.85 (d, 1H, J = 11.2 Hz), 4.96 (m, 1H), 5.19 (m, 1H), 6.45 (m, 1H), 7.07-7.60 (m, 21H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.7, 22.7, 28.3, 29.0, 29.1, 29.4, 29.6, 29.7, 31.9, 56.1, 64.7 (d, J_{CP} = 22.0 Hz), 71.3, 71.4, 72.6, 73.9, 74.6, 91.5, 127.8, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9, 129.3, 129.7, 130.9, 131.6, 135.6, 137.2; ^{31}P NMR ($CDCl_3$) δ 13.1; HR-MS (FAB, MNa^+) m/z calcd for $C_{47}H_{60}NO_5PNa^+$ 772.4101, found 772.4132.

(2*S*,3*S*,4*S*)-Phytosphingosine 1-Phosphonate [(-)-3]. To a solution of 116 mg (0.17 mmol) of unsaturated phosphonic acid **22** in 90 mL of MeOH was added 18 mg (0.034 mmol) of 20% $Pd(OH)_2$/C. The resulting suspension was purged with H_2 for approximately 10 min and then stirred with a balloon filled with H_2 overnight, when TLC indicated the complete consumption of the starting material. The crude reaction mixture was filtered through a short pad of Celite, and the solvent was evaporated to provide 49 mg (80%) of **3** as a white solid; mp 214.0-214.8 °C; $[\alpha]^{25}_D$ –6.6° (*c* 0.64, $CHCl_3$/MeOH 1:1); R_f 0.45 ($CHCl_3$/MeOH/H_2O/HOAc 65:25:4:1); ^{1}H NMR ($CDCl_3$/CD_3OD 1:1) δ 0.88 (t, 3H, J = 6.4 Hz), 1.22-1.35 (m, 24H), 1.60 (m, 2H), 1.89 (m, 2H), 2.06 (m, 2H), 3.47 (m, 1H), 3.68 (m, 1H), 3.79 (m, 1H); ^{13}C NMR ($CDCl_3$:CD_3OD 1:1) δ 14.3, 23.1, 23.4, 23.8, 26.1, 29.8, 30.1, 30.2, 32.4, 34.0, 56.7 (d, J_{CP} = 13.0 Hz), 70.6, 71.5; ^{31}P NMR ($CDCl_3$/CD_3OD 1:1) δ 27.9; HR-MS (FAB, MNa^+) m/z calcd for $C_{19}H_{42}NO_5PNa^+$ 418.2693, found 418.2712.

For data on compounds **23**, **24**, **26**, **27**, **28**, and **29**, see footnotes 32, 34, 35, 36, and 37, respectively.

References

1. (a) Karlsson, K.-A.; Martensson, E. *Biochim. Biophys. Acta* **1968**, *152*, 230-233. (b) Carter, H. E.; Hirschberg, C. B. *Biochemistry* **1968**, *7*, 2291-2300. (c) Bouhours, D.;

Bouhours, J.-F. *Biochim. Biophys. Acta* **1984**, *794*, 169-171. (d) Okabe, K.; Keenan, R. W.; Schmidt, G. *Biochem. Biophys. Res. Commun.* **1986**, *31*, 137-143. (e) Higuchi, R.; Kagoshima, M.; Komori, T. *Liebigs Ann. Chem.* **1990**, 659-663.

2. For reviews, see: (a) Dickson, R. C.; Lester, R. L. *Biochim. Biophys. Acta* **2003**, *1583*, 13-25. (b) Jenkins, G. M. *Cell. Mol. Life Sci.* **2003**, *60*, 701-710.

3. Park, M. T.; Kang, J. A.; Choi, J. A.; Kang, C. M.; Kim, T. H.; Bae, S.; Kang, S.; Kim, S.; Choi, W. I.; Cho, C. K.; Chung, H. Y.; Lee, Y. S.; Lee, S. *J. Clin. Cancer Res.* **2003**, *9*, 878-885.

4. (a) Wertz, P. W.; Miethke, M. C.; Long, S. A.; Strauss, J. S.; Downing, D. T. *J. Invest. Dermatol.* **1985**, *84*, 410-412. (b) Motta, S.; Monti, M.; Sesana, S.; Caputo, R.; Carelli, S.; Ghidoni, R. *Biochim. Biophys. Acta* **1993**, *1182*, 147-151. (c) Ponec, M.; Weerheim, A.; Lankhorst, P.; Wertz, P. *J. Invest. Dermatol.* **2003**, *120*, 581-588. (d) Rerek, M. E.; Chen, H.-C.; Markovic, B.; Van Wyck, D.; Garidel, P.; Mendelsohn, R.; Moore, D. J. *J. Phys. Chem. B* **2001**, *105*, 9355-9362.

5. (a) Kobayashi, E.; Motoki, K.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Oncol. Res*. **1995**, *7*, 529-534. (b) Nishi, N.; Hizuka, Y.; Natori, T. *Molec. Med.* **2002**, *39*, 478-483.

6. (a) Lester, R. L.; Dickson, R. C. *Adv. Lipid Res.* **1993**, *26*, 253-274. (b) Fankhauser, C.; Homans, S. W.; Thomas-Oates, J. E.; McConville, M. J.; Desponds, C.; Conzelmann, A.; Ferguson, M. A. *J. Biol. Chem.* **1993**, *268*, 26365-26374. (c) Fontaine, T.; Magnin, T.; Melhert, A.; Lamont, D.; Latge, J.-P.; Ferguson, M. A. *Glycobiology* **2003**, *13*, 169-177.

7. For a recent review, see: Howell, A. R.; Ndakala, A. J. *Curr. Org. Chem.* **2002**, *6*, 365-391.

8. (a) Murakami, T.; Taguchi, K. *Tetrahedron* **1999**, *55*, 989-1004. (b) Chiu, H.-Y.; Tzou, D.-L. M.; Patkar, L. N.; Lin, C.-C. *J. Org. Chem.* **2003**, *68*, 5788-5791.

9. (a) Dondoni, A.; Fantin, G.; Fogagnolo, M.; Pedrini, P. *J. Org. Chem.* **1990**, *55*, 1439-1446. (b) Imashiro, R.; Sakurai, O.; Yamashita, T.; Horikawa, H. *Tetrahedron* **1998**, *54*, 10657-10670. (c) Ndakala, A. J.; Hashemzadeh, M.; So, R. C.; Howell, A. R. *Org. Lett.* **2002**, *4*, 1719-1722.

10. (a) Lin, G.-Q.; Shi, Z.-E. *Tetrahedron* **1996**, *52*, 2187-2192. (b) Li, S.; Pang, J.; Wilson, W. K.; Schroepfer, G. J., Jr. *Tetrahedron Lett.* **2000**, *41*, 10309-10312.

11. He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618-7626.

12. Kobayashi, S.; Hayashi, T.; Kawasuji, T. *Tetrahedron Lett.* **1994**, *35*, 9573-9576.

13. (a) Ayad, T.; Genisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582. (b) Raghavan, S.; Rajender, A.; Yadav, J. S. *Tetrahedron: Asymmetry* **2003**, *14*, 2093-2099.

14. For other methods to synthesize L-*lyxo*-PHS, see ref. 11 and: (a) Wild, R.; Schmidt, R. R. *Tetrahedron: Asymmetry* **1994**, *11*, 2195-2208. (b) Li, Y.; Mao, X.; Wu, Y. *J. Chem. Soc. Perkin Trans. 1* **1995**, 559-663. (c) Nakamura, T.; Shiozaki, M. *Tetrahedron* **2001**, *44*, 9087-9092. (d) Lee, H. K.; Kim, E.; Pak, C. S. *Tetrahedron Lett*. **2002**, *43*, 9641-9644.

15. Candelore, M. R.; Wright, M. J.; Tota, L. M.; Milligan, J.; Shei, G.-J.; Bergstrom, J. D.; Mandala, S. M. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 600-606.

16. For recent examples of synthetic studies to prepare phosphonate analogues of sphingolipids, see: (a) McClure, K. C.; Mishra, P. K.; Grote, C. W. *J. Org. Chem.* **1997**, *62*, 2437-2441. (b) McClure, K. C.; Mishra, P. K. *Tetrahedron Lett.* **2002**, *43*, 5249-5253.

17. (a) Bittman, R.; Byun, H.-S.; Mercier, B.; Salari, H. *J. Med. Chem.* **1994**, *37*, 425-430. (b) Lin, W.; Leung, L. W.; Bae, Y. S.; Bittman, R.; Arthur, G. *Biochem. Pharmacol.* **1999**, *57*, 1153-1158. (c) Samadder, P.; Bittman, R.; Byun, H.-S.; Arthur, G. *J. Med. Chem.* **2004**, *47*, 2710-2713.

18. Watanabe, Y.; Nakajima, M.; Hoshino, T.; Jayasimhulu, K.; Brooks, E. E.; Kaneshiro, E. S. *Lipids* **2001**, *36*, 513-519.

19. Kariotoglou, D. M.; Mastronicolis, S. K. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2003**, *136*, 27-44.

20. For a review, see: Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2002**, *102*, 2523-2584.

21. For reviews, see: (a) Gawroński, J.; Gawrońska, K. *Tartaric and Malic Acids in Synthesis*; John Wiley & Sons: New York, 1999. (b) Ghosh, A. K.; Koltun, E. S.; Bilcer, G. *Synthesis* **2001**, 1281-1301.

22. Byun, H.-S.; Bittman, R. *Syn. Commun.* **1993**, *23*, 3201-3204.

23. Steuer, B.; Wehner, V.; Lieberknecht, A.; Jager, V. *Org. Synth.* **1997**, *74*, 1-12.

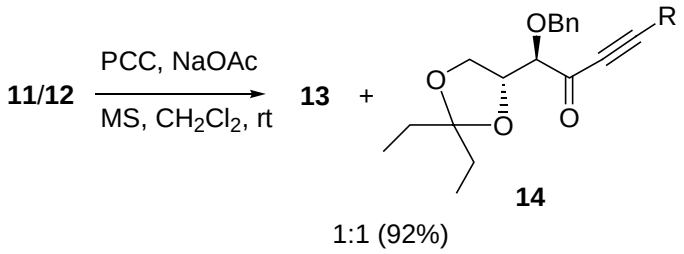
24. For examples, see: (a) Backstrom, A. D.; Austin, R.; McMordie, A. S.; Begley, T. P. *J. Carbohydr. Chem.* **1995**, *14*, 171-175. (b) Yoshino, T.; Nagata, Y.; Itoh, E.; Hashimoto, M.; Katoh, T.; Terashima, S. *Tetrahedron* **1997**, *53*, 10239-10252. (c) Matsumura, R.; Suzuki, T.; Hagiwara, H.; Hoshi, T.; Ando, M. *Tetrahedron Lett.* **2001**, *42*, 1543-1546.

25. For methods to prepare 3,3'-dimethoxypentane, see: (a) Napolitano, E.; Fiaschi, R.; Mastrorilli, E. *Synthesis* **1986**, 122-125. (b) Linclau, B.; Boydell, A. J.; Clarke, P. J.; Horan, R.; Jacquet, C. *J. Org. Chem.* **2003**, *68*, 1821-1826.

26. The configuration at C2 was not altered on PCC oxidation of chiral 2-*O*-benzyl-3,4-*O*-isopropylidene-1-butanol to the isopropylidene aldehyde analogue of **10**: (a) Williams, D. R.; Klingler, F. D. *Tetrahedron Lett.* **1987**, *28*, 869-872. (b) Valverde, S.; Herradon, B.; Martin-Lomas, M. *Tetrahedron Lett*. **1985**, *26*, 3731-3734. (c) Sánchez-Sancho, F.; Valverde, S.; Herradón, B. *Tetrahedron: Asymmetry* **1996**, *7*, 3209-3246.

27. Propargylation of aldehyde **10** may be improved under a variety of asymmetric alkynylmetal addition conditions in order to increase the ratio of **11**:**12** and also to construct the stereochemistry leading to the D-*ribo*-PHS backbone.

28. PCC oxidation of the mixture of compounds **11** and **12** gave a mixture of diastereomers **13** and **14** in a 1:1 ratio.



1:1 (92%)

29. The configuration at C4 of compound **11** was confirmed by the 4*S* configuration in product **2**.

30. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) For a postulated mechanism to account for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion, see Scheme 1 of ref. 30a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

31. For the Dess-Martin oxidation of a *N*,*N*-dibenzyl α-amino alcohol, see: Cooke, J. W. B.; Davies, S. G.; Naylor, A. *Tetrahedron* **1993**, 49, 7955-7966.

32. Experimental details on addition of aldehyde **10** to $C_{14}H_{29}MgBr$ and isolation of **23** and **24**: To a solution of aldehyde **10** (1.39 g, 5.0 mmol) in 50 mL of Et_2O at 0 °C was quickly added freshly prepared $C_{12}H_{25}MgBr$ (15 mmol) in 50 mL of Et_2O. The solution was stirred at 0 °C for 3 h, then warmed to rt and stirred overnight. The reaction was quenched by adding 50 mL of water, the organic phase was separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed by vacuum evaporation. The residue was purified by chromatography (hexane/EtOAc 3:1) to give 1.61 g of **23** (68%) as a colorless oil: R_f 0.54 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ +18.6° (*c* 2.36, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86-0.95 (m, 9H), 1.25-1.35 (m, 24H), 1.62-1.72 (m, 6H), 1.82 (s, 1H), 3.31 (m, 1H), 3.39 (m, 1H), 3.62 (dd, 1H, *J* = 8.0, 0.4 Hz), 4.08 (dd, 1H, *J* = 8.0, 6.4 Hz), 4.40 (m, 1H), 4.67 (d, 1H, *J* = 11.2 Hz), 4.92 (d, 1H, *J* = 11.2 Hz), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.12, 8.31, 14.1, 22.7, 25.9, 29.3, 29.6, 29.7, 31.9, 34.8, 68.2, 72.1, 74.1, 78.3, 81.2, 113.2, 127.8, 128.3, 128.4, 138.4. Also obtained was 168 mg of **24** (7%): R_f 0.58 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ +17.0° (*c* 0.37, $CHCl_3$);^{1}H NMR ($CDCl_3$) δ 0.86-0.94 (m, 9H), 1.25-1.35 (m, 24H), 1.48-1.70 (m, 6H), 2.48 (m, 1H), 3.42 (m, 1H), 3.65 (m, 1H), 3.75 (m, 1H), 3.99 (m, 1H), 4.30 (m, 1H), 4.66 (d, 1H, *J* = 11.2 Hz), 4.76 (d, 1H, *J* = 11.2 Hz), 7.25-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.14, 8.31, 14.1, 22.7, 25.9, 29.3, 29.6, 29.7, 31.9, 33.1, 66.4, 72.1, 74.1, 78.3, 81.3, 113.1, 127.4, 127.8, 128.3, 138.4.

33. For an example of chelation-controlled Grignard addition, see: Ndakala, A. J.; Hashemzadeh, M.; So, R. C.; Howell, A. R. *Org. Lett*. **2002**, *4*, 1719-1722.

34. Data for **(–)-26**: R_f 0.24 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –11.6° (*c* 1.08, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.33-1.71 (m, 26H), 2.60 (s, 2H), 3.49 (m, 1H), 3.60 (m, 3H), 3.78 (m, 1H), 4.56 (m, 3H), 4.71 (d, 1H, *J* = 11.2 Hz), 7.28-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.2, 22.7, 26.0, 29.4, 29.6, 30.4, 32.0, 64.3, 71.3, 72.9, 74.2, 77.3, 79.6, 127.8, 128.0, 128.1, 128.2, 128.5, 128.6, 137.9, 138.0.

35. Data for **(–)-27**: R_f 0.58 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –2.4° (*c* 1.15, $CHCl_3$); R_f 0.70 (hexane/EtOAc 3:1); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.33-1.51 (m, 24H), 1.62 (m, 2H); 1.91 (brs, 1H), 3.56 (m, 1H), 3.68 (m, 2H), 3.87 (m, 2H), 4.59 (m, 2H), 4.65 (m, 2H), 7.28-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.2, 22.7, 25.9, 29.4, 29.6, 30.4, 32.0, 62.5, 63.3, 72.6, 74.4, 79.2, 79.8, 79.6, 127.8, 128.0, 128.1, 128.2, 128.5, 128.6, 137.7, 138.2.

36. Data for (–)-**28**: R_f 0.29 (hexane/EtOAc 1:1); mp 129.5-131.2 °C; $[\alpha]^{25}_D$ –7.9° (*c* 0.35, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.33-1.71 (m, 33H), 1.62 (m, 2H), 3.32 (m, 1H), 3.51 (m, 1H); 3.72 (m, 1H), 4.06 (m, 1H), 5.21 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.3, 29.4, 29.6, 32.0, 53.5, 61.9, 69.7, 72.8, 80.4, 157.3.

37. Data for (–)-**29**: R_f 0.69 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –15.0° (*c* 1.33, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86-0.93 (m, 9H), 1.23-1.55 (m, 24H), 1.60-1.69 (m, 6H), 3.67 (m, 1H), 3.77 (m, 1H), 4.01 (m, 1H), 4.25 (m, 1H), 4.75 (d, 1H, *J* = 11.2 Hz), 4.79 (d, 1H, *J* = 11.2 Hz), 5.04 (m, 1H), 7.26-7.37 (m, 5H), 8.14 (d, 2H, *J* = 7.2 Hz), 8.29 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 8.14, 8.23, 14.1, 22.7, 25.7, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 66.7, 73.9, 76.2, 77.6, 80.7, 113.4, 123.5, 127.7, 128.1, 128.3, 130.8, 135.5, 138.2, 150.6, 164.2.

38. Data for (–)-**31**: R_f 0.24 (hexane/EtOAc 3:1): $[\alpha]^{25}_D$ –9.8° (*c* 1.39, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.33-1.71 (m, 26H), 2.51 (s, 1H), 3.34 (m, 1H), 3.53 (m, 1H), 3.65 (m, 3H), 3.87 (m, 1H), 4.54 (m, 2H), 4.63 (d, 1H, *J* = 11.2 Hz), 4.71 (d, 1H, *J* = 11.2 Hz), 7.28-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.5, 25.6, 29.4, 29.6, 30.8, 31.9, 63.7, 71.4, 72.7, 73.6, 77.4, 79.7, 127.5, 127.8, 128.0, 128.1, 128.2, 128.5, 128.6, 137.9, 138.0.

39. Data for azido alcohol (–)-**32**: R_f 0.58 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –3.71° (*c* 4.15, $CHCl_3$); R_f 0.70 (hexane:EtOAc 3:1); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.33-1.51 (m, 24H), 1.60 (m, 2H); 2.61(m, 1H), 3.63 (m, 3H), 3.78 (m, 1H), 3.87 (m, 1H), 4.59 (m, 2H), 4.67 (m, 2H), 7.28-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.5, 29.4, 29.6, 30.2, 31.9, 62.2, 63.1, 72.5, 73.6, 79.1, 80.4, 127.8, 128.0, 128.1, 128.2, 128.4, 128.5, 137.7, 138.2.

40. Data for (+)-**33**: R_f 0.29 (hexane/EtOAc 1:1); mp 89.2-90.4 °C; $[\alpha]^{25}_D$ +7.5° (*c* 0.51, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.16-1.78 (m, 35H), 3.64 (m, 3H); 3.82 (m, 2H), 4.09 (m, 1H), 4.16 (m, 1H), 4.44 (m, 1H), 5.62 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.3, 29.4, 29.6, 29.7, 31.9, 52.6, 61.7, 73.0, 75.6, 79.9, 156.3.

41. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

42. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 3165-3168.

Chapter 4

Synthesis of a Novel Ceramide Analogue via Tebbe Methylenation and Evaluation of Its Antiproliferative Activity

Abstract

A new analogue of (2*S*,3*R*)-ceramide (**2**) with a methylene group at C4 has been synthesized from D-tartaric acid (**3**) by using Tebbe methylenation as the key step. Compound **2** exhibited markedly higher antiproliferative activity on mouse embryonic fibroblast (MEF) cells than natural ceramide (**1**).

Introduction

Ceramide (*N*-acylsphingosine, **1**) is a long-chain aliphatic 2-amido-1,3-diol with a C(4),C(5)-trans double bond (Figure 1).[1] As a key intermediate in the biosynthesis of many sphingolipid mediators, ceramide has been implicated in many physiological events, including the regulation of cell growth and differentiation, inflammation, and in cellular responses to stress stimuli (such as exposure to heat, radiation, oxidative conditions, and chemotherapeutic agents).[2] Ceramide is a messenger for induction of apoptosis, the cell's intrinsic death program.[3]

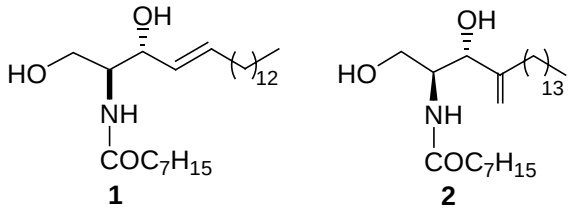


Figure 1. Structures of D-*erythro*-*N*-octanoylceramide (**1**) and its C4-methylene analogue (**2**).

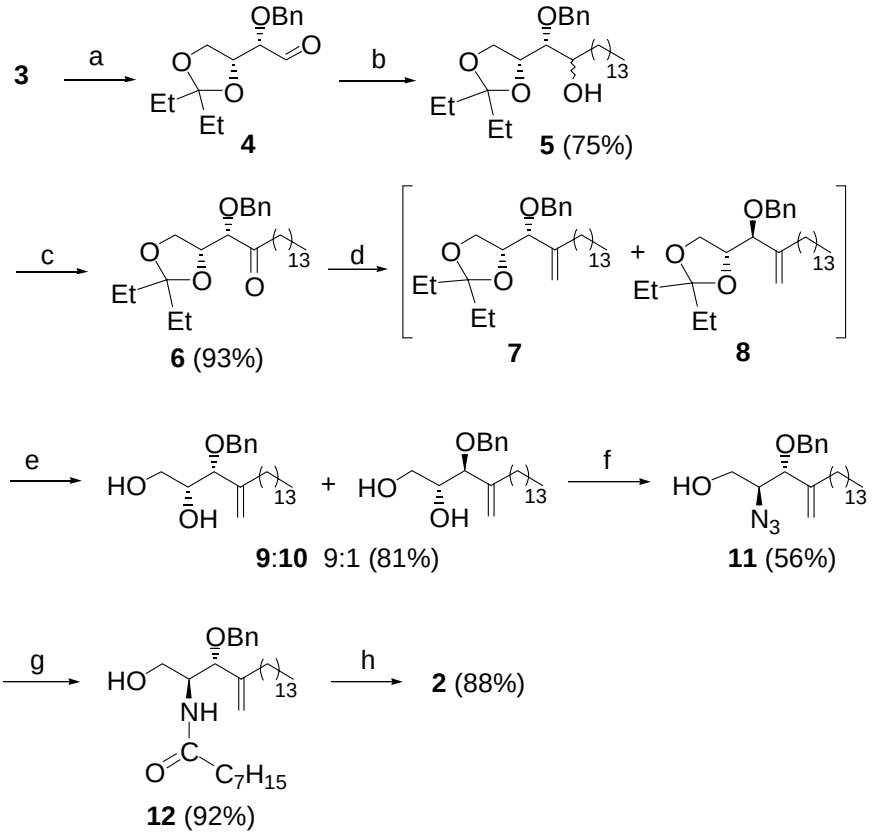
The well-known biological importance of ceramide has inspired the chemical synthesis of analogues to examine the structural features responsible for some of its physiological functions. In a recent monolayer study of 14 synthetic analogues of **1**, it was demonstrated that the (*E*)-C(4)-C(5) double bond of **1** regulates its dipole potential, elastic properties, and packing behavior,[4] and appears to be crucial for ceramide's capacity to modulate various fundamental biological functions. Analogues that lack this double bond have reduced apoptotic activity.[5] The ability of **1** to induce apoptosis has been postulated to also require the allylic alcohol group at C3.[6,7]

A deficiency in induction of apoptosis may result in aberrant cell proliferation, and may culminate in tumor formation.[8] An experimental approach in anticancer chemotherapy is to employ unnatural analogues of **1** that escape recognition by endogenous ceramide-metabolizing enzymes, and thus are not converted to sphingolipids that stimulate cell proliferation,[9] or inhibit enzymes involved in ceramide turnover.[10] The resulting high intracellular levels of ceramide or its analogues are expected to amplify apoptosis.[11] The interest in Professor Bittman's laboratory in cell-permeable (*N*-octanoyl) analogues of ceramide that can be added exogenously to cells to stimulate apoptosis[5b,12] prompted us to prepare the novel C4-exomethylene ceramide analogue **2**, in which both the unsaturation at C4 and allylic nature of the C3-hydroxy group are

preserved. In collaboration with Professor Gilbert Arthur (University of Manitoba), the properties of **2** as an antiproliferative agent were examined in cells having normal or dysfunctional apoptosis.[13]

Results and Discussion

Scheme 1. Synthesis of C4-methylene-ceramide analogue 2



Reagents and conditions: (a) reference 14; (b) $C_{14}H_{29}MgBr$, Et_2O; (c) Dess-Martin periodinane, CH_2Cl_2; (d) Tebbe reagent (see Table 1); (e) 5% H_2SO_4, MeOH; (f) (i) PPh_3, DIAD, CH_2Cl_2, 0 ^oC; (ii) $TMSN_3$, 0 ^oC – rt, (iii) TBAF, THF; (g) $C_7H_{15}CO_2C_6H_4NO_2$-4, Ph_3P, THF/H_2O 9/1; (h) Na, NH_3, THF, -78 ^oC. See reference 27.

Scheme 1 outlines the synthesis of C4-methylene-ceramide analogue **2**. Aldehyde **4**, which was prepared from D-tartaric acid as previously reported,[14] reacted with tetradecylmagnesium bromide to give a diastereomeric mixture of alcohol **5**. After the mixture was oxidized to ketone **6** with the Dess-Martin reagent[15] various methods were tested to carry out the methylenation reaction (Table 1). Wittig reaction gave a low yield of (2*R*,3*R*)-alkene **7** (without any accompanying 2*R*,3*S* diastereomer, epimer **8**), and changing the base (KOBu-*t* or NaH) or refluxing the reaction mixture did not improve the yield (entry 1). When 2 equiv of Tebbe reagent[16] was added slowly to a solution of ketone **6** in THF at –78 ^{o}C, and the reaction mixture was allowed to warm with stirring for 3 h, the yield was high but epimerization occurred at C3 to give a mixture of alkenes **7** and **8** in 1:1 ratio (entry 2). The identification of compounds **7** and **8** were established by the pure sample of **7** obtained in the Wittig reaction (entry 1). Since epimerization may have resulted from the slow addition of the Tebbe reagent to the carbonyl group of compound **6** at low temperature, we added 2 equiv of Tebbe reagent to ketone **7** at room temperature. Entry 3 shows that the ratio of **7** to **8** was increased to 3:1. A high diastereoselectivity was attained when ketone **6** was added to 2 equiv of Tebbe reagent slowly at room temperature for 30 min, with stirring at room temperature for an additional 3 h; a 9:1 ratio of alkenes **7** and **8** was obtained (entry 4). The mixture of **7**/**8** was treated with 5% H_2SO_4 to give diols **9** and **10** in 81% yield for the two steps.[17] Then, diol **9** was converted to azido alcohol **11** in a one-pot reaction.[18] This was accomplished by adding the diol to a mixture of diisopropyl azodicarboxylate (DIAD) and Ph_3P at 0 ^{o}C. After 3 h, $TMSN_3$ was added to accomplish the azide substitution reaction. Hydrolysis of the concomitant silyl ether of the primary hydroxy group and purification by column

chromatography provided azido alcohol **11** in 56% yield. Reduction of the azido group with PPh_3, followed by amide formation in a one-pot reaction, gave amide **12** in 92% yield. The benzyl group of amide **12** was removed by Birch reduction (Na, liquid NH_3, 30 min) to give product **2** in 88% yield.

Table 1. Methylenation of Ketone **6** (See reference 27)

entry		conditions	ratio of **7:8**	overall yield (%)
1	Wittig reaction	CH_2PPh_3, THF, -78 °C to reflux	1.0:0	29
2	Tebbe reagent	2 equiv Tebbe reagent added to ketone **6** at -78 °C, then warmed to rt, 3 h	1.0:1.0	84
3	Tebbe reagent	2 equiv Tebbe reagent added to ketone **6** at rt, 3 h	3.0:1.0	81
4	Tebbe reagent	ketone **6** added to 2 equiv Tebbe reagent at rt, 3 h	9.0:1.0	81

Activation of the cellular apoptosis machinery with exogenous agents may provide a viable therapeutic strategy for the elimination of tumor cells.[19] Since the antiproliferative effects of exogenous short-chain, cell-permeable ceramides have been widely reported,[20] Professor Arthur investigated the effect of **2** on the proliferation of wild-type mouse embryonic fibroblasts (MEFs) and MEFs lacking Apaf-1, a component of the apoptosome (a large complex that activates the caspase cascade in the intrinsic mitochondrial apoptosis pathway).[13c,21] As shown in Figure 2, analogue **2** inhibited the growth of wild-type MEFs with an IC_{50} of 5.9 μM and Apaf-1-/- cells with an IC_{50} of 11 μM. (The loss of Apaf-1 in the latter cells leads to defects in the execution of cell death by the intrinsic apoptosis pathway.)[13c,21] In contrast, the IC_{50} value of **1** was >>20 μM for

both the wild-type and Apaf-1 null cells. The higher antiproliferative activity of ceramide analogue **2** against these cell lines indicates that variations in the structural features of **1** can lead to significantly enhanced apoptogenic activity.[22] While the sensitivity of the Apaf-1 -/- cells to **2** was less than that of the wild type, the ability of **2** to inhibit the growth of these cells suggests that its mechanism of action is not critically dependent on the formation of the apoptosome to induce apoptosis.

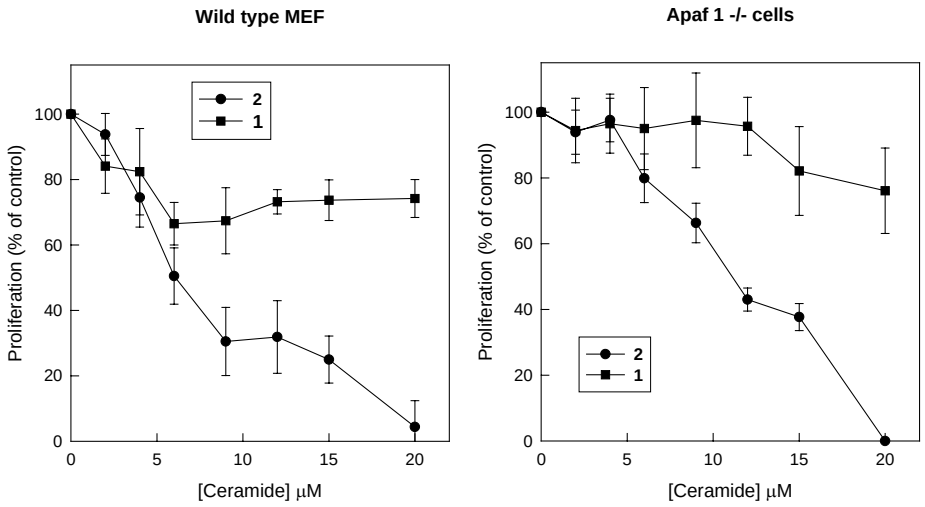


Figure 2. Antiproliferative Activities of **1** and **2**.[23] See reference 27.

In summary, (2*S*,3*R*)-ceramide analogue **2**, which bears an exomethylene group at C4 and an allylic hydroxyl group at C3, was synthesized from D-threitol acetal aldehyde **4**. The key step was Tebbe methylenation of ketone **6** with careful control of the conditions to avoid epimerization at C3. The utility of **2** for inhibition of growth was demonstrated by using mouse embryonic fibroblast cells.

Experimental Section

3-*O*-Benzyl-1,2-*O*-(3'-pentylidene)-octadecane-1,2,3,4-tetrol [5]. To a solution of aldehyde **6** (1.39 g, 5.0 mmol) in 50 mL of Et_2O at 0 °C was quickly added freshly prepared $C_{14}H_{29}MgBr$ (15 mmol) in 50 mL of Et_2O. The solution was stirred at 0 °C for 3 h, then warmed to rt and stirred overnight. The reaction was quenched by adding 50 mL of water, the organic phase was separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed by vacuum evaporation. The residue was purified by chromatography (hexane/EtOAc 3:1) to give 1.78 g (75%) of diastereomeric alcohol **5** as a colorless oil; R_f 0.54 (hexane/EtOAc 3:1).

(2*R*,3*S*)-3-*O*-Benzyl-4-oxo-1,2-*O*-(3'-pentylidene)-octadecane-1,2,3-triol [(-)-6]. A solution of 1.60 g (3.36 mmol) of alcohol **5** in 100 mL of dry CH_2Cl_2 at room temperature was treated with 1.69 g (4.03 mmol) of Dess-Martin periodinane. After 1 h, TLC analysis indicated the complete consumption of starting material. An aqueous solution of 10% $Na_2S_2O_3$ (50 mL) was added. The mixture was stirred until both layers became clear. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution, water, and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by chromatography (hexane/EtOAc 9:1) to afford 1.48 g (93%) of ketone **6** as a colorless liquid: R_f 0.85 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –23.0° (*c* 1.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85-0.91 (m, 9H), 1.25-1.42 (m, 22H), 1.57-1.68 (m, 6H), 2.56 (m, 2H), 3.79 (m, 2H), 4.00 (dd, 1H, *J* = 8.0, 0.4 Hz), 4.28 (dd, 1H, *J* = 8.0, 6.4 Hz), 4.58 (d, 1H, *J* = 11.2 Hz), 4.70 (d, 1H, *J* = 11.2 Hz), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.15, 14.1, 22.7, 22.9, 29.0, 29.2, 29.3, 29.4, 29.6, 31.9, 39.6, 66.2, 73.6, 84.7, 113.5,

127.9, 128.0, 128.5, 137.4, 211.6. HR-MS (FAB, MNa^+) *m/z* calcd for $C_{30}H_{46}O_4Na^+$ 493.3288, found 493.3288.

(2*R*,3*R*)-3-*O*-Benzyl-4-methylene-octadecane-1,2,3-triol [(-)-9]. To a solution of Tebbe reagent (a 0.5 M solution in toluene, 2 mmol) in 25 mL of toluene at rt was added 474 mg of ketone **6** (1.0 mmol) in 25 mL of THF dropwise over 30 min. The solution was stirred for 3 h at rt. The reaction was quenched by adding 50 mL of a 1 M aqueous NaOH solution. The organic phase was separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed. To the dry residue of **7** and **8** in 100 mL of MeOH was added 5 mL of 5% aqueous H_2SO_4. The mixture was stirred overnight at rt. After 5.0 g (36 mmol) of K_2CO_3 was added, the mixture was filtered. The filtrate was evaporated to provide a residue that was purified by chromatography (hexane/EtOAc 3:1), affording 328 mg (81% for the two steps) of a 9:1 mixture of **9** and **10** as a colorless oil: R_f 0.24 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –25.5° (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86-0.90 (t, 1H, *J* = 6.4 Hz), 1.25-1.42 (m, 22H), 1.50 (m, 2H), 2.01 (m, 2H), 2.20 (brs, 2H), 3.53 (m, 1H), 3.62 (m, 1H), 3.65 (m, 1H), 4.56 (d, 1H, *J* = 7.2 Hz), 4.25 (d, 1H, *J* = 11.2 Hz), 4.56 (d, 1H, *J* = 11.2 Hz), 5.14 (d, 2H, *J* = 12.4 Hz), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.7, 29.4, 29.6, 29.7, 30.9, 31.9, 63.1, 70.5, 72.5, 84.0, 114.8, 127.9, 128.0, 128.5, 137.8, 145.7.

(2*S*,3*R*)-2-Azido-3-*O*-benzyl-4-methylene-octadecane-1,3-diol [(-)-11]. To a solution of 289 mg (1.1 mmol) of Ph_3P (thoroughly dried overnight at 0.7 Torr) in 20 mL of dry CH_2Cl_2 was added 0.23 mL (1.1 mmol) of DIAD at 0 °C, with stirring for 10 min, before 300 mg (0.74 mmol) of **9** was added. After the reaction mixture was stirred at 0

°C for 3 h under nitrogen, 66 mg (0.57 mmol) of Me_3SiN_3 was added. The reaction mixture was stirred at the same temperature for 3 h and then allowed to warm to rt overnight. After removal of the solvent, the residue was dissolved in 10 mL of THF and treated with 1 mL (1 mmol) of *n*-Bu_4NF (TBAF) (a 1 M solution in THF containing 5 wt % H_2O). The brown reaction mixture was stirred at rt until the silyloxy azides were consumed completely (TLC). Concentration gave a slurry that was dissolved in CH_2Cl_2 and passed through a pad of silica gel in a sintered glass funnel to remove $Ph_3P(O)$ and salts. The pad was washed with a mixture of hexanes/EtOAc (6:1). The crude products were purified by silica gel chromatography (hexanes/EtOAc 3:1) to give 178 mg (56%) of **11** as a colorless oil: R_f 0.53 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –36.7° (*c* 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86-0.90 (t, 3H, *J* = 6.4 Hz), 1.25-1.42 (m, 22H), 1.50 (m, 2H), 2.01 (m, 2H), 3.48 (m, 1H), 3.81 (m, 2H), 3.90 (d, 1H, *J* = 7.2 Hz), 4.27 (d, 1H, *J* = 11.2 Hz), 4.56 (d, 1H, *J* = 11.2 Hz), 5.20 (d, 2H, *J* = 8.4 Hz), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.7, 29.4, 29.7, 31.9, 63.1, 63.5, 70.6, 72.5, 83.0, 115.2, 127.9, 128.0, 128.5, 138.2, 145.7.

(2*S*,3*R*)-2-[Octanoylamido]-3-*O*-benzyl-4-methylene-octadecane-1,3-diol [(-)-12]. To a solution of 202 mg (0.50 mmol) of **11** and 332 mg (1.25 mmol) of *p*-nitrophenyl caprylate in 30 mL of THF/H_2O 9:1 was added 262 mg (1.0 mmol) of Ph_3P. The reaction mixture was stirred at rt under nitrogen for 48 h. After the solvents were removed (2-PrOH was used to remove the residual water), the light yellow residue was dissolved in 100 mL of Et_2O and washed with 1% aqueous Na_2CO_3 solution (4 × 20 mL) to remove the 4-nitrophenol by-product. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc 3:1)

to give 243 mg (92%) of **12** as a colorless liquid: R_f 0.10 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ – 50.7° (*c* 1.25, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86-0.90 (m, 6H), 1.25-1.55 (m, 34H), 2.01 (m, 4H), 3.54 (m, 1H), 3.97 (m, 3H), 4.20 (d, 1H, *J* = 11.2 Hz), 4.63 (d, 1H, *J* = 11.2 Hz), 5.14 (s, 1H), 5.21 (s, 1H), 6.17 (m, 1H), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.7, 29.4, 29.7, 31.9, 36.7, 50.9, 61.6, 71.6, 83.9, 112.1, 128.0, 128.2, 128.6, 137.7, 145.7, 173.2.

(2*S*,3*R*)-2-[Octanoylamido]-4-methylene-octadecane-1,3-diol [(-)-2]. To a blue solution prepared from sodium (42 mg, 6.0 mmol) and dry liquid NH_3 (10 mL) was added a solution of **12** (160 mg, 0.30 mmol) in THF (5 mL) at -78 °C. The reaction mixture was stirred for 30 min at -78 °C and then quenched with solid NH_4Cl. After NH_3 was evaporated, 10 mL of water was added. The aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (EtOAc/hexane 1:1), providing **2** (115 mg, 88%) as a white wax: R_f 0.74 (hexane/EtOAc 1:1); $[\boldsymbol{\alpha}]^{25}_D$ –8.2° (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86-0.90 (m, 6H), 1.25-1.55 (m, 34H), 2.01 (m, 2H), 2.25 (t, 2H, *J* = 7.6 Hz), 2.70 (brs, 2H), 3.65 (m, 1H), 3.97 (m, 1H), 4.08 (m, 1H), 4.42 (m, 1H), 5.04 (s, 1H), 5.21 (s, 1H), 6.43 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.9, 29.0, 29.2, 29.4, 29.5, 29.6, 29.7, 31.7, 32.4, 36.8, 51.3, 62.0, 110.3, 149.4, 173.6.

References

1. Karlsson, K.-A. *Chem. Phys. Lipids* **1970**, *5*, 6-43.

2. (a) Hannun, Y. A. *Science* **1996**, *274*, 1855-1859. (b) Ariga, T.; Jarvis, W. D.; Yu, R. K. *J. Lipid Res*. **1998**, *39*, 1-16. (c) Perry, D. K.; Hannun, Y. A. *Biochim. Biophys.*

Acta **1998**, *1436*, 233-243. (d) Hannun, Y. A.; Luberto, C. *Trends Cell Biol.* **2000**, *10*, 73-80.

3. Reynolds, C. P.; Maurer, B. J.; Kolesnick, R. N. *Cancer Lett.* **2004**, *206*, 169-180.

4. Brockman, H. L.; Momsen, M. M.; Brown, R. E.; He, L.; Chun, J.; Byun, H.-S.; Bittman, R. *Biophys. J.* **2004**, *87*, 1722-1731.

5. (a) Obeid, L. M.; Linardic, C. M.; Karolak, L. A.; Hannun, Y. A. *Science* **1993**, *259*,1769-1771. (b) Karasavvas, N.; Erukulla, R. K.; Bittman, R.; Lockshin, R.; Zakeri, Z. *Eur. J. Biochem.* **1996**, *236*, 729-737. (c) Ahn, E. H.; Schroeder, J. J. *Exp. Biol. Med. (Maywood)* **2002**, *227*, 345-353. (d) Chang, Y.-T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J.-M.; Chung, S.-K.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1856-1857.

6. Radin, N. S. *Eksp. Onkol.* **2004**, *26*, 3-10.

7. (a) An allylic hydroxy group or ketone is present in many anticancer agents, as reported in Radin, N. S. *Bioorg. Med. Chem.* **2003**, *11*, 2123-2142. (b) The allylic hydroxyl group of **1** is postulated to be oxidized in cells to an α,β-unsaturated ketone, which undergoes Michael addition with glutathione.

8. Debatin, K. M.; Krammer, P. H. *Oncogene* **2004**, *23*, 2950-2966.

9. (a) Maceyka, M.; Payne, S. G.; Milstien, S.; Spiegel, S. *Biochim. Biophys. Acta* **2002**, *1585*, 193-201. (b) Bektas, M.; Jolly, P. S.; Müller, C.; Eberle, J.; Spiegel, S.; Geilen, C. C. *Oncogene* **2005**, *24*, 178-187.

10. Selzner, M.; Bielawska, A.; Morse, M. A.; Rudiger, H. A.; Sindram, D.; Hannun, Y. A.; Clavien, P. A. *Cancer Res.* **2001**, *61*, 1233-1240.

11. Bieberich, E.; Hu, B.; Silva, J.; MacKinnon, S.; Yu, R. K.; Fillmore, H.; Broaddus, W. C.; Ottenbrite, R. M. *Cancer Lett.* **2002**, 181, 55-64.

12. (a) Bittman, R. *Chem. Phys. Lipids* **2004**, *129*, 111-131. (b) Struckhoff, A. P.; Bittman, R.; Burow, M. E.; Clejan, S.; Elliott, S.; Hammond, T.; Tang, Y.; Beckman, B. S. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 523-532.

13. (a) Cain, K. *Drug Metab. Rev.* **2003**, *35*, 337-363. (b) Sanchis, D.; Mayorga, M.; Ballester, M.; Comella, J. X. *Cell Death Differ.* **2003**, *10*, 977-986. (c) Hajra, K. M.; Liu, J. R. *Apoptosis* **2004**, *9*, 691-704.

14. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

15. For a review of the Dess-Martin oxidation, see: Tohma, H.; Kita, Y. *Adv. Synth. Catal.* **2004**, *346*, 111-124.

16. For a review of Tebbe methylenation, see: Hartley, R. C.; McKiernan, G. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2763-2793.

17. Representative procedure for the preparation of **9**: A solution of 474 mg (1.0 mmol) of ketone **6** in 25 mL of THF was added dropwise over 30 min to a solution of Tebbe reagent (a 0.5 M solution in toluene, 2 mmol) in 25 mL of toluene at rt. After the solution had been stirred for 3 h at rt, 50 mL of a 1 M aqueous NaOH solution was added, the mixture was extracted with Et_2O (3 x 20 mL), and the extracts were dried (Na_2SO_4). The solvent was evaporated, and the resulting residue was dissolved in 100 mL of MeOH and treated with 5 mL of 5% aqueous H_2SO_4. After the mixture had stirred overnight at rt, 5.0 g (36 mmol) of K_2CO_3 was added, the mixture was filtered, the filtrate was evaporated, and the residue was purified by column chromatography on silica gel

(hexane/EtOAc 3:1) to give 328 mg (81% for the two steps) of a 9:1 mixture of **9** and **10** as a colorless oil.

18. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) A mechanism for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion has been proposed: Scheme 1 of ref 18a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

19. (a) Lowe, S. W.; Lin, A. W. *Carcinogenesis* **2000**, *21*, 485-495. (b) Johnstone, R. W.; Ruefli, A. A.; Lowe, S.W. *Cell* **2002**, *108*, 153-164.

20. Ogretmen, B.; Hannun, Y.A. *Nature Rev. Cancer* **2004**, *4*, 604-616.

21. (a) Volm, M.; Rittgen, W. *Anticancer Res.* **2002**, *20*, 3449-3458. (b) Gallego, M.-A.; Joseph, B.; Hemstrom, T. H.; Tamiji, S.; Mortier, L.; Kroemer, G.; Formstecher, P.; Zhivotovsky, B.; Marchetti, P. *Oncogene* **2004**, *23*, 6282-6291.

22. Results shown in Figure 2 were obtained with a 9:1 mixture of (2*S*,3*R*):(2*S*,3*S*)-**2** vs (2*S*,3*R*)-**1**. To address the possible contribution of the C3 diastereomer to the potency of **2**, we make note of several recent studies that suggest a minimal role of the configuration at C3 in the activation of apoptotic cell death by **1**: (1) One of the best characterized direct targets of ceramide-mediated apoptosis, ceramide-activated protein phosphatase (CAPP), was activated by (2*S*,3*R*)-**1** but not by the (2*S*,3*S*)-diastereomer of **1**.[24] (2) Ceramidase, which regulates the endogenous level of **1** and its subsequent apoptotic responses in cells, catalyzed the hydrolysis of the amide linkage in the natural (2*S*,3*R*)-isomer of **1** but not in the other three stereoisomers; moreover, only a modest stereoselectivity was found for inhibition of ceramidase by the three unnatural isomers.[25] (3) Ceramide-activated protein kinase (CAPK, which has been identified as the kinase

suppressor of Ras, KSR, an activator of stress pathways mediated by MAP kinase cascades) was activated by the four stereoisomers of **1** to an equal extent.[5b] In addition, CERT, a cytosolic protein that facilitates intracellular trafficking of (2*S*,3*R*)-**1**, did not recognize the three unnatural stereoisomers of ceramide in a cell-free assay system.[26] These findings indicate that there is no marked selectivity with respect to the configuration at C3 of **1** for interaction with several target proteins and suggest that the presence of the minor amount of the C3 epimer does not account for the improved antiproliferative potency of **2**.

23. Proliferating cells in 48-well plates were incubated with compounds **1** and **2** at 37 °C for 48 h in a CO_2 incubator. The media was removed and the plates were frozen at -80 °C for 3 days. The cell numbers at day 0 and after 48 h incubation with the ceramides were determined by the CyQuant assay (Molecular Probes). Each point is the average of 6 independent experiments.

24. Usta, J.; El Bawab, S.; Roddy, P.; Szulc, Z. M.; Hannun, Y. A.; Bielawska, A. *Biochemistry* **2001**, *40*, 9657-9668.

25. (a) Chalfant, C. E.; Kishikawa, K.; Bielawska, A.; Hannun, Y. A. *Methods Enzymol.* **2000**, *312*, 420-428. (b) Chalfant, C. E.; Szulc, Z.; Roddy, P.; Bielawska, A.; Hannun, Y. A. *J. Lipid Res.* **2004**, *45*, 496-506.

26. Kumagai, K.; Yasuda, S.; Okemoto, K.; Nishijima, M.; Kobayashi, S.; Hanada, K. *J. Biol. Chem.* **2005**, *280*, 6488-6495.

27. Lu, X.; Arthur, G.; Bittman, R. *Org. Lett.* **2005**, *7*, 1645-1648.

Chapter 5

A New Route to (2*S*,3*R*)-2-Azido-3-*O*-Benzylsphingosine and (2*S*,3*R*)-Sphingosine from D-Tartaric Acid

Abstract

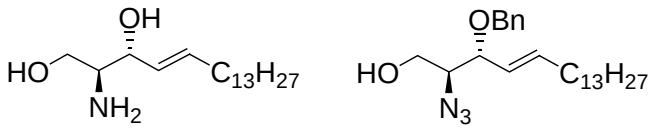
The title compounds (**1**,**2**) were synthesized from (2*R*,3*S*)-2-*O*-benzyl-3,4-*O*-(3'-pentylidene)-2,3,4-trihydroxybutanal (**5**), which was prepared from D-tartaric acid (**3**). Installation of the E-double bond and aliphatic chain into the sphingosine base was effected by a sequence of Horner-Wadsworth-Emmons olefination of **5**, conversion to allylic acetate **8**, and copper-mediated Grignard coupling.

Introduction

Sphingolipids are structural components of eukaryotic cell membranes, are key contributors to the stability of "lipid raft' microdomains, and are a source of lipid messengers that regulate a wide variety of biological processes ranging from inflammation to cell proliferation and apoptosis.[1] Sphingoid bases, which form the backbone of sphingolipids, are aliphatic 2-amino-1,3-diols. The most prevalent base is (2*S*,3*R*)-2-amino-4*E*-1,3-octadecenediol, known as D-*erythro*-sphingosine (**1**).

A great deal of effort has been devoted toward the synthesis of **1** and its derivatives for use in biological and pharmacological studies.[2] Many synthetic efforts have utilized starting materials derived from the chiral pool, in particular carbohydrate,

serine and tartaric acid precursors,[3] whereas other syntheses have employed asymmetric induction, including Sharpless asymmetric epoxidation, asymmetric aldol, and other stereoselective reactions, to achieve high diastereoselectivity.[4] When chiral substrates are used as the starting materials, the characteristic trans double bond of sphingosine is often generated by Wittig reaction[4e] or Julia olefination.[5] When an *E*,*Z* mixture is obtained, photoisomerization has been used to convert the *Z* unsaturation to the desired *E* configuration.[4e]

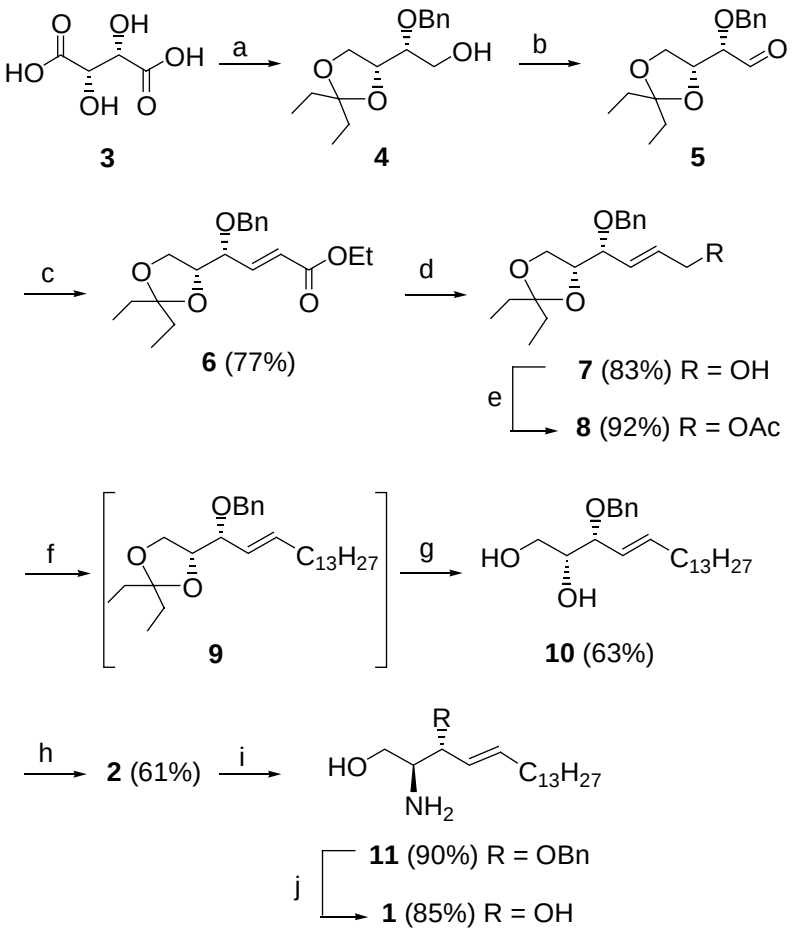


1 **2**

Chart 1. Structures of (2*S*,3*R*)-sphingosine (**1**) and (2*S*,3*R*)-2-azido-3-*O*-benzylsphingosine (**2**).

Compound **2**, the 3-*O*-benzyl ether derivative of sphingosine (**1**),[6] has been used frequently as a glycosyl acceptor in the synthesis of glycosphingolipids.[6,7] In this communication, we describe an efficient method for the preparation of (2*S*,3*R*)-sphingosine (**1**) and (2*S*,3*R*)-2-azido-3-*O*-benzyl-sphingosine (**2**) that utilizes D-tartaric acid (**3**) as the starting material for the protected D-threose synthon **4**, and a Horner-Wadsworth-Emmons (HWE) reaction followed by coupling of an allylic acetate with dodecylorganocuprate to introduce the 4,5-trans double bond and the long chain.

Scheme 1. Synthesis of (2*S*,3*R*)-sphingosine (1) and intermediate 2



Reagents and conditions: (a) reference 9; (b) PCC, NaOAc, CH_2Cl_2, molecular sieves; (c) $(i\text{-PrO})_2P(O)CH_2CO_2Et$, NEt_3, LiBr, THF, rt; (d) DIBAL, -78 °C, CH_2Cl_2; (e) AcCl, *i*-Pr_2NEt, CH_2Cl_2, -40 °C - rt; (f) $C_{12}H_{25}MgBr$, Li_2CuCl_4, Et_2O, -78 °C - rt; (g) 5% H_2SO_4, MeOH; (h) (i) PPh_3, DIAD, CH_2Cl_2, 0 °C, (ii) $TMSN_3$, 0 °C - rt, (iii) TBAF, THF; (i) PPh_3, THF/H_2O 9:1; and (j) Na, NH_3, THF, -78 °C, 30 min. See reference 16.

Results and Discussion

As illustrated in Scheme 1, the synthesis of sphingosine **1** described in this chapter started with readily available D-(-)-tartaric acid (**3**),[8] which was converted to pentylidene-protected D-threitol derivative **4** as described previously.[9] The advantage of pentylidene acetal **4** is that it is less acid sensitive and thus more stable than the corresponding isopropylidene acetal. Alcohol **4** was oxidized with PCC to afford aldehyde **5**, which was used directly in the next step without further purification. After the trans double bond was formed using the HWE reaction with diisopropyl (ethoxycarbonylmethyl)phosphonate in the presence of lithium bromide and triethylamine, the resulting ester **6** was converted via alcohol **7**[10] to allylic acetate **8**, which was coupled with an organocuprate to install the aliphatic chain of sphingosine **1**.

In the initial attempt to install the aliphatic chain, allylic alcohol **7** was activated by mesylation and then subjected to copper-mediated Grignard reaction. Unfortunately, the coupling reaction was not regioselective, giving a 1:2 mixture of the desired coupling product **9** (via α attack) and product **13** (via γ attack).[11] Therefore, allylic alcohol **7** was converted to acetate derivative **8** by treatment with acetyl chloride in the presence of *i*-Pr_2NEt. Coupling of acetate **8** with freshly prepared $C_{12}H_{25}MgBr$ in the presence of catalytic Li_2CuCl_4 in Et_2O at –78 °C was regioselective, affording intermediate **9** exclusively. Deprotection of the acetal with 5% H_2SO_4 in methanol provided diol **10** (65% yield for the two steps). The secondary hydroxy group of **10** was converted to an azido group in a one-pot reaction,[12] which was accomplished by adding diol **10** to a mixture of diisopropyl azodicarboxylate (DIAD) and Ph_3P at 0 °C. After 3 h, $TMSN_3$ was added to give the azide substitution product with concomitant transfer of the silyl

group to the primary hydroxyl group. Hydrolysis of the silyl ether and purification by column chromatography provided azidosphingosine derivative **2** in 61% yield. Although Birch reduction would reduce the azido group and cleave the *O*-benzyl group of **2** in one pot, it was reported that the yield is low for this conversion.[13] Therefore, the azide was first reduced to an amino group by the Staudinger reaction (PPh_3, THF/H_2O (9:1))[14] to afford α-amino alcohol **11** in high yield, and then the benzyl group was removed by Birch reduction (-78 °C, 30 min) to give sphingosine **1** in 85% yield. The structure of **1** was confirmed by the identity of its physical data (^1H and ^{13}C NMR, $[\alpha]^{25}_D$, mp) with those reported in the literature.[3,4,15]

In conclusion, the synthesis of sphingosine (**1**) from D-tartaric acid (**3**) via aldehyde **5** in 8 steps (from **5**) in 17% overall yield has been described. 2-Azido-3-*O*-benzylsphingosine **2** was also prepared by this method (6 steps from **5**, 23% overall yield). If L-tartaric acid is used as the starting material, the method shown here would provide a convenient route to the enantiomer of **1**.

Experimental Section

(2*E*,4*R*,5*R*)-Ethyl 4-*O*-Benzyl-5,6-*O*-(3'-pentylidene)-4,5,6-trihydroxy-2-hexenoate [(–)-6]. A mixture of 5.6 g (20.0 mmol) of triol **4** and 5 g of 3Å molecular sieves in 200 mL of dry CH_2Cl_2 was stirred at rt for 2 h (mixture A). A slurry of 11.5 g (140 mmol) of NaOAc, 15.8 g (73.3 mmol) of PCC, and 5 g of 3Å molecular sieves in 200 mL of CH_2Cl_2 was stirred at rt for 2 h (mixture B). Mixture B was added to mixture A, with stirring at rt for 4 h. After 400 mL of dry Et_2O was added, stirring was continued for 30 min. The resulting precipitate was removed by filtration over a short pad of silica gel and thoroughly washed with Et_2O. The solvent was evaporated and the residue was

dried to give 5.0 g (91%) of **5** as a pale yellow liquid, which was used in the next reaction without further purification.

To a nitrogen-flushed solution of 7.5 g (87 mmol) of LiBr in 100 mL of dry THF was injected 5.1 mL (5.4 g, 21 mmol) of $(i\text{-PrO})_2P(O)CH_2CO_2Et$ at rt. After the solution was stirred at rt for 10 min, 5.0 mL (35 mmol) of Et_3N was added, and stirring was continued for 10 min. A solution of aldehyde **5** (5.0 g, 17.9 mmol) in 20 mL of dry THF was added. The reaction mixture was stirred vigorously at rt until the full consumption of the aldehyde **5** was observed (TLC). The precipitate was removed by passing the reaction mixture through a pad of silica gel in a sintered glass funnel. The pad was washed with hexane/EtOAc 10:1. Concentration gave a pale yellow oil that was purified by column chromatography (hexane/EtOAc 20:1), providing 4.8 g (77%) of ester **6** as a colorless oil; R_f 0.72 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –11.0° (*c* 2.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84-0.92 (m, 6H), 1.32 (t, 3H, *J* = 6.8 Hz), 1.58-1.66 (m, 4H), 3.68 (t, 1H, *J* = 7.6 Hz), 3.98 (t, 1H, *J* = 6.8 Hz), 4.13 (m, 1H), 4.22 (m, 3H), 4.52 (d, 1H, *J* = 12.0 Hz), 4.70 (d, 1H, *J* = 12.0 Hz), 6.10 (d, 1H, *J* = 16.0 Hz), 6.85 (dd, 1H, *J* = 6.0, 7.6 Hz), 7.25-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.1, 8.2, 14.2, 28.9, 29.4, 60.6, 65.9, 71.5, 77.2, 79.0, 83.1, 113.8, 124.4, 127.8, 128.4, 137.7, 143.4, 165.8.

(2*R*,3*R*,4*E*)-1,2-*O*-(3'-Pentylidene)-3-*O*-benzyl-4-hexene-1,2,3,6-tetrol [(–)-7]. To a solution of 5.0 g (14.4 mmol) of ester **6** in 150 mL of dry THF was added 48 mL (a 1.5 M solution in toluene, 72 mmol) of DIBAL-H at -78 °C under argon. After 5 h, the reaction was quenched by slow addition of 5 mL of MeOH and allowed to warm to rt, followed by addition of 20 mL of cold 5% aqueous potassium sodium tartrate solution. The product was extracted with EtOAc (3 × 20 mL), and the combined organic layers

were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (hexane/EtOAc 3:1) gave 3.7 g (83%) of alcohol **7** as a colorless oil; R_f 0.21 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –18.6° (*c* 2.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84-0.92 (m, 6H), 1.70-1.86 (m, 4H), 3.77 (t, 1H, *J* = 8.0 Hz), 3.92 (m, 4H), 4.28 (m, 1H), 4.48 (d, 1H, *J* = 12.4 Hz), 4.70 (d, 1H, *J* = 12.0 Hz), 5.73 (m, 2H), 7.20 (m, 1H), 7.25-7.47 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 8.4, 8.5, 29.6, 30.1, 62.5, 66.5, 70.6, 78.7, 80.9, 113.6, 126.7, 134.8, 139.2.

(2*R*,3*R*,4*E*)-1,2-*O*-(3'-Pentylidene)-3-*O*-benzyl-6-acetyl-4-hexene-1,2,3,6-tetrol [(–)-8]. To a solution of allylic alcohol **7** (3.06 g, 10 mmol) and *i*-Pr_2NEt (5.2 mL, 30 mmol) in CH_2Cl_2 at –40 °C was added 1.1 mL of acetyl chloride (15 mmol). After the reaction mixture was stirred for 3 h, a second portion of acetyl chloride (3.5 mL, 5 mmol) was added. The mixture was stirred for 1 h at –40 °C and for 1 h at rt, and then quenched with saturated aqueous $NaHCO_3$ solution. After addition of CH_2Cl_2 and phase separation, the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered, and the solvents were removed in vacuo. Flash chromatography (hexanes/EtOAc 6:1) afforded 3.2 g (92%) of **8** as a colorless oil; R_f 0.64 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –13.5° (*c* 4.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84-0.91 (m, 6H), 1.60-1.65 (m, 4H), 2.07 (s, 3H), 3.65 (t, 1H, *J* = 8.0 Hz), 3.92 (m, 2H), 4.20 (dd, 1H, *J* = 6.8, 13.6 Hz), 4.48 (d, 1H, *J* = 12.4 Hz), 4.59 (m, 1H), 4.70 (d, 1H, *J* = 12.4 Hz), 5.66 (dd, 1H, *J* = 7.2, 15.6 Hz), 5.85 (dt, 1H, *J* = 5.6, 15.6 Hz), 7.25-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.0, 8.2, 20.9, 29.1, 29.6, 63.9, 66.2, 70.6, 77.8, 79.7, 113.4, 127.6, 128.0, 128.4, 129.4, 129.9, 138.1, 170.7.

(2*R*,3*R*,4*E*)-3-*O*-Benzyl-4-octadecene-1,2,3-triol [(–)-10]. To a solution of acetate **8** (1.74 g, 5.0 mmol) and Li_2CuCl_4 (0.1 mmol, 1 mL of a 0.1 M solution) in 50 mL of Et_2O at –78 °C was quickly added freshly prepared $C_{12}H_{25}MgBr$ (15 mmol) in 50 mL of Et_2O. The solution was stirred at –78 °C for 3 h, then warmed to rt and stirred overnight. The reaction was quenched by adding 50 mL of water, the organic phase was separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed by vacuum evaporation. To the residue was added 100 mL of MeOH, followed by 10 mL of 5% aqueous H_2SO_4. After the mixture was stirred overnight at ambient temperature, 5 g of K_2CO_3 was added, the mixture was filtered, and the filtrate was removed. The residue was purified by chromatography (hexane/EtOAc 2:1) to give 1.23 g of **10** (63% for two steps) as a colorless oil; R_f 0.15 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –23.7° (*c* 4.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.41 (m, 22H), 2.10 (m, 2H), 3.54 (m, 1H), 3.65 (m, 2H), 3.77 (m, 1H), 4.30 (d, 1H, *J* = 11.6 Hz), 4.60 (d, 1H, *J* = 11.6 Hz), 5.38 (m, 1H), 5.76 (m, 1H), 7.27-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) 14.2, 19.5, 29.0, 29.3, 29.5, 29.8, 29.9, 30.0, 30.1, 31.9, 32.3, 63.3, 70.0, 74.1, 81.1, 126.3, 127.9, 128.0, 128.6, 138.0, 138.2. MS (ESI) *m*/*z* 408.3 (MNH_4^+).

(2*S*,3*R*,4*E*)-2-Azido-3-*O*-benzylsphingosine [(–)-2]. To a solution of 1.17 g (3.0 mmol) of **10** and 1.18 g (4.5 mmol) of Ph_3P in 100 mL of dry CH_2Cl_2 was added 0.94 mL (4.5 mmol) of DIAD at 0 °C. After the reaction mixture was stirred at this temperature for 3 h under nitrogen, 0.59 mL (4.4 mmol) of Me_3SiN_3 was added. The reaction mixture was stirred 0 °C for 3 h and then allowed to warm to rt. After removal of the solvent, the residue was dissolved in 10 mL of THF and treated with 7.5 mL (7.5 mmol, a 1 M

solution in THF containing 5 wt % H_2O) of *n*-Bu_4NF (TBAF). The brown reaction mixture was stirred at rt for 5 h. Concentration gave a slurry that was dissolved in CH_2Cl_2 and passed through a pad of silica gel in a sintered glass funnel to remove $Ph_3P(O)$ and salts. The pad was washed with a mixture of hexanes/EtOAc (usually 6:1). The crude products were purified by chromatography (hexanes/EtOAc 3:1) to give 0.76 g (61%) of **2** as a colorless oil; R_f 0.51 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –55.2° (*c* 1.34, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.41 (m, 22H), 2.10 (m, 2H), 3.50 (m, 1H), 3.71 (m, 2H), 3.90 (m, 1H), 4.34 (d, 1H, *J* = 11.6 Hz), 4.62 (d, 1H, *J* = 11.6 Hz), 5.48 (m, 1H), 5.80 (m, 1H), 7.27-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) 14.1, 22.7, 28.9, 29.2, 29.3, 29.6, 31.9, 32.4, 62.7, 66.0, 70.1, 80.7, 126.3, 127.7, 128.5, 137.8, 138.4. MS (ESI) *m*/*z* 433.4 (MNH_4^+). (The ^1H NMR spectrum was in full agreement with the literature data, but no ^{13}C NMR spectrum and specific rotation were reported.)[6]

(2*S*,3*R*,4*E*)-3-*O*-Benzylsphingosine [(–)-11]. To a solution of 0.62 g (1.5 mmol) of **2** in 30 mL of THF/H_2O 9:1 was added 0.49 g (1.8 mmol) of Ph_3P. The reaction mixture was stirred at rt under nitrogen for 48 h. After the solvents were removed in vacuo, purification by silica gel chromatography ($CHCl_3$/MeOH 9:1) gave **11** (0.52 g, 90%) as a colorless wax; R_f 0.38 ($CHCl_3$/MeOH 9:1); $[\alpha]^{25}_D$ –35.3° (*c* 0.75, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.41 (m, 22H), 2.10 (m, 2H), 3.10 (m, 4H), 3.71 (m, 3H), 4.31 (d, 1H, *J* = 12.0 Hz), 4.65 (d, 1H, *J* = 12.0 Hz), 5.38 (m, 1H), 5.76 (m, 1H), 7.27-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) 14.1, 22.7, 29.2, 29.3, 29.5, 29.8, 32.8, 32.9, 55.9, 63.0, 70.1, 74.1, 81.2, 126.6, 127.7, 127.8, 128.4, 138.1. MS (ESI) *m*/*z* 390.3 (MH^+).

(2*S*,3*R*,4*E*)-Sphingosine [(–)-1]. To a solution of **11** (312 mg, 0.8 mmol) in liquid NH_3 (15 mL) was added Na (200 mg, 80 mmol), and the mixture was stirred for 30 min. Solid NH_4Cl was added to the mixture and NH_3 was allowed to evaporate at rt. Extraction and purification by column chromatography gave 204 mg (85%) of **1** as a white solid; R_f 0.35 ($CHCl_3$/MeOH/NH_4OH 135:25:4); mp 81.0-82.3 °C [lit.[4b] mp 76-77 °C, lit.[4c] mp 72-75 °C, lit.[4d] mp 80.7-82.1 °C]; $[\alpha]^{25}_D$ -2.9° (*c* 1.0, $CHCl_3$) [lit.[3a] $[\alpha]^{22}_D$ -2.5° (*c* 6, $CHCl_3$), lit.[4c] $[\alpha]^{21}_D$ -1.3° (*c* 3.5, $CHCl_3$), lit.[4d] $[\alpha]^{25}_D$ -2.7° (*c* 1.2, $CHCl_3$), lit.[4f] $[\alpha]^{20}_D$ –1.4° (*c* 0.42, $CHCl_3$), lit.[4g] $[\alpha]^{20}_D$ –1.6° (*c* 1.0, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, *J* = 7.0 Hz), 1.23 (m, 20H), 1.35 (m, 2H), 2.02 (q, 2H, *J* = 7.0 Hz), 2.64 (br s, 4H), 2.84 (q, 1H, *J* = 5.2 Hz), 3.64 (m, 2H), 4.04 (t, 1H, *J* = 6.0 Hz), 5.44 (dd, 1H, *J* = 15.4, 7.2 Hz), 5.71 (dt, 1H, *J* = 15.4, 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.2, 29.3, 29.4, 29.5, 29.62, 29.65, 29.68, 31.9, 32.4, 56.2, 63.7, 75.1, 129.1, 134.7. MS (ESI) *m*/*z* 300.3 (MH^+).

(2*R*,3*R*,4*E*)-1,2-*O*-(3'-Pentylidene)-3-*O*-benzyl-6-mesyl-4-hexene-1,2,3,6-tetrol [(–)-12]. To a solution of allylic alcohol **7** (3.06 g, 10 mmol) and *i*-Pr_2NEt (5.2 mL, 30 mmol) in CH_2Cl_2 at –40 °C was added 1.1 mL (15 mmol) of acetyl chloride. After the reaction mixture was stirred for 3 h, a second portion of mesyl chloride (3.5 mL, 5.0 mmol) was added. The mixture was stirred for 1 h at –40 °C and then for 1 h at rt, and then quenched with saturated aqueous $NaHCO_3$ solution. After addition of CH_2Cl_2 and phase separation, the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered, and the solvents were removed in vacuo. Flash chromatography (hexanes/EtOAc 6:1) afforded **12** (3.2 g, 92%) as a colorless oil; R_f 0.70 (hexane/EtOAc 3:1); $[\alpha]^{25}_D$ –16.4° (*c* 1.35, $CHCl_3$); ^{1}H NMR

($CDCl_3$) δ 0.84-0.92 (m, 6H), 1.70-1.86 (m, 4H), 3.77 (t, 1H, *J* = 8.0 Hz), 3.92 (m, 4H), 4.28 (m, 1H), 4.48 (d, 1H, *J* = 12.4 Hz), 4.70 (d, 1H, *J* = 12.0 Hz), 5.73 (m, 2H), 7.20(m, 1H), 7.25-7.47 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 8.4, 8.5, 29.6, 30.1, 62.5, 66.5, 70.6, 78.7, 80.9, 113.6, 126.7, 134.8, 139.2.

References

1. (a) 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., Jr. *Pharmacol Res.* **2003**, *47*, 373-381. (b) Futerman, A. H.; Hannun, Y. A. *EMBO Reports* **2004**, *5*, 777-782.

2. For reviews of the synthesis of sphingosine, see: (a) Byun, H.-S.; Bittman, R. In *Phospholipids Handbook*, Cevc, G., Ed.; Dekker: New York, 1993, pp 97-140. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, *8*, 1075-1091. (c) Curfman, C.; Liotta, D. *Methods Enzymol.* **1999**, *311*, 391-440. (d) Jung, K.-H.; Schmidt, R. R. in *Lipid Synthesis and Manufacture*, Gunstone, F. D., Ed.; Sheffield Academic Press: Sheffield, U. K., 1999, pp 208-249. (e) Koskinen, P. M.; Koskinen, A. M. P. *Methods Enzymol.* **1999**, *311*, 458-479.

3. (a) Zimmermann, P.; Schmidt, R. R. *Liebigs Ann. Chem.* **1988**, 633-667. (b) Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395-4398. (c) Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362. (d) Murakami, T.; Hato, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 823-827. (e) Somfai, P.; Olsson, R. *Tetrahedron* **1993**, *49*, 6645-50.

4. (a) Julina, R.; Herzig, T.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 368-373. (b) Solladié-Cavallo, A.; Koessler, J. L. *J. Org. Chem.* **1994**, *59*, 3240-3242. (c)

Boutin, R. H.; Rapoport, H. *J. Org. Chem.* **1986**, *51*, 5320-5327. (d) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem*. **2000**, *65*, 7627-7633. (e) Nugent, T. C.; Hudlicky, T. *J. Org. Chem.* **1998**, *63*, 510-520. (f) van den Berg, R. J. B. H. N.; Korevaar, C. G. N.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem*. **2004**, *69*, 5699-5704. (g) Torsell, S.; Somfai, P. *Org. Biomol. Chem.* **2004**, *2*, 1643-1646.

5. Compostella, F.; Franchini, L.; Panza, L.; Prosperi, D.; Ronchetti, F. *Tetrahedron* **2002**, *58*, 4425-4428.

6. Tsunoda, H.; Ogawa, S. *Liebigs Ann. Chem.* **1995**, *2*, 267-277.

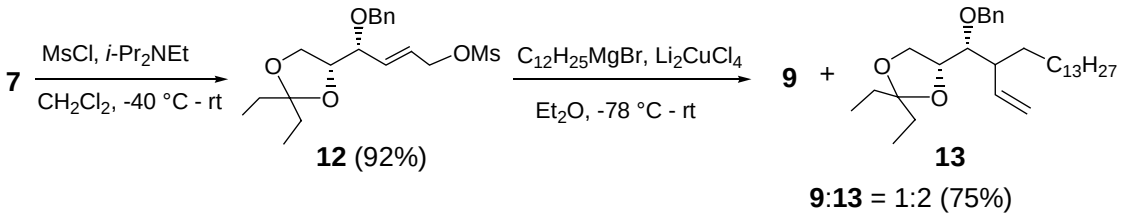
7. For uses of **2** as a glycosyl acceptor, see: (a) Park, T. K.; Lim, I. J.; Danishefsky, S. J. *Tetrahedron Lett.* **1995**, *36*, 9089-9092. (b) Park, T. K.; Kim, I. J.; Hu, S.; Bilodeau, M. T.; Randolph, J. T.; Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc*, **1996**, *118*, 11488-11500. (c) Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1588-1599. (d) Deshpande, P. P.; Kim, H. M.; Zatorski, A.; Park, T.-K.; Ragupathi, G.; Livingston, P. O.; Live, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1600-1614.

8. For reviews of the uses of **3** and its derivatives as synthons, see: (a) Gawroński, J.; Gawrońska, K. *Tartaric and Malic Acids in Synthesis*; John Wiley & Sons: New York, 1999. (b) Ghosh, A. K.; Koltun, E. S.; Bilcer, G. *Synthesis* **2001**, 1281-1301.

9. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

10. Reduction of ester **6** to allylic alcohol **7** with DIBAL was carried out at –78 °C because it was found that the acetal was reduced to release the primary hydroxy group when the temperature was raised to 0 °C.

11. Copper-mediated Grignard reaction of allylic mesylate **12** gave a mixture of **9** and **13**:



12. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) For a postulated mechanism to account for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion, see Scheme 1 of ref. 12a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

13. Wang, X.-Z.; Wu, Y.-L.; Jiang, S.; Singh, G. *Tetrahedron Lett.* **1999**, *40*, 8911-8914.

14. For a review of the Staudinger reaction, see: Gololobov, Yu. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353-1406.

15. It should be noted that the ^{13}C NMR spectrum and specific rotation of **2**, a widely used sphingosine derivative in glycosphingolipid synthesis, have not been reported previously.

16. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 1873-1875.

Chapter 6

Total Synthesis of the Novel Immunosuppressive Agent FTY720 and Its Chiral Regioisomers, Phosphate, and Phosphonate Analogues

Abstract

This chapter presents a concise synthesis of the immunosuppressive agent FTY720 and the first syntheses of its chiral phosphonate and regioisomeric analogues. A 2,3-epoxy alcohol, which was prepared by Sharpless epoxidation, served as a common precursor of these targets. An intramolecular reaction of a 2,3-epoxytrichloroacetimidate mediated by Et_2AlCl gave a five-membered oxazoline ring, which created the quaternary center.

Introduction

FTY720 (2-amino-[2-(4-*n*-octylphenyl)ethyl]-1,3-propanediol, **1**) is a synthetic analogue of the well-known chiral sphingolipid myriocin (**2**).[1] FTY720 alters the migration and homing of lymphocytes, causing them to be sequestered in secondary lymphoid organs,[2] and is currently in phase III clinical studies to evaluate its effects with respect to kidney graft rejection in humans. In addition to its apparent efficacy against graft and transplant rejection, FTY720 is efficacious against autoimmune diseases.[3]

As an analogue of sphingosine, FTY720 is phosphorylated in vivo by sphingosine kinases, converting the prodrug to a chiral FTY720-phosphate ((*R*)- or (*S*)-**3**).[4] FTY720,

or its phosphorylated derivative, down-regulates sphingosine 1-phosphate (S1P) G protein-coupled receptors in thymocytes and lymphocytes by a noncompetitive mechanism of inhibition.[5] Internalization of the receptor renders the cells unresponsive to S1P; thus, lymphocytes are not capable of recirculation to peripheral inflammatory tissues and sites of the transplant.[6]

Several syntheses of FTY720 have been reported recently.[7] Previous preparations of the enantiomers of **3** were accomplished by a difficult chiral HPLC separation of the fully protected derivatives,[8] by a formal asymmetric synthesis starting with a serine-derived oxazolidine,[8] and by a lipase-catalyzed asymmetric acylation.[9] A racemic mixture of the nonhydrolyzable FTY720-phosphonate analogue **4**, in which the carbon-oxygen-phosphorus bond is replaced with a carbon-phosphorus bond, was prepared chemically and was biologically active in vivo, confirming that phosphorylation of FTY720 is essential for S1P receptor internalization.[6]

This chapter describes a novel synthesis of FTY720 (**1**) and the first asymmetric synthesis of its chiral phosphonate (**4**) analogues. Our interest in establishing structure-activity relationships of FTY720 prompted us to also synthesize the enantiomers of the unnatural regioisomer **5** in which the positions of the amino group and one of the hydroxymethyl groups are interchanged. To prepare the chiral phosphonate derivatives **4**, it is obviously crucial to control the stereochemistry of reaction at one of the prochiral hydroxymethyl groups. We chose the asymmetric Sharpless epoxidation, which of course has found numerous applications in synthesis,[10] as a key step in the preparation of a 2,3-epoxy alcohol, which served as a common precursor of our synthetic targets, compounds **1**, **4**, and **5**. A Lewis acid catalyzed cyclization of a 2,3-

epoxytrichloroacetimidate was used to create the quaternary center of FTY720 (**1**) and its phosphonate analogues (**4**), whereas a regioselective epoxide opening reaction was employed to obtain the (2-aminomethyl)-1,2-diol analogue **5**.

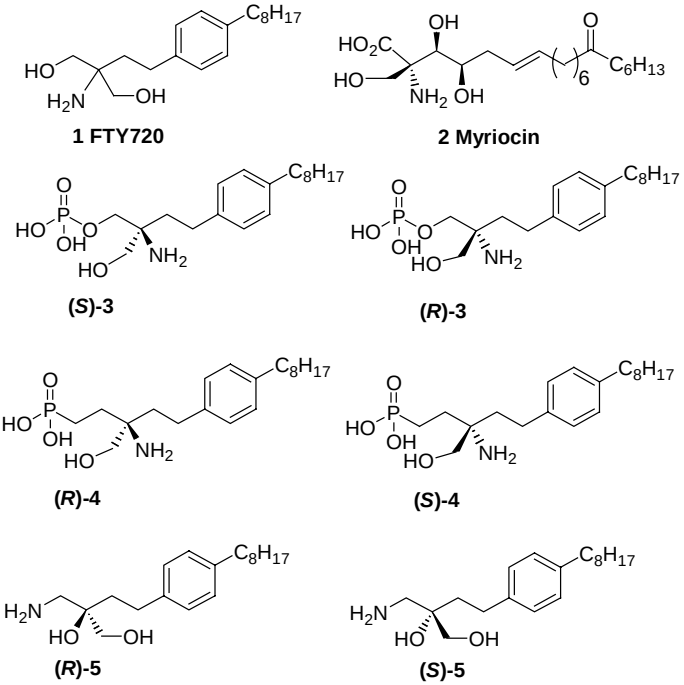


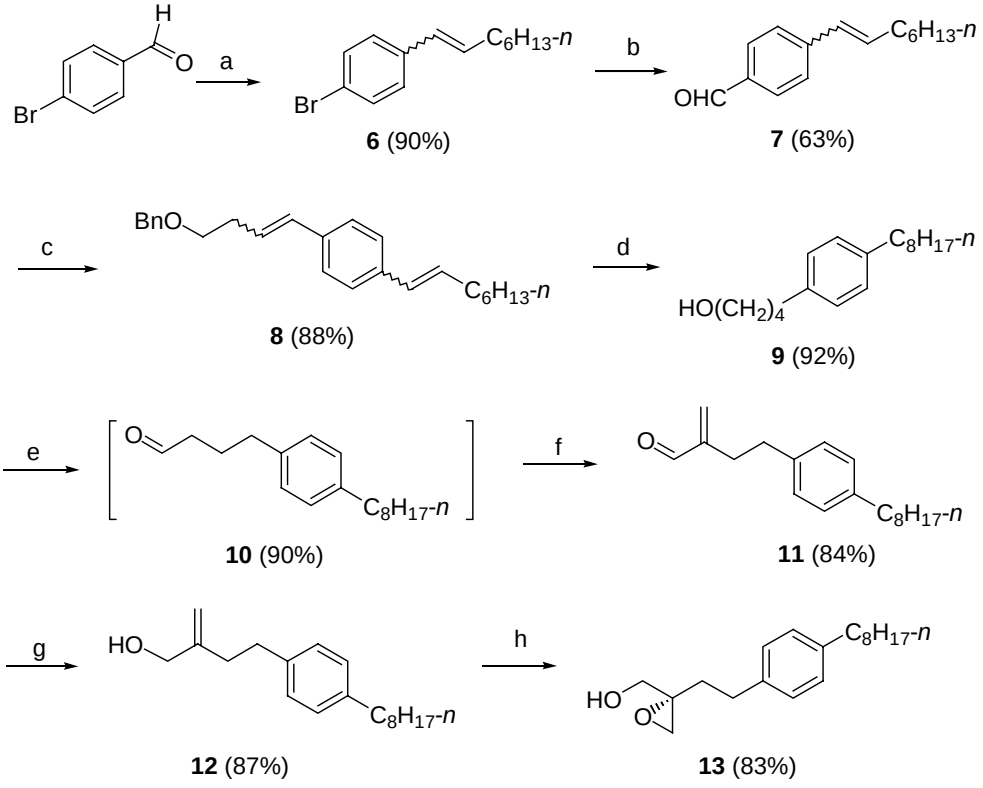
Chart 1. Structures of FTY720 (**1**), myriocin (**2**), and FTY720 analogues **3-5**.

Results and Discussion

Synthesis of Epoxide (*S*)-13. As outlined in Scheme 1, Wittig reaction of 4-bromobenzaldehyde with the ylide of *n*-heptyltriphenylphosphonium bromide gave arylalkene **6** as an *E,Z* mixture in 90% yield. After reaction of the aryllithium derived from **6** with DMF afforded aldehyde **7**, a Wittig reaction with (3-(benzyloxy)propyl)triphenylphosphonium bromide, which was obtained from 1,3-propanediol in three steps,[11] delivered diene **8**. Alcohol **9** was obtained in 92% yield on reduction of the double bonds and hydrogenolysis of the *O*-benzyl group in the presence of Pearlman's catalyst. After PCC oxidation of alcohol **9** provided aldehyde **10**, an

exocyclic methylene group was introduced by use of a Mannich reagent, Eschenmoser's salt,[12] in the presence of excess Et_3N, affording aldehyde **11** in 84% yield. Reduction of α-methylene aldehyde **11** with $NaBH_4$ in the presence of $CeCl_3$ (to suppress conjugate reduction) gave allylic alcohol **12** in 87% yield.[13] We found that the rather expensive anhydrous $CeCl_3$ is not required, since CsCl also provided **12** as the only product. Asymmetric Sharpless epoxidation of allylic alcohol **12** with cumene hydroperoxide in the presence of 0.5 equiv (+)-DIPT, 0.5 equiv of Ti(OPr-*i*)$_4$, and molecular sieves gave epoxide (*S*)-**13** in 83% yield. The synthesis of (*S*)-13 was accomplished in 8 steps and proceeded in an overall yield of 25%. It should be noted that when the amounts of (+)-DIPT and Ti(OPr-*i*)$_4$ were each increased to 1.2 equiv, epoxide opening with Ti(OPr-*i*)$_4$ took place to give a mixture of epoxide **13** and isoproproxy-diol **13'** in a ratio of 1:2.[14]

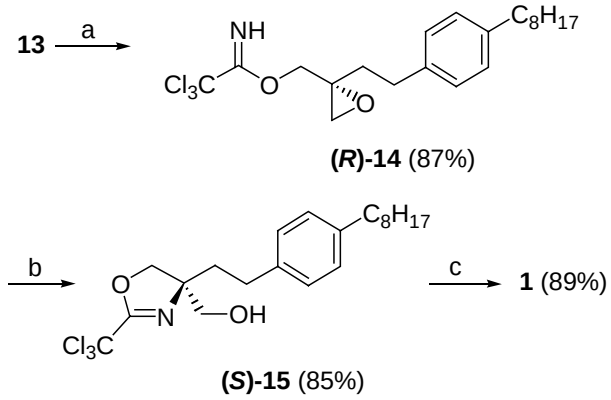
Scheme 1. Synthesis of epoxide (*S*)-13



Reagents and conditions: (a) *n*-$C_7H_{15}PPh_3Br$, *n*-BuLi, THF, -78 °C - rt; (b) *n*-BuLi, THF, DMF, -78 °C - rt; (c) $BnO(CH_2)_3PPh_3Br$, *n*-BuLi, THF, -78 °C - rt; (d) H_2, $Pd(OH)_2/C$, MeOH; (e) PCC, NaOAc, 3 Å molecular sieves, CH_2Cl_2, rt; (f) $[(CH_3)_2N{=}CH_2]^+I^-$ (2.2 equiv), Et_3N, CH_2Cl_2, rt, overnight; (g) $NaBH_4$ (1.5 equiv), CsCl (1.5 equiv), MeOH, 0 °C, 3 h; (h) (+)-DIPT (0.5 equiv), $Ti(OPr\text{-}i)_4$ (0.5 equiv), CH_2Cl_2, cumene hydroperoxide, 4 Å molecular sieves, -20 ºC.

Synthesis of FTY720 (1). Reaction of epoxide (*S*)-**13** with trichloroacetonitrile in the presence of DBU gave 2,3-epoxy-1-trichloroacetimidate (*R*)-**14** in good yield. The tetrasubstituted carbon was set up bearing the desired nitrogen substituent by the reaction of **14** with Et_2AlCl, which afforded oxazoline **15** in 74% yield for the two steps (Scheme 2).[15] The hydroxy and amino groups were released by treatment with 2 M HCl to give FTY720 (**1**) in 89% yield.

Scheme 2. Synthesis of FTY720 (1) from 13

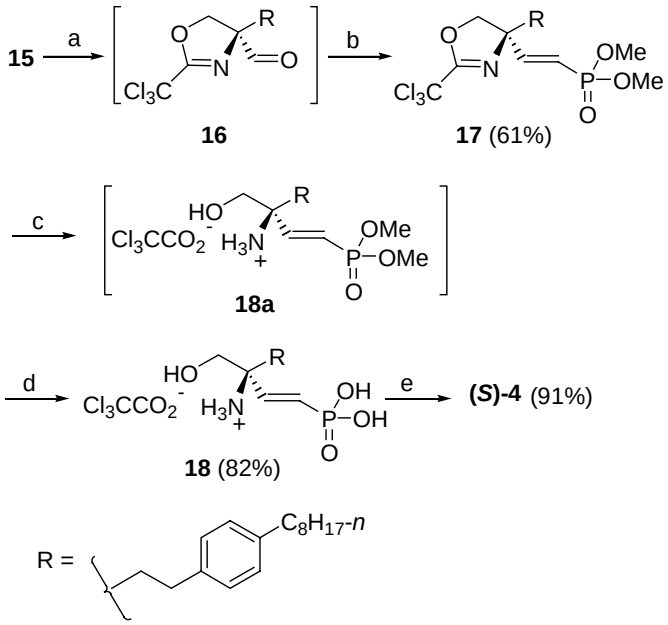


Reagents and conditions: (a) Cl_3CCN, DBU (0.1 equiv), CH_2Cl_2, 0 °C; (b) Et_2AlCl (0.6 equiv), CH_2Cl_2, 0 °C; (c) 2 N HCl, THF, rt.

Synthesis of (*S*)-4. Dess-Martin oxidation of oxazoline **15** in the presence of pyridine gave oxazoline aldehyde **16** (Scheme 3),[16] which on Wittig reaction with

tetramethyl methylenediphosphonate afforded the dimethyl ester of ene-phosphonate **17** in 61% overall yield. Simultaneous demethylation and release of the hydroxy and amino groups by treatment with trimethylsilyl bromide provided ene-phosphonate **18**, but the yield was low. Therefore, the hydroxy and amino groups were first released by treatment with 2 M HCl to give amino alcohol **18a**, which was converted to **18** by treatment with trimethylsilyl bromide and then with 95% methanol (82% yield for the two steps). Reduction of the double bond of ene-phosphonate **18** with Pearlman's catalyst gave (*S*)-**4** in 91% yield.

Scheme 3. Synthesis of (*S*)-4 from (*S*)-15

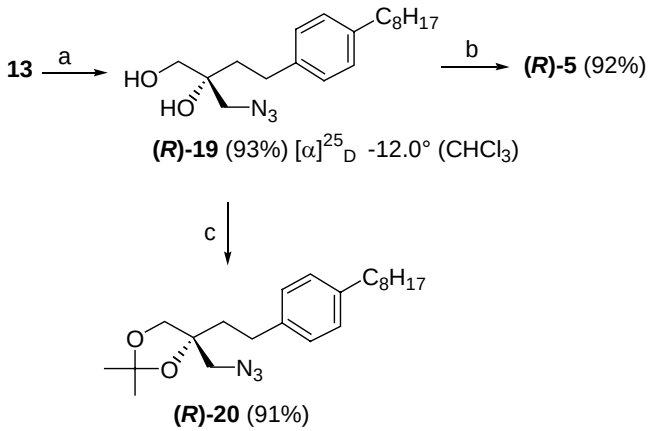


Reagents and conditions: (a) Dess-Martin reagent, py, CH_2Cl_2, rt; (b) $CH_2[P(O)(OMe)_2]_2$, NaH, THF, 0 °C; (c) 2 M HCl, THF, rt; (d) (i) TMSBr, CH_2Cl_2, rt, 4 h; (ii) MeOH, H_2O, rt, 1 h; (e) $Pd(OH)_2/C$, MeOH, H_2.

Synthesis of (*R*)-5. Our strategy for the synthesis of regioisomer (*R*)-**5** is illustrated in Scheme 4. Regioselective opening of 2,3-epoxy alcohol (*S*)-**13** with NaN_3

in the presence of NH_4Cl in aqueous MeOH gave azido diol (*R*)-**19** as the only product in 93% yield (Scheme 4).[17] The azido group of **19** was reduced to an amino group with Pearlman's catalyst to give the desired regioisomer (*R*)-**5** in 92% yield. To confirm the 1,2 relationship of the hydroxy groups in **19**, we derivatized the diol as acetonide **20** (Scheme 4). The ^{13}C NMR spectrum of the quaternary carbon of the acetal is δ 110.2 ppm, which is consistent with the chemical shift reported for five-membered ring acetonides.[18] In a 6-membered acetonide ring, the chemical shift of the quaternary carbon is found to be upfield, at about δ 100 to 101 ppm.[18] Thus the 1,2 relationship of the two hydroxy groups of azido diol **19** was confirmed.

Scheme 4. Syntheses of (*R*)-5 and acetonide 20

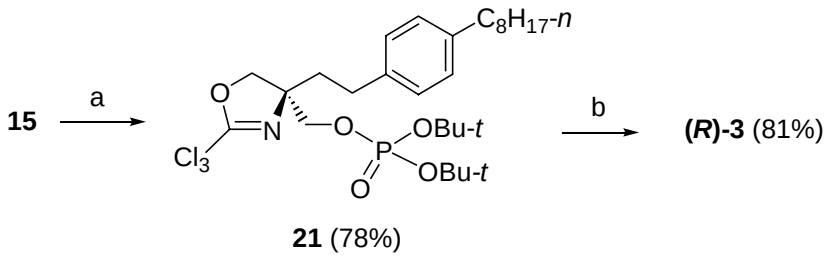


Reagents and conditions: (a) NaN_3 (5 equiv), NH_4Cl (5 equiv), $MeOH/H_2O$ (8:1), 85 °C, overnight; (b) $Pd(OH)_2/C$, H_2, MeOH; (c) 3,3-dimethoxypropane, CSA (cat.), THF, reflux, 2 h.

Synthesis of (*R*)-3. As depicted in Scheme 3, the unmasked hydroxy group of chiral oxazoline **15** was converted to phosphate ester **21** with di-*tert*-butyl diisopropylphosphoramidite in the presence of 1*H*-tetrazole, followed by oxidation of the

phosphite triester with *tert*-butyl hydroperoxide. Finally, a one-pot reaction to hydrolyze the *tert*-butyl ester groups and release the hydroxy and amino groups provided (*R*)-**3** in 63% overall yield from **15**.

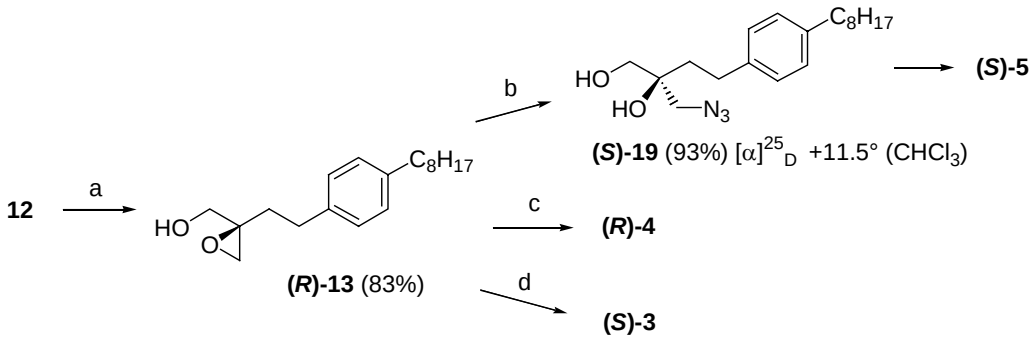
Scheme 5. Conversion of 15 to (*R*)-3



Reagents and conditions: (a) (i) $(t\text{-BuO})_2\text{PN}(\text{Pr-}i)_2$, 1*H*-tetrazole, CH_2Cl_2/CH_3CN, 6 h; (ii) *t*-BuOOH, rt, overnight; (b) 2 M HCl, THF, rt.

Conversion of 13 to (*S*)-5 and (*R*)-4. Sharpless epoxidation of allylic alcohol **12** with (-)-DIPT gave epoxide (*R*)-**13**, which was converted to (*S*)-**5**, to (*R*)-**4**, and to (*S*)-**3** as outlined in Scheme 5.

Scheme 6. Syntheses of (*S*)-5, (*R*)-4, and (*S*)-3



Reagents and conditions: (a) (-)-DIPT, $\text{Ti}(\text{OPr-}i)_4$, CH_2Cl_2, cumene peroxide, molecular sieves, -20 °C; (b) see Scheme 4; (c) see Scheme 3; (d) see Scheme 5.

In conclusion, a concise synthesis of FTY720 (**1**) has been developed that involves 11 steps from *p*-bromobenzaldehyde and proceeds in 16.5% overall yield. Also described in this chapter are the first syntheses of the enantiomers of its phosphonate analogue **4** and regioisomeric analogue **5** in which the stereochemistry at C(2) is controlled by the asymmetric Sharpless epoxidation of allylic alcohol **12**. Alcohol **12** was synthesized via treatment of aldehyde **10** with Eschenmoser's salt, which installed an exo double bond, followed by chemoselective reduction of the aldehyde functionality of enal **11** with $NaBH_4$ and CsCl (or $CeCl_3$). For the synthesis of FTY720 (**1**), 2,3-epoxy alcohol **13** was treated with trichloroacetonitrile and DBU to give trichloroacetimidate **14**, which on intramolecular epoxide opening mediated by Et_2AlCl afforded oxazoline **15**. The synthesis was completed by treatment of oxazoline **15** with HCl. Phosphonate analogue **4** was obtained by condensation of the aldehyde derived from **15** with tetramethyl methylenediphosphonate, followed by ester hydrolysis and reduction of the double bond. The synthesis of FTY720 regioisomer **5** was accomplished by regioselective opening of epoxy alcohol **13** with NaN_3/NH_4Cl in aqueous methanol followed by azide reduction.

Experimental Section

1-Bromo-4-(oct-1-enyl)benzene (6). To a mixture of *n*-heptyltriphenylphosphonium bromide (3.9 g, 8.8 mmol) in anhydrous THF (80 mL) at −78 °C was added *n*-BuLi (4.2 mL, 10.6 mmol, 2.5 M solution in hexane) with stirring. The reaction mixture was warmed to rt for 30 min before being re-cooled to −78 °C, followed by the addition of 1.5 g (8.15 mmol) of 4-bromobenzaldehyde. After the

reaction mixture was stirred at –78 °C for 30 min, and then at rt overnight, 50 mL of a 10% aqueous ammonium chloride solution was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (3 × 50 mL), the organic phase washed with water (50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexane/EtOAc 6:1) to give 1.95 g (90%) of **6** as a colorless liquid; R_f 0.84 (EtOAc/hexane 1:3).

4-(Oct-1-enyl)benzaldehyde (7). To a solution of 1.86 g (7.0 mmol) of bromide **6** in 30 mL of THF at -78 °C under nitrogen was added dropwise *n*-BuLi (3.36 mL, 8.4 mmol, 2.5 M solution in hexane). After the reaction mixture was stirred at -78 °C for 1 h, DMF (0.66 mL, 8.4 mmol), predried over molecular sieves, was added. The reaction mixture was stirred at -78 °C for 1 h and then at rt overnight, and then was diluted with water (50 mL) and extracted with Et_2O (3 x 30 mL). The combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (hexanes/EtOAc 3:1) afforded 0.95 g (63%) of **7** as a colorless liquid; R_f 0.68 (EtOAc/hexane 1:3).

1-(4-(Benzyloxy)but-1-enyl)-4-(oct-1-enyl)benzene (8). To a mixture of Wittig reagent (3-(benzyloxy)propyl)triphenylphosphonium bromide (2.45 g, 5.0 mmol) in anhydrous THF (50 mL) at −78 °C was added *n*-BuLi (2.0 mL, 5.0 mmol, 2.5 M solution in hexane) with stirring. The reaction mixture was warmed to rt for 30 min before being re-cooled to −78 °C, followed by the addition of 0.90 g (4.16 mmol) of aldehyde **7**. After the reaction mixture was stirred at –78 °C for 30 min, and then at rt overnight, 30 mL of a 10% aqueous ammonium chloride solution was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (3 × 30 mL), the organic phase washed with water

(50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexane/EtOAc 6:1) to give 1.27 g (88%) of **8** as a colorless oil; R_f 0.86 (EtOAc/hexane 1:3).

4-(4-Octylphenyl)butan-1-ol (9). To a solution of 1.20 g (3.45 mmol) of diene **8** in 50 mL of MeOH was added 55 mg of 20% $Pd(OH)_2/C$. The suspension was purged with H_2 for approximately 10 min and then stirred overnight under a balloon filled with H_2, when TLC (EtOAc/hexane 1:3, R_f 0.39) indicated the complete consumption of the starting material. The crude reaction mixture was filtered through a short pad of Celite, washed with EtOAc, and concentrated. The residue was purified by flash chromatography (hexane/EtOAc 3:1) to give 0.83 g (92%) of **9** as a colorless oil: R_f 0.39 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.28-1.38 (m, 10H), 1.57-1.69 (m, 6H), 2.56 (t, 2H, J = 7.6 Hz), 2.61 (t, 2H, J = 7.6 Hz), 3.65 (t, 2H, J = 6.4 Hz), 7.08 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.6, 29.3, 29.4, 29.5, 31.6, 31.9, 32.4, 35.2, 35.6, 62.9, 128.3, 139.4, 140.4. HR-MS (FAB, MNa^+) m/z calcd for $C_{18}H_{60}ONa^+$ 285.2189, found 285.2194.

2-Methylene-4-(4-octylphenyl)butanal (11). A mixture of 820 mg (3.13 mmol) of alcohol **9** and 150 mg of 3 Å molecular sieves in 20 mL of dry CH_2Cl_2 was stirred at rt for 2 h (mixture A). A slurry of 1.50 g (18.8 mmol) of NaOAc, 2.70 g (12.5 mmol) of PCC, and 200 mg of 3 Å molecular sieves in 50 mL of CH_2Cl_2 was stirred at rt for 2 h (mixture B). Mixture A was added to mixture B, with stirring at rt for 4 h. After 70 mL of dry Et_2O was added, stirring was continued for 30 min. The resulting precipitate was removed by filtration over a short pad of silica gel, which was washed thoroughly with Et_2O. The solvents were evaporated, and the residue was dried to give 733 mg (90%) of

10 as a pale yellow liquid, which was used in the next reaction without further purification. To a stirred solution of aldehyde **10** (2.82 mmol) and Et_3N (1.30 mL, 9.39 mmol) in 50 mL of CH_2Cl_2 was added 1.16 g (6.26 mmol) of Eschenmoser's salt at rt, and the resulting mixture was stirred overnight. After addition of saturated aqueous $NaHCO_3$ solution (30 mL), the mixture was extracted with CH_2Cl_2 (3 x 30 mL), and the extract was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc 6:1) to afford 644 mg (84%) of **11** as a colorless oil: R_f 0.76 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 2.56 (m, 4H), 2.73 (m, 2H), 5.98 (s, 1H), 6.19 (s, 1H), 7.08 (s, 4H), 9.54 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.8, 31.6, 31.9, 33.6, 35.6, 128.3, 134.5, 138.3, 140.6, 149.5, 194.5. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{19}H_{28}ONa^+$ 295.2032, found 295.2040.

2-Methylene-4-(4-octylphenyl)butan-1-ol (12). To a solution of 0.65 g (3.47 mmol) of CsCl and 112 mg (3.47 mmol) of $NaBH_4$ in 30 mL of MeOH was added a solution of 630 mg (2.31 mmol) of α-methylene aldehyde **11** in 10 mL of MeOH at 0 °C. The mixture was stirred for 3 h at 0 °C, diluted with 100 mL of EtOAc, and filtered through a pad of silica gel, which was rinsed with 100 mL of EtOAc. The filtrate was concentrated, and the residue was purified by chromatography (hexane/EtOAc 3:1) to give 0.55 g (87%) of allylic alcohol **12** as a colorless oil: R_f 0.46 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.42 (s, 1H), 1.58 (m, 2H), 2.36 (t, 2H, *J* = 7.6 Hz), 2.56 (t, 2H, *J* = 8.0 Hz), 2.75 (t, 2H, *J* = 8.4 Hz), 4.08 (s, 2H), 4.92 (s, 1H), 5.06 (s, 1H), 7.10 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5,

31.6, 31.9, 33.6, 34.7, 35.6, 66.1, 109.7, 128.2, 128.3, 138.9, 140.5, 148.6. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{19}H_{30}ONa^+$ 297.2189, found 297.2179.

(2*S*)-2-(Oxiranyl)-4-(4-octylphenyl)butan-1-ol [(-)-13]. To a solution of 0.22 mL (1.0 mmol) of D-(+)-diisopropyl tartrate in 100 mL of dry CH_2Cl_2 was added 500 mg of 4 Å molecular sieves, and the mixture was stirred at rt for 30 min before it was cooled to –40 °C. To the reaction mixture was added Ti(OPr-*i*)$_4$ (0.30 mL, 1.0 mmol), with stirring for 30 min. Cumene hydroperoxide (608 mg, 4.0 mmol) was added, and the reaction mixture was stirred for 30 min. A solution of 548 mg (2.0 mmol) of alcohol **12** in 5 mL of dry CH_2Cl_2 was added, and the reaction mixture was stirred overnight at −20 °C. After 50 mL of a 10% aqueous solution of tartaric acid was added dropwise, the mixture was allowed to warm to rt over 1 h, at which time the solution was transparent. The organic layer was separated, washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was dissolved in 50 mL of Et_2O at 0 °C, and 30 mL of 5% aqueous NaOH was added. The mixture was stirred for 30 min, the organic layer was separated, washed with brine (50 mL), and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by chromatography (hexane/EtOAc 3:1) to afford 482 mg (83%) of epoxide **13** as a colorless oil: R_f 0.18 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –2.5° (*c* 2.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.82 (m, 1H), 1.86 (m, 1H), 2.54 (m, 2H), 2.69 (m, 4H), 2.83 (d, 1H, *J* = 4.8 Hz), 3.59 (m, 1H), 3.77 (m, 1H), 7.08 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 30.5, 31.6, 31.9, 33.7, 35.6, 50.0, 59.8, 63.2, 128.1, 128.5, 138.4, 140.7. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{19}H_{30}O_2Na^+$ 313.2138, found 313.2130.

(2*S*)-2-(2-(Trichloromethyl)-4,5-dihydrooxazol-5-yl)-4-(4-octylphenyl)-butan-1-ol [(+)-15]. To a solution of 435 mg (1.5 mmol) of epoxide (*S*)-**13** in 25 mL of CH_2Cl_2 at 0 °C were added trichloroacetonitrile (0.17 mL, 1.65 mmol) and DBU (0.023 mL, 0.15 mmol). After being stirred at 0 °C for 1.5 h, the reaction mixture was diluted with Et_2O (20 mL) and quenched with water (20 mL). The organic layer was separated, washed with brine (20 mL), and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by chromatography (hexane/EtOAc 20:1) to give 565 mg (87%) of trichloroacetimidate **14**; R_f 0.58 (EtOAc/hexane 1:3). To an ice-cold solution of 565 mg (1.31 mmol) of **14** in 20 mL of CH_2Cl_2 was added Et_2AlCl (0.75 mL, 0.75 mmol, 1.0 M solution in hexane). After the mixture was stirred at 0 °C for 20 min and then at rt for 3 h, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated, washed with brine (20 mL), and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by chromatography (hexane/EtOAc 3:1) to give 480 mg (85%) of (*S*)-**15** as a white solid; mp 56.7-57.5 °C; R_f 0.29 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +24.9° (*c* 1.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.84 (m, 1H), 2.01 (m, 1H), 2.57 (m, 4H), 3.30 (s, 1H), 3.52 (d, 1H, *J* = 11.6 Hz), 3.71 (d, 1H, *J* = 11.6 Hz), 4.39 (d, 1H, *J* = 8.4 Hz), 4.65 (d, 1H, *J* = 8.4 Hz), 7.08 (s, 4H); ^{13}C NMR ($CDCl_3$) 14.2, 22.7, 29.3, 29.4, 29.5, 29.8, 31.6, 32.0, 35.6, 37.6, 66.8, 76.0, 86.5, 128.2, 128.9, 138.2, 140.8, 163.2. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{21}H_{30}Cl_3NO_2Na^+$ 456.1234, found 456.1241. Data for [(*R*)-(-)-**15**]: $[\alpha]^{25}_D$ –25.0° (*c* 2.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.85 (m, 1H), 2.01 (m, 1H), 2.14 (s, 1H), 2.60 (m, 4H), 3.54 (d, 1H, *J* = 11.6 Hz), 3.83 (d, 1H, *J* = 11.6 Hz), 4.45 (d, 1H, *J* = 8.4 Hz), 4.63 (d, 1H,

J = 8.4 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.0, 29.3, 29.5, 29.7, 31.6, 31.9, 35.5, 37.5, 67.0, 75.9, 86.4, 128.1, 128.6, 138.1, 140.9, 163.3.

2-Amino-[2-(4-octylphenyl)ethyl]-1,3-propanediol (1). To a solution of 217 mg (0.50 mmol) of alcohol **15** in 10 mL of THF at rt was added 3 mL of 1 M HCl. After the reaction mixture was stirred overnight, 10 mL of saturated aqueous $NaHCO_3$ solution was added, and the organic layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography ($CHCl_3$/MeOH/NH_4OH 135:25:4) to give 137 mg (89%) of FTY720 (**1**) as a white solid: mp 104.7-106.3 °C; R_f 0.38 ($CHCl_3$/MeOH/H_2O/AcOH 135:25:4); ^{1}H NMR ($CDCl_3$/CD_3OD 9:1) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.72 (m, 2H), 2.55 (m, 2H), 2.61 (m, 2H), 3.50 (d, 2H, J = 11.6 Hz), 3.60 (d, 1H, J = 11.6 Hz), 3.87 (s, 4H), 7.10 (s, 4H); ^{13}C NMR ($CDCl_3$) 14.1, 22.7, 28.6, 29.3, 29.4, 29.5, 31.5, 31.9, 34.4, 35.5, 60.0, 65.5, 128.0, 128.6, 137.7, 140.9.

Dimethyl (3*S*)-3-(2-(Trichloromethyl)-4,5-dihydrooxazol-5-yl)-5-(4-octyl-phenyl)-pent-(1*E*)-enylphosphonate [(+)-17]. A solution of 433 mg (1.0 mmol) of alcohol **15** in 1 mL of pyridine and 50 mL of dry CH_2Cl_2 at rt was treated with 504 mg (1.20 mmol) of Dess-Martin periodinane. After 1 h, an aqueous solution of 10% $Na_2S_2O_3$ (50 mL) was added, and the mixture was stirred until both layers became clear. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (25 mL), water, and dried ($MgSO_4$). The solvent was evaporated, and the residue was dried to afford crude aldehyde **16**. To a mixture of 36 mg (1.5 mmol) of NaH and 50 mL of THF was added a solution of 348 mg (1.5 mmol) of tetramethyl methylenediphosphonate in 10 mL of THF at 0 °C. After the mixture was stirred for 10 min, a solution of crude

aldehyde **16** in 10 mL of THF was added. The reaction mixture was stirred for 2 h, diluted with Et_2O (20 mL), and extracted in successive order with 1 M NaOH/MeOH (7:3) (2 x 30 mL) (to remove the excess diphosphonate), H_2O/brine (1:1) (1 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (hexane/EtOAc 1:2) to give 328 mg (61% for the two steps) of (*S*)-**17** as a colorless oil; R_f 0.37 (EtOAc/hexane 2:1); $[\alpha]^{25}_D$ +24.8° (*c* 4.30, $CHCl_3$);. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.23-1.35 (m, 10H), 1.56 (m, 2H), 2.10 (m, 2H), 2.59 (m, 3H), 2.71 (m, 1H), 3.71 (s, 3H), 3.74 (s, 3H), 4.45(dd, 2H, *J* = 8.8, 20.4 Hz), 5.89 (dd, 1H, *J* = 17.2, 19.2 Hz), 6.87 (dd, 1H, *J* = 17.2, 22.4 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 21.0, 29.3, 29.5, 29.7, 31.5, 31.9, 35.5, 40.9, 52.5, 61.1, 75.6, 75.8, 78.7, 86.3, 115.7, 117.6, 128.1, 128.6, 137.8, 140.9, 153.1, 171.0; ^{31}P NMR ($CDCl_3$) δ 20.6. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{24}H_{35}Cl_3NO_4PNa^+$ 560.1261, found 560.1272. Data for [(*R*)-(-)-**17**]: $[\alpha]^{25}_D$ –29.3° (*c* 6.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.23-1.35 (m, 10H), 1.58 (m, 2H), 2.10 (m, 2H), 2.57 (m, 3H), 2.72 (m, 1H), 3.72 (s, 3H), 3.75 (s, 3H), 4.45(dd, 2H, *J* = 8.8, 20.4 Hz), 5.92 (dd, 1H, *J* = 17.2, 19.2 Hz), 6.89 (dd, 1H, *J* = 17.2, 22.4 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 21.0, 29.3, 29.5, 29.7, 31.5, 31.9, 35.5, 40.9, 52.5, 61.1, 75.6, 75.8, 78.7, 86.3, 115.7, 117.6, 128.1, 128.6, 137.8, 140.9, 153.1, 171.0; ^{31}P NMR ($CDCl_3$) δ 20.7.

(3*S*)-3-(Aminomethyl)-3-hydroxy-5-(4-octylphenyl)pent-(1*E*)-enylphosphonic Acid [(+)-18]. To a solution of 269 mg (0.50 mmol) of dimethyl phosphonate **17** in 10 mL of THF at rt was added 3 mL of 1 M HCl. After the reaction mixture was stirred overnight, the solvent was evaporated to give crude **18a**. To a solution of dry crude **18a** in 10 mL of dry CH_2Cl_2 at rt was added 0.66 mL (5.0 mmol) of bromotrimethylsilane.

After the reaction mixture was stirred for 4 h, the solvent was removed, and the residue was dried under vacuum. The residue was dissolved in 2 mL of 95% MeOH with stirring for 1 h, and the solvent was removed to afford 224 mg (82% for 2 steps) of (*S*)-**18** as a white solid: mp 159.2-161.1 °C; R_f 0.14 ($CHCl_3$/MeOH/H_2O/AcOH 65:25:4:1); $[\alpha]^{25}_D$ +8.1° (*c* 6.45, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 9:1) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.23-1.35 (m, 10H), 1.57 (m, 2H), 2.07 (m, 2H), 2.57 (m, 4H), 3.86 (s, 3H), 6.01 (br s, 5H), 6.20 (m, 1H), 6.59 (m, 1H), 7.09 (m, 4H); ^{13}C NMR ($CDCl_3$/CD_3OD 9:1) δ 14.1, 22.9, 29.1, 29.5, 29.6, 31.8, 32.3, 35.8, 36.5, 62.1, 62.3, 64.4, 123.3, 125.2, 128.3, 128.9, 137.6, 141.4, 144.2; ^{31}P NMR ($CDCl_3$/CD_3OD 9:1) δ 13.1. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{20}H_{34}NO_4PNa^+$ 406.2118, found 406.2106. Data for [(*R*)-(-)-**18**]: $[\alpha]^{25}_D$ –12.0° (*c* 2.65, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 9:1) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.23-1.35 (m, 10H), 1.57 (m, 2H), 2.07 (m, 2H), 2.57 (m, 4H), 3.77 (s, 2H), 4.83 (br s, 5H), 6.20 (m, 1H), 6.59 (m, 1H), 7.09 (m, 4H); ^{13}C NMR ($CDCl_3$/CD_3OD 9:1) δ 14.2, 22.9, 29.0, 29.5, 29.6, 29.7, 29.9, 31.8, 32.3, 35.8, 36.5, 62.1, 62.3, 64.4, 123.4, 125.2, 128.3, 128.9, 137.6, 141.4, 144.2; ^{31}P NMR ($CDCl_3$/CD_3OD 9:1) δ 13.1.

(3*S*)-3-(Aminomethyl)-3-hydroxy-5-(4-octylphenyl)pentylphosphonic Acid [(-)-4]. To a solution of 164 mg (0.30 mmol) of unsaturated phosphonic acid **18** in 20 mL of MeOH was added 16 mg of 20% $Pd(OH)_2$/C. The resulting suspension was purged with H_2 for approximately 10 min and then stirred overnight under a balloon filled with H_2. The reaction mixture was filtered through a short pad of Celite, and the solvent was evaporated to provide 150 mg (91%) of (*S*)-**4** as a white solid: mp 160.1-161.5 °C; R_f 0.14 ($CHCl_3$/MeOH/H_2O/AcOH 65:25:4:1); $[\alpha]^{25}_D$ -0.30° (*c* 5.17, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 9:1) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.23-1.35 (m, 10H), 1.55 (m, 2H),

2.07 (m, 6H), 2.52 (t, 2H, J = 7.2 Hz), 2.64 (m, 2H), 3.75 (s, 2H), 5.95 (br s, 5H), 7.06 (m, 4H); ^{13}C NMR ($CDCl_3/CD_3OD$ 9:1) δ 14.1, 22.9, 26.0, 28.6, 29.4, 29.5, 29.6, 31.7, 32.0, 34.8, 35.7, 60.7, 63.5, 128.3, 128.7, 137.5, 141.1; ^{31}P NMR ($CDCl_3/CD_3OD$ 9:1) δ 29.6. HR-MS (FAB, MNa^+) m/z calcd for $C_{20}H_{36}NO_4PNa^+$ 408.2274, found 408.2274.

(3*R*)-3-(Aminomethyl)-3-hydroxy-5-(4-octylphenyl)pentylphosphonic Acid [(+)-4]. $[\alpha]^{25}_D$ +0.54° (*c* 2.06, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3/CD_3OD$ 9:1) δ 0.88 (t, 3H, J = 6.4 Hz), 1.23-1.35 (m, 10H), 1.55 (m, 2H), 2.07 (m, 6H), 2.52 (m, 2H), 2.64 (m, 2H), 3.75 (s, 2H), 4.68 (br s, 5H), 7.06 (m, 4H); ^{13}C NMR ($CDCl_3/CD_3OD$ 9:1) δ 14.1, 22.9, 26.0, 28.6, 29.4, 29.5, 29.6, 31.7, 32.0, 34.8, 35.7, 60.5, 63.5, 128.3, 128.7, 137.5, 141.1; ^{31}P NMR ($CDCl_3/CD_3OD$ 9:1) δ 29.6.

(2*R*)-2-(Azidomethyl)-4-(4-octylphenyl)butane-1,2-diol [(-)-19]. To a stirred solution of 290 mg (1.0 mmol) of epoxy alcohol **13** in 9 mL of MeOH/H_2O (8:1) were added sodium azide (0.33 g, 5.0 mmol) and ammonium chloride (269 mg, 5.0 mmol). The reaction mixture was heated at 85 °C overnight. The solvents were evaporated, and the residue was dissolved in 10 mL of H_2O and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with brine (20 mL), dried (Na_2SO_4), concentrated, and purified by chromatography (hexane/EtOAc 1:1) to give 310 mg (93%) of **19** as a colorless oil: R_f 0.17 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –12.0° (*c* 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.81 (m, 2H), 2.54 (t, 2H, J = 7.6 Hz), 2.62 (m, 2H), 2.70 (s, 1H), 2.83 (s, 1H), 3.40 (s, 2H), 3.54 (dd, 2H, J = 13.6, 21.2 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) 14.1, 22.7, 28.8, 29.0, 29.3, 29.4, 29.5, 29.8, 31.6, 31.9, 35.6, 36.9, 55.9, 66.0, 74.4, 128.2, 128.6, 138.7, 140.8. Data for [(*S*)-(+)-**19**]: $[\alpha]^{25}_D$ +11.5° (*c* 3.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26-

1.38 (m, 10H), 1.58 (m, 2H), 1.81 (m, 2H), 2.54 (t, 2H, J = 7.6 Hz), 2.62 (t, 2H, J = 8.4 Hz), 3.12 (s, 2H), 3.39 (s, 2H), 3.54 (dd, 2H, J = 13.6, 21.2 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.8, 29.0, 29.3, 29.4, 29.5, 29.8, 31.6, 31.9, 35.6, 36.9, 55.7, 66.0, 74.6, 128.2, 128.6, 138.7, 140.8. HR-MS (FAB, MNa^+) m/z calcd for $C_{19}H_{31}N_3O_2Na^+$ 356.2308, found 356.2317.

(4*R*)-4-(4-Octylphenethyl)-4-(azidomethyl)-2,2-dimethyl-1,3-dioxolane [(-)-20]. To a solution of 100 mg (0.30 mmol) of diol (*R*)-**19** and 7 mg (0.030 mmol) of CSA in 10 mL of dry THF was added 38 mg (0.36 mmol) of 3,3-dimethoxypropane. After the solution was stirred at reflux for 2 h, the reaction was quenched with 138 mg (1.0 mmol) of anhydrous K_2CO_3. The solid residue was removed by filtration and washed with Et_2O (2 x 10 mL). The solvent was evaporated, and the residue was purified by chromatography (EtOAc/hexane 1:6) to give 84 mg (91%) of (*R*)-**20** as a colorless liquid: R_f 0.81 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –11.5° (*c* 2.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 3H, J = 6.8 Hz), 1.26-1.38 (m, 10H), 1.42 (s, 3H), 1.47 (s, 3H), 1.58 (m, 2H), 1.94 (t, 2H, J = 8.4 Hz), 2.62 (m, 4H), 3.34 (s, 2H), 3.78 (d, 1H, J = 8.8 Hz), 3.89 (d, 1H, J = 8.8 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.8, 27.1, 27.5, 29.3, 29.4, 29.5, 29.8, 31.6, 31.9, 35.6, 37.9, 55.8, 70.7, 82.7, 110.2, 128.1, 128.6, 138.6, 140.7. HR-MS (FAB, MNa^+) m/z calcd for $C_{22}H_{35}N_3O_2Na^+$ 396.2621, found 396.2411.

(2*R*)-2-(Aminomethyl)-4-(4-octylphenyl)butane-1,2-diol [(-)-5]. To a solution of 100 mg (0.30 mmol) of diol (*R*)-**19** in 10 mL of MeOH was added 16 mg of 20% $Pd(OH)_2$/C. The resulting suspension was purged with H_2 for approximately 10 min, and then equipped with a balloon filled with H_2 and stirred overnight, when TLC ($CHCl_3$/MeOH/NH_4OH 135:25:4, R_f 0.30) indicated the complete consumption of the

starting material. The crude reaction mixture was filtered through a short pad of Celite, and the solvent was evaporated to provide 85 mg (92%) of (*R*)-**5** as a white wax: R_f 0.30 ($CHCl_3$/MeOH/NH_4OH 135:25:4); $[\alpha]^{25}_D$ –2.2° (*c* 0.29, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.70 (m, 2H), 2.46 (br s, 4H), 2.55 (t, 2H, *J* = 7.6 Hz), 2.64 (m, 2H), 2.88 (m, 2H), 3.62 (dd, 2H, *J* = 11.2, 36.0 Hz), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.1, 29.3, 29.4, 29.5, 29.8, 31.6, 31.9, 35.6, 38.2, 48.5, 68.9, 72.3, 128.1, 128.5, 139.3, 140.5. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{19}H_{33}NO_2Na^+$ 330.2403, found 330.2412.

(2*S*)-2-(Aminomethyl)-4-(4-octylphenyl)butane-1,2-diol [(+)-5]. $[\alpha]^{25}_D$ +1.0° (*c* 1.84, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.70 (m, 2H), 2.55 (t, 2H, *J* = 7.6 Hz), 2.64 (m, 2H), 2.96 (s, 2H), 3.64 (m, 2H), 3.71 (br s, 4H), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.0, 29.3, 29.4, 29.5, 31.6, 31.9, 35.6, 38.3, 48.0, 68.5, 72.3, 128.1, 128.4, 139.1, 140.5. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{19}H_{33}NO_2Na^+$ 330.2403, found 330.2407.

((*R*)-4-(4-Octylphenethyl)-2-(trichloromethyl)-4,5-dihydrooxazol-4-yl)methyl Di-*tert*-butyl Phosphate (21). A solution of oxazoline **15** (109 mg, 0.25 mmol) and di-*tert*-butyl diisopropylphosphoramidite (111 mg, 0.40 mmol) in CH_2Cl_2 (40 mL) at 0 °C was treated with 1*H*-tetrazole (1.12 mL, 0.45 M, 0.50 mmol). The resulting mixture was stirred at rt for 6 h, then cooled to −78 °C. After *tert*-butyl hydroperoxide (90 mg, 1.0 mmol) was added, the cooling bath was removed and the reaction mixture was stirred at rt overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (15 mL), and then extracted with CH_2Cl_2 (3 x 15 mL). The organic extract was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography

(hexane/EtOAc 3:1) to give 119 mg (78%) of **21** as a colorless oil: R_f 0.15 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ –10.8° (*c* 3.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.22-1.36 (m, 10H), 1.54-1.64 (m, 20H), 1.86-2.04 (m, 2H), 2.57 (m, 2H), 2.64 (m, 2H), 4.06 (m, 2H), 4.42 (d, 1H, *J* = 8.8 Hz), 4.72 (d, 1H, *J* = 8.8 Hz), 7.11 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.9, 29.3, 29.5, 29.9, 31.6, 31.9, 35.5, 37.8, 61.2, 73.9, 74.7, 82.8, 100.0, 128.2, 128.6, 138.2, 140.8, 162.5; ^{31}P NMR ($CDCl_3$) δ -9.80.

FTY720-Phosphate (3). To a solution of 92 mg (0.15 mmol) of alcohol **11** in 5 mL of THF at rt was added 2 mL of 2 M HCl. After the reaction mixture was stirred overnight, 375 mg (4.46 mmol) of $NaHCO_3$ was added. The reaction mixture was concentrated under vacuum and the residue was purified by chromatography ($CHCl_3$/MeOH/H_2O/AcOH 65:25:4:1) to give 47 mg (81%) of phosphate **3** as a white solid. Data for (*R*)-**3** and (*S*)-**3**: R_f 0.30 ($CHCl_3$/MeOH/H_2O/AcOH 65:25:4:1); ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.22-1.36 (m, 10H), 1.54-1.64 (m, 2H), 1.86-2.04 (m, 2H), 2.52 (m, 2H), 2.68 (m, 2H), 3.60 (m, 2H), 3.90 (m, 2H), 7.07 (m, 4H); ^{31}P NMR (CD_3OD) δ 0.28; MS (ESI): m/z 388.2 (MH^+).

(2*R*)-2-(Oxiranyl)-4-(4-octylphenyl)butan-1-ol [(+)-13a]. This compound was prepared in 83% yield by using (-)-DIPT in the Sharpless epoxidation reaction (Scheme 1, step h): $[\alpha]^{25}_D$ +3.0° (*c* 2.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.82 (m, 1H), 1.86 (m, 1H), 2.54 (m, 2H), 2.66 (m, 3H), 2.74 (s, 1H), 2.83 (d, 1H, *J* = 4.8 Hz), 3.59 (d, 1H, *J* = 10.0 Hz), 3.77 (d, 1H, *J* = 12.4 Hz), 7.08 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 30.5, 31.6, 31.9, 33.7, 35.6, 50.0, 59.8, 63.2, 128.1, 128.5, 138.4, 140.7.

References

1. Fujita, T.; Inoue, K.; Yamamoto, S.; Ikumoto, T.; Sasaki, S.; Toyama, R.; Chiba, K.; Hoshino, Y.; Okumoto, T. *J. Antibiot. (Tokyo)* **1994**, *47*, 208-215.

2. For recent reviews, see: (a) Brinkmann, V. *Yonsei Med. J.* **2004**, *45*, 991-997. (b) Chiba, K. *Pharmacol. Ther.* **2005**, in press (e-pub).

3. Gonsette, R. E. *J. Neurol. Sci.* **2004**, *223*, 87-93.

4. Brinkmann, V.; Lynch, K. R. *Curr. Opin. Immunol.* **2002**, *14*, 569-575.

5. Graler, M. H.; Goetzl, E. J. *FASEB J.* **2004**, *18*, 551-553.

6. Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. *Science* **2002**, *296*, 346-349.

7. (a) Sugiyama, S.; Arai, S.; Kiriyama, M.; Ishii, K. *Chem. Pharm. Bull.* **2005**, *53*, 100-102. (b) Seidel, G.; Laurich, D.; Fürstner, A. *J. Org. Chem.* **2004**, *69*, 3950-3952. (c) Adachi, K.; Kohara, T.; Nakao, N.; Arita, M.; Chiba, K.; Mishina, T.; Sasaki, S.; Fujita, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 853-856. (d) Kiuchi, M.; Adachi, K.; Kohara, T.; Minoguchi, M.; Hanano, T.; Aoki, Y.; Mishina, T.; Arita, M.; Nakao, N.; Ohtsuki, M.; Hoshino, Y.; Teshima, K.; Chiba, K.; Sasaki, S.; Fujita, T. *J. Med. Chem.* **2000**, *43*, 2946-2961. (e) Durand, P.; Peralba, P.; Sierra, F.; Renaut, P. *Synthesis* **2000**, 505-506. (f) Kalita, B.; Barua, N. C.; Bezbarua, M.; Bez, G. *Synlett* **2001**, 1411-1414.

8. Hale, J. J.; Yan, L.; Neway, W. E.; Hajdu, R.; Bergstrom, J. D.; Milligan, J. A.; Shei, G.-J.; Chrebet, G. L.; Thornton, R. A.; Card, D.; Rosenbach, M.; Rosen, H.; Mandala, S. *Bioorg. Med. Chem.* **2004**, *12*, 4803-4807.

9. Kiuchi, M.; Adachi, K.; Tomatsu, A.; Chino, M.; Takeda, S.; Tanaka, Y.; Maeda, Y.; Sato, N.; Mitsutomi, N.; Sugahara, K.; Chiba, K. *Bioorg. Med. Chem.* **2005**, *13*, 425-432.

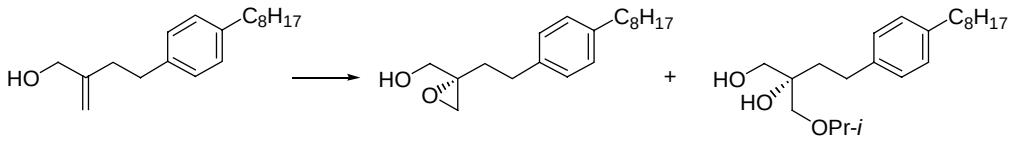
10. For a review of the Sharpless epoxidation, see: Katsuki, T.; Martin, V. S. *Org. React.* **1996**, *48*, 1-299.

11. Ziegler, F. E.; Klein, S. I.; Pati, U. K.; Wang, T. F. *J. Am. Chem. Soc.* **1985**, *107*, 2730-2737. 1,3-Propanediol reacted with 1 equiv of benzyl bromide in the presence of NaH to give 3-(benzyloxy)-1-propanol, which was converted to the bromide (NBS, PPh_3).

12. (a) Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Intl. Ed. Engl.* **1971**, *10*, 330-331. (b) Roberts, J. L.; Borromeo, P. S.; Poulter, C. D. *Tetrahedron Lett.* **1977**, *18*, 1621-1623.

13. Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226-2227.

14. Sharpless epoxidation gave a mixture of epoxide **13** and *O*-isopropyl triol **13'** in a ratio of 1:2 when 1.2 equiv of (+)-DIPT and Ti(OPr-*i*)$_4$ were used.



12 **13** **13'**

ratio of 13:13' = 1:2 (83%)

15. Hatakeyama, S.; Matsumoto, H.; Fukuyama, H.; Mukugi, Y.; Irie, H. *J. Org. Chem.* **1997**, *62*, 2275–2279. For an example of the application of this method to prepare a chiral α-substituted alanine substructure by intramolecular opening of a 2-methyl-2,3-epoxy-1-trichloroacetimidate intermediate with $BF_3{\cdot}OEt_2$, see: Liu, D.-G.; Wang, B.; Lin, G.-Q. *J. Org. Chem.* **2000**, *65*, 9114-9119.

16. For a review of the Dess-Martin oxidation, see: Tohma, H.; Kita, Y. *Adv. Synth. Catal.* **2004**, *346*, 111-124.

17. Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696-5704.

18. Pihlaja, K.; Kleinpeter, E. In: *Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis*, VCH: New York 1994, pp. 55–56. For a recent example of the chemical shifts of the quaternary carbon of 5- and 6-membered ring acetonides, see: Ayad, T.; Génisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582.

Chapter 7

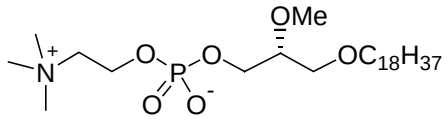
Synthesis of the *β-C*-Galactosyl Analogue of ET-18-OCH_3 from a Galactonolactone

Abstract

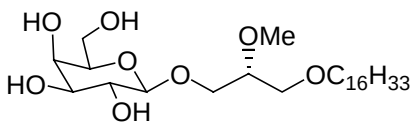
A short synthesis of a new β-*C*-glycoside analogue the of the antitumor ether lipid ET-18-OCH_3 is described. The key C-C bond forming reaction was accomplished by the addition of a chiral propargylic alcohol to 2,3,4,6-tetra-*O*-benzylgalacto-δ-lactone.

Introduction

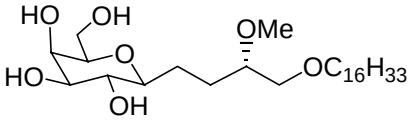
The antitumor ether lipid (AEL) 1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine (**1**, Figure 1), also known as ET-18-OCH_3, inhibits tumor cell growth by a multitude of processes, but does not interact directly with DNA and is this not mutagenic.[1] Many AELs have been synthesized and analyzed for antitumor activity, including glycosylated antitumor ether lipids in which the *sn*-3-phosphocholine residue of **1** has been replaced by a *O*- or *S*-glycoside, such as *O*-*α*- and *O*-*β*-D-glucosyl,[2a] *O*-β-2'-*O*-methyl-D-glucosyl,[2b] *O*-*α*-D-mannosyl,[2b] *O*-*β*-D-maltosyl,[3,4] *O*-*β*-D-lactosyl,[4] and *S*-*α*- and *S*-*β*-D-glucosyl.[2a] Some of these glycolipid conjugates displayed antiproliferative activities against epithelial[5] and mouse-derived[2] cancer cell lines that varied with the cell type.



1 ET-18-OCH$_3$



2



3

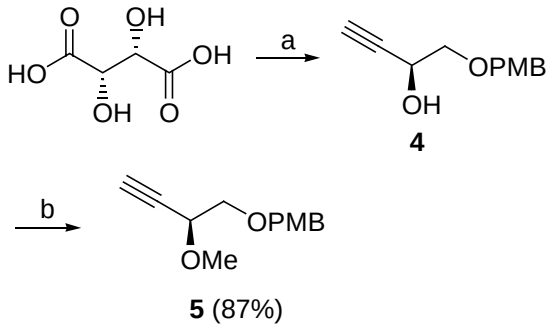
Figure 1. Structures of ET-18-OCH$_3$ (**1**) and its *O*- and *C*-β-D-galactopyranoside analogues **2** and **3**.

C-Glycoside analogues are resistant to degradation by glycosidases; therefore, these compounds may exhibit longer lifetimes in cells. A large number of *C*-glycoside analogues of biologically active *O*-glycosides have been synthesized.[6] Previous syntheses of isosteric *C*-glycoside analogues of ET-18-OCH$_3$, in which the glycoside oxygen has been replaced by a methylene group, have been reported with 2'-deoxy-2'-aminoglucose or 2'-deoxyglucose as the polar head group.[7] Both analogues have antiproliferative properties similar to those of the corresponding *O*-glycoside analogue.[7] In both syntheses the Ramberg-Bäcklund rearrangement was a key step, and 8 or 12 steps were required to complete the synthesis. This chapter describes a highly efficient and versatile synthesis of the *β-C*-glycoside (**3**) of the *β-O*-galactoside ET-18-OCH$_3$ analogue **2** in only 5 steps from the known propargylic alcohol **4**,[8] which may be obtained from D-tartaric acid.

Results and Discussion

As shown in Scheme 1, alkyne **5** was prepared from D-tartaric acid, which was converted to propargylic alcohol **4**.[8] The hydroxy group of **4** was converted to a methyl ether to afford alkyne **5**[9] in 87% yield.

Scheme 1. Synthesis of alkyne 5

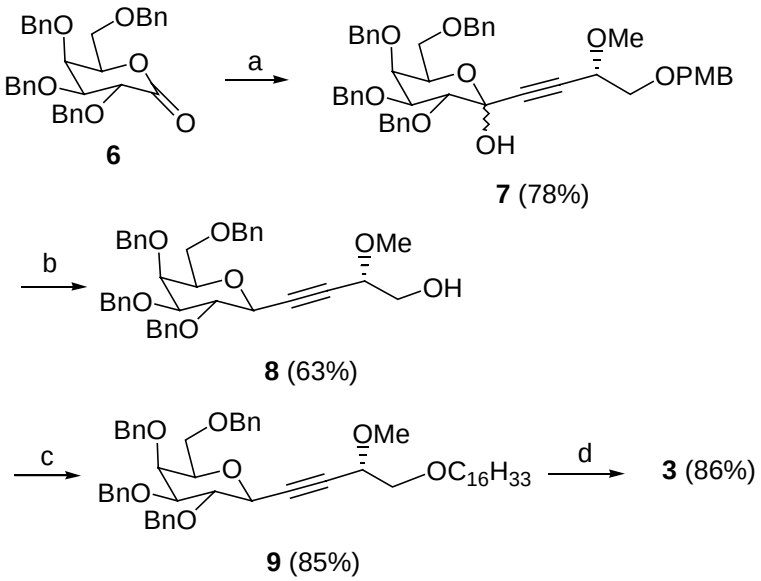


Reagents and conditions: (a) Ref. 7; (b) NaH, THF, MeI, (*n*-Bu)$_4$NBr, reflux.

Scheme 2 outlines the synthesis of the *β-C*-galactoside analogue of ET-18-OCH$_3$ (**3**) via the reaction of alkyne **5** with protected galactopyrano-δ-lactone **6**. The strategy for forming the *β-C*-glycosidic linkage is based on the aldose reduction process introduced independently by Gray[10] and Kishi[11] with Et$_3$SiH and a Lewis acid. Reaction of 2,3,4,6-tetra-*O*-benzylgalacto-δ-lactone (**6**)[12] with lithiated alkyne **5** furnished coupling product **7** as an α/*β* mixture. Reduction of hemiketal **7** with Et$_3$SiH in the presence of $BF_3 \cdot Et_2O$ and deprotection of the PMB group, followed by purification by column chromatography, afforded *β-C*-glycosidic alcohol **8**[13] in 71% overall yield. The long aliphatic chain was installed by treatment of alcohol **8** with hexadecyl bromide in the presence of NaH and (*n*-Bu)$_4$NBr, giving compound **9**[14] in 85% yield. Hydrogenation

of the triple bond and catalytic hydrogenolysis of the *O*-benzyl groups of compound **9** with Pearlman's catalyst afforded product **3**[15] in 86% yield.

Scheme 2. Synthesis of 3



Reagents and conditions: (a) *n*-BuLi, HMPA, **5**, THF; (b) Et_3SiH, $BF_3{\cdot}Et_2O$, -40 °C, CH_3CN/CH_2Cl_2; (c) NaH, $C_{16}H_{33}Br$, $(n\text{-}Bu)_4NBr$, THF, reflux; (d) H_2, $Pd(OH)_2/C$, MeOH.

In summary, a *β-C*-galactosyl analogue of ET-18-OCH_3 (**3**) was synthesized in only five steps from propargylic alcohol **4**, with an overall yield of 31%. The key step is the reaction of lithiated alkyne **5** with protected galactosyl lactone **6**, which was found to be an excellent glycosyl donor for the synthesis of **3**. Other *β-C*-saccharide conjugates can be made by modification of this methodology. For example, the C2-epimer of **3** can be prepared by using L-tartaric acid, and analogues of **3** with a different sugar head group

or long aliphatic chain can be prepared by using a different glycosyl lactone or alkyl bromide, respectively.

References

1. (a) Lohmeyer, M.; Bittman, R. *Drugs Future* **1994**, *19*, 1021–1037. (b) Houlihan, W. J.; Lohmeyer, M.; Workman, P.; Chen, S. H. *Med. Res. Rev.* **1995**, *15*, 157–223. (c) Bittman, R.; Arthur, G. Antitumor ether lipids: biological and biochemical effects. In: *Liposomes: Rational Design*, A. S. Janoff, Ed.; Marcel Dekker, New York, 1999, pp 125–144. (d) Gajate, C.; Mollinedo, F. *Curr. Drug Metab.* **2002**, *3*, 491-525. (e) Mollinedo, F.; Gajate, C.; Martin-Santamaria, S.; Gago, F. *Curr. Med. Chem.* **2004**, *11*, 3163-3184.

2. (a) Guivisdalsky, P. N.; Bittman, R.; Smith, Z.; Blank, M. L.; Snyder, F.; Howard, S.; Salari, H. *J. Med. Chem.* **1990**, *33*, 2614-2621. (b) Marino-Albernas, J. R.; Bittman, R.; Peters, A.; Mayhew, E. *J. Med. Chem.* **1996**, *39*, 3241-3247.

3. Bauer, F.; Ruess, K.-P.; Liefländer, M. *Liebigs Ann. Chem.* **1991**, 765-768.

4. Erukulla, R. K.; Bittman, R. *Synth. Commun.* **1994**, *24*, 2765-2770.

5. Lu, X.; Rengan, K.; Bittman, R.; Arthur, G. *Oncol. Rep.* **1994**, *1*, 933-936.

6. For reviews on the synthesis of *C*-glycosides, see: (a) Beau, J.-M.; Gallagher, T. *Top. Curr. Chem.* **1997**, *187*, 1-54. (b) Nicotra, F. *Top. Curr. Chem.* **1997**, *187*, 55-83. (c) Du, Y.; Linhardt, R. J.; Vlahov, I. R. *Tetrahedron* **1998**, *54*, 9913-9959.

7. (a) Yang, G.; Franck, R. W.; Bittman, R.; Samadder, P.; Arthur, G. *Org. Lett.* **2001**, *3*, 197-200. (b) Yang, G.; Franck, R. W.; Byun, H.-S.; Bittman, R.; Samadder, P.; Arthur, G. *Org. Lett.* **1999**, *1*, 2149-2151.

8. Yadav, J. S.; Chander, M. C.; Joshi, B. V. *Tetrahedron Lett.* **1988**, *29*, 2737-2740.

9. Data for (*S*)-1-*O*-(4'-methoxybenzyl)-2-*O*-methylbut-3-yn-1,2-diol (**5**): R_f 0.50 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 2.48 (d, 1H, *J* = 2.4 Hz), 3.48 (s, 3H), 3.63 (m, 2H), 3.82 (s, 3H), 4.20 (m, 1H), 4.53 (d, 1H, *J* = 12.0 Hz), 4.59 (d, 1H, *J* = 12.0 Hz), 6.90 (m, 2H), 7.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 55.3, 56.8, 70.7, 71.7, 73.1, 74.9, 80.0, 113.7, 129.6, 129.9, 159.2.

10. Rolf, D.; Gray, G. R. *J. Am. Chem. Soc.* **1982**, *104*, 3539-3541.

11. Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976-4978.

12. Overkleeft, H. S.; van Wiltenburg, J.; Pandit, U. K. *Tetrahedron* **1994**, *50*, 4215–4224.

13. Preparation of (*S*)-4-(2',3',4',6'-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-2-methoxybut-3-yn-1-ol (**8**): To a solution of **7** (1.52 g, 2.0 mmol) in 20 mL of MeCN/CH_2Cl_2 (1:1) at –40 C was added 3.4 mL (21.4 mmol) of Et_3SiH, followed by 2.67 mL (21.4 mmol) of $BF_3{\cdot}Et_2O$. After the reaction mixture was stirred for 6 h, solid $NaHCO_3$ (1.68 g, 20 mmol) and saturated aqueous $NaHCO_3$ solution (20 mL) were added. The organic layer was extracted with CH_2Cl_2 (3 x 10 mL), the combined organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated. Purification of the residue by chromatography (hexane/EtOAc 3:1) gave 834 mg (63%) of **8** as a colorless liquid; $[\alpha]^{25}_D$ +25.7° (*c* 1.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.81 (m, 1H), 3.52 (s, 3H), 3.67 (m, 4H), 3.80 (t, 2H, *J* = 5.6 Hz), 4.06 (d, 1H, *J* = 2.4 Hz), 4.13 (t, 1H, *J* = 9.6 Hz), 4.20 (m, 2H), 4.53 (dd, 2H, *J* = 8.0, 26.0 Hz), 4.74 (d, 1H, *J* = 10.8 Hz), 4.83 (s, 2H), 5.04 (m, 3H); 7.37-7.50 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 57.0, 65.1, 68.7, 70.3, 72.4,

72.8, 73.7, 73.8, 74.8, 75.9, 77.6, 79.0, 81.4, 83.5, 84.7, 127.0, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 129.0, 137.9, 138.3, 138.4, 138.6.

14. Data for (*S*)-1-(2',3',4',6'-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-3-methoxy-4-(hexadecyloxy)but-1-yne (**9**): R_f 0.54 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +20.4° (*c* 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.26-1.28 (m, 26H), 1.54 (m, 2H), 3.42 (m, 2H), 3.45 (s, 3H), 3.69 (m, 6H), 3.95 (d, 1H, *J* = 2.4 Hz), 4.99 (m, 1H), 4.07 (m, 1H), 4.24 (m, 1H), 4.42 (dd, 2H, *J* = 11.6, 24.0 Hz), 4.62 (d, 1H, *J* = 11.6 Hz), 4.63 (s, 2H), 4.87 (d, 1H, *J* = 11.6 Hz), 4.97 (m, 2H), 7.26-7.40 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.1, 26.6, 29.5, 29.6, 29.7, 29.8, 32.0, 57.0, 68.6, 70.3, 71.0, 71.8, 72.8, 73.1, 73.6, 73.7, 74.7, 75.8, 77.4, 79.0, 81.7, 83.4, 84.2, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 137.8, 138.3, 138.4, 138.6.

15. Data for (*S*)-1-(*β*-D-galactopyranosyl)-3-methoxy-4-(hexadecyloxy)butane (**3**): R_f 0.78 ($CHCl_3$/MeOH 9:1); $[\alpha]^{25}_D$ +43.4° (*c* 1.60, $CHCl_3$/MeOH 9:1); ^{1}H NMR ($CDCl_3$/CD_3OD 9:1) δ 0.82 (m, 3H), 1.13-1.21 (m, 24H), 1.33 (m, 2H), 1.43 (m, 4H), 1.75 (m, 2H), 3.01 (m, 2H), 3.30-4.00 (m, 11H); ^{13}C NMR ($CDCl_3$/CD_3OD 9:1) δ 14.1, 22.7, 27.8, 29.4, 29.6, 29.7, 29.8, 31.4, 32.0, 57.8, 61.8, 70.4, 71.5, 71.9, 73.1, 75.2, 80.3. MS (APCI): *m/z* 525.3 ($M+^{35}Cl^-$).

Chapter 8

An Efficient Synthesis of a *C*-Glycoside Analogue of the α-Galactosylceramide KRN7000.

Abstract

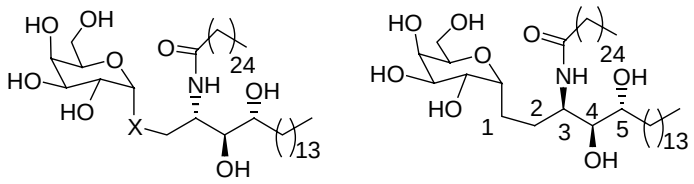
The first synthesis of a new α-*C*-galactosylceramide analogue tentatively assighed to bear a D-*arabino*-(3*R*,4*S*,5*R*)-phytosphingosine backbone (compound **2**) is described. The method has only 11 steps (or 13 steps with the final acylation and deacylation) in the longest pathway and an overall yield of 8.8%, and can be applied to the preparation of other analogues, especially those with different sphingosine chain lengths and different sugar heads. An unexpected retention of configuration during ring opening of a glycosyl epoxide with NaN_3/NH_4Cl at reflux is proposed to take place via a double inversion mechanism. The configuration at C(3) of the α-*C*-galactosylceramide appears to play an important role in the in vitro bioactivity.

Introduction

The glycolipid called KRN7000 (Figure 1), which was isolated from a marine sponge and is distributed to researchers by Kirin Pharmaceuticals,[1] has a 18-carbon D-*ribo*-phytosphingosine (PHS) backbone, a 26-carbon amide chain, and an α-galactosyl head group. KRN7000 binds tightly to human and mouse CD1d and specifically stimulates a subset of T cells called natural killer T (NKT) cells. NKT cells are potent producers of several immunoregulatory cytokines and are thought to contribute to

immune responses in a variety of diseases including infection, cancer, autoimmunity, and atherosclerosis.[2] *C*-Glycoside analogues of biologically active *O*-glycosides are, in general, of considerable interest, since transformation of the anomeric carbon into a hydrolytically stable ether makes *C*-glycosides resistant to degradation by glycosidases. Thus, *C*-glycosides may exhibit longer lifetimes in cells. Surprisingly, the cellular responses elicited by *O*- vs. *C*-glycosides of α-galactosylceramide differ distinctly with respect to the cytokine profiles they induce.[3] Isosteric *C*-glycoside analogue **1** has been shown to possess a 1000-fold higher activity than the natural agonist of NKT cells (KRN7000) in a mouse malaria model and a 100-fold higher activity in a mouse melanoma model.[4]

Figure 1. Structures of KRN7000 and its α-*C*-galacosyl analogues **1** and **2**



KRN7000: X = O **2**: *N*-epimer (3*R*,4*S*,5*R*) of analogue **1**

1: X = CH_2

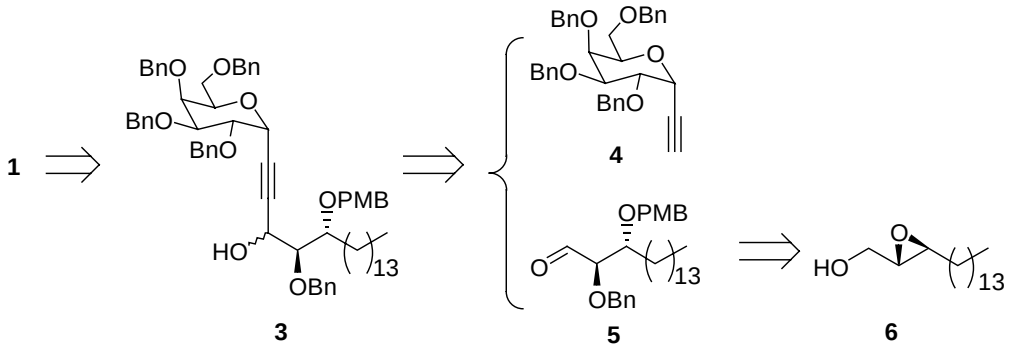
There have been several reports on the synthesis of **1** to date. One synthetic method utilized the Ramberg–Bäcklund reaction followed by β-selective hydrogen transfer from a diisopropylsilyl protecting group.[4] Other approaches involved an olefin cross-metathesis for the installation of the *C*-glycoside linkage,[5] a Wittig reaction of a protected lipid aldehyde with a protected sugar followed by deprotection;[6] and addition of a lithiated *C*-glycoside alkyne to an aldehyde derived from arabinose.[7] The first two syntheses[3,4] used commercially available D-*ribo*-PHS as the starting material, and thus

variations of the lipid chain length and hydroxy group positions in the sphingosine base are precluded. The third synthesis[5] has a very low degree of stereoselectivity in the key step. In an attempt to develop a new route to α-*C*-galactosylceramide **1**, we serendipitously accomplished the first synthesis of a compound that we tentatively assign as its *N*-epimer **2**, which has a D-*arabino*-PHS backbone. An unusual double inversion of configuration is proposed to give rise to compound **2**. The short asymmetric synthesis of analogue **2** we describe here is versatile, permitting a variety of α-*C*-glycoside analogues to be prepared with different sugar head groups and sphingosine chain length or degree of unsaturation, and with different hydroxy-group substitution patterns.

Results and Discussion

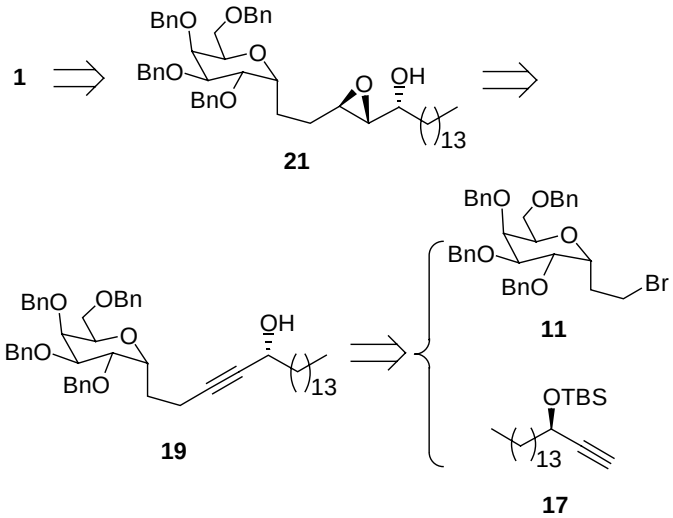
A retrosynthetic analysis of our first attempt to prepare *C*-KRN7000 (**1**) is illustrated in Scheme 1. We envisaged that addition of alkyne **4**[8] to aldehyde **5**, which can be prepared from epoxide **6** in 4 steps,[9] would give compound **3**. Subsequently, the last chiral center would be installed by an oxidation-reduction procedure.[10] The resulting (*R*)-hydroxy group would be converted to an (*S*)-azido group, then to an *N*-acyl group, followed by reduction of the alkyne to give final product **1**. Unfortunately, the attempted addition of the lithium salt of acetylene **4** to aldehyde **5** in THF/HMPA at -78 ºC did not afford compound **3** but instead yielded an elimination product, α,β-unsaturated aldehyde **7**.[11]

Scheme 1. Retrosynthetic plan of the first attempt to prepare 1



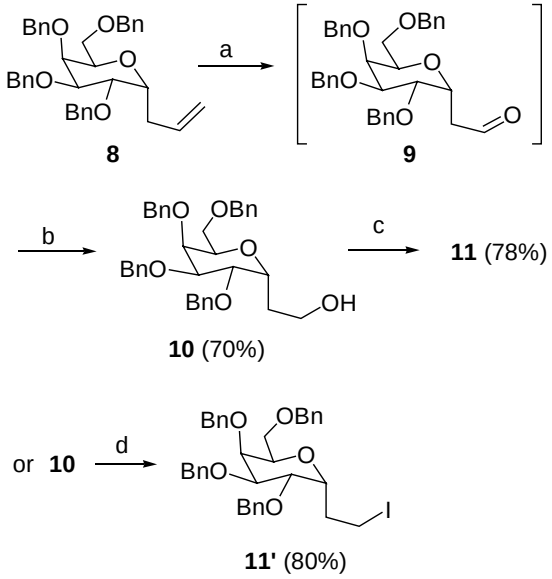
Another approach to **1** is illustrated in Scheme 2. Bromide **11** and TBS-protected propargylic alcohol **17** would be used as the starting materials, with the long-chain protected propargylic alcohol functioning as a nucleophile. After S_N2 substitution, the TBS group would be removed, and the resulting propargylic alcohol **19** would be reduced to an allylic alcohol (**20**), which on asymmetric Sharpless epoxidation gave epoxy alcohol **21**. Chelation-controlled opening of the epoxide with NaN_3/NH_4Cl would give an azido diol, which would be reduced and deprotected simultaneously, followed by *N*-acylation, to give **1**.

Scheme 2. Retrosynthetic analysis



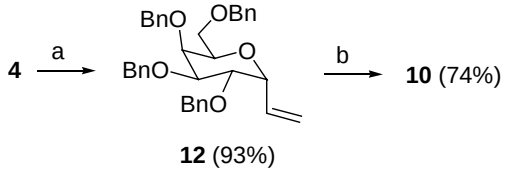
Synthesis of Bromide 11. Two methods were used to prepare bromide **11**. As illustrated in Scheme 3, one approach to alcohol **10** started with alkene **8**, which was available by deacetylation and benzylation of *C*-allyl tetra-*O*-acetylglycosides.[12] Alkene **8** was oxidatively cleaved using catalytic osmium tetroxide and sodium periodate to cleanly provide the corresponding aldehyde, which was immediately reduced using $NaBH_4$ in methanol to give the corresponding alcohol **10**.[13] Primary alcohol **10** was converted to the corresponding bromide **11** (CBr_4, PPh_3) or iodide **11'** (PPh_3, I_2). The other method (Scheme 4) started with acetylene **4**, which was reduced to alkene **12** with Lindlar catalyst in 93% yield.[5] Hydroboration of alkene **12** with $BH_3.SMe_2$, followed by oxidation with H_2O_2, gave alcohol **10** in 74% yield.

Scheme 3. Synthesis of compounds 11 and 11'



Reagents and conditions: (a) OsO_4, $NaIO_4$; (b) $NaBH_4$, MeOH; (c) CBr_4, PPh_3, CH_2Cl_2; (d) I_2, PPh_3, imidazole, CH_2Cl_2/CH_3CN.

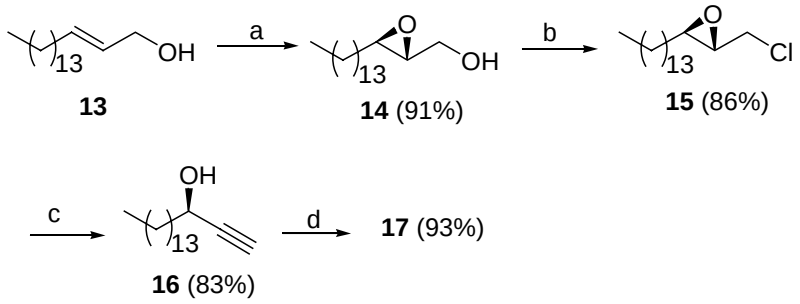
Scheme 4. Another route to compound 10



Reagents and conditions: (a) Lindlar catalyst, H_2, MeOH; (b) (i) $BH_3 \cdot SMe_2$, THF; (ii) H_2O_2, NaOH.

Synthesis of Alcohol 17. Alcohol **13** has been prepared by alkylation of a propargylic alcohol followed by reduction with $LiAlH_4$[14] or by Knoevenagel condensation of an aldehyde with ethyl hydrogen malonate and reduction of the resulting (*E*)-α,β-unsaturated ester with DIBAL.[15] As illustrated in Scheme 5, asymmetric epoxidation of **13** gave epoxy alcohol **14** in 91% yield.[16] After **14** was converted to chloride **15** (Ph_3P, NCS, 86% yield), propargylic alcohol **16** was obtained in 83% yield by treatment of **15** with excess *n*-BuLi and HMPA in THF at low temperature.[17] TBS protection gave alkyne **17** in 93% yield.

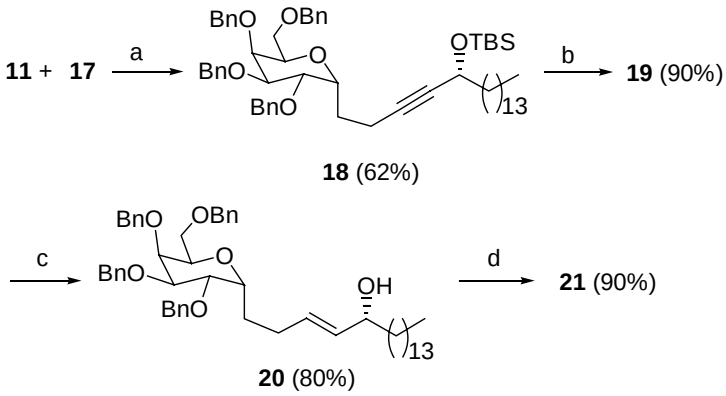
Scheme 5. Synthesis of TBS-protected propargylic alcohol 17



Reagents and conditions: (a) (-)-DIPT, cumene hydroperoxide, Ti(OPr-*i*)$_4$, CH_2Cl_2, molecular sieves, -20 °C; (b) NCS, PPh_3, CH_2Cl_2; (c) *n*-BuLi, HMPA, THF, -78 °C; (d) TBSCl, imidazole, CH_2Cl_2.

Synthesis of Azido Diol 22. The reaction of dilithiated propargylic alcohol **16** with **11** or **11'** in THF/HMPA failed to afford product **19**; the only product was alkene **12**. On the other hand, S_N2 reaction of TBS ether **17** with α-bromoethyl *C*-galactoside **11** gave compound **18** in 62% yield (Scheme 6). When α-iodoethyl *C*-galactoside **11'** was used, elimination was again the main reaction, with alkene **12** being formed in 52% yield. Deprotection of the TBS ether with TBAF gave propargylic alcohol **19**, which was reduced to allylic alcohol **20** with Red-Al. Sharpless epoxidation of allylic alcohol **20** provided epoxy alcohol **21** as the only product in 90% yield.

Scheme 6. Synthesis of epoxide 21



Reagents and conditions: (a) *n*-BuLi, HMPA, THF, -78 °C ; (b) TBAF, THF, rt; (c) Red-Al, Et_2O, rt; (d) (-)-DIPT, cumene hydroperoxide, $Ti(OPr\text{-}i)_4$, CH_2Cl_2, molecular sieves, -20 °C.

Confirmation of the Structure of Compound 22. As shown in Scheme 7, chelation-controlled opening of **21** with excess NaN_3/NH_4Cl[18] in aqueous MeOH (reflux, two days) gave **22** as the only product in 65% yield, with 28% of unreacted starting material **21** recovered. Et_2AlN_3[19] and $Ti(O\text{-}Pr\text{-}i)_2(N_3)_2$[20] failed to open epoxy alcohol **21**. The functional group sequence of azido diol **22** was confirmed by ^{13}C-NMR analysis of acetonide **23**, which was prepared by treatment of **22** with 2,2'-dimethoxypropane

(Scheme 7). The quaternary ketal carbon signal at δ 108.4 ppm, which is characteristic of a five-membered acetonide,[21] unambiguously indicated the 1,2 relationship of the two hydroxy groups.

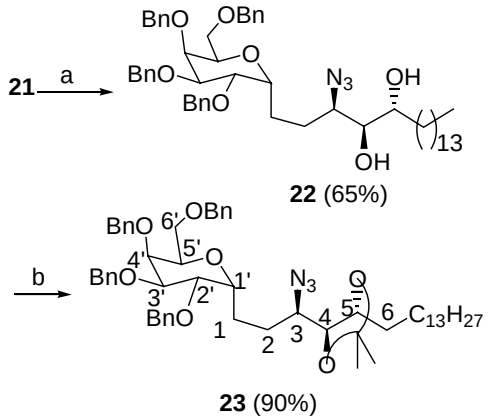
However, after diol **19** was converted to final product **2**, we unexpectedly found that the ^{1}H and ^{13}C NMR spectra differed from those previously reported for *C*-KRN7000 (**1**).[4] Therefore, we carried out the following detailed NMR analysis in an attempt to elucidate the structure of compound **2**.

First, we compared the 500-MHz ^{1}H NMR spectrum of **2** (2 mg in C_5D_5N) with the spectrum of the *N*-epimer of compound **1** prepared by Dr. Jun Pu in the laboratory of Professor Richard W. Franck (Hunter College of CUNY). The two spectra are strikingly similar.

Next, the proton and carbon chemical shifts of the methylene groups of the sugar ring were identified in a DEPT-135 ^{13}C spectrum (Figure 2) and a ^{13}C-^{1}H heterocorrelation spectrum (Figure 2). In the DEPT-135 spectrum, these carbons appear with negative phase in the region δ 60 - 80 ppm. Based on this chemical shift, the methylene protons at 3.62 and 3.80 ppm, which correlates to carbon at δ 67.6 ppm, is assigned to the protons and the carbon at the 6' position. The unambiguous assignment of the H-6' protons allowed us to assign the remaining protons of the sugar ring, from H-5' to H-1', and the side chain protons, H-1 to H-6, by following the connectivity through the bonds in the COSY spectrum (Figure 2). The finding that the H-3 resonance is at δ 3.38 ppm and the C-3 resonance is at δ 60.9 ppm, which are upfield from the resonances of H-4, H-5, C-4, and C-5, may be taken to suggest that the azido group is at the C3 position. This suggests that a Payne rearrangement did not occur during the ring-opening reaction

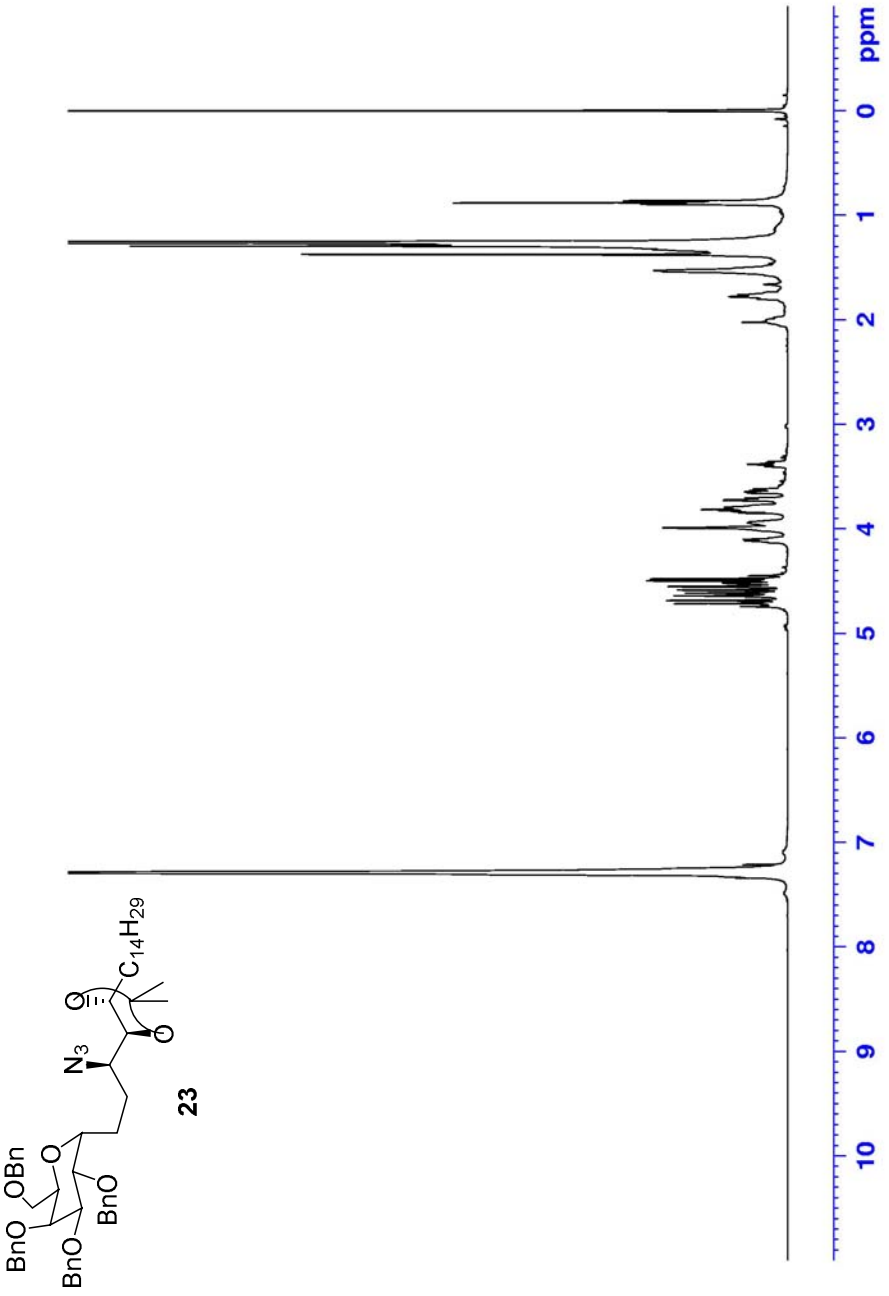
of epoxy alcohol **21** with azide ion. The anti ketal geometry for compound **23** predicts an NOE between the H-6 and H-3 protons; however, this NOE could not be uniquely identified in Figure 2 because there was an overlap of the H-6 protons with one of the H-2 protons.

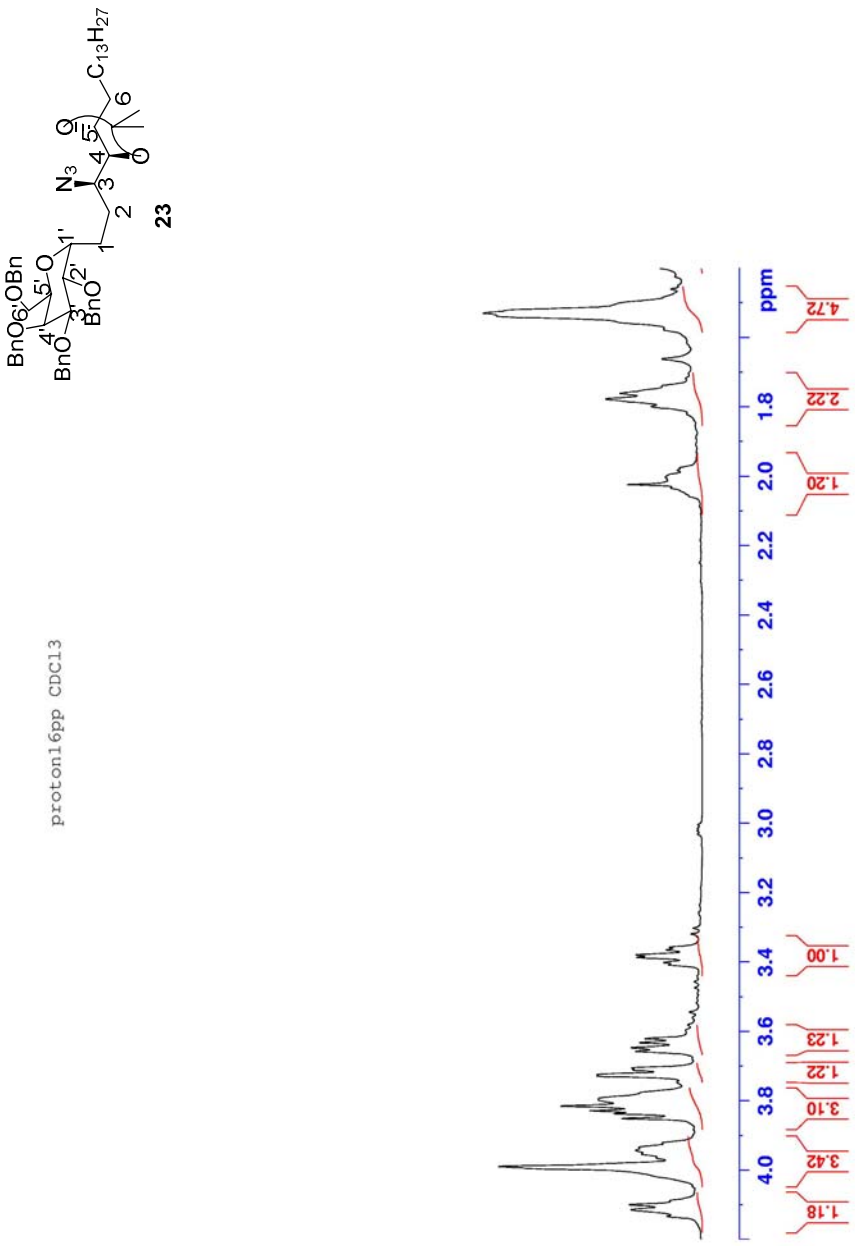
Scheme 7. Acetonide formation



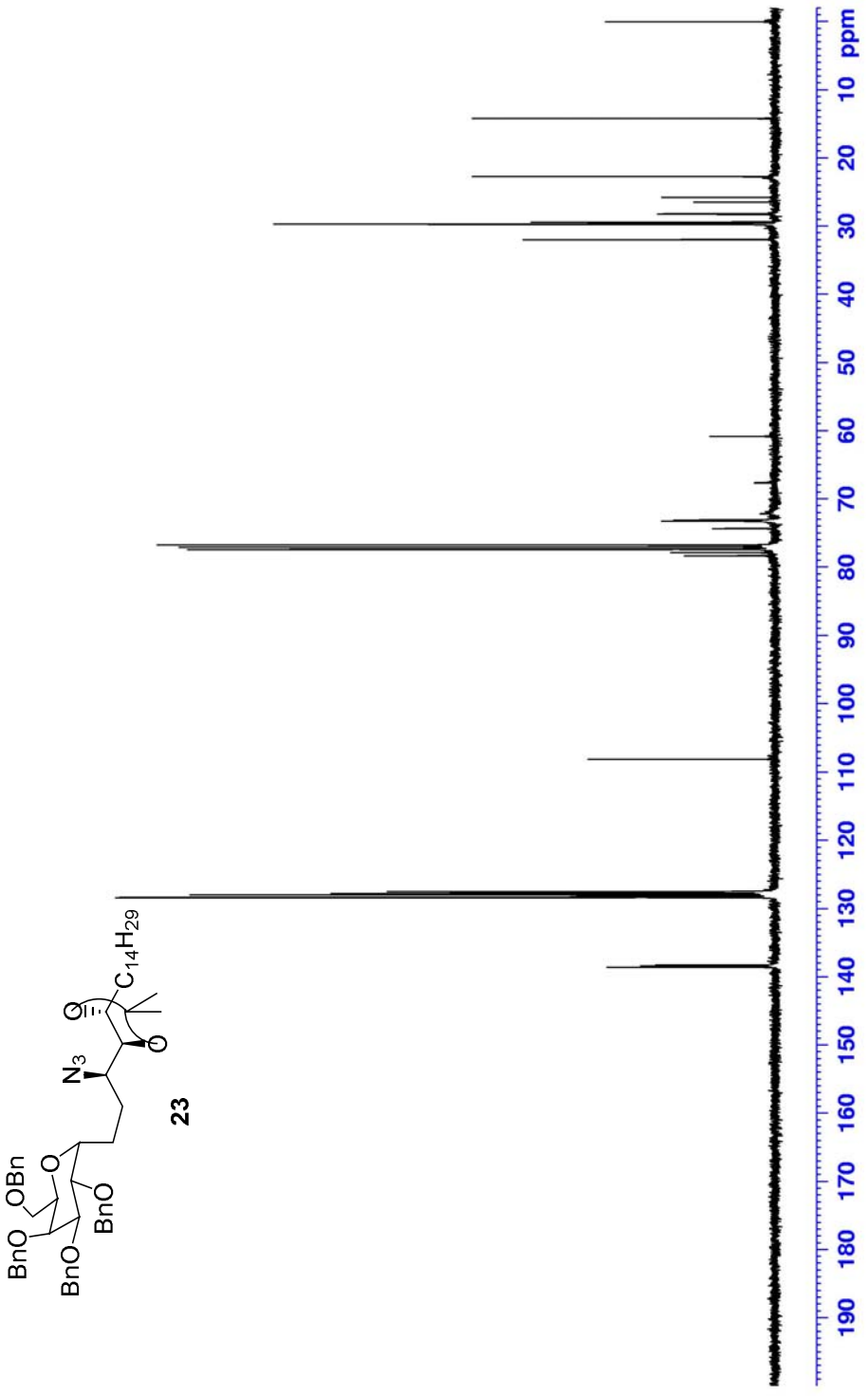
Reagents and conditions: (a) NaN_3, NH_4Cl, $MeOH/H_2O$ (8:1), reflux; (b) 2,2'-dimethoxypropane, CSA (cat.), THF, reflux.

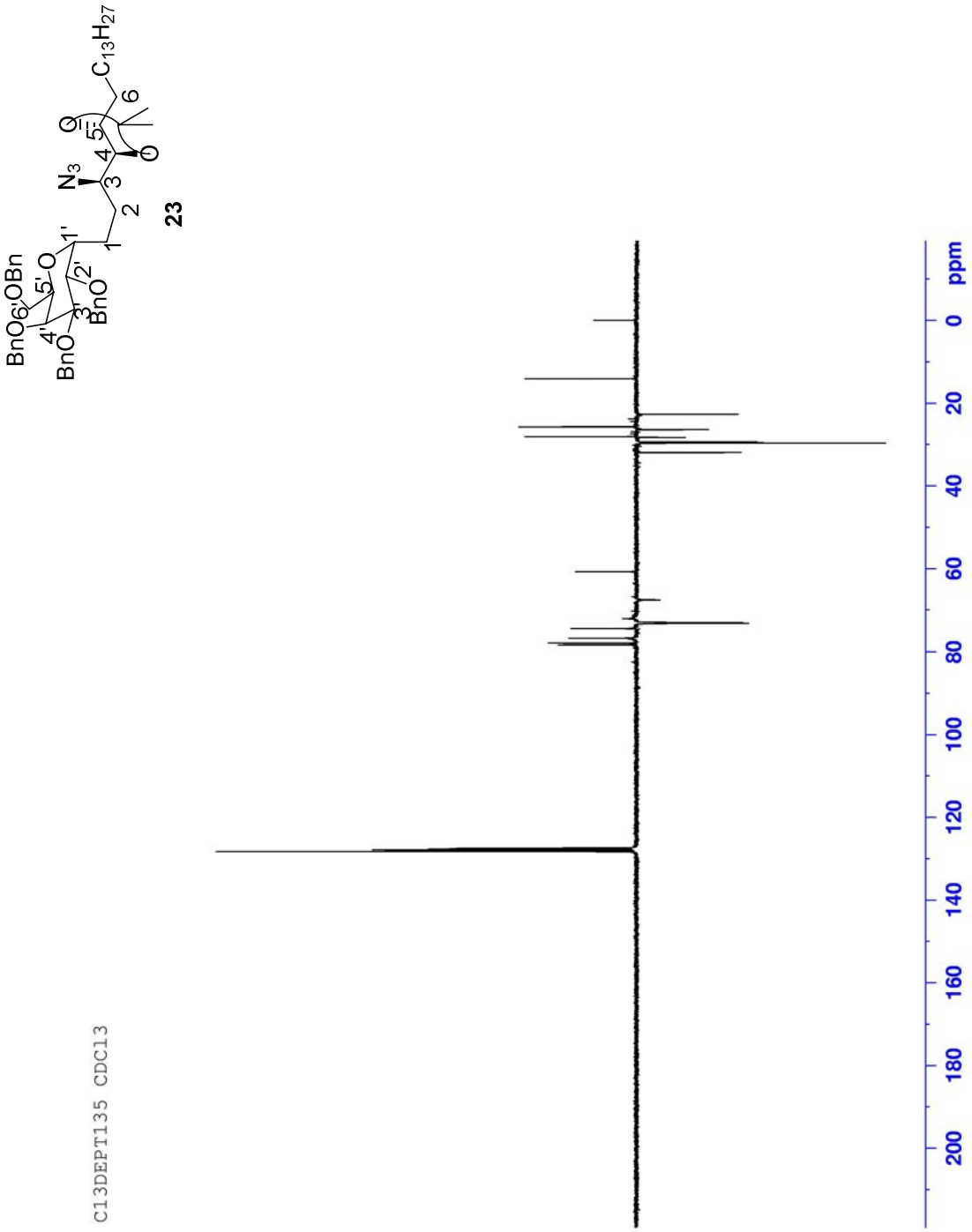
Figure 2. ^{1}H, ^{13}C, ^{13}C DEPT135, HCCOSW, COSY45SW, and NOESY NMR spectra for compound **23**.

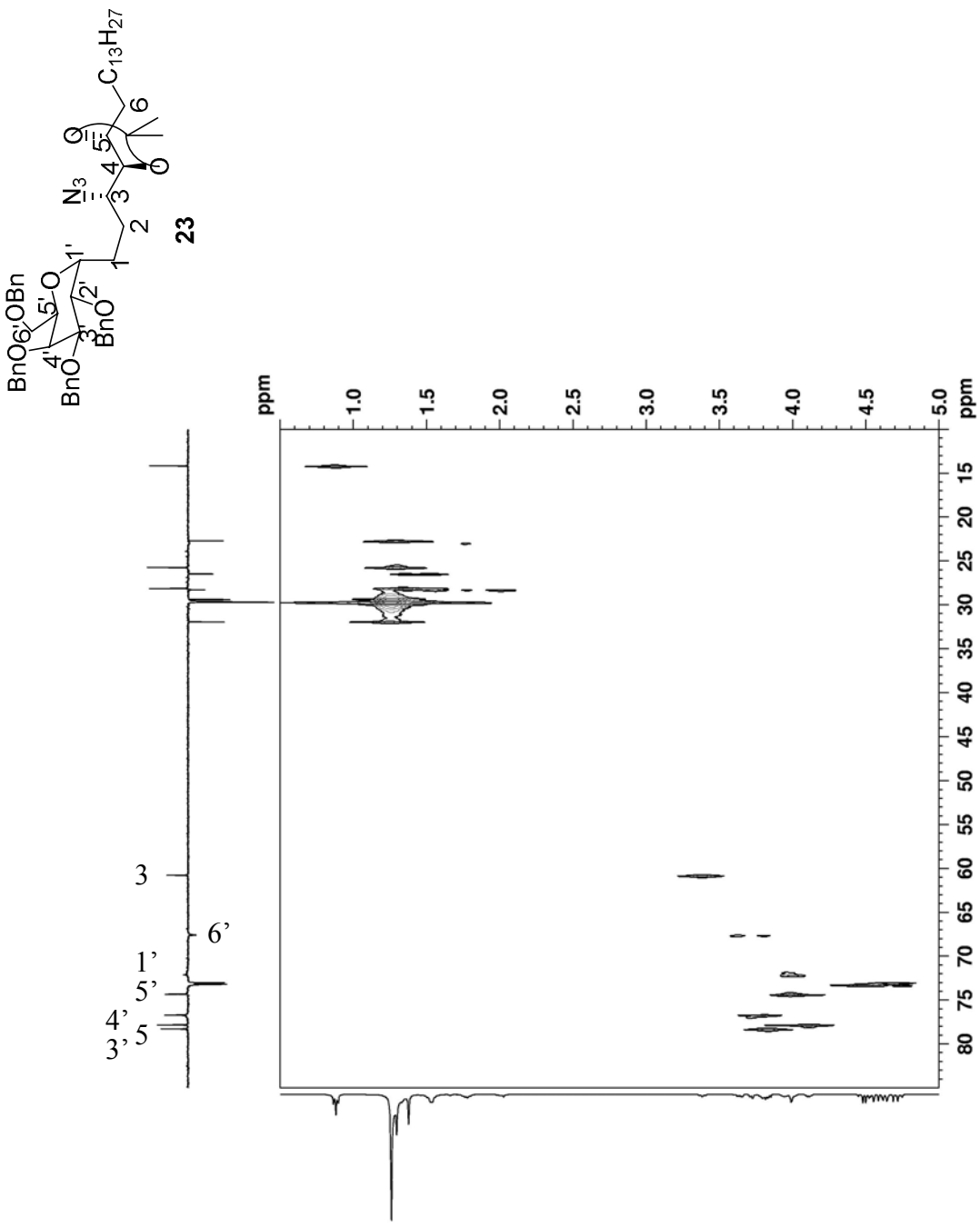


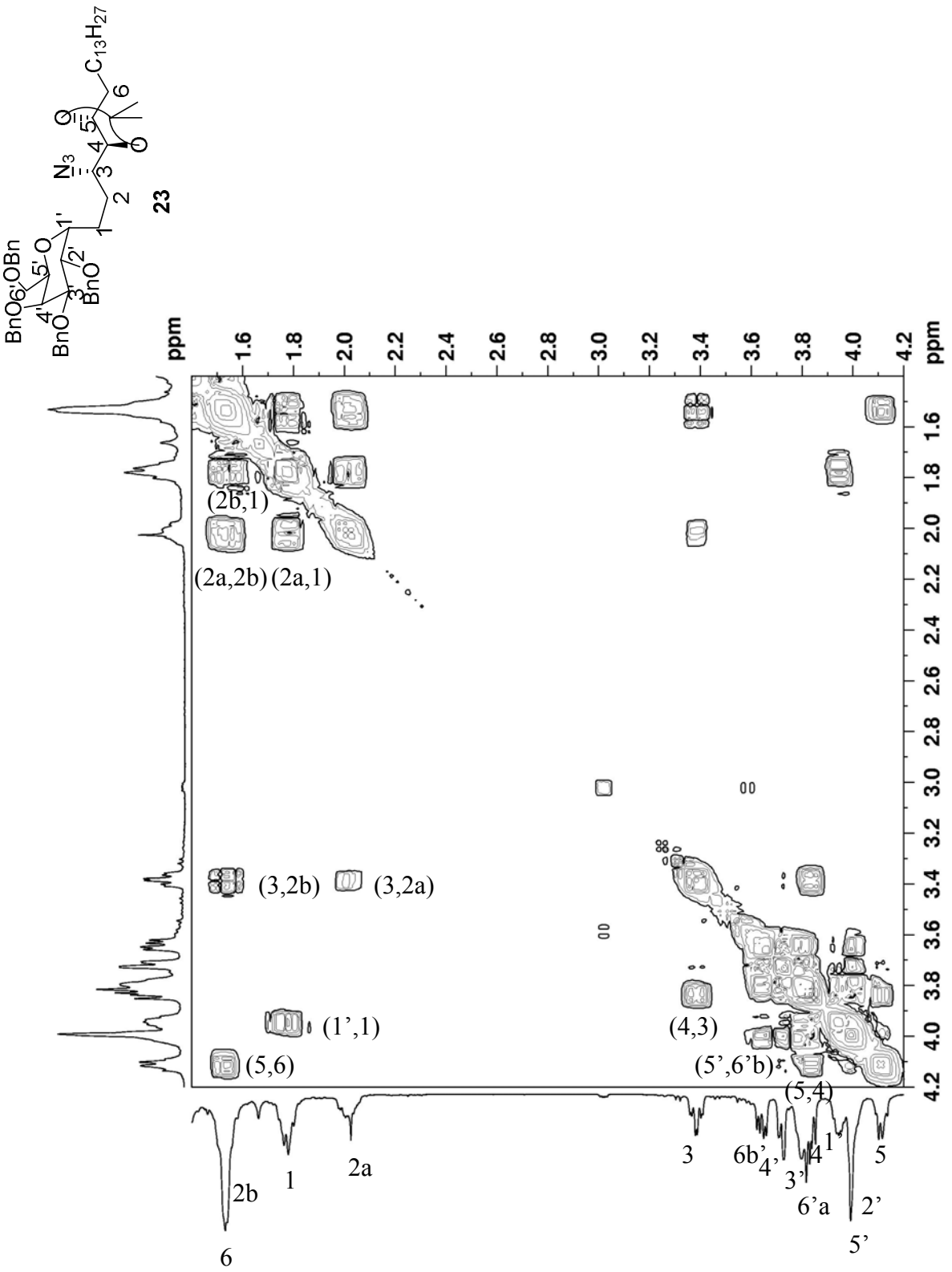


Assignments of the Protons in Compound **23**. H6': 3.62 and 3.80 ppm, H5': 3.99 ppm, H4': 3.73 ppm, H3': 3.82 ppm, H2': 3.99 ppm; H1': 3.90 ppm, H1: 1.78 ppm, H2: 1.51 and 2.01 ppm, H3: 3.38 ppm, H4: 3.84 ppm, H5: 4.11 ppm, H6: 1.53 ppm.

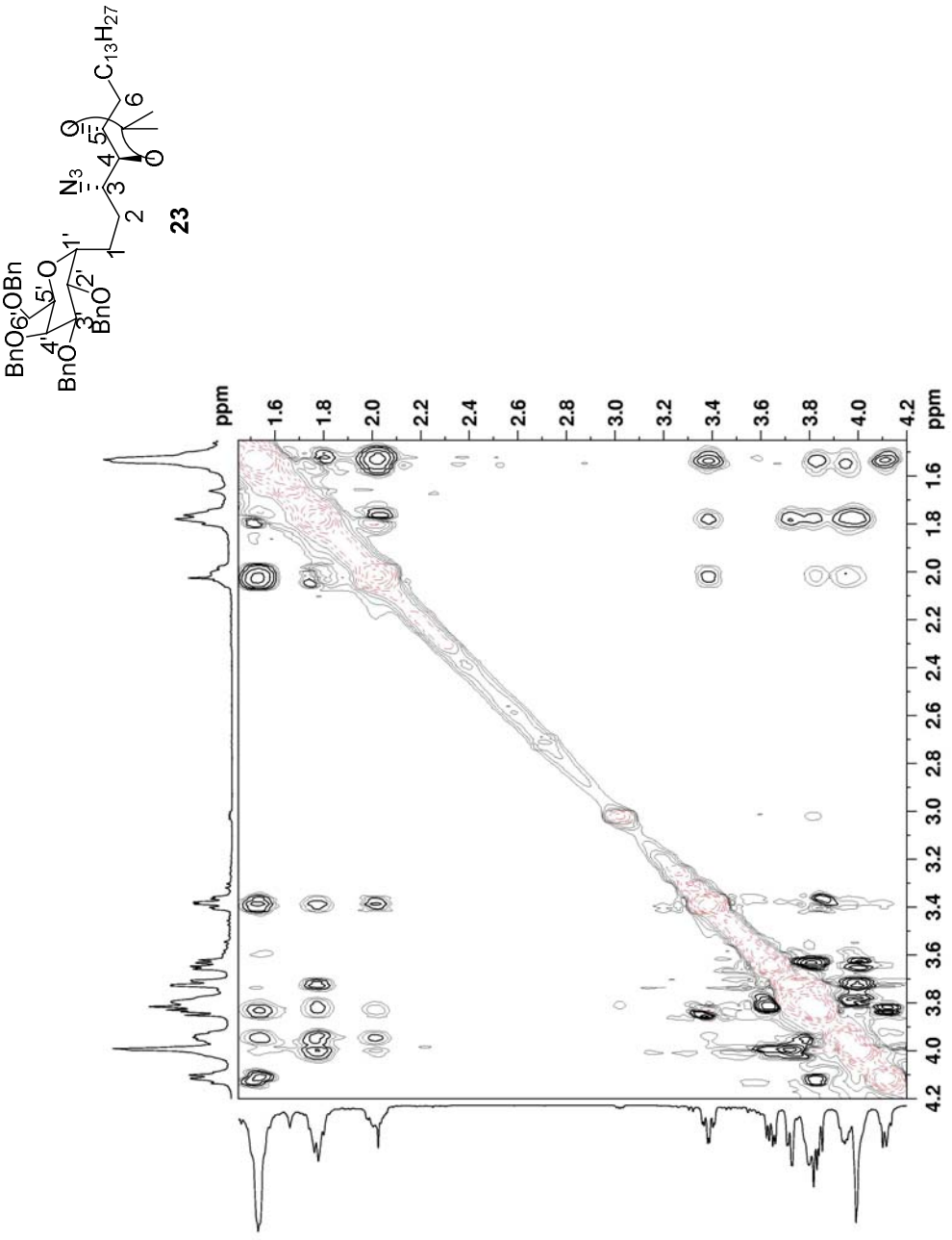

BnO
OBn
O
BnO
BnO
N3
O
O
C14H29
23
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


BnO
6'
OBn
5'
O
4'
BnO
3'
2'
BnO
1'
1
2
3
N3
4
5
O
O
6
C13H27
23
C13DEPT135 CDCl3
ppm
0
20
40
60
80
100
120
140
160
180
200


BnOBnO
OBn
5'
O
4'
3'
2'
1'
BnO
N3
1
2
3
4
5
O
O
6
C13H27
23
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
ppm
15
20
25
30
35
40
45
50
55
60
65
70
75
80
3
6'
1'
5'
4'
3'
5


BnO6'OBn
5'-O
4'
BnO
3'
2'
BnO
1'
1
2
3
N3
4
5
O
6
C13H27
23
ppm
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
(2b,1)
(2a,2b)
(2a,1)
(3,2b)
(3,2a)
(1',1)
(5,6)
(4,3)
(5',6'b)
(5,4)
6
2b
1
2a
3
6b'
4'
3'
6'a
4
1'
2'
5'
5

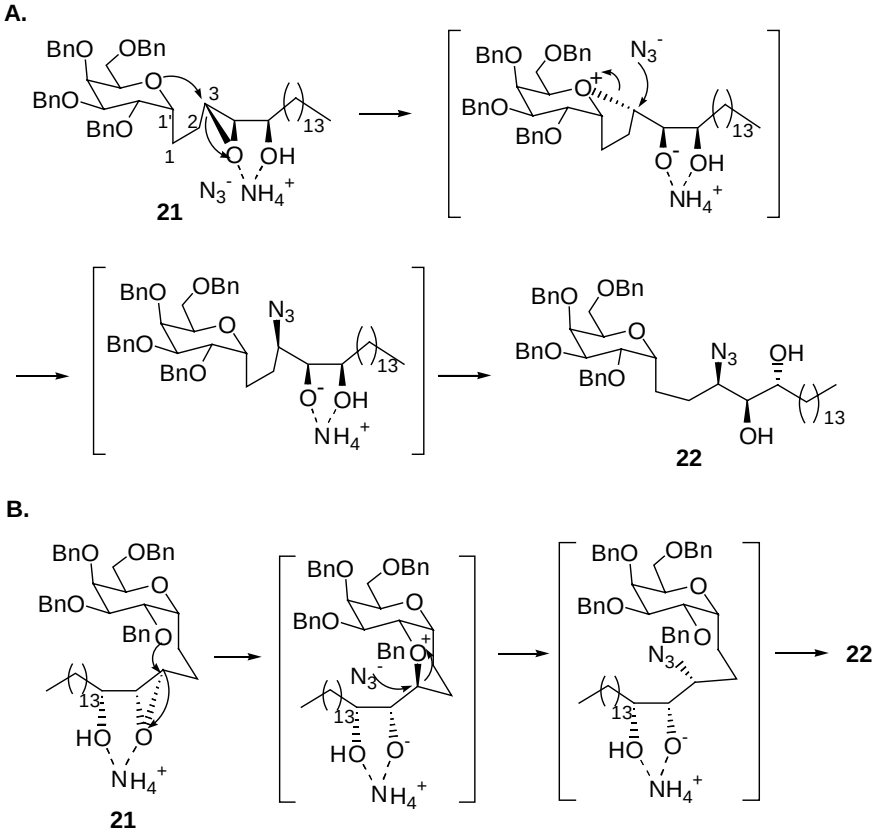
COSY correlation: (H6', H5'): (3.62, 3.99 ppm) and (3.80, 3.99 ppm); (H5', H4'): (3.99, 3.73 ppm); (H4', H3'): (3.73, 3.82 ppm); (H3', H2'): (3.82, 3.99 ppm); (H2', H1'): (3.99, 3.90 ppm); (H1', H1): (3.90, 1.78 ppm); (H1, H2): (1.78, 1.51 ppm) and (1.78, 2.01 ppm); (H2, H3): (1.51, 3.38 ppm) and (2.01, 3.38 ppm); (H3, H4): (3.38, 3.84 ppm); (H4, H5): (3.84, 4.11 ppm); (H5, H6): (4.11, 1.53 ppm).



This finding prompted us to consider other possible structures of product **2**.[22] The ^{1}H NMR spectrum of product **2** is very similar to that of the *N*-3 epimer of an analogue of **1** bearing a truncated long-chain base.[7]

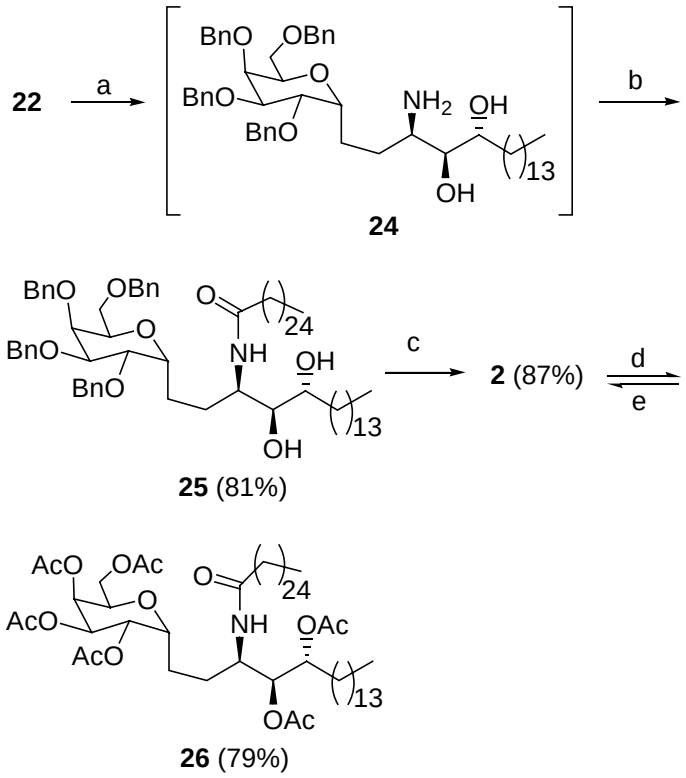
Since a Payne rearrangement appears unlikely on the basis of a COSY experiment, we tentatively conclude that *N*-epimer **2** was formed. Two possible mechanisms for the retention of configuration during the conversion of **21** to **22**, which can explain the high regioselectivity of the epoxide opening reaction, are depicted in Figure 3. We postulate that a double inversion of configuration takes place in which the pyranose oxygen (mechanism A) or the 2'-*O*-benzyl oxygen (mechanism B) first attacks C-3 of **21** in the presence of excess NH_4Cl in aqueous methanol at reflux; in the second step, azide ion attacks the intermediate. The precedent for pyranose oxygen participation in intramolecular reactions to form an oxonium ion intermediate was demonstrated about twenty years ago;[23] however, the second step in such reactions is different than the azide opening reaction we have utilized. Examples of an intramolecular attack of the 2'-*O*-benzyl oxygen of an α-*C*-glycoside on an iodonium ion[24] or a α-iodoethyl group[13] to form a bicyclic product are also known.

Figure 3. Proposed mechanisms of the double inversion



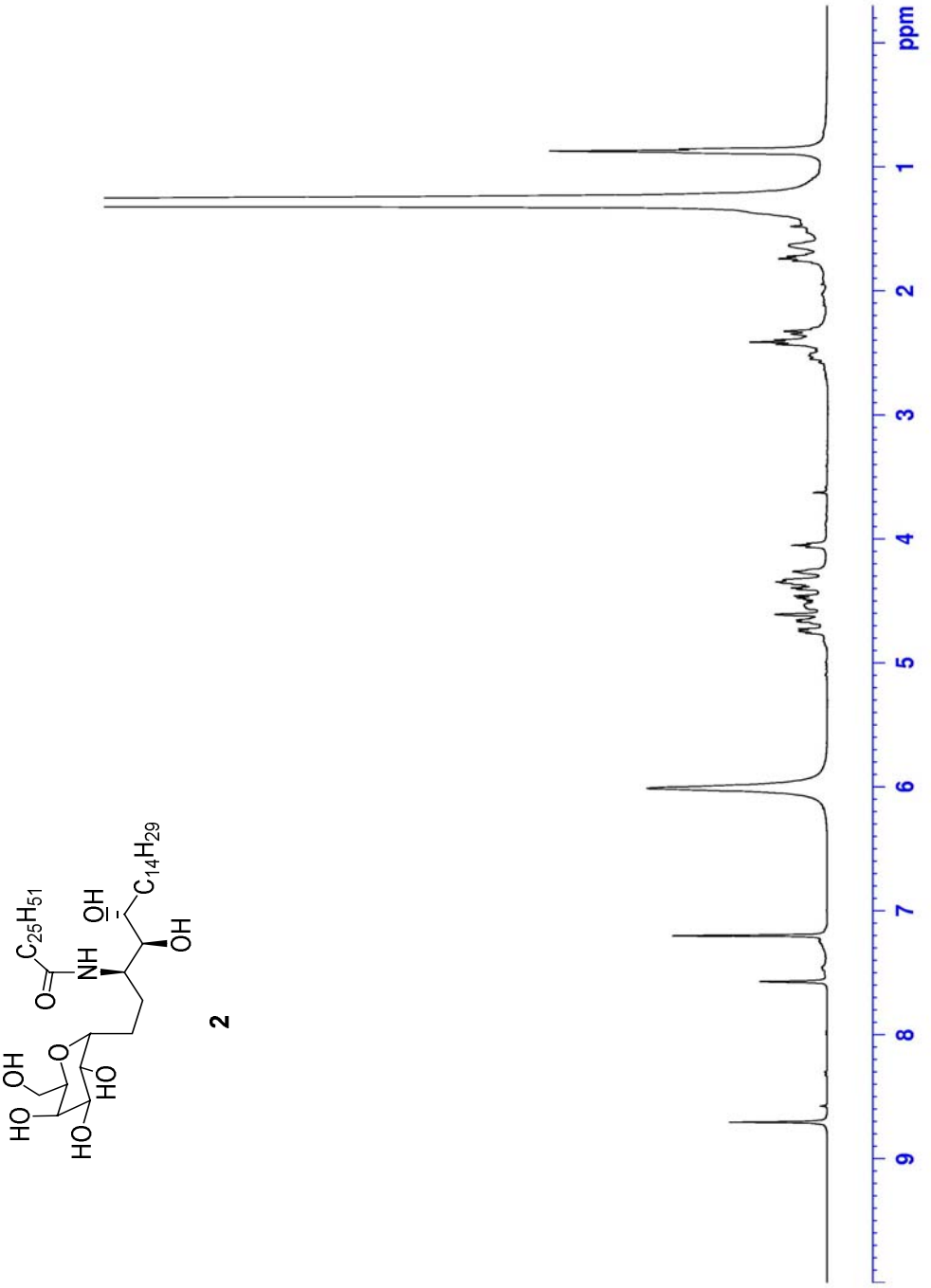
Conversion of 22 to Product 2. The completion of the synthesis of product **2** is illustrated in Scheme 8. Azido diol **22** was converted to amide **25** by reduction of the azido group to an amino group (PPh_3, THF/H_2O), followed by *N*-acylation with *p*-nitrophenyl hexacosanoate (80% for the two steps). Deprotection of the benzyl groups by hydrogenolysis in the presence of Pearlman's catalyst gave final product **2**. However, the yield was dramatically decreased on chromatography on a short column of silica gel. Therefore, crude **2** was converted to peracetate **26** (Ac_2O, pyridine, DMAP), which was purified by chromatography (EtOAc/pentane 1:3) and treated with NaOMe in MeOH to furnish product **2**, which was pure according to the NMR spectra (Figure 4).

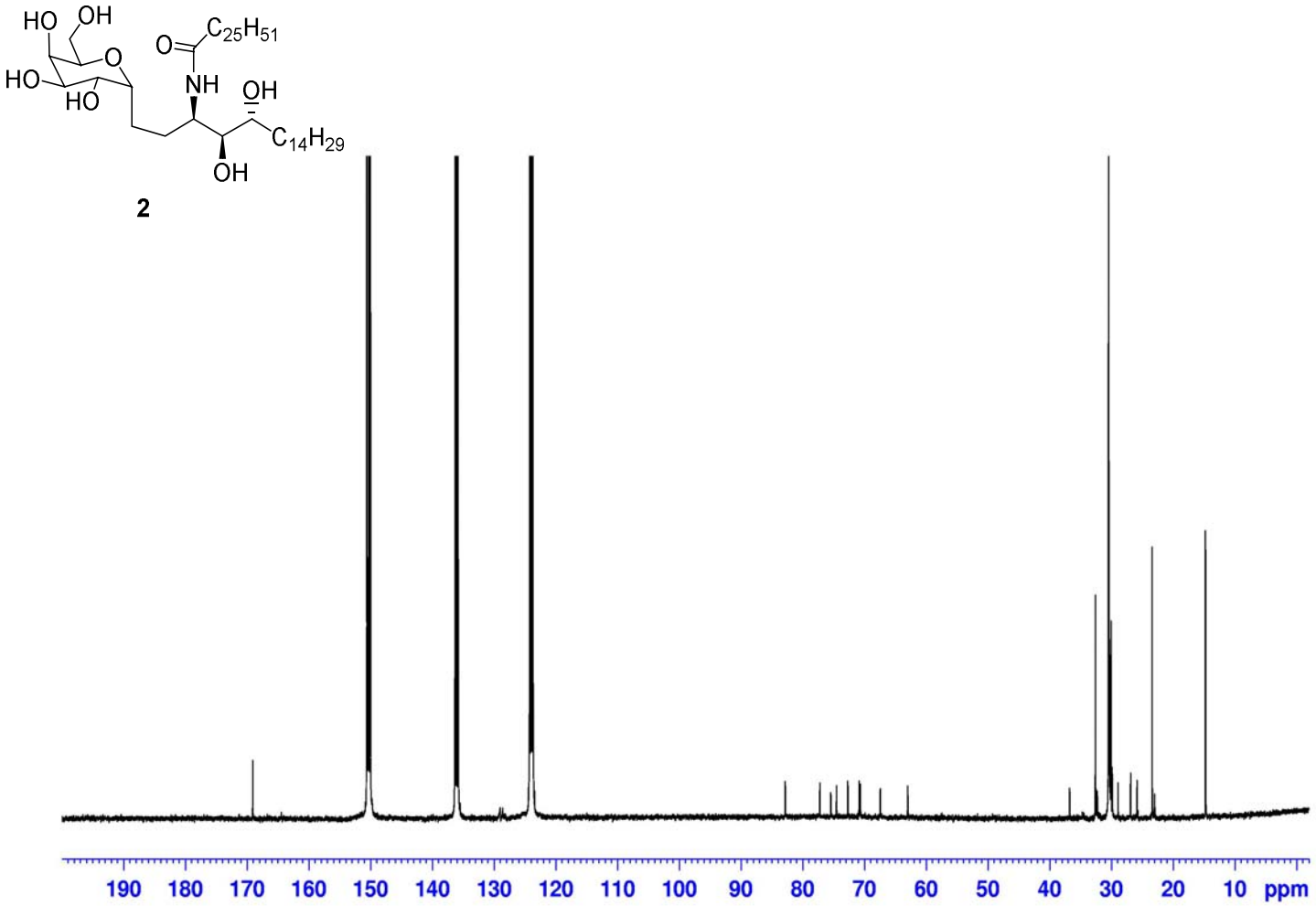
Scheme 8. Completion of the synthesis of 2



Reagents and conditions: (a) PPh_3, THF/H_2O (9:1), rt; (b) *p*-nitrophenyl hexacosanoate, THF/H_2O (9:1); (c) $Pd(OH)_2/C$, H_2, $CHCl_3$/MeOH; (d) Ac_2O, Py, DMAP, CH_2Cl_2, rt; (e) MeOH, NaOMe.

Figure 4. ^{1}H and ^{13}C NMR spectra for compound **2**.




HO
OH
O
HO
HO
O
C25H51
NH
OH
C14H29
OH
2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

In summary, this chapter describes the first synthesis of a new α-*C*-galactosylceramide analogue tentatively assigned to bear a D-*arabino*-phytosphingosine backbone (**2**). An unexpected retention of configuration during epoxide ring opening of **21** with NaN_3/NH_4Cl at reflux is proposed to take place via a double inversion of configuration involving either the 2-*O*-benzyl or pyronose oxygen in an initial intramolecular attack on epoxide **21**. The total synthesis of **2** is efficient; only 11 steps (or 13 steps with the final acylation and deacylation) are required in the longest pathway, and an overall yield of 8.8% was achieved. The synthetic methodology can be applied to the preparation of other α-*C*-galactosylceramide analogues; for example, different sphingosine chain lengths and different sugar heads can be accessed by changing the structures of propargylic alcohol **17** and bromoethyl α-*C*-glycoside **11**.

To compare the in vitro activity of arabino epimer **2** with that of α-C-galctosylceramide **1**, tests of cytokine production were carried out in human NKT cells by Dr. Leonid Metelitsa (Children's Hospital of Los Angeles). Compound **2** was less effective than **1** in the amounts of IFNγ, TNFα, and IL4 produced by factors of 4, 6, and 20, respectively. This assay indicates the importance of configuration at C-3 of the PHS backbone in the in vitro response of NKT cells to α-*C*-galactosylceramides.

Experimental Section

(*Z*)-2-(Benzyloxy)heptadec-2-enal (7). To a cooled (-78 ^{o}C), stirred solution of **4** (0.83 g, 1.50 mmol) in anhydrous THF (25 mL) was slowly added *n*-BuLi (0.62 mL, 1.8 mmol, a 2.89 M solution in hexane). Stirring was continued for 30 min, and then a solution of **5** (0.75 g, 1.5 mmol) in 1 mL of HMPA and 4 mL of anhydrous THF was

added dropwise. The reaction mixture was stirred at -78 °C for an additional 3 h, and then was warmed to rt and stirring was continued overnight. The reaction mixture was quenched with 30 mL of saturated aqueous NH_4Cl solution. The suspension was diluted with Et_2O (40 mL), the phases were separated, and the aqueous layer was extracted twice with Et_2O (40 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel (hexane/EtOAc 6:1) to afford 0.46 g (86%) of **7** as a colorless liquid: R_f 0.80 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24-1.33 (m, 24H), 2.25 (dt, 2H, *J* = 7.2, 7.6 Hz), 5.04 (s, 2H), 5.99 (t, 2H, *J* = 7.6 Hz), 7.33 (m, 5H), 9.23 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.4, 28.4, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 72.9, 128.2, 128.4, 137.2, 142.3, 153.8, 189.3.

(2*R*,3*R*)-3-(4-Methoxybenzyloxy)-2-(benzyloxy)heptadecanal (5). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24-1.33 (m, 24H), 1.50 (m, 1H), 1.72 (m, 1H), 3.72 (m, 1H), 3.79 (s, 3H), 3.88 (m, 1H), 4.46 (d, 1H, *J* = 11.2 Hz), 4.54 (d, 1H, *J* = 11.2 Hz), 4.60 (d, 1H, *J* = 11.2 Hz), 4.71 (d, 1H, *J* = 11.2 Hz), 6.85 (d, 2H, *J* = 8.8 Hz), 7.23 (d, 2H, *J* = 8.8 Hz), 7.32 (m, 5H), 9.71 (d, 1H, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.3, 29.4, 29.5, 29.7, 31.0, 31.9, 55.2, 71.9, 72.8, 79.4, 84.6, 113.8, 128.0, 128.5, 129.5, 130.2, 137.3, 159.3, 203.0.

2-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1-bromoethane (11). To a solution of compound **10** (3.0 g, 5.28 mmol) and CBr_4 (4.39 g, 13.2 mmol) in 100 mL of dry CH_2Cl_2 at 0 °C was slowly added Ph_3P (3.46 g, 13.2 mmol). After the reaction mixture was stirred at rt overnight, the reaction mixture was concentrated under vacuum, and the resulting residue was purified by chromatography to give 2.60 g (78%) of **11** as a colorless oil: R_f 0.55 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 1H), 2.24 (m,

1H), 3.44 (m, 2H), 3.69 (m, 2H), 3.71 (m, 1H), 3.82 (m, 1H), 3.98 (m, 2H), 4.18 (m, 1H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 30.4, 67.7, 68.9, 72.9, 73.1, 73.2, 73.5, 74.2, 77.2, 127.5, 127.6, 127.8, 127.9, 128.0, 128.3, 128.4, 128.8, 138.1, 138.4, 138.5.

(2*S*,3*R*)-2-(Chloromethyl)-3-tetradecyloxirane (15). To a solution of 2.30 g (8.5 mmol) of alcohol **14**[16] and 2.68 g (10.2 mmol) of Ph_3P in 100 mL of dry CH_2Cl_2 was added 1.38 g (10.2 mmol) of NCS at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 1 h, and then allowed to warm to rt, and stirred for 2 h. The mixture was diluted with 50 mL of hexane and passed through a pad of silica gel with suction to remove the precipitate of $Ph_3P(O)$. The filtrate was concentrated, and the resulting residue was purified by chromatography (EtOAc/hexane 1:6) to give 2.11 g (86%) of allylic chloride **15** as a white solid: R_f 0.88 (EtOAc/hexane 1:3); mp 47.4-48.5 °C; $[\alpha]^{25}_D$ +4.8° (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24-1.61 (m, 24H), 2.95 (m, 1H), 2.99 (m, 1H), 3.48 (m, 1H), 3.55 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.8, 29.3, 29.4, 29.5, 29.6, 29.7, 31.5, 32.0, 44.8, 57.2, 59.2.

(*R*)-Heptadec-1-yn-3-ol (16). To a stirred solution of 4.0 mL (23.2 mmol) of HMPA in 20 mL of dry THF was added 7.2 mL (20.8 mmol) of *n*-BuLi (a 2.89 M solution in hexane) at -78 °C under N_2. After 10 min, a solution of 2.0 g (6.94 mmol) of chloride **15** in 10 mL of THF was added dropwise over 5 min. After 0.5 h, the reaction mixture was warmed to rt and stirred for another 2 h. Saturated aqueous NH_4Cl solution was added to quench the reaction. The product was extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by chromatography (hexane/EtOAc 3:1) gave

1.45 g (83%) of **16** as a white solid: R_f 0.54 (EtOAc/hexane 1:3); mp 48.8-49.3 °C; $[\alpha]^{25}_D$ +2.1° (*c* 1.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24-1.48 (m, 24H), 1.70 (m, 2H), 1.78 (m, 1H), 2.45 (d, 1H, *J* = 2.0 Hz), 4.48 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.0, 29.3, 29.4, 29.5, 29.6, 29.7, 32.0, 37.7, 62.4, 72.8, 85.1.

(*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-heptadecyne (17). To a solution of *tert*-butyldimethylsilyl chloride (1.25 g, 8.33 mmol) in dry CH_2Cl_2 (20 mL) was added imidazole (1.14 g, 16.7 mmol) at rt under a N_2 atmosphere. The mixture was stirred for 1 h before a solution of propargylic alcohol **16** (1.40 g, 5.55 mmol) in dry CH_2Cl_2 (10 mL) was added. The mixture was stirred overnight, and then quenched with water (10 mL). After the mixture was extracted with CH_2Cl_2 (3 × 25 mL), the combined organic phases were washed with brine (40 mL) and dried ($MgSO_4$). The residue was purified by flash chromatography (EtOAc/hexane 1:6) to afford **17** (1.89 g, 93%) as a colorless oil: R_f 0.88 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +23.4° (*c* 1.82, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 1H), 0.13 (s, 1H), 0.88 (m, 12H), 1.24-1.35 (m, 22H), 1.42 (m, 2H), 1.53 (m, 2H), 2.37 (d, 1H, *J* = 2.0 Hz), 4.32 (m, 1H). ^{13}C NMR ($CDCl_3$) δ -5.04, -4.55, 14.1, 18.3, 22.7, 25.1, 25.8, 29.3, 29.4, 29.5, 29.6, 29.7, 32.0, 38.6, 62.8, 71.8, 85.9.

(*R*)-1-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)-5-*O*-(*tert*-butyldimethylsilyl)nonadec-3-yn-5-ol (18). To a solution of alkyne **17** (878 mg, 2.4 mmol) in dry THF (25 mL) was added *n*-BuLi (1.0 mL, 2.89 mmol, 2.89 M in hexane) at -78 °C under a N_2 atmosphere. The mixture was stirred for 2 h before HMPA (10 mL) was added. After the mixture was stirred for another 30 min, a solution of bromide **11** (1.00 g, 1.6 mmol) in 5 mL of HMPA was added slowly. The solution was stirred at -78 °C for 2.5 h, and then at rt overnight, before being quenched with saturated aqueous NH_4Cl

solution (40 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography to afford **18** (909 mg, 62%) as a colorless oil: R_f 0.72 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +33.7° (*c* 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 3H), 0.11 (s, 1H), 0.88 (m, 12H), 1.25-1.46 (m, 24H), 1.60 (m, 2H), 1.70 (m, 1H), 1.87 (m, 1H), 2.17 (m, 1H), 2.33 (m, 1H), 3.68 (m, 2H), 3.78 (m, 2H), 3.96 (m, 2H), 4.08 (m, 1H), 4.30 (m, 1H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ -4.93, -4.31, 14.1, 15.3, 18.3, 22.7, 25.0, 25.4, 25.9, 29.3, 29.7, 31.9, 39.1, 63.2, 67.9, 70.5, 72.3, 73.0, 73.4, 74.5, 82.4, 83.7, 127.5, 127.6, 127.7, 127.8, 127.9, 128.3, 138.3, 138.4, 138.6. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{59}H_{84}O_6SiNa^+$ 939.5929, found 939.5914.

(*R*)-1-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)nonadec-3-yn-5-ol (19). A solution of TBAF (2.7 mL, 2.7 mmol, 1 M in THF) was added to a solution of **18** (824 mg, 0.90 mmol) in THF (20 mL). After the mixture was stirred at rt overnight, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane 1:6), providing **19** (650 mg, 90%) as a colorless oil: R_f 0.42 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +23.1° (*c* 1.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.46 (m, 24H), 1.61 (m, 2H), 1.68 (m, 1H), 1.87 (m, 1H), 1.98 (m, 1H), 2.26 (m, 2H), 3.66 (m, 1H), 3.70 (m, 1H), 3.82 (m, 2H), 3.94 (m, 2H), 4.11 (m, 1H), 4.28 (m, 1H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.1, 15.3, 22.7, 25.3, 26.3, 29.4, 29.6, 29.7, 31.9, 38.1, 62.6, 67.9, 70.6, 72.2, 73.0, 73.3, 73.4, 74.5, 81.9, 84.7, 127.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 138.3, 138.5, 138.6. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{53}H_{70}O_6Na^+$ 825.5065, found 825.5094.

(*R*,*E*)-1-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)nonadec-3-en-5-ol (20). To a solution of **19** (540 mg, 0.67 mmol) in anhydrous Et_2O (20 mL) at 0 °C was added dropwise a solution of Red-Al (2.9 mL, 10 mmol, a 70% solution in toluene). After 10 min, the cooling bath was removed, and the reaction mixture was stirred at rt overnight. An aqueous saturated solution of NH_4Cl (2 mL) was slowly added (*Caution! very exothermic*). The resulting white slurry was diluted with Et_2O (10 mL), 1 N NaOH (5 mL), and water (5 mL), and the layers were separated. The aqueous phase was re-extracted with Et_2O (3 × 5 mL), and the combined organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc/hexane 1:3) to afford 431 mg (80%) of alkene **20** as a colorless oil: R_f 0.41 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +19.3° (*c* 1.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.36 (m, 24H), 1.46 (m, 2H), 1.59 (m, 1H), 1.62 (m, 1H), 1.97 (m, 1H), 2.12 (m, 1H), 3.60 (m, 1H), 3.70 (m, 1H), 3.76 (m, 2H), 3.94 (m, 4H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.5, 28.5, 29.4, 29.6, 29.7, 31.9, 37.3, 67.9, 70.6, 72.2, 73.0, 73.1, 73.2, 73.4, 74.5, 127.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 131.2, 133.7, 138.3, 138.4, 138.5, 138.6. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{53}H_{72}O_6Na^+$ 827.5221, found 827.5236.

(*R*)-((2*R*,3*R*)-3-(2-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)ethyl)-oxiran-2-yl)pentadecan-1-ol (21). To a solution of 0.13 mL (0.60 mmol) of (-)-diisopropyl D-tartrate in 10 mL of dry CH_2Cl_2 was added 50 mg of 4 Å molecular sieves. The mixture was stirred at rt for 30 min before it was cooled to –40 °C. To the reaction mixture was added Ti(OPr-*i*)$_4$ (0.18 mL, 0.60 mmol), with stirring for 30 min. Cumene hydroperoxide (152 mg, 1.0 mmol) was added, and the reaction mixture was stirred for 30 min. A solution of 403 mg (0.50 mmol) of alcohol **20** in 3 mL of dry CH_2Cl_2 was

added, and the reaction mixture was stirred overnight at −20 °C. After 10 mL of a 10% aqueous solution of tartaric acid was added dropwise, the mixture was allowed to warm to rt over 1 h, at which time the solution was transparent. The organic layer was separated, washed with brine (10 mL), dried ($MgSO_4$), and concentrated. The residue was dissolved in 10 mL of Et_2O at 0 °C, and 10 mL of 5% aqueous NaOH was added. The mixture was stirred for 30 min, the organic layer was separated, washed with brine (10 mL), and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by chromatography (hexane/EtOAc 3:1) to afford 369 mg (90%) of **21** as a colorless oil: R_f 0.29 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +19.1° (*c* 2.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.51 (m, 26H), 1.60-1.75 (m, 4H), 2.15 (s, 1H), 2.67 (m, 1H), 3.01 (m, 1H), 3.53 (m, 2H), 3.70 (m, 1H), 3.82 (m, 2H), 3.95 (m, 3H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.4, 28.5, 29.4, 29.6, 29.7, 31.9, 33.8, 54.8, 61.1, 65.3, 68.0, 68.4, 72.1, 73.1, 73.3, 74.5, 127.0, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 138.1, 138.3, 138.4, 138.5. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{53}H_{72}O_7Na^+$ 843.5170, found 843.5148.

(3*R*,4*S*,5*R*)-1-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3-azido-nonadecane-4,5-diol (22). To epoxy alcohol **21** (328 mg, 0.40 mmol) in 9 mL of MeOH/H_2O (8:1) were added NH_4Cl (249 mg, 4.7 mmol) and NaN_3 (605 mg, 9.3 mmol). The reaction mixture was heated at reflux for two days (external bath temperature, 105 °C). The reaction mixture was allowed to cool to rt, and the solvents were evaporated. The residue was extracted with EtOAc (2 × 10 mL). The crude reaction mixture was purified by chromatography (EtOAc/hexane 1:1) to afford 225 mg (65%) of azido diol **22** as a colorless wax: R_f 0.27 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +22.8° (*c* 1.65, $CHCl_3$); ^{1}H NMR

($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.36 (m, 24H), 1.42 (m, 1H), 1.55 (m, 2H), 1.73, (m, 2H), 1.88 (m, 1H), 2.48 (m, 2H), 3.53 (m, 4H), 3.72 (m, 2H), 3.98 (m, 4H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.4, 29.7, 29.8, 31.9, 32.3, 64.1, 67.8, 71.6, 71.8, 72.3, 73.1, 73.3, 74.4, 76.2, 127.0, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 138.0, 138.2, 138.3, 138.5.

(*R*)-((4*S*,5*R*)-4-((*R*)-1-Azido-3-(2',3',4',6'-tetra-*O*-benzyl-α-D-galacto-pyranosyl))propyl)-2,2-dimethyl-5-tetradecyl-1,3-dioxolane (23). To a solution of 86 mg (0.10 mmol) of diol **22** and 12 mg (0.050 mmol) of CSA in 10 mL of dry THF was added 27 mg (0.20 mmol) of 3,3-dimethoxypentane. The solution was stirred at reflux for 2 h and then quenched by adding 69 mg (0.50 mmol) of anhydrous K_2CO_3. The solid residue was removed by filtration and washed with Et_2O (2 × 100 mL). The solvent was evaporated, and the residue was purified by chromatography (EtOAc/hexane 1:6) to give 81 mg (90%) of **23** as a colorless wax: R_f 0.65 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +27.9° (*c* 1.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25-1.36 (m, 30H), 1.53 (m, 3H), 1.78 (m, 2H), 2.01 (m, 1H), 3.38 (m, 1H), 3.62 (m, 1H), 3.73 (m, 1H), 3.82 (m, 3H), 3.99 (m, 3H), 4.11 (m, 1H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.2, 22.7, 25.7, 26.4, 28.1, 28.2, 28.3, 29.4, 29.7, 29.8, 30.4, 32.0, 60.9, 67.6, 72.1, 72.2, 73.1, 73.2, 73.3, 74.4, 77.9, 78.3, 108.4, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 138.3, 138.4, 138.5, 138.6.

***N*-((3*R*,4*S*,5*R*)-1-(2',3',4',6'-Tetra-*O*-benzyl-α-D-galactopyranosyl)-4,5-dihydroxynonadecan-3-yl)hexacosanamide (25).** To a solution of 172 mg (0.20 mmol) of compound **22** in 6 mL of THF/H_2O (9:1) was added 105 mg (0.40 mmol) of PPh_3. After the reaction mixture was stirred overnight, 196 mg (0.40 mmol) of *p*-nitrophenyl

hexacosanoate was added, and the reaction mixture was stirred for two days. The reaction mixture was concentrated, the residue was dissolved in 25 mL of CH_2Cl_2 and washed with saturated aqueous Na_2CO_3 solution (15 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (EtOAc/hexane 1:3) to give 192 mg (81%) of compound **25** as a wax: R_f 0.18 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ +19.1° (*c* 5.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.25-1.88 (m, 74H), 2.36 (m, 2H), 3.56 (m, 2H), 3.85 (m, 4H), 3.98 (m, 2H), 4.02 (m, 2H), 4.42-4.76 (m, 8H), 7.29 (m, 20H). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.1, 25.3, 29.2, 29.3, 29.4, 29.6, 29.7, 29.8, 32.0, 33.0, 34.5, 73.1, 73.2, 73.3, 74.5, 127.6, 127.7, 127.8, 128.0, 128.3, 128.4, 138.1, 138.3, 138.4, 138.6, 173.9.

***N*-((3*R*,4*S*,5*R*)-1-(2',3',4',6'-Tetra-*O*-acyl-α-D-galactopyranosyl)-4,5-di-*O*-acyl-nonadecane-4,5-diol-3-yl)hexacosanamide (26).** To a solution of 119 mg (0.10 mmol) of diol **25** in 90 mL of $CHCl_3$/MeOH (9:1) was added 26 mg (0.050 mmol) of 20% $Pd(OH)_2$/C. The resulting suspension was purged with H_2 for approximately 10 min and then stirred with a balloon filled with H_2 overnight. The crude reaction mixture was filtered through a short pad of silica gel, and the solvent was evaporated to provide crude **26** as a white solid. Compound **26** was dried and dissolved in pyridine (4.0 mL, 50 mmol) and Ac_2O (2.0 mL, 21 mmol), and allowed to stand overnight. Coevaporation three times with toluene and chromatography (EtOAc/pentane 1:3) yielded 85 mg (79%, two steps) of **26** as a white solid: R_f 0.39 (EtOAc/hexane 1:3); mp 38.2-39.6 °C; $[\alpha]^{25}_D$ +61.6° (*c* 1.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.25-1.36 (m, 68H), 1.59 (m, 6H), 2.02-2.13 (m, 18H), 2.28 (m, 2H), 3.74 (m, 1H), 4.13 (m, 3H), 4.22 (m, 1H), 4.43 (m, 1H), 5.20 (m, 1H), 5.30 (m, 1H), 5.42 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 14.2, 20.6, 20.7,

20.8, 21.0, 22.7, 24.9, 26.1, 28.2, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 30.6, 31.9, 35.8, 60.4, 61.6, 65.0, 67.7, 68.0, 68.2, 71.6, 75.7, 82.8, 169.8, 169.9, 170.0, 170.1, 170.4.

***N*-((3*R*,4*S*,5*R*)-1-(α-D-Galactopyranosyl)-nonadecane-4,5-diol-3-yl)-hexacosanamide (2).** To a solution of 70 mg (0.065 mmol) of peracetate **26** in 10 mL of MeOH was added 10 mg (0.18 mmol) of NaOMe. After stirring overnight at 20 °C, the reaction mixture was neutralized by adding 200 mg of Dowex 50W-X8 resin, with stirring for 30 min. The resin was removed by filtration, and the filtrate was concentrated and dried under vacuum to give 47 mg (87%) of **2** as a white solid: mp 92.4-93.7 °C; $[\alpha]^{25}_{D}$ +62.9° (*c* 1.35, pyridine); [lit.[4] $[\alpha]^{25}_{D}$ +40.8° (*c* 0.13, pyridine)]; ^{1}H NMR (pyridine-d_5) δ 0.88 (m, 6H), 1.25-1.36 (m, 66H), 1.66 (m, 2H), 1.75 (m, 2H), 2.32 (m, 2H), 2.41 (m, 3H), 2.53 (m, 1H), 4.05 (m, 1H), 4.23 (m, 1H), 4.32 (m, 2H), 4.48 (m, 1H), 4.95 (m, 1H), 4.54 (m, 1H), 4.61 (m, 1H), 4.67 (m, 1H), 4.73 (m, 1H), 6.02 (br s, 7H); ^{13}C NMR ($CDCl_3$) δ 14.8, 23.0, 23.4, 25.7, 25.9, 27.0, 29.0, 29.9, 30.1, 30.2, 30.3, 30.4, 30.5, 32.3, 32.6, 36.8, 63.0, 67.5, 70.7, 70.9, 72.7, 74.5, 75.5, 77.3, 82.9, 169.1. HR-MS (FAB, MNa^+) *m*/*z* calcd for $C_{51}H_{101}NO_8Na^+$ 878.7419, found 878.7349. [lit. data for *C*-KRN7000 (**1**)[5] ^{1}H NMR (500 MHz, pyridine-d_5): δ 0.89 (t, 6H, *J* = 6.8 Hz), 1.17-1.48 (m, 68H), 1.70 (m, 1H), 1.85 (m, 2H), 1.93 (m, 2H), 2.22 (m, 1H), 2.32 (m, 2H), 2.45 (m, 2H), 2.58 (m, 1H), 2.72 (m, 1H), 4.22 (m, 4H), 4.36 (m, 1H), 4.52 (m, 3H), 4.72 (m, 1H, *J* = 9.3 Hz), 5.12 (m, 1H), 5.98 (d, 1H, *J* = 4.7 Hz), 6.16 (d, 1H, *J* = 4.4 Hz), 6.37 (m, 2H), 6.49 (d, 1H, *J* = 4.7 Hz), 6.65 (d, 1H, *J* = 4.7 Hz), 8.43 (d, 1H, *J* = 9.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) 14.6, 23.0, 23.3, 26.9, 27.0, 30.0, 30.2, 30.3, 30.4, 30.6, 30.8, 32.5, 34.8, 37.4, 53.1, 63.1, 70.8, 71.0, 72.6, 73.1, 74.1, 77.4, 78.9, 173.9.] [lit. data for *C*-KRN7000 (**1**)[6]: ^{1}H ($CDCl_3$): δ 0.90 (t, 6H, *J* = 6.8 Hz), 1.29 (m, 74H), 1.63-1.65 (m,

2H), 1.70-1.86 (m, 2H), 2.39 (m, 1H), 3.30 (d, 1H, *J* = 8.6 Hz), 3.40-3.52 (m, 1H), 3.70-3.74 (m, 3H), 3.79 (dd, 1H, *J* = 3.8, 9.0 Hz), 3.80-3.92 (m, 2H), 4.11 (d, 1H, *J* = 8.3 Hz), 5.89 (m, 2H).]

References

1. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771-2784.

2. For recent reviews, see: (a) Van Kaer, L. *Nat. Rev. Immunol.* **2005**, *5*, 31-42. (b) Hayakawa, Y.; Godfrey, D. I.; Smyth, M. J. *Curr. Med. Chem.* **2004**, *11*, 241-252.

3. Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631-1641.

4. Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Intl. Ed. Engl.* **2004**, *43*, 3818-3822.

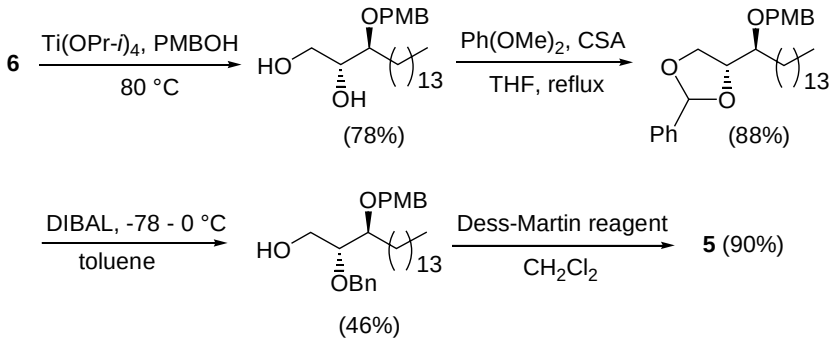
5. Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077-4080.

6. Tomiyama, H.; Yanagisawa, T.; Nimura, M.; Noda, A.; Tomiyama, T. US Patent 6,635,622, 2001.

7. Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. *Tetrahedron Lett.* **2005**, *46*, 5043-5047.

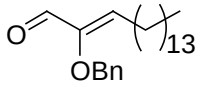
8. Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, 67, 4475-4486. Acetylene **4** was obtained by *C*-glycosidation of the sugar acetate using 1-tri-*n*-butylstannyl-2-trimethylsilylacetylene in the presence of trimethylsilyl triflate, followed by desilylation of the initial product.

9. Compound **5** was synthesized as shown below:



10. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

11. The structure of compound **7** is:



12. (a) Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976-4978. (b) Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479-1482.

13. Nolen, E. G.; Watts, M. M.; Fowler, D. J. *Org. Lett.* **2002**, *4*, 3963-3965.

14. Wang, T.-L.; Hu, X. E.; Cassady, J. M. *Tetrahedron Lett.* **1995**, *36*, 9301-9304.

15. Kokotos, G.; Padron, J. M.; Noula, C.; Gibbons, W. A.; Martin, V. S. *Tetrahedron: Asymmetry* **1996**, *7*, 857-866.

16. Padron, J. M.; Martin, V. S.; Hadjipavlou-Litina, D.; Noula, C.; Constantinou-Kokotou, V.; Peters, G. J.; Kokotos, G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 821-826.

17. Yadav, J. S.; Deshpande, P. K.; Sharma, G. V. M. *Tetrahedron* **1990**, *46*, 7033-7046.

18. Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696-5704.

19. Benedetti, F.; Berti, F.; Norbedo, S. *Tetrahedron Lett.* **1998**, *39*, 7971-7974.

20. Caron, M.; Carlier, P. R.; Sharpless, K. B. *J. Org. Chem.* **1988**, *53*, 5185-5187.

21. Pihlaja, K.; Kleinpeter, E. In: *Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis*, VCH: New York, 1994, pp. 55–56. For a recent example of the

chemical shifts of the quaternary carbon of 5- and 6-membered ring acetonides, see: Ayad, T.; Génisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582.

22. The positions and configurations of the functional groups of the compounds shown in Scheme 8 cannot undergo changes during these reactions.

23. Mootoo, D. R.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1986**, 1570-1571.

24. Cipolla, L.; Lay, L.; Nicotra, F. *J. Org. Chem.* **1997**, *62*, 6678-6681. For other examples of a benzyloxy group acting as a nucleophile, see: (a) Dehmlow, H.; Mulzer, J.; Seilz, C.; Strecker, A. R.; Kohlmann, A. *Tetrahedron Lett.* **1992**, *33*, 3607-3610. (b) Yang, B.-H.; Jiang, J.-Q.; Ma, K. and Wu, H.-M. *Tetrahedron Lett.* **1995**, *36*, 2831-2834.

List of References for Chapters 1-8

Chapter 1

1. (a) Pyne, S.; Pyne, N. J. *Biochem. J.* **2000**, *349*, 385-402. (b) Spiegel, S.; Milstien, S. *J. Biol. Chem.* **2002**, *277*, 25851-25854. (c) Hla, T. *Pharmacol. Res.* **2003**, *47*, 401-407.

2. (a) Spiegel, S.; Milstien, S. *Biochim. Biophys. Acta* **2000**, *1484*, 107-116. (b) Spiegel, S.; English, D.; Milstien, S. *Trends Cell Biol.* **2002**, *12*, 236-242.

3. (a) Maceyka, M.; Payne, S. G.; Milstien, S.; Spiegel, S. *Biochim. Biophys. Acta* **2002**, *1585*, 193-201. (b) Liu, H.; Chakravarty, D.; Maceyka, M.; Milstien, S.; Spiegel, S. *Prog. Nucleic Acid Res. Mol. Biol.* **2002**, *71*, 493-511.

4. Parrill, A. L.; Wang, D.; Bautista, D. L.; Van Brocklyn, J. R.; Lorincz, Z.; Fischer, D. J.; Baker, D. L.; Liliom, K.; Spiegel, S.; Tigyi, G. *J. Biol. Chem.* **2000**, *275*, 39379-39384.

5. (a) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Xia, P.; Gamble, J. R.; Vadas, M. A.; D'Andrea, R. J.; Wattenberg, B. W. *J. Biol. Chem.* **2000**, *275*, 33945-33950. (b) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Vadas, M. A.; D'Andrea, R. J.; Wattenberg, B. W. *FEBS Lett.* **2001**, *509*, 169-173. (c) Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Zareie, R.; Derian, C. K.; Darrow, A. L.; Qi, J.; D'Andrea, R. J.; Bagley, C. J.; Vadas, M. A.; Wattenberg, B. W. *J. Biol. Chem.* **2002**, *277*, 49545-49553.

6. Murata, N.; Sato, K.; Kon, J.; Tomura, H.; Yanagita, M.; Kuwabara, A.; Ui, M.; Okajima, F. *Biochem. J.* **2000**, *352*, 809-815.

7. (a) Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687. (b) Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.

8. (a) Garner, P.; Park, J. M. *Org. Synth*. **1991**, *70*, 18-27. (b) Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136-2157.

9. Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362.

10. For a review of the Mitsunobu reaction, see: Hughes, D. L. *Org. React*. **1992**, *42*, 335-656.

11. Li, G.; Samadder, P.; Arthur, G.; Bittman, R. *Tetrahedron* **2001**, *57*, 8925-8932.

12. Wang, D. A.; Lorincz, Z.; Bautista, D. L.; Liliom, K.; Tigyi, G.; Parrill, A. L. *J. Biol. Chem.* **2001**, *276*, 49213-49220.

13. Nagase, S.; Shimamune, K.; Shumiya, S. *Science* **1979**, *205*, 590-591.

14. Kalivretenos, A.; Stille, J. K.; Hegedus, L. S. *J. Org. Chem.* **1991**, *56*, 2883-2894.

15. Lu, X.; Cseh, S.; Byun, H.-S.; Tigyi; G.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 7046-7050.

Chapter 2

1. Ito, M.; Kita, K.; Kurita, T.; Sueyoshi, N.; Izu, H. *Methods Enzymol.* **2000**, *311*, 297-303.

2. Liliom, K.; Sun, G.; Bunemann, M.; Virag, T.; Nusser, N.; Baker, D. L.; Wang, D. A.; Fabian, M. J.; Brandts, B.; Bender, K.; Eickel, A.; Malik, K. U.; Miller, D. D.; Desiderio, D. M.; Tigyi, G.; Pott, L. *Biochem. J.* **2001**, *355*, 189-197.

3. Nofer, J. R.; Geigenmuller, S.; Gopfert, C.; Assmann, G.; Buddecke, E.; Schmidt, A. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 98-103.

4. Rodriguez-Lafrasse, C.; Vanier, M. T. *Neurochem. Res.* **1999**, *24*, 199-205.

5. Xu, Y.; Casey, G.; Mills, G. B. *J. Cell Physiol.* **1995**, *163*, 441-450.

6. (a) Xu, Y. *Biochim. Biophys. Acta* **2002**, *1582*, 81-88. (b) Meyer zu Heringdorf, D. M.; Himmel, H. M.; Jakobs, K. H. *Biochim. Biophys. Acta* **2002**, *1582*, 178-189. (c) Kye, K. C.; Chae, E. K.; Piao, Y. J.; Park, S.; Park, J. K.; Kim, C. D.; Lee, J. H.; Suhr, K. B. *J. Invest. Dermatol.* **2004**, *122*, 1365-1371.

7. Kim, K.-S.; Ren, J.; Jiang, Y.; Ebrahem, Q.; Tipps, R.; Cristina, K.; Xiao, Y.-J.; Qiao, J.; Taylor, K. L.; Lum, H.; Anand-Apte, B.; Xu, Y. *FASEB J.* **2005**, *19*, 819-821.

8. Beil, M.; Micoulet, A.; von Wichert, G.; Paschke, S.; Walther, P.; Omary, M. B.; Van Veldhoven, P. P.; Gern, U.; Wolff-Hieber, E.; Eggermann, J.; Waltenberger, J.; Adler, G.; Spatz, J.; Seufferlein, T. *Nat. Cell Biol.* **2003**, *5*, 803-811.

9. (a) Xu, Y.; Zhu, K.; Hong, G.; Wu, W.; Baudhuin, L. M.; Xiao, Y.; Damron, D. S. *Nat. Cell Biol.* **2000**, *2*, 261-267. (b) Ignatov, A.; Lintzel, J.; Hermans-Borgmeyer, I.; Kreienkamp, H. J.; Joost, P.; Thomsen, S.; Methner, A.; Schaller, H. C. *J. Neurosci.* **2003**, *23*, 907-914.

10. Zhu, K.; Baudhuin, L. M.; Hong, G.; Williams, F. S.; Cristina, K. L.; Kabarowski, J. H.; Witte, O. N.; Xu, Y. *J. Biol. Chem.* **2001**, *276*, 41325-41335.

11. Witte, O. N.; Kabarowski, J. H.; Xu, Y.; Le, L. Q.; Zhu, K. *Science* **2005**, *307*, 206.

12. (a) Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687. (b) Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.

13. Lu, X.; Cseh, S.; Byun, H.-S.; Tigyi, G.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 7046-7050.

14. Parrill, A. L.; Sardar, V. M.; Yuan, H. *Seminars Cell Develop. Biol.* **2004**, *15*, 467-476.

15. (a) Garner, P.; Park, J. M. *Org. Synth.* **1991**, *70*, 18-27. (b) Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136-2157.

16. Pfaendler, H. R.; Weimar, V. *Synthesis* **1996**, 1345-1349.

17. Wang, Y.; Dong, X.; Larock, R. C. *J. Org. Chem.* **2003**, *68*, 3090-3098.

18. For reviews of the Suzuki reaction, see: (a) Chemler, S. R.; Trauner, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 4544-4568. (b) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633-9695. (c) Bellina, F.; Carpita, A.; Rossi, R. *Synthesis* **2004**, 2419-2440.

19. Khiar, N.; Singh, K.; Garcia, M.; Martin-Lomas, M. *Tetrahedron Lett.* **1999**, *40*, 5779-5782.

20. Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, *19*, 3633-3634.

21. Lu, X.; Bittman, R. *J. Org. Chem.* **2005**, *70*, 4746-4750.

Chapter 3

1. Karlsson, K.-A.; Martensson, E. *Biochim. Biophys. Acta* **1968**, *152*, 230-233. (b) Carter, H. E.; Hirschberg, C. B. *Biochemistry* **1968**, *7*, 2291-2300. (c) Bouhours, D.; Bouhours, J.-F. *Biochim. Biophys. Acta* **1984**, *794*, 169-171. (d) Okabe, K.; Keenan, R. W.; Schmidt, G. *Biochem. Biophys. Res. Commun.* **1986**, *31*, 137-143. (e) Higuchi, R.; Kagoshima, M.; Komori, T. *Liebigs Ann. Chem.* **1990**, 659-663.

2. For reviews, see: (a) Dickson, R. C.; Lester, R. L. *Biochim. Biophys. Acta* **2003**, *1583*, 13-25. (b) Jenkins, G. M. *Cell. Mol. Life Sci.* **2003**, *60*, 701-710.

3. Park, M. T.; Kang, J. A.; Choi, J. A.; Kang, C. M.; Kim, T. H.; Bae, S.; Kang, S.; Kim, S.; Choi, W. I.; Cho, C. K.; Chung, H. Y.; Lee, Y. S.; Lee, S. *J. Clin. Cancer Res.* **2003**, *9*, 878-885.

4. (a) Wertz, P. W.; Miethke, M. C.; Long, S. A.; Strauss, J. S.; Downing, D. T. *J. Invest. Dermatol.* **1985**, *84*, 410-412. (b) Motta, S.; Monti, M.; Sesana, S.; Caputo, R.; Carelli, S.; Ghidoni, R. *Biochim. Biophys. Acta* **1993**, *1182*, 147-151. (c) Ponec, M.; Weerheim, A.; Lankhorst, P.; Wertz, P. *J. Invest. Dermatol.* **2003**, *120*, 581-588. (d) Rerek, M. E.; Chen, H.-C.; Markovic, B.; Van Wyck, D.; Garidel, P.; Mendelsohn, R.; Moore, D. J. *J. Phys. Chem. B* **2001**, *105*, 9355-9362.

5. (a) Kobayashi, E.; Motoki, K.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Oncol. Res*. **1995**, *7*, 529-534. (b) Nishi, N.; Hizuka, Y.; Natori, T. *Molec. Med.* **2002**, *39*, 478-483.

6. (a) Lester, R. L.; Dickson, R. C. *Adv. Lipid Res.* **1993**, *26*, 253-274. (b) Fankhauser, C.; Homans, S. W.; Thomas-Oates, J. E.; McConville, M. J.; Desponds, C.; Conzelmann, A.; Ferguson, M. A. *J. Biol. Chem.* **1993**, *268*, 26365-26374. (c) Fontaine, T.; Magnin, T.; Melhert, A.; Lamont, D.; Latge, J.-P.; Ferguson, M. A. *Glycobiology* **2003**, *13*, 169-177.

7. For a recent review, see: Howell, A. R.; Ndakala, A. J. *Curr. Org. Chem.* **2002**, *6*, 365-391.

8. (a) Murakami, T.; Taguchi, K. *Tetrahedron* **1999**, *55*, 989-1004. (b) Chiu, H.-Y.; Tzou, D.-L. M.; Patkar, L. N.; Lin, C.-C. *J. Org. Chem.* **2003**, *68*, 5788-5791.

9. (a) Dondoni, A.; Fantin, G.; Fogagnolo, M.; Pedrini, P. *J. Org. Chem.* **1990**, *55*, 1439-1446. (b) Imashiro, R.; Sakurai, O.; Yamashita, T.; Horikawa, H. *Tetrahedron*

1998, *54*, 10657-10670. (c) Ndakala, A. J.; Hashemzadeh, M.; So, R. C.; Howell, A. R. *Org. Lett.* **2002**, *4*, 1719-1722.

10. (a) Lin, G.-Q.; Shi, Z.-E. *Tetrahedron* **1996**, *52*, 2187-2192. (b) Li, S.; Pang, J.; Wilson, W. K.; Schroepfer, G. J., Jr. *Tetrahedron Lett.* **2000**, *41*, 10309-10312.

11. He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618-7626.

12. Kobayashi, S.; Hayashi, T.; Kawasuji, T. *Tetrahedron Lett.* **1994**, *35*, 9573-9576.

13. (a) Ayad, T.; Genisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582. (b) Raghavan, S.; Rajender, A.; Yadav, J. S. *Tetrahedron: Asymmetry* **2003**, *14*, 2093-2099.

14. For other methods to synthesize L-*lyxo*-PHS, see ref. 11 and: (a) Wild, R.; Schmidt, R. R. *Tetrahedron: Asymmetry* **1994**, *11*, 2195-2208. (b) Li, Y.; Mao, X.; Wu, Y. *J. Chem. Soc. Perkin Trans. 1* **1995**, 559-663. (c) Nakamura, T.; Shiozaki, M. *Tetrahedron* **2001**, *44*, 9087-9092. (d) Lee, H. K.; Kim, E.; Pak, C. S. *Tetrahedron Lett*. **2002**, *43*, 9641-9644.

15. Candelore, M. R.; Wright, M. J.; Tota, L. M.; Milligan, J.; Shei, G.-J.; Bergstrom, J. D.; Mandala, S. M. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 600-606.

16. For recent examples of synthetic studies to prepare phosphonate analogues of sphingolipids, see: (a) McClure, K. C.; Mishra, P. K.; Grote, C. W. *J. Org. Chem.* **1997**, *62*, 2437-2441. (b) McClure, K. C.; Mishra, P. K. *Tetrahedron Lett.* **2002**, *43*, 5249-5253.

17. (a) Bittman, R.; Byun, H.-S.; Mercier, B.; Salari, H. *J. Med. Chem.* **1994**, *37*, 425-430. (b) Lin, W.; Leung, L. W.; Bae, Y. S.; Bittman, R.; Arthur, G. *Biochem.*

Pharmacol. **1999**, *57*, 1153-1158. (c) Samadder, P.; Bittman, R.; Byun, H.-S.; Arthur, G. *J. Med. Chem.* **2004**, *47*, 2710-2713.

18. Watanabe, Y.; Nakajima, M.; Hoshino, T.; Jayasimhulu, K.; Brooks, E. E.; Kaneshiro, E. S. *Lipids* **2001**, *36*, 513-519.

19. Kariotoglou, D. M.; Mastronicolis, S. K. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2003**, *136*, 27-44.

20. For a review, see: Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2002**, *102*, 2523-2584.

21. For reviews, see: (a) Gawroński, J.; Gawrońska, K. *Tartaric and Malic Acids in Synthesis*; John Wiley & Sons: New York, 1999. (b) Ghosh, A. K.; Koltun, E. S.; Bilcer, G. *Synthesis* **2001**, 1281-1301.

22. Byun, H.-S.; Bittman, R. *Syn. Commun.* **1993**, *23*, 3201-3204.

23. Steuer, B.; Wehner, V.; Lieberknecht, A.; Jager, V. *Org. Synth.* **1997**, *74*, 1-12.

24. For examples, see: (a) Backstrom, A. D.; Austin, R.; McMordie, A. S.; Begley, T. P. *J. Carbohydr. Chem.* **1995**, *14*, 171-175. (b) Yoshino, T.; Nagata, Y.; Itoh, E.; Hashimoto, M.; Katoh, T.; Terashima, S. *Tetrahedron* **1997**, *53*, 10239-10252. (c) Matsumura, R.; Suzuki, T.; Hagiwara, H.; Hoshi, T.; Ando, M. *Tetrahedron Lett.* **2001**, *42*, 1543-1546.

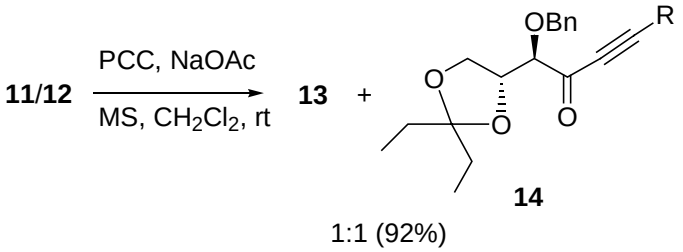
25. For methods to prepare 3,3'-dimethoxypentane, see: (a) Napolitano, E.; Fiaschi, R.; Mastrorilli, E. *Synthesis* **1986**, 122-125. (b) Linclau, B.; Boydell, A. J.; Clarke, P. J.; Horan, R.; Jacquet, C. *J. Org. Chem.* **2003**, *68*, 1821-1826.

26. The configuration at C2 was not altered on PCC oxidation of chiral 2-*O*-benzyl-3,4-*O*-isopropylidene-1-butanol to the isopropylidene aldehyde analogue of **10**: (a)

Williams, D. R.; Klingler, F. D. *Tetrahedron Lett.* **1987**, *28*, 869-872. (b) Valverde, S.; Herradon, B.; Martin-Lomas, M. *Tetrahedron Lett*. **1985**, *26*, 3731-3734. (c) Sánchez-Sancho, F.; Valverde, S.; Herradón, B. *Tetrahedron: Asymmetry* **1996**, *7*, 3209-3246.

27. Propargylation of aldehyde **10** may be improved under a variety of asymmetric alkynylmetal addition conditions in order to increase the ratio of **11**:**12** and also to construct the stereochemistry leading to the D-*ribo*-PHS backbone.

28. PCC oxidation of the mixture of compounds **11** and **12** gave a mixture of diastereomers **13** and **14** in a 1:1 ratio.



29. The configuration at C4 of compound **11** was confirmed by the 4*S* configuration in product **2**.

30. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) For a postulated mechanism to account for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion, see Scheme 1 of ref. 30a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

31. For the Dess-Martin oxidation of a *N*,*N*-dibenzyl α-amino alcohol, see: Cooke, J. W. B.; Davies, S. G.; Naylor, A. *Tetrahedron* **1993**, 49, 7955-7966.

32. For details on addition of **10** to $C_{14}H_{29}MgBr$ and isolation of **23** and **24**: see p. 69.

33. For an example of chelation-controlled Grignard addition, see: Ndakala, A. J.; Hashemzadeh, M.; So, R. C.; Howell, A. R. *Org. Lett*. **2002**, *4*, 1719-1722.

34. Data for (–)-**26**: see page 70.

35. Data for (–)-**27**: see page 70

36. Data for (–)-**28**: see page 70

37. Data for (–)-**29**: see page 70.

38. Data for (–)-**31**: see page 71.

39. Data for azido alcohol (–)-**32**: see page 71.

40. Data for (+)-**33**: see page 71.

41. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

42. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 3165-3168.

Chapter 4

1. Karlsson, K.-A. *Chem. Phys. Lipids* **1970**, *5*, 6-43.

2. (a) Hannun, Y. A. *Science* **1996**, *274*, 1855-1859. (b) Ariga, T.; Jarvis, W. D.; Yu, R. K. *J. Lipid Res*. **1998**, *39*, 1-16. (c) Perry, D. K.; Hannun, Y. A. *Biochim. Biophys. Acta* **1998**, *1436*, 233-243. (d) Hannun, Y. A.; Luberto, C. *Trends Cell Biol.* **2000**, *10*, 73-80.

3. Reynolds, C. P.; Maurer, B. J.; Kolesnick, R. N. *Cancer Lett.* **2004**, *206*, 169-180.

4. Brockman, H. L.; Momsen, M. M.; Brown, R. E.; He, L.; Chun, J.; Byun, H.-S.; Bittman, R. *Biophys. J.* **2004**, *87*, 1722-1731.

5. (a) Obeid, L. M.; Linardic, C. M.; Karolak, L. A.; Hannun, Y. A. *Science* **1993**, *259*,1769-1771. (b) Karasavvas, N.; Erukulla, R. K.; Bittman, R.; Lockshin, R.; Zakeri, Z. *Eur. J. Biochem.* **1996**, *236*, 729-737. (c) Ahn, E. H.; Schroeder, J. J. *Exp. Biol. Med. (Maywood)* **2002**, *227*, 345-353. (d) Chang, Y.-T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J.-M.; Chung, S.-K.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1856-1857.

6. Radin, N. S. *Eksp. Onkol.* **2004**, *26*, 3-10.

7. (a) An allylic hydroxy group or ketone is present in many anticancer agents, as reported in Radin, N. S. *Bioorg. Med. Chem.* **2003**, *11*, 2123-2142. (b) The allylic hydroxyl group of **1** is postulated to be oxidized in cells to an α,β-unsaturated ketone, which undergoes Michael addition with glutathione.

8. Debatin, K. M.; Krammer, P. H. *Oncogene* **2004**, *23*, 2950-2966.

9. (a) Maceyka, M.; Payne, S. G.; Milstien, S.; Spiegel, S. *Biochim. Biophys. Acta* **2002**, *1585*, 193-201. (b) Bektas, M.; Jolly, P. S.; Müller, C.; Eberle, J.; Spiegel, S.; Geilen, C. C. *Oncogene* **2005**, *24*, 178-187.

10. Selzner, M.; Bielawska, A.; Morse, M. A.; Rudiger, H. A.; Sindram, D.; Hannun, Y. A.; Clavien, P. A. *Cancer Res.* **2001**, *61*, 1233-1240.

11. Bieberich, E.; Hu, B.; Silva, J.; MacKinnon, S.; Yu, R. K.; Fillmore, H.; Broaddus, W. C.; Ottenbrite, R. M. *Cancer Lett.* **2002**, 181, 55-64.

12. (a) Bittman, R. *Chem. Phys. Lipids* **2004**, *129*, 111-131. (b) Struckhoff, A. P.; Bittman, R.; Burow, M. E.; Clejan, S.; Elliott, S.; Hammond, T.; Tang, Y.; Beckman, B. S. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 523-532.

13. (a) Cain, K. *Drug Metab. Rev.* **2003**, *35*, 337-363. (b) Sanchis, D.; Mayorga, M.; Ballester, M.; Comella, J. X. *Cell Death Differ.* **2003**, *10*, 977-986. (c) Hajra, K. M.; Liu, J. R. *Apoptosis* **2004**, *9*, 691-704.

14. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

15. For a review of the Dess-Martin oxidation, see: Tohma, H.; Kita, Y. *Adv. Synth. Catal.* **2004**, *346*, 111-124.

16. For a review of Tebbe methylenation, see: Hartley, R. C.; McKiernan, G. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2763-2793.

17. Representative procedure for the preparation of **9**: see page 88.

18. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) A mechanism for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion has been proposed: Scheme 1 of ref 18a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

19. (a) Lowe, S. W.; Lin, A. W. *Carcinogenesis* **2000**, *21*, 485-495. (b) Johnstone, R. W.; Ruefli, A. A.; Lowe, S.W. *Cell* **2002**, *108*, 153-164.

20. Ogretmen, B.; Hannun, Y.A. *Nature Rev. Cancer* **2004**, *4*, 604-616.

21. (a) Volm, M.; Rittgen, W. *Anticancer Res.* **2002**, *20*, 3449-3458. (b) Gallego, M.-A.; Joseph, B.; Hemstrom, T. H.; Tamiji, S.; Mortier, L.; Kroemer, G.; Formstecher, P.; Zhivotovsky, B.; Marchetti, P. *Oncogene* **2004**, *23*, 6282-6291.

22. The results shown in Figure 2 were obtained with a 9:1 mixture of (2*S*,3*R*):(2*S*,3*S*)-**2** vs (2*S*,3*R*)-**1**.

23. Proliferating cells in 48-well plates were incubated with compounds **1** and **2** at 37 °C for 48 h in a CO_2 incubator.

24. Usta, J.; El Bawab, S.; Roddy, P.; Szulc, Z. M.; Hannun, Y. A.; Bielawska, A. *Biochemistry* **2001**, *40*, 9657-9668.

25. (a) Chalfant, C. E.; Kishikawa, K.; Bielawska, A.; Hannun, Y. A. *Methods Enzymol.* **2000**, *312*, 420-428. (b) Chalfant, C. E.; Szulc, Z.; Roddy, P.; Bielawska, A.; Hannun, Y. A. *J. Lipid Res.* **2004**, *45*, 496-506.

26. Kumagai, K.; Yasuda, S.; Okemoto, K.; Nishijima, M.; Kobayashi, S.; Hanada, K. *J. Biol. Chem.* **2005**, *280*, 6488-6495.

27. Lu, X.; Arthur, G.; Bittman, R. *Org. Lett.* **2005**, *7*, 1645-1648.

Chapter 5

1. (a) 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., Jr. *Pharmacol Res.* **2003**, *47*, 373-381. (b) Futerman, A. H.; Hannun, Y. A. *EMBO Reports* **2004**, *5*, 777-782.

2. For reviews of the synthesis of sphingosine, see: (a) Byun, H.-S.; Bittman, R. In *Phospholipids Handbook*, Cevc, G., Ed.; Dekker: New York, 1993, pp 97-140. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, *8*, 1075-1091. (c) Curfman, C.; Liotta, D. *Methods Enzymol.* **1999**, *311*, 391-440. (d) Jung, K.-H.; Schmidt, R. R. in *Lipid Synthesis and Manufacture*, Gunstone, F. D., Ed.; Sheffield Academic Press: Sheffield, U. K., 1999, pp 208-249. (e) Koskinen, P. M.; Koskinen, A. M. P. *Methods Enzymol.* **1999**, *311*, 458-479.

3. (a) Zimmermann, P.; Schmidt, R. R. *Liebigs Ann. Chem.* **1988**, 633-667. (b) Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395-4398. (c) Herold, P.

Helv. Chim. Acta **1988**, *71*, 354-362. (d) Murakami, T.; Hato, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 823-827. (e) Somfai, P.; Olsson, R. *Tetrahedron* **1993**, *49*, 6645-50.

4. (a) Julina, R.; Herzig, T.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 368-373. (b) Solladié-Cavallo, A.; Koessler, J. L. *J. Org. Chem.* **1994**, *59*, 3240-3242. (c) Boutin, R. H.; Rapoport, H. *J. Org. Chem.* **1986**, *51*, 5320-5327. (d) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem*. **2000**, *65*, 7627-7633. (e) Nugent, T. C.; Hudlicky, T. *J. Org. Chem.* **1998**, *63*, 510-520. (f) van den Berg, R. J. B. H. N.; Korevaar, C. G. N.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem*. **2004**, *69*, 5699-5704. (g) Torsell, S.; Somfai, P. *Org. Biomol. Chem.* **2004**, *2*, 1643-1646.

5. Compostella, F.; Franchini, L.; Panza, L.; Prosperi, D.; Ronchetti, F. *Tetrahedron* **2002**, *58*, 4425-4428.

6. Tsunoda, H.; Ogawa, S. *Liebigs Ann. Chem.* **1995**, *2*, 267-277.

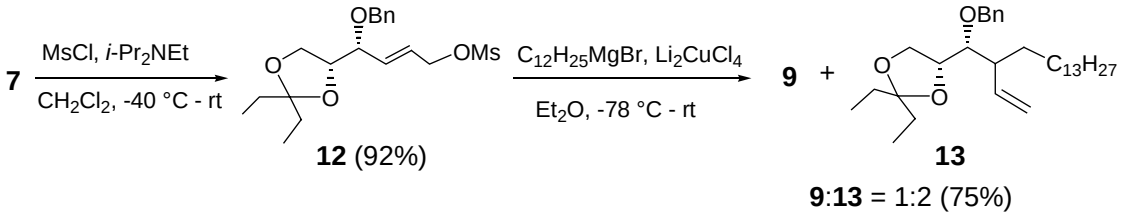
7. For uses of **2** as a glycosyl acceptor, see: (a) Park, T. K.; Lim, I. J.; Danishefsky, S. J. *Tetrahedron Lett.* **1995**, *36*, 9089-9092. (b) Park, T. K.; Kim, I. J.; Hu, S.; Bilodeau, M. T.; Randolph, J. T.; Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc*, **1996**, *118*, 11488-11500. (c) Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1588-1599. (d) Deshpande, P. P.; Kim, H. M.; Zatorski, A.; Park, T.-K.; Ragupathi, G.; Livingston, P. O.; Live, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1600-1614.

8. For reviews of the uses of **3** and its derivatives as synthons, see: (a) Gawroński, J.; Gawrońska, K. *Tartaric and Malic Acids in Synthesis*; John Wiley & Sons: New York, 1999. (b) Ghosh, A. K.; Koltun, E. S.; Bilcer, G. *Synthesis* **2001**, 1281-1301.

9. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

10. Reduction of ester **6** to allylic alcohol **7** with DIBAL was carried out at –78 °C because it was found that the acetal was reduced to release the primary hydroxy group when the temperature was raised to 0 °C.

11. Copper-mediated Grignard reaction of allylic mesylate **12** gave a mixture of **9** and **13**:



12. (a) He, L.; Wanunu, M.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1999**, *64*, 6049-6055. (b) For a postulated mechanism to account for the conversion of a 1,2-diol to a 2-azido-1-ol with inversion, see Scheme 1 of ref. 12a and Mathieu-Pelta, I.; Evans, S. A., Jr. *J. Org. Chem.* **1992**, *57*, 3409-3413.

13. Wang, X.-Z.; Wu, Y.-L.; Jiang, S.; Singh, G. *Tetrahedron Lett.* **1999**, *40*, 8911-8914.

14. For a review of the Staudinger reaction, see: Gololobov, Yu. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353-1406.

15. It should be noted that the ^{13}C NMR spectrum and specific rotation of **2**, a widely used sphingosine derivative in glycosphingolipid synthesis, have not been reported previously.

16. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 1873-1875.

Chapter 6

1. Fujita, T.; Inoue, K.; Yamamoto, S.; Ikumoto, T.; Sasaki, S.; Toyama, R.; Chiba, K.; Hoshino, Y.; Okumoto, T. *J. Antibiot. (Tokyo)* **1994**, *47*, 208-215.

2. For recent reviews, see: (a) Brinkmann, V. *Yonsei Med. J.* **2004**, *45*, 991-997. (b) Chiba, K. *Pharmacol. Ther.* **2005**, in press (e-pub).

3. Gonsette, R. E. *J. Neurol. Sci.* **2004**, *223*, 87-93.

4. Brinkmann, V.; Lynch, K. R. *Curr. Opin. Immunol.* **2002**, *14*, 569-575.

5. Graler, M. H.; Goetzl, E. J. *FASEB J.* **2004**, *18*, 551-553.

6. Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. *Science* **2002**, *296*, 346-349.

7. (a) Sugiyama, S.; Arai, S.; Kiriyama, M.; Ishii, K. *Chem. Pharm. Bull.* **2005**, *53*, 100-102. (b) Seidel, G.; Laurich, D.; Fürstner, A. *J. Org. Chem.* **2004**, *69*, 3950-3952. (c) Adachi, K.; Kohara, T.; Nakao, N.; Arita, M.; Chiba, K.; Mishina, T.; Sasaki, S.; Fujita, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 853-856. (d) Kiuchi, M.; Adachi, K.; Kohara, T.; Minoguchi, M.; Hanano, T.; Aoki, Y.; Mishina, T.; Arita, M.; Nakao, N.; Ohtsuki, M.; Hoshino, Y.; Teshima, K.; Chiba, K.; Sasaki, S.; Fujita, T. *J. Med. Chem.* **2000**, *43*, 2946-2961. (e) Durand, P.; Peralba, P.; Sierra, F.; Renaut, P. *Synthesis* **2000**, 505-506. (f) Kalita, B.; Barua, N. C.; Bezbarua, M.; Bez, G. *Synlett* **2001**, 1411-1414.

8. Hale, J. J.; Yan, L.; Neway, W. E.; Hajdu, R.; Bergstrom, J. D.; Milligan, J. A.; Shei, G.-J.; Chrebet, G. L.; Thornton, R. A.; Card, D.; Rosenbach, M.; Rosen, H.; Mandala, S. *Bioorg. Med. Chem.* **2004**, *12*, 4803-4807.

9. Kiuchi, M.; Adachi, K.; Tomatsu, A.; Chino, M.; Takeda, S.; Tanaka, Y.; Maeda, Y.; Sato, N.; Mitsutomi, N.; Sugahara, K.; Chiba, K. *Bioorg. Med. Chem.* **2005**, *13*, 425-432.

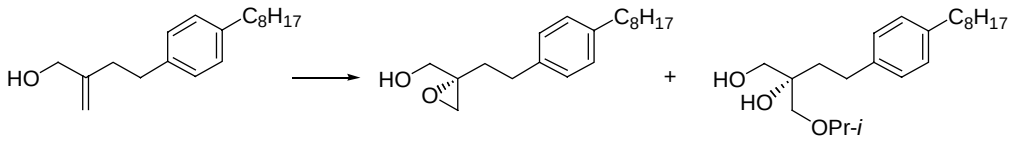
10. For a review of the Sharpless epoxidation, see: Katsuki, T.; Martin, V. S. *Org. React.* **1996**, *48*, 1-299.

11. Ziegler, F. E.; Klein, S. I.; Pati, U. K.; Wang, T. F. *J. Am. Chem. Soc.* **1985**, *107*, 2730-2737. 1,3-Propanediol reacted with 1 equiv of benzyl bromide in the presence of NaH to give 3-(benzyloxy)-1-propanol, which was converted to the bromide (NBS, PPh_3).

12. (a) Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Intl. Ed. Engl.* **1971**, *10*, 330-331. (b) Roberts, J. L.; Borromeo, P. S.; Poulter, C. D. *Tetrahedron Lett.* **1977**, *18*, 1621-1623.

13. Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226-2227.

14. Sharpless epoxidation gave a mixture of epoxide **13** and *O*-isopropyl triol **13'** in a ratio of 1:2 when 1.2 equiv of (+)-DIPT and Ti(OPr-*i*)$_4$ were used.



12 **13** **13'**

ratio of 13:13' = 1:2 (83%)

15. Hatakeyama, S.; Matsumoto, H.; Fukuyama, H.; Mukugi, Y.; Irie, H. *J. Org. Chem.* **1997**, *62*, 2275–2279. For an example of the application of this method to prepare a chiral α-substituted alanine substructure by intramolecular opening of a 2-methyl-2,3-epoxy-1-trichloroacetimidate intermediate with $BF_3{\cdot}OEt_2$, see: Liu, D.-G.; Wang, B.; Lin, G.-Q. *J. Org. Chem.* **2000**, *65*, 9114-9119.

16. For a review of the Dess-Martin oxidation, see: Tohma, H.; Kita, Y. *Adv. Synth. Catal.* **2004**, *346*, 111-124.

17. Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696-5704.

18. Pihlaja, K.; Kleinpeter, E. In: *Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis*, VCH: New York 1994, pp. 55–56. For a recent example of the chemical shifts of the quaternary carbon of 5- and 6-membered ring acetonides, see: Ayad, T.; Génisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582.

Chapter 7

1. (a) Lohmeyer, M.; Bittman, R. *Drugs Future* **1994**, *19*, 1021–1037. (b) Houlihan, W. J.; Lohmeyer, M.; Workman, P.; Chen, S. H. *Med. Res. Rev.* **1995**, *15*, 157–223. (c) Bittman, R.; Arthur, G. Antitumor ether lipids: biological and biochemical effects. In: *Liposomes: Rational Design*, A. S. Janoff, Ed.; Marcel Dekker, New York, 1999, pp 125–144. (d) Gajate, C.; Mollinedo, F. *Curr. Drug Metab.* **2002**, *3*, 491-525. (e) Mollinedo, F.; Gajate, C.; Martin-Santamaria, S.; Gago, F. *Curr. Med. Chem.* **2004**, *11*, 3163-3184.

2. (a) Guivisdalsky, P. N.; Bittman, R.; Smith, Z.; Blank, M. L.; Snyder, F.; Howard, S.; Salari, H. *J. Med. Chem.* **1990**, *33*, 2614-2621. (b) Marino-Albernas, J. R.; Bittman, R.; Peters, A.; Mayhew, E. *J. Med. Chem.* **1996**, *39*, 3241-3247.

3. Bauer, F.; Ruess, K.-P.; Liefländer, M. *Liebigs Ann. Chem.* **1991**, 765-768.

4. Erukulla, R. K.; Bittman, R. *Synth. Commun.* **1994**, *24*, 2765-2770.

5. Lu, X.; Rengan, K.; Bittman, R.; Arthur, G. *Oncol. Rep.* **1994**, *1*, 933-936.

6. For reviews on the synthesis of *C*-glycosides, see: (a) Beau, J.-M.; Gallagher, T. *Top. Curr. Chem.* **1997**, *187*, 1-54. (b) Nicotra, F. *Top. Curr. Chem.* **1997**, *187*, 55-83. (c) Du, Y.; Linhardt, R. J.; Vlahov, I. R. *Tetrahedron* **1998**, *54*, 9913-9959.

7. (a) Yang, G.; Franck, R. W.; Bittman, R.; Samadder, P.; Arthur, G. *Org. Lett.* **2001**, *3*, 197-200. (b) Yang, G.; Franck, R. W.; Byun, H.-S.; Bittman, R.; Samadder, P.; Arthur, G. *Org. Lett.* **1999**, *1*, 2149-2151.

8. Yadav, J. S.; Chander, M. C.; Joshi, B. V. *Tetrahedron Lett.* **1988**, *29*, 2737-2740.

9. Data for (*S*)-1-*O*-(4'-methoxybenzyl)-2-*O*-methylbut-3-yn-1,2-diol (**5**): see page 128.

10. Rolf, D.; Gray, G. R. *J. Am. Chem. Soc.* **1982**, *104*, 3539-3541.

11. Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976-4978.

12. Overkleeft, H. S.; van Wiltenburg, J.; Pandit, U. K. *Tetrahedron* **1994**, *50*, 4215–4224.

13. Preparation of (*S*)-4-(2',3',4',6'-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-2-methoxybut-3-yn-1-ol (**8**): see page 128.

14. Data for (*S*)-1-(2',3',4',6'-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-3-methoxy-4-(hexadecyloxy)but-1-yne (**9**): see page 129.

15. Data for (*S*)-1-(*β*-D-galactopyranosyl)-3-methoxy-4-(hexadecyloxy)butane (**3**): see page 129.

Chapter 8

1. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771-2784.

2. For recent reviews, see: (a) Van Kaer, L. *Nat. Rev. Immunol.* **2005**, *5*, 31-42. (b) Hayakawa, Y.; Godfrey, D. I.; Smyth, M. J. *Curr. Med. Chem.* **2004**, *11*, 241-252.

3. Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631-1641.

4. Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Intl. Ed. Engl.* **2004**, *43*, 3818-3822.

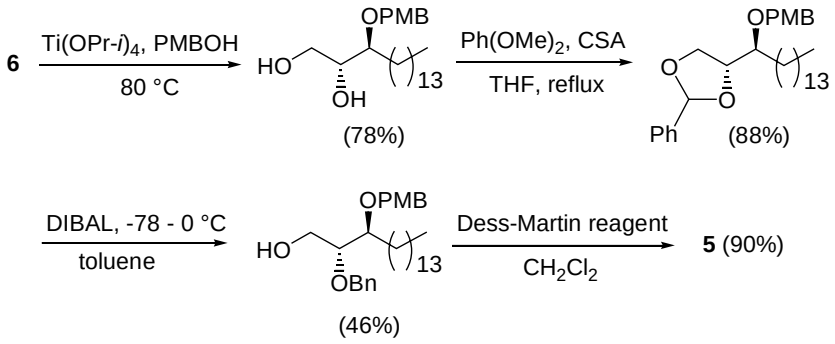
5. Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077-4080.

6. Tomiyama, H.; Yanagisawa, T.; Nimura, M.; Noda, A.; Tomiyama, T. US Patent 6,635,622, 2001.

7. Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. *Tetrahedron Lett.* **2005**, *46*, 5043-5047.

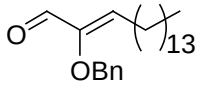
8. Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, 67, 4475-4486. Acetylene **4** was obtained by *C*-glycosidation of the sugar acetate using 1-tri-*n*-butylstannyl-2-trimethylsilylacetylene in the presence of trimethylsilyl triflate, followed by desilylation of the initial product.

9. Compound **5** was synthesized as shown below:



10. Lu, X.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 5433-5438.

11. The structure of compound **7** is:



12. (a) Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976-4978. (b) Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479-1482.

13. Nolen, E. G.; Watts, M. M.; Fowler, D. J. *Org. Lett.* **2002**, *4*, 3963-3965.

14. Wang, T.-L.; Hu, X. E.; Cassady, J. M. *Tetrahedron Lett.* **1995**, *36*, 9301-9304.

15. Kokotos, G.; Padron, J. M.; Noula, C.; Gibbons, W. A.; Martin, V. S. *Tetrahedron: Asymmetry* **1996**, *7*, 857-866.

16. Padron, J. M.; Martin, V. S.; Hadjipavlou-Litina, D.; Noula, C.; Constantinou-Kokotou, V.; Peters, G. J.; Kokotos, G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 821-826.

17. Yadav, J. S.; Deshpande, P. K.; Sharma, G. V. M. *Tetrahedron* **1990**, *46*, 7033-7046.

18. Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696-5704.

19. Benedetti, F.; Berti, F.; Norbedo, S. *Tetrahedron Lett.* **1998**, *39*, 7971-7974.

20. Caron, M.; Carlier, P. R.; Sharpless, K. B. *J. Org. Chem.* **1988**, *53*, 5185-5187.

21. Pihlaja, K.; Kleinpeter, E. In: *Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis*, VCH: New York, 1994, pp. 55–56. For a recent example of the

chemical shifts of the quaternary carbon of 5- and 6-membered ring acetonides, see: Ayad, T.; Génisson, Y.; Verdu, A.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 579-582.

22. The positions and configurations of the functional groups of the compounds shown in Scheme 8 cannot undergo changes during these reactions.

23. Mootoo, D. R.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1986**, 1570-1571.

24. Cipolla, L.; Lay, L.; Nicotra, F. *J. Org. Chem.* **1997**, *62*, 6678-6681. For other examples of a benzyloxy group acting as a nucleophile, see: (a) Dehmlow, H.; Mulzer, J.; Seilz, C.; Strecker, A. R.; Kohlmann, A. *Tetrahedron Lett.* **1992**, *33*, 3607-3610. (b) Yang, B.-H.; Jiang, J.-Q.; Ma, K. and Wu, H.-M. *Tetrahedron Lett.* **1995**, *36*, 2831-2834.

25. Lu, X.; Arthur, G.; Bittman, R. *Org. Lett.* **2005**, *7*, 1645-1648.