Asymmetric Syntheses of New Analogues of Ceramide

by

Jiong Chun

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.

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Abstract

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Research Adviser: Professor Robert Bittman

Abstract

This dissertation presents the asymmetric total syntheses of natural occurring ceramides and analogues bearing modifications in the sphingoid backbone. Also included in this dissertation is a novel methodology for the preparation of chiral propargylic alcohols.

Chapter 1 presents the syntheses of ceramide analogues in which the trans double bond of natural occurring ceramide is incorporated a benzene or pyridine ring. Two efficient and stereoselective routes are described. The first route involves the addition of an aryllithium or a heteroaryllithium reagent to an L-serine-derived aldehyde, followed by hydrolysis of the oxazolidine, liberation of the amino group, and *N*-acylation. The second route utilizes a Heck reaction to afford an (*E*)-α,β-unsaturated ester, then Sharpless asymmetric dihydroxylation for the construction of the desired chirality. Regioselective azide followed by simultaneous reduction of the azido and ester groups

with lithium aluminum hydride and *N*-acylation complete the synthesis of an aryl-ceramide analogue in eight steps and 28% overall yield.

Chapter 2 presents a simple and convenient method for the regiospecific introduction of unsaturation (both as a single trans double bond and as a conjugated diene) into the sphingoid backbone of ceramide by employing organosulfur chemistry. *C*-Alkylation of a β-ketosulfoxide and elimination of PhS(O)H provided a conjugated ketone. Alkylation of a β-ketosulfone and desulfonation with Al(Hg) gave a ketone which bears a double bond. Diastereoselective reduction, hydrolysis of the oxazolidine ring, liberation of the amino group, and *N*-acylation provided the (*E*)-$\Delta^{4,6}$- and (*E*)-Δ^{6}-ceramide analogues.

Chapter 3 presents the synthesis of a new analogue of ceramide with deuterium in the sphingoid backbone for quantification of sphingosine 1-phosphate in biological samples by stable isotope dilution mass spectrometry. Addition of lithium pentadecyne-d_{27} to Garner aldehyde in the presence of HMPA resulted in high erythro selectivity. Red-Al reduction and acid hydrolysis provided sphingosine-d_{27}. Sphingosine-d_{27} phosphate was obtained by treatment of sphingosine-d_{27} with ATP in the presence of sphingosine kinase.

Chapter 4 presents the first synthesis of the 5*R* and 5*S* diastereoisomers of the *N*-acyl derivatives of 5-hydroxy-3-sphingenine, which represent regioisomers of natural occurring 2*S*,3*R*-ceramide. The key steps include the synthesis of α,β-unsaturated ketone intermediates from *N*-Cbz- and *N*-Boc-L-serine and diastereoselective reduction of the enones. The configuration at the new carbinol center was deduced by proton NMR

analysis of (*R*)- and (*S*)-Mosher [methoxy(trifluoromethyl)phenylacetate] ester derivatives.

Chapter 5 presents the first synthesis of the 6*S* and 6*R* diastereoisomers of 6-hydroxy-4-sphingenine-containing ceramides, which were found recently in human skin. Chiral propargylic alcohols were prepared via Sharpless asymmetric dihydroxylation of an α,β–unsaturated ester and allylic chloride. The key oxazolidine intermediates were obtained by nucleophilic addition of lithiated tert-butyldimethyl-protected propargylic ethers to L-serine-derived aldehyde, respectively. Acid-mediated deprotection of the oxazolidine, followed by *N*-acylation and Birch reduction, complete the syntheses of the target molecures.

Chapter 6 presents a simple and efficient method for the preparation of chiral propargylic alcohols which are important chiral building blocks for the synthesis of natural and unnatural products. High enantiomeric excess was attained by asymmetric dihydroxylation of α,β-unsaturated esters and conversion to 4-chloromethyl-1,3-dioxalane intermediates, followed by base-mediated double elimination.

Chapter 7 presents the syntheses of ceramide analogues in which the trans double bound of naturally occurring ceramide was changed to an allene. Hydride ($LiAlH_4$) addition to propargylic alcohols (S_N2' reaction) and elimination installed the allene group.

Dedicated to those who made this thesis possible:

My parents, my wife

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Abbreviations

AD	asymmetric dihydroxylation
DCC	*N,N'*-dicyclohexylcarbodiimide
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
ee	enantiomeric excess
HMPA	hexamethylphosphoramide
HWE	Horner-Wadsworth-Emmons
PCC	pyridinium chlorochromate
PMP	*p*-methoxyphenyl
SFV	Semliki Forest virus
TBAF	tetra-*n*-butylammonium fluoride
THF	tetrahydrofuran
TMS	trimethylsilyl
TBS	*tert*-butyldimethylsilyl
TLC	thin-layer chromatography

Chapter 1

Synthesis of Ceramide Analogues Having the C(4)-C(5) Bond of the Long-chain Base As Part of an Aromatic or Heteroaromatic System

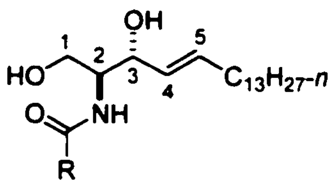
Abstract

Two efficient and stereoselective methods are described for the preparation of aryl and heteroaryl ceramide analogues **2** and **3**. The first route involves the addition of an aryllithium or a heteroaryllithium reagent (**6a** or **26a**, respectively) to the L-serine-derived aldehyde **5**, followed by hydrolysis of the oxazolidine, liberation of the amino group, and *N*-acylation. The second route, which was used to prepare aryl-ceramide analogue **2** in eight steps and 28% overall yield starting with 3-bromobenzaldehyde, utilizes a Heck reaction to afford (*E*)-α,β-unsaturated ester **19**, then Sharpless asymmetric dihydroxylation for the introduction of the desired chirality at C-2 and C-3. Regioselective α-azidation of α-*O*-nosyl-β-hydroxyester **21** with sodium azide, followed by $LiAlH_4$ reduction of the azido and ester groups and *N*-acylation, complete the synthesis of aryl-ceramide analogue **2**.

Introduction

Ceramide (*N*-acylsphingosine, **1**) is a long-chain aliphatic 2-amido-1,3-diol with a C(4),C(5)-trans double bond; the predominant long-chain base found in mammalian cells has 18 carbons (Chart 1).[1] Ceramide is an important signaling molecule that has been implicated in a myriad of physiological events, including the regulation of cell growth and differentiation, inflammation, and apotosis.[2] The mechanisms by which ceramide regulates cellular functions involve its ability to stimulate kinases and protein phosphatases.[2]

Chart 1



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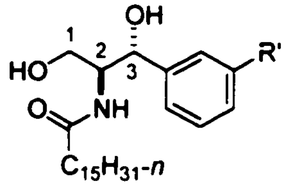
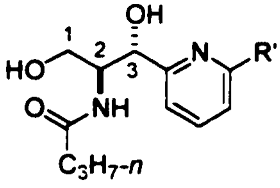


2 R' = $C_{12}H_{25}$-n



3

Ceramide is one of the major lipid components of human skin. It comprises about 35% of the total lipids in human stratum corneum (the apical skin layer).[3] Its role in normal skin tissue function includes maintenance of the permeability barrier and water-binding properties of the outer layer.[4]

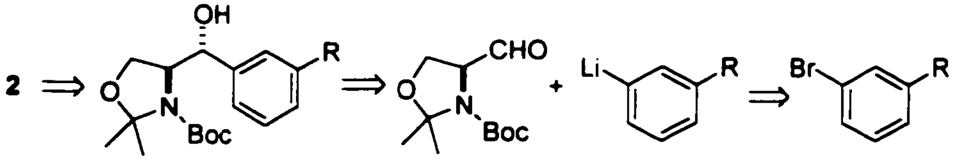
Ceramides are the minimally required sphingolipids for activation of membrane fusion of Semliki Forest virus (SFV).[5] The C(4)-C(5) trans double bond in the sphingoid base of naturally occurring **1** may be crucial for the ceramide's capacity to modulate various fundamental biological functions.[6] Indeed, it was shown very recently that an unnatural ceramide analogue having a trans C(5)-C(6) double bond is unable to support fusion of SFV.[7] In order to obtain more information about viral fusion at the molecular level, we set out to prepare a series of new ceramide analogues in which unsaturated functional groups are incorporated. In the present study, the C(4)-C(5) double bond of the long-chain base was incorporated into an aromatic ring such as benzene and pyridine

(Chart 1, compounds **2** and **3**). These analogues will allow us to test whether a C(4)-C(5)-double bond that is part of an aromatic or heteroaromatic system can substitute for the aliphatic double bond in the long-chain base of **1** in the activation of fusion of SFV with target membranes.

Results and Discussion

A. Synthesis of Ceramide Analogue 2.

Scheme 1. Retrosynthetic Plan

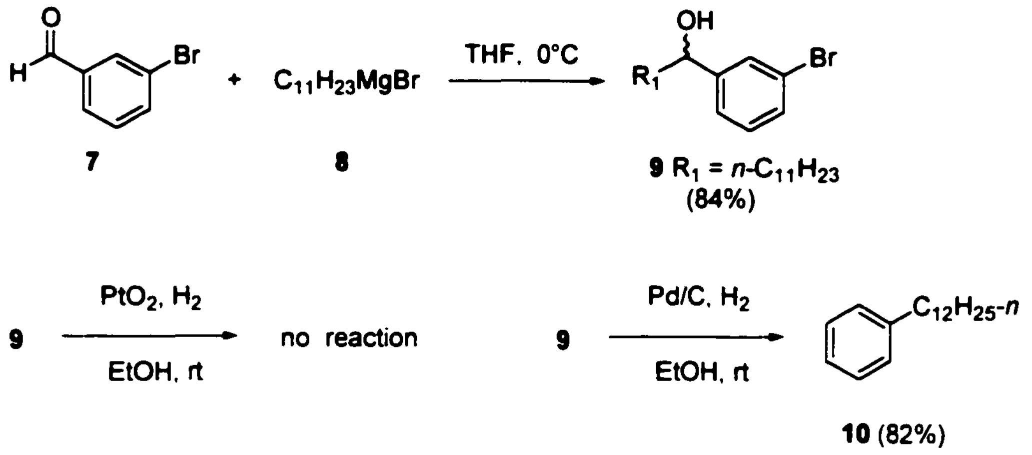


Oxazolidine Intermediate 4 **Aldehyde 5** **6a** **Aryl Bromide 6**

(1) Retrosynthetic Plan: As illustrated in Scheme 1, the synthesis of ceramide analogue **2** involves the key oxazolidine intermediate **4**, which can be prepared by the addition of an aryllithium **6a** to the L-serine-derived aldehyde **5**. Aryllithium **6a** can be prepared by the halogen-lithium exchange reaction from aryl bromide **6**.

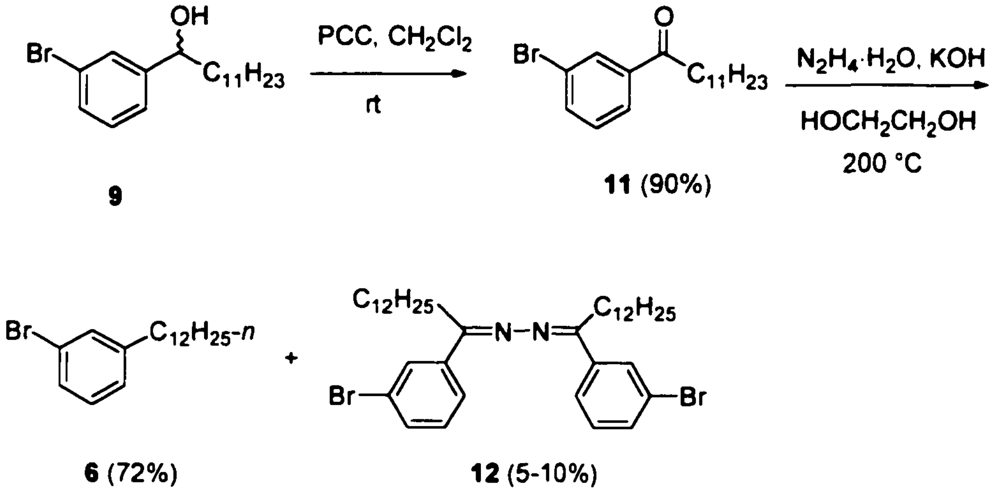
(2) Synthesis of Aryl Bromide 6. The initial attempt to prepare compound **6** by using plantium oxide as catalyst was not successful. No reaction took place under H_2 for two days (Scheme 2). However, when Pd/C was used as the catalyst, both the hydroxy and bromine groups were removed to afford compound **10**.

Scheme 2. Attempted Synthesis of Aryl Bromide 6 by Catalytic Hydrogenolysis



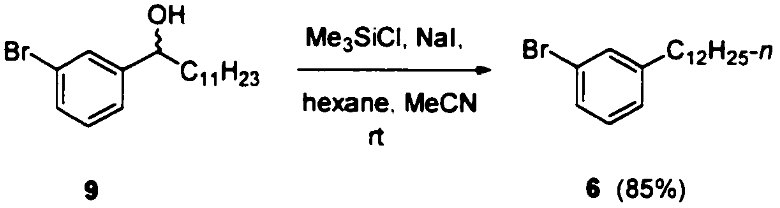
A successful synthesis of **6** is shown in Scheme 3. After benzylic alcohol **9** was oxidized to ketone **11**. Wolff-Kishner reduction[8] of the carbonyl group was carried out. Aryl bromide **6** was obtained in 72% yield accompanied by small amount of byproduct **12**.

Scheme 3. Synthesis of Aryl Bromide 6 by Wolff-Kishner Reduction



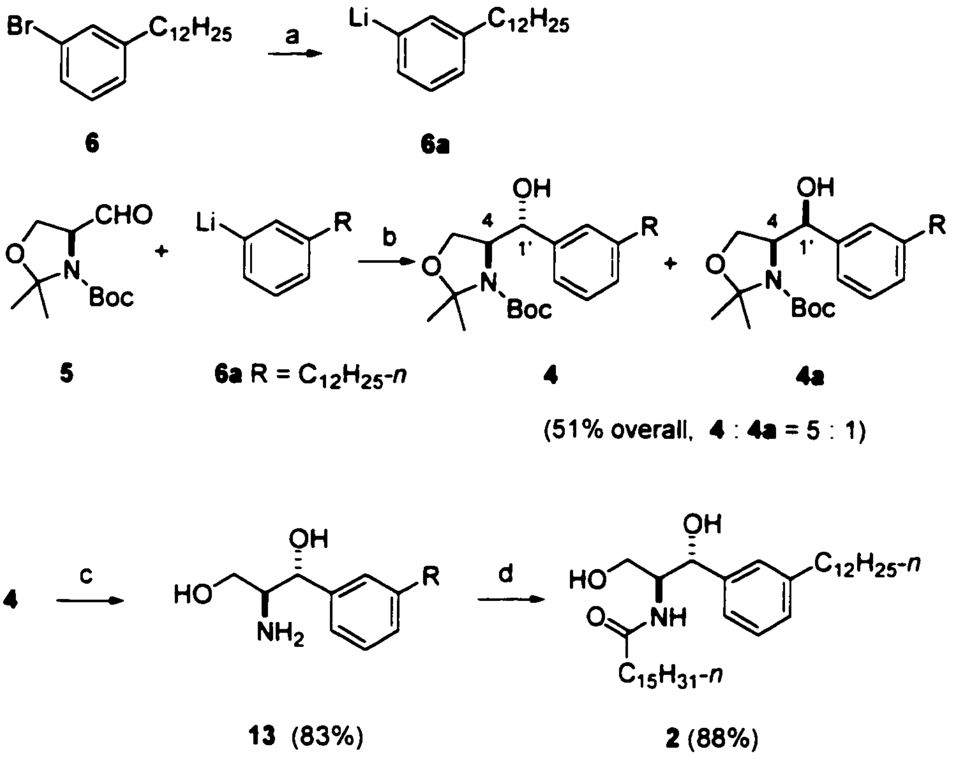
Since the Wolff-Kishner reduction was carried out at very high temperature (200 °C) and for a long time (over 12 h) and the yield was only 72%, another method to prepare **6** was sought. As shown in Scheme 4, treatment of alcohol **9** with chlorotrimethylsilane in the presence of sodium iodide in acetonitrile-hexane[9] afforded aryl bromide **6**. This reaction was very mild, and the yield (85%) was good.

Scheme 4. An efficient Method to Prepare Aryl Bromide 6



(3) Synthesis of Aryl-ceramide Analogue 2. The development of new methods for the synthesis of sphingosine and its stereoisomers has been an active field of research.[10] In 1988, Garner and coworkers used L-serine aldehyde **5** ("Garner aldehyde," which is prepared from L-(*N*-Boc) serine methyl ester) for the synthesis of sphingosine.[11a] One of the routes used in the present work started with Garner aldehyde **5** as the chiral synthon to prepare the target compounds **2** and **3**. In this route the stereochemistry at C-2 of the target ceramide analogue is introduced from the L-serine-derived aldehyde **5**. Our approach to **2**, which represents an analogue of natural ceramide (**1**) in which the aliphatic trans double bond is incorporated into a benzene ring, is shown in Scheme 5. Aryl bromide **6** was lithiated (*n*-BuLi, THF, -42 °C) and reacted with aldehyde **5** to give a mixture of *erythro*-**4** and *threo*-**4a** in 51% overall yield. The ratio of **4**:**4a** was found to be 5:1 by high-temperature ^{1}H-NMR spectroscopy.[12] Addition of 2 equiv of HMPA[13] did not improve the diastereoselectivity significantly. After diastereomers **4** and **4a** were separated chromatographically, acid hydrolysis of **4** (1 M HCl in THF, 70 °C) provided D-*erythro*-sphingosine **13**. Ceramide analogue **2** was obtained by *N*-acylation of sphingosine **13** with *p*-nitrophenyl palmitate.

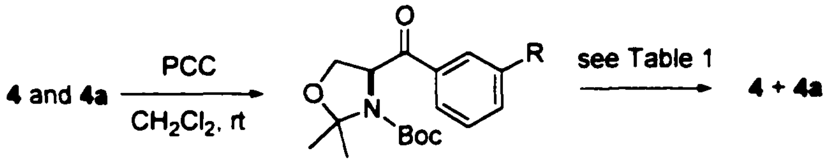
Scheme 5. Synthesis of Ceramide Analogue 2[a]



[a]Reagents and conditions: (a) *n*-BuLi, THF, -42 °C; (b) THF, -42 °C-rt; (c) 1 M HCl, THF, 70 °C; (d) p-$O_2NC_6H_4CO_2C_{15}H_{31}$-*n*, THF, rt.

(4) Diastereoselective Reduction of L-(*N*-Boc)-oxazolidine Ketone 11. Scheme 6 outlines an improved chirospecific route to **2**. For induction of stereochemistry at C-3 of the target ceramide analogue, ketone **14** was prepared by PCC oxidation of a mixture of **4** and **4a**, and several reducing agents were screened for attempted diastereoselective reduction. As shown in Table 1, high erythro selectivity was observed with DIBAL, which gave *erythro*-**4**/*threo*-**4a** in a ratio of 26:1. However, the undesired threo diastereomer was formed as the major product with the other reducing agents we used. $LiBH_4$ and $NaBH_4$ gave a ~3:1 ratio of threo/erythro isomers; Red-Al also resulted in threo selectivity (**4a**:**4**, 2.3:1.0 ratio), in agreement with previous studies on the reduction of a ketone linked to an *N*-Boc-protected oxazolidine.[8c,15]

Scheme 6. Oxidation of 4, 4a and Diastereoselective Reduction of 14



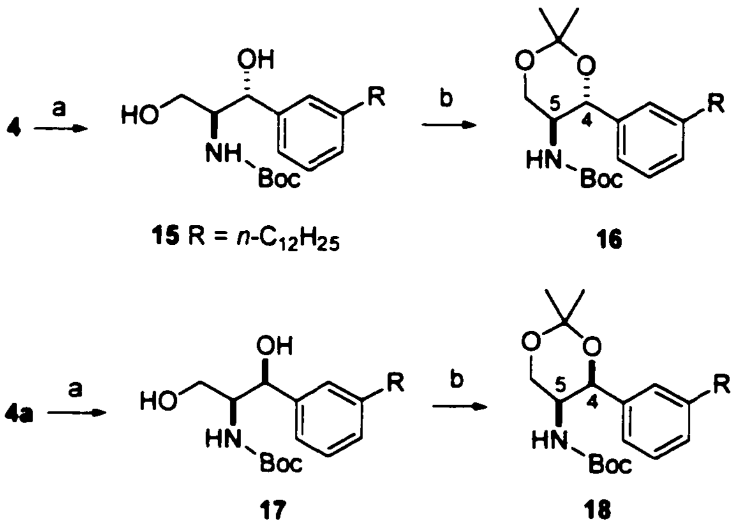
14 R = n-$C_{12}H_{25}$ (85%)

Table 1. Diastereoselective Reduction of 14

reducing agent	solvent	temp (°C)	time (h)	overall yield (%)	ratio (**4**:**4a**)
DIBAL	THF	0	0.5	94	26:1
$NaBH_4$	MeOH	0-rt	2	90	1.0:3.2
$LiBH_4$	MeOH	0-rt	2	93	1.0:2.9
Red-Al	toluene	0	2	92	1.0:2.3

(5) Stereochemistry at C(3) of Ceramide Analogue 2. To establish the configurations of **4** and **4a**. **4** was converted to the corresponding acetonide **16** (and **4a** afforded **18**) in two steps (Scheme 7).[14,16] First, the oxazolidine group was removed by treatment with an acidic resin (Amberlyst 15) in methanol, forming **15**; then, acetalization (2,2-dimethoxypropane, PPTS, CH_2Cl_2) afforded **16**. Since the protons on C(4) and C(5) occupy the axial position in the chair conformation of **16**, erythro derivative **16** is expected to have a larger coupling constant ($J_{4,5}$ ~10 Hz) than threo derivative **18** ($J_{4,5}$ ~1-3 Hz).[14] The configurations of **16** and **18** were confirmed by their coupling constants ($J_{4,5}$ = 9.3 Hz in **16** and $J_{4,5}$ = 1.3 Hz in **18**).

Scheme 7. Synthesis of Acetonide 16 and 18[a]



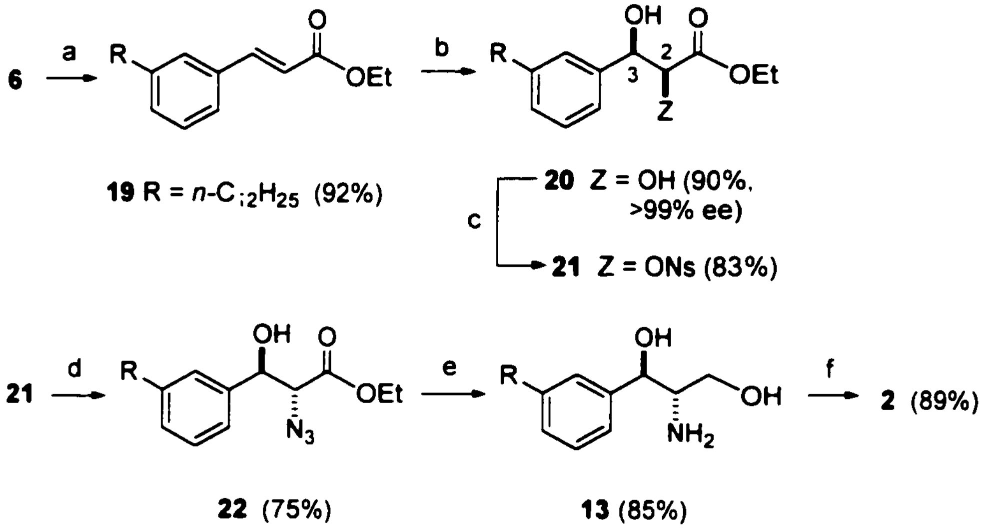
15 R = *n*-$C_{12}H_{25}$ **16**

17 **18**

[a]Reagents and conditions: (a) Amberlyst 15, MeOH, rt; (b) $Me_2C(OMe)_2$, PPTS, CH_2Cl_2, rt.

(6) Conversion of 3-Bromobenzaldehyde (7) to 2. Scheme 8 outlines a route to aryl-ceramide analogue **2** from α,β-unsaturated ester **19** via a Heck reaction.[17] Treatment of *m*-bromododecylbenzene **6** with ethyl acrylate in the presence of a catalytic amount of tetrakis(triphenylphosphine)Pd(0) at 120 °C afforded cinnamate derivative **19** in 92% yield. Sharpless asymmetric dihydroxylation[18] of **19** provided diol ester **20** in 90% yield and >99% ee.[19] Regioselective α-azidation of diol ester **20** was achieved via nosylate intermediate **21**. The selectivity of the mononosylation reaction at C-2 of diol **20** may arise because of intramolecular hydrogen-bonding between the C-3 hydroxy group and the carbonyl group[20a] or the difference in acidity of the two hydroxy groups.[20b] Nosylate **21** was obtained in 83% yield and no β-mononosylate was formed. Nucleophilic substitution of nosylate **21** with NaN_3 in DMF at 55 °C provided azido ester **22** in 75% yield.

Scheme 8. Syntheses of Aryl-Substituted Ceramide Analogue 2[a]

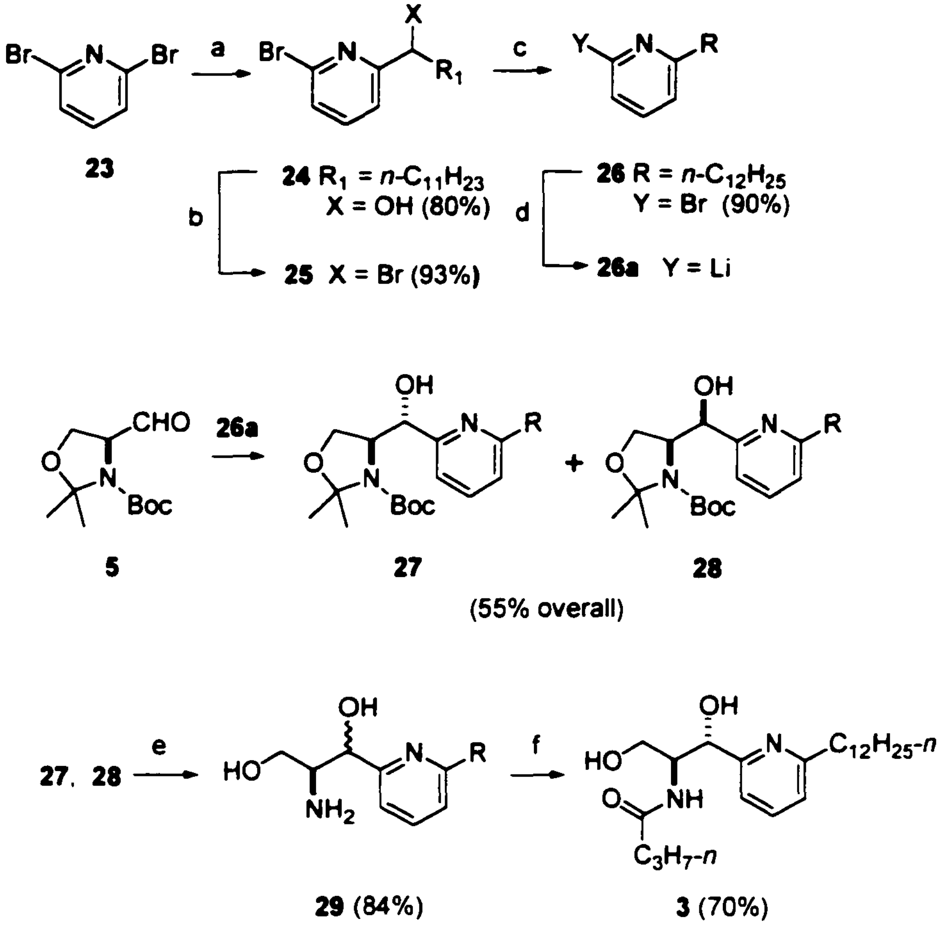


[a]Reagents and conditions: (a) $CH_2=CHCO_2Et$, $(Ph_3P)_4Pd(0)$, Et_3N, DMF, 120 °C; (b) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH/H_2O 1/1, rt; (c) *p*-$O_2NC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C; (d) NaN_3, DMF, 55 °C; (e) $LiAlH_4$, THF, 65 °C; (f) *p*-$O_2NC_6H_4CO_2C_{15}H_{31}$-*n*, THF, rt.

The conversion of **22** to **2** was achieved in 76% overall yield via the corresponding sphingosine **13**. The latter was obtained in 85% yield by $LiAlH_4$ reduction of azido ester **22**. This eight-step synthesis starting from commercially available 3-bromobenzaldehyde provided ceramide analogue **2** in 28% overall yield.

B. Synthesis of Heteroaryl-ceramide Analogue 3. Compound **3** represents an analogue of natural ceramide in which the trans double bond is incorporated into a pyridine ring. The synthesis of **3** (Scheme 9) started with commercially available 2,6-dibromopyridine (**23**), which was lithiated at –78 °C and reacted with dodecanal to give alcohol **24** in 80% yield. The alcohol was converted to bromide **25** in 93% yield by Mitsunobu reaction.[21] Reduction of the benzylic bromide with sodium borohydride in DMSO provided **26** in 90% yield. Lithiation of **26** followed by reaction with Garner aldehyde **5** gave a mixture of *erythro*-**27** and *threo*-**28**; the ratio of **27**:**28** was estimated to be ~5:1 by ^{1}H-NMR spectroscopy. Diastereoisomers **27** and **28** could not be separated by column chromatography. Hydrolysis of **27** and **28** (1 M HCl, THF, 70 °C, 5 h) resulted in the formation of diastereomeric sphingoid alcohol **29**. Since chromatographic separation of the diastereoisomers at this stage was still difficult, sphingosine analogue **29** was *N*-acylated with *p*-nitrophenyl butyrate. Fortunately, the ceramide diastereoisomers were separated by column chromatography, and the desired D-*erythro* stereoisomer **3** was obtained in 70% yield.[22]

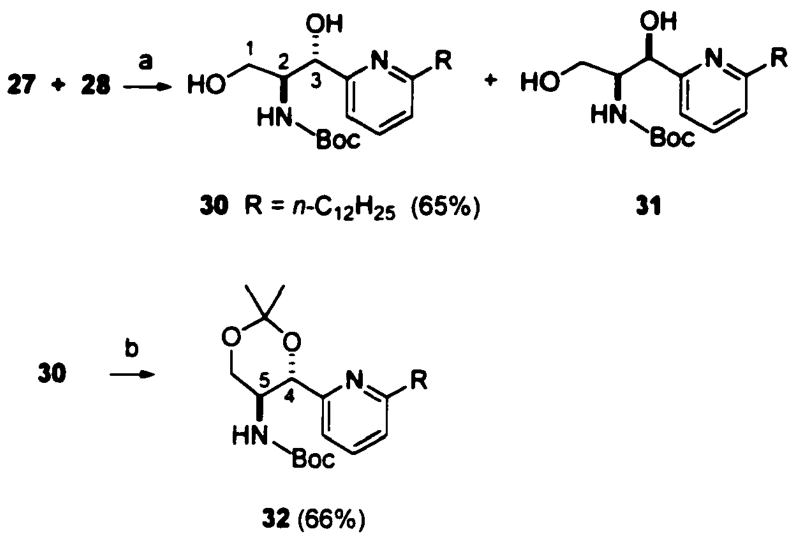
Scheme 9. Synthesis of Ceramide Analogue 3[a]



[a]Reagents and conditions: (a) (i) n-BuLi. THF. -78 °C: (ii) $n\text{-}C_{11}H_{23}CHO$. -78 °C–rt: (b) NBS. Ph_3P. CH_2Cl_2. 0 °C–rt: (c) $NaBH_4$. DMSO. rt: (d) n-BuLi. THF. -78 °C: (e) 1 M HCl. THF. 70 °C: (f) $p\text{-}O_2NC_6H_4CO_2C_3H_7\text{-}n$. THF. rt.

In order to establish the configuration at C(3) in **30**, a mixture of **27** and **28** was converted to **30** and **31** (Scheme 10). Careful separation of the diastereomers by column chromatography gave **30** in 65% yield. Conversion of **30** to the corresponding acetonide **32** and NMR analysis ($J_{4,5}$ = 9.6 Hz) confirmed the absolute configuration of **30**.

Scheme 10. Synthesis of Acetonide 32[a]



[a]Reagents and conditions: (a) Amberlyst 15, MeOH, rt; (b) $Me_2C(OMe)_2$, PPTS, CH_2Cl_2, rt.

Experimental Section

General Information. Melting points were measured on a Hoover capillary melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded at 400-MHz and 100-MHz on a Bruker spectrometer, respectively, and were referenced to the residual $CHCl_3$ at 7.24 (^1H) and 77.00 ppm (^{13}C). $CDCl_3$ was the only solvent used for the NMR analyses unless otherwise indicated. IR spectra were recorded on a Perkin-Elmer 1600-series FT-IR spectrophotometer, and $CHCl_3$ was used as the sole solvent. Optical rotations were measured in a 1.0-dm cell on a JASCO Model DIP-140 digital polarimeter. High-resolution mass spectra were recorded the University of California at Riverside. THF was distilled from sodium and benzophenone immediately before use. CH_2Cl_2 and DMF were dried over CaH_2. Spectral-grade benzene was distilled from sodium wire. Flash chromatography and TLC were carried out with Merck silica gel 60 (230-400 ASTM mesh) and Merck $60F_{254}$ (0.25-mm thick) sheets, respectively. $LiAlH_4$, $NaBH_4$, AD-mix-α/β, and *p*-nitrophenyl butyrate were purchased from Sigma-Aldrich. Sodium azide and 2,6-dibromopyridine were purchased from Lancaster and Acros, respectively. Garner aldehyde **5** was prepared from *N*-Boc-L-serine methyl ester as described previously.[11b,c] Mosher esters were prepared as described previously.[23] *p*-Nitrophenyl palmitate was prepared by the reaction of palmitic acid with *p*-nitrophenol in CH_2Cl_2 in the presence of DCC and DMAP. Tetrakis(triphenylphosphine)Pd(0) was prepared according to a reported procedure.[24]

1-Bromo-3-(1'-hydroxydodecyl)benzene (9). A suspension of 0.48 g (20 mmol) of magnesium powder in 15 mL of dry THF was treated dropwise with a solution of 5.0 g (21 mmol) of 1-bromoundecane in 20 mL of dry THF under Ar at rt. The reaction mixture was stirred until almost all of the magnesium metal reacted (~3 h). After the reaction mixture was chilled to 0 °C, a solution of 1.9 g (10 mmol) of 3-bromobenzaldehyde in 20 mL of THF was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 3 h, and then poured into 20 mL of ice-cold 1 M HCl solution. The product was extracted with Et_2O (3 × 40 mL). The combined organic phase was washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (hexane/EtOAc 4:1) gave 2.84 g (84%) of the desired alcohol **9** as a low-melting white solid: IR 3601, 3456 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.12-1.50 (m, 18H), 1.55-1.90 (m, 2H), 2.30 (s, 1H), 4.56 (t, 1H, J = 5.9 Hz), 7.05-7.24 (m, 2H), 7.30-7.40 (m, 1H), 7.45 (s, 1H); ^{13}C NMR δ 14.08, 22.64, 25.63, 29.30, 29.42, 29.48. 29.54. 29.57. 29.59. 31.87. 39.06. 73.89. 122.47. 124.46. 128.94. 129.90. 130.36. 147.24.

1-Bromo-3-dodecylbenzene (6). To a mixture of Me_3SiCl (1.3 g. 12 mmol). NaI (1.8 g. 12 mmol). and MeCN (0.59 g. 12 mmol) was added a solution of 0.68 g (2.0 mmol) of benzylic alcohol **6** in 2 mL of hexane at rt. The reaction mixture was stirred at this temperature under N_2 until the disappearance of alcohol **9** was noticed by TLC (~48 h). The reaction mixture was then diluted with 20 mL of water. extracted with hexane (3 × 30 mL). and dried (Na_2SO_4). Concentration gave a liquid residue that was purified by flash chromatography (hexane). giving 0.55 g (85%) of product **7** as a colorless oil: ^1H NMR δ 0.86 (t. 3H. J = 7.0 Hz). 1.10-1.40 (m. 18H). 1.50-1.60 (m. 2H). 2.54 (t. 2H. J = 7.8 Hz). 7.06-7.14 (m. 2H). 7.23-7.31 (m. 2H): ^{13}C NMR δ 14.13. 22.69. 29.21. 29.35. 29.45. 29.55. 29.64. 29.66. 31.26. 31.92. 35.63. 122.29. 127.06. 128.65. 129.75. 131.42. 145.29.

***N-tert*-Butoxycarbonyl (4*S*)-4-[1'-(3''-Dodecylphenyl)hydroxymethyl]-2,2-dimethyl-1,3-oxazolidine (4, 4a).** To a solution of 0.39 g (1.2 mmol) of **6** in 10 mL of dry THF at –42 °C was added 0.5 mL (1.25 mmol) of *n*-BuLi (a 2.5 M solution in hexane) dropwise under N_2. After the mixture was stirred for 1 h. a solution of 0.23 g (1.0 mmol) of aldehyde **5** in 5 mL of dry THF was added dropwise over a 5-min period. The mixture was stirred at –42 °C for 3 h. The reaction was quenched by addition of aqueous saturated $NaHCO_3$ solution (5 mL). The organic phase was separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organic phase was washed twice with brine. dried ($MgSO_4$). and evaporated. Column chromatography (hexane/EtOAc 4:1) gave 242 mg (51%) of a mixture of **4** and **4a**. Pure **4** and **4a** were obtained by chromatography (hexane/EtOAc 4:1) on multiple columns. **4**: $[\alpha]^{25}{}_D$ −11.3° (*c* 10.9, $CHCl_3$); IR 3613, 3366, 1690 1390, 1167 cm^{-1}; ^1H NMR (C_6D_6, 60 °C) δ 0.88 (t, 3H, *J* = 6.7 Hz), 1.10-1.80 (m, 35H), 2.55 (t, 2H, *J* = 7.7 Hz), 3.52 (t, 1H, *J* = 8.9 Hz), 3.97 (d, 1H, *J* = 8.9 Hz), 4.11 (br s, 1H), 5.10 (br s, 1H), 6.99 (d, 1H, *J* = 7.2 Hz), 7.15 (t, 1H, *J* = 7.7 Hz), 7.23 (d, 1H, *J* = 7.5 Hz), 7.31 (s, 1H). **4a**: ^1H NMR (C_6D_6, 60 °C) δ 0.90 (t, 3H, *J* = 5.5 Hz), 1.10-1.80 (m, 35H), 2.53 (t, 2H, *J* = 7.7 Hz), 3.46 (t, 1H, *J* = 7.8 Hz), 3.74 (d, 1H, *J* = 8.9 Hz), 4.19 (br s, 1H), 4.92 (d, 1H, *J* = 7.8 Hz), 7.02 (d, 1H, *J* = 7.3 Hz), 7.15 (1H, overlap with C_6H_6 peak), 7.22 (d, 1H, *J* = 7.5 Hz), 7.30 (s, 1H).

(2*S*,3*R*)-3-(3'-Dodecylphenyl)-2-aminopropane-1,3-diol [(-)-13]. Method A (Scheme 4): A solution of 48 mg (1.0 mmol) of **4** in 3 mL of 1 M HCl and 3 mL of THF was heated at 70 °C with stirring for 5 h under N_2. The reaction mixture was cooled to rt, and neutralized with 1 M NaOH (3 mL). The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Flash chromatography ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4) gave 28 mg (83%) of sphingosine analogue **13** as a white solid: mp 55.2-56.2 °C. **Method B (Scheme 7):** To an ice-cooled suspension of 57 mg (1.5 mmol) of $LiAlH_4$ in 15 mL of dry THF under nitrogen was injected a solution of 101 mg (0.25 mmol) of azido ester **22** in 4 mL of THF. The reaction mixture was stirred at rt for 2 h and then at 65 °C until the full consumption of the azido ester was noticed by TLC (~2 h). After being chilled to 0 °C, the reaction mixture was filtered through a pad of silica gel in a sintered glass funnel to remove the salt and the excess $LiAlH_4$. The pad was washed with $CHCl_3$/MeOH/conc. NH_4OH 130:25:4 to collect the product. After concentration, the residue was purified by flash chromatography ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4). A solution of the product in $CHCl_3$ was passed through a Cameo filter (Fisher Scientific) to remove the dissolved silica gel. Concentration gave 71 mg (85%) of **13** as a white solid: mp 55.0-56.0 °C; $[\alpha]^{25}_{D}$ -22.0° (*c* 3.4, $CHCl_3$); IR 3609, 3383, 1604, 1462, 1233, 1035, 893 cm^{-1}; ^{1}H NMR δ 0.86 (t, 3H, *J* = 7.0 Hz), 1.10-1.32 (m, 18H), 1.56 (m, 2H), 2.56 (t, 2H, *J* = 7.9 Hz), 2.79 (br s, 4H), 2.98 (dt, 1H, *J* = 6.1, 5.1 Hz), 3.62 (m, 2H), 4.58 (d, 1H, *J* = 6.1 Hz), 7.08 (m, 3H), 7.21 (t, 1H, *J* = 7.5 Hz); ^{13}C NMR δ 14.07, 22.65, 29.32, 29.42, 29.48, 29.59, 29.62, 29.65, 31.52, 31.88, 35.97, 57.22, 63.50, 76.45, 123.66, 126.37, 127.88, 128.38, 141.51, 143.32.

(2*S*,3*R*)-3-(3'-Dodecylphenyl)-2-palmitoylamidopropane-1,3-diol [(-)-2]. To a solution of 30 mg (0.09 mmol) of **13** in 3 mL of dry THF was added 68 mg (0.18 mmol) of *p*-nitrophenyl palmitate at rt. The mixture was stirred for 48 h and then concentrated under reduced pressure. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 45 mg (88%) of **2** as a white solid: mp 92.1-92.8 °C; $[\alpha]^{25}_{D}$ –5.4° (*c* 1.7, $CHCl_3$); IR 3599, 3429, 1655, 1500 cm^{-1}; ^{1}H NMR δ 0.86 (t, 6H, *J* = 7.0 Hz), 1.23 (m, 42H), 1.58 (m, 4H), 2.19 (t, 2H, *J* = 7.8 Hz), 2.57 (t, 2H, *J* = 7.9 Hz), 2.77 (br s, 1H), 3.57 (dd, 1H, *J* = 11.5, 3.5 Hz), 3.79 (dd, 1H, *J* = 11.5, 3.3 Hz), 4.04 (m, 1H), 4.99 (d, 1H, *J* = 3.6 Hz), 6.32 (d, 1H, *J* = 7.8 Hz), 7.08 (d, 1H, *J* = 7.4 Hz), 7.17 (s, 1H), 7.17 (d, 1H, *J* = 6.6 Hz), 7.24 (t, 1H, *J* = 7.5 Hz); ^{13}C NMR δ 14.09, 22.67, 25.71, 29.28, 29.35, 29.37, 29.43, 29.51, 29.61, 29.64, 29.67, 29.69, 31.60, 31.91, 36.01, 36.82, 55.46, 61.74, 76.05, 122.99, 125.83, 127.93, 128.46, 140.79, 143.42, 173.98; HR-MS (DCI, MH^+) *m/z* calcd for $C_{37}H_{68}NO_3$ 574.5199, found 574.5187.

***N-tert*-Butoxycarbonyl (4*S*)-4-(3'-Dodecylbenzoyl)-2,2-dimethyl-1,3-oxazolidine [(-)-14].** To a mixture of 0.48 g (1.0 mmol) of **4** and **4a** in 15 mL of CH_2Cl_2

was added 0.43 g (2.0 mmol) of PCC. The reaction mixture was stirred at rt for 5 h. The mixture was filtered through a pad of silica gel, which was washed with CH_2Cl_2, and the solvent was evaporated. Flash chromatography (hexane/EtOAc 9:1) of the residue afforded 0.41 g (85%) of ketone **14** as a white solid: mp 60.0-61.0 °C; $[\alpha]^{25}_D$ –42.0° (*c* 3.1, $CHCl_3$); IR 1702, 1390, 1243, 1173 cm^{-1}; ^1H NMR[25] (C_6D_6) δ 0.90 (t, 3H, *J* = 6.5 Hz), 1.10-1.50 (m, 29H), 1.55 and 1.72 (two sets of s, 3H), 1.92 and 2.04 (two sets of s, 3H), 2.36 (m, 2H), 3.66 (two sets of m, 2H), 5.08 (dd, 0.67H, *J* = 7.6, 3.7 Hz), 5.32 (dd, 0.33H, *J* = 7.3, 3.0 Hz), 7.06 (m, 2H), 7.50 (d, 0.67H, *J* = 7.3 Hz), 7.55 (d, 0.33H, *J* = 7.5 Hz), 7.81 (s, 1H); ^{13}C NMR (C_6D_6) δ 14.34, 23.08, 25.04, 25.21, 25.89, 26.32, 28.29, 28.35, 29.56, 29.62, 29.79, 29.85, 30.00, 30.08, 30.10, 31.62, 31.68, 32.30, 36.01, 62.07, 62.39, 65.75, 66.11, 79.63, 79.99, 94.51, 95.48, 125.86, 126.10, 128.68, 128.83, 133.21, 133.46, 135.91, 135.96, 143.70, 143.92, 151.47, 152.00, 195.15, 195.61.

DIBAL Reduction of Ketone 14. To a solution of 0.10 g (0.21 mmol) of ketone **14** in dry THF (10 mL) was added, dropwise, ~0.30 mL (~0.45 mmol) of DIBALH (a 1.5 M solution in toluene) at 0 °C under Ar. The rate of addition was very slow to maintain the low temperature. The reaction mixture was stirred for 0.5 h at 0 °C until TLC analysis showed the reaction to be complete. The reaction was quenched by slow addition of 0.5 mL of MeOH followed by 5.0 mL of cold 5% aqueous potassium sodium tartrate solution. The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (hexane/EtOAc 4:1) gave 94 mg (94%) of **4** and **4a** in a ratio of 26:1, determined by NMR spectroscopy.

(2*S*,3*R*)-3-(3'-Dodecylphenyl)-2-*tert*-butoxycarbonylaminopropane-1,3-diol [(-)-15]. To a solution of 0.10 g (0.21 mmol) of **4** in 5 mL of MeOH was added 0.15 g of Amberlyst 15, and the heterogeneous mixture was stirred at rt for 48 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated. Flash chromatography ($CHCl_3$/MeOH 9:1) of the residue afforded 73 mg (80%) of **15** as a white solid: mp 76.5-77.5 °C; $[\alpha]^{25}_{D}$ -5.8° (*c* 5.5, $CHCl_3$); IR 3612, 3436, 1699, 1496 cm^{-1}; ^1H NMR (C_6D_6) δ 0.86 (t, 3H, *J* = 8.0 Hz), 1.10-1.50 (m, 29H), 1.59 (s, 2H), 2.54 (t, 2H, *J* = 8.0 Hz), 3.58 (d, 1H, *J* = 6.6 Hz), 3.63 (s, 1H), 3.82 (d, 1H, *J* = 10.3 Hz), 3.98 (d, 1H, *J* = 3.4 Hz), 4.20 (d, 1H, *J* = 4.4 Hz), 5.03 (t, 1H, *J* = 4.7 Hz), 5.58 (d, 1H, *J* = 8.3 Hz), 7.01 (d, 1H, *J* = 7.3 Hz), 7.15-7.40 (m, 3H); ^{13}C NMR (C_6D_6) δ 14.36, 23.07, 28.45, 29.81, 29.89, 30.02, 30.12, 30.16, 32.09, 32.32, 36.44, 57.32, 62.03, 75.82, 79.38, 124.00, 126.68, 127.89, 128.12, 128.53, 142.01, 143.09, 156.48.

(4*R*,5*S*)-5-*tert*-Butoxycarbonylamino-4-(3'-dodecylphenyl)-2,2-dimethyl-1,3-dioxane [(+)-16]. To a solution of 43 mg (0.10 mmol) of **15** and 100 mg (1.0 mmol) of 2,2-dimethoxypropane in 5 mL of dry CH_2Cl_2 was added 25 mg (0.10 mmol) of PPTS. The mixture was stirred for 2 d at rt, concentrated under reduced pressure, and purified by flash chromatography (hexane/EtOAc 4:1), yielding 32 mg (67%) of **16** as a white solid: mp 68.2-69.6 °C; $[\alpha]^{25}_{D}$ +10.9° (*c* 7.9, $CHCl_3$); IR 3436, 1701, 1496, 1366, 1243, 1161 cm^{-1}; ^1H NMR (C_6D_6) δ 0.88 (m, 3H), 1.10-1.0 (m, 37H), 2.53 (t, 2H, *J* = 7.6 Hz), 3.60 (br s, 1H), 3.70-4.00 (m, 2H), 4.90 (d, 1H, $J_{4,5}$ = 9.3 Hz), 7.10-7.50 (m, 4H); ^{13}C NMR (C_6D_6) δ 14.34, 19.45, 23.09, 28.28, 29.37, 29.80, 29.98, 30.06, 30.09, 30.13, 32.05, 32.30, 36.42, 51.48, 63.09, 74.68, 78.82, 99.00, 125.26, 127.89, 128.30, 139.94, 142.91, 154.86.

Ethyl *m*-Dodecylcinnamate (19). A solution of 1.0 g (3.1 mmol) of bromide **6**, 0.60 g (6.0 mmol) of ethyl acrylate, 0.60 g (6.0 mmol) of Et_3N and 30 mg (26 μmol) of $(Ph_3P)_4Pd(0)$ in 10 mL of DMF was stirred at 120 °C under Ar for 6 h. After the reaction mixture was allowed to cool to rt, 15 mL of aqueous 1 M HCl solution was added, and stirring was continued for 5 min. The product was extracted with Et_2O (3 × 30 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), and concentrated. Column chromatography (hexane/EtOAc 9:1) yielded 0.98 g (92%) of cinnamate derivative **19** as a colorless oil: IR 1704, 1637 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, *J* = 7.0 Hz), 1.10-1.40 (m, 21H), 1.50-1.70 (m, 2H), 2.59 (t, 2H, *J* = 7.8 Hz), 4.25 (q, 2H, *J* = 7.1 Hz), 6.41 (d, 1H, *J* = 16.0 Hz), 7.17 (d, 1H, *J* = 7.4 Hz), 7.26 (t, 1H, *J* = 7.5 Hz), 7.30 (s, 1H), 7.32 (d, 1H, *J* = 7.4 Hz), 7.65 (d, 1H, *J* = 16.0 Hz); ^{13}C NMR δ 14.14, 14.33, 22.71, 29.29, 29.37, 29.51, 29.59, 29.67, 31.40, 31.93, 35.65, 35.81, 60.44, 117.94, 125.44, 127.51, 128.12, 128.75, 128.88, 130.48, 134.38, 143.60, 144.91, 167.10; HR-MS (DEI, M^+) *m/z* calcd for $C_{23}H_{36}O_2$ 344.2715, found 344.2709.

Ethyl (2*S*,3*R*)-3-(3'-Dodecylphenyl)-2,3-dihydroxypropionate [(-)-20]. After a solution of 1.4 g of AD-mix-β and 95 mg (1.0 mmol) of $MeSO_2NH_2$ in 30 mL of *t*-BuOH/H_2O 1/1 was stirred vigorously at rt for 30 min, 0.35 g (1.0 mmol) of α,β-unsaturated ester **19** was added. The reaction mixture was stirred vigorously until the disappearance of the α,β-unsaturated ester was noted. Sodium sulfite (1.50 g, 1.46 mmol) was added to quench the reaction. Stirring was continued for another 30 min. The product was extracted with EtOAc (3 × 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a yellow solid residue, which was dissolved in minimum volume of EtOAc and passed through a pad of silica gel in a sintered glass funnel to remove the ligand. The pad was washed with hexane/EtOAc 2:1 to collect the product. Concentration of the filtrate provided an almost pure product, which was purified by column chromatography (hexane/EtOAc 2:1), giving 0.34 g (90%) of diol **20** as a white solid: mp 49.0-49.5 °C. Compound (-)-**20** was formed in >99% ee, as estimated by ^{1}H NMR analysis of the Mosher esters[23] derived from both enantiomers; (+)-**20** was prepared by reaction of **20** with AD-mix-α. $[\alpha]^{25}_D$ -4.4° (*c* 2.3, $CHCl_3$): IR 3549, 1725 cm^{-1}; ^{1}H NMR δ 0.86 (t, 3H, *J* = 7.0 Hz), 1.16-1.31 (m, 21H), 1.58 (m, 2H), 2.58 (t, 2H, *J* = 8.0 Hz), 2.83 (br s, 2H), 4.21 (dq, 2H, *J* = 7.3, 1.3 Hz), 4.31 (d, 1H, *J* = 3.1 Hz), 4.94 (d, 1H, *J* = 3.1 Hz), 7.10 (d, 1H, *J* = 7.4 Hz), 7.18 (m, 2H), 7.23 (m, 1H); ^{13}C NMR δ 14.02, 14.09, 22.65, 29.32, 29.37, 29.48, 29.57, 29.61, 29.64, 31.50, 31.88, 35.97, 62.06, 74.62, 74.74, 123.46, 126.26, 128.07, 128.26, 139.79, 143.13, 172.76; HR-MS [DCI, MNH_4^+] *m/z* calcd for $C_{23}H_{42}NO_4$ 396.3114, found 396.3120.

Ethyl (2*S*,3*R*)-3-(3'-Dodecylphenyl)-2-nosyloxy-3-hydroxypropionate [(-)-21]. To an ice-cooled solution of 0.38 g (1.0 mmol) of diol ester **20** in 25 mL of CH_2Cl_2 was added 0.51 g (5.0 mmol) of Et_3N, followed by 0.26 g (1.2 mmol) of 4-nitrobenzenesulfonyl chloride. The yellow solution was stirred at 0 °C under Ar until the full consumption of diol ester **20** was observed (TLC). Methanol (0.5 mL) was added to quench the reaction. After the solvents were removed under reduced pressure, the yellow residue was purified by column chromatography (hexane/EtOAc 3:1), giving 0.47 g (83%) of α-nosylate ester **21** as a pale yellow oil: $[\alpha]^{25}_D$ −37.8° (*c* 1.93, $CHCl_3$): IR 3606, 1745, 1536 cm^{-1}; ^{1}H NMR δ 0.85 (t, 3H, *J* = 7.5 Hz), 1.17 (t, 3H, *J* = 7.2 Hz), 1.24 (m, 19H), 1.49 (m, 2H), 2.45 (t, 2H, *J* = 8.0 Hz), 4.16 (t, 2H, *J* = 7.2 Hz), 4.98 (d, 1H, *J* = 3.8 Hz), 5.16 (d, 1H, *J* = 3.8 Hz), 6.95-7.20 (m, 5H), 7.76 (d, 2H, *J* = 7.0 Hz), 8.15 (d, 2H, *J*

= 7.0 Hz); ^{13}C NMR δ 13.74, 14.02, 22.58, 29.24, 29.28, 29.37, 29.49, 29.53, 29.56, 31.42, 31.80, 35.70, 62.41, 73.34, 82.51, 123.27, 123.95, 125.93, 128.36, 128.38, 128.87, 137.37, 141.21, 143.31, 150.32, 166.50; HR-MS (DCI, MNH_4^+) calcd for *m/z* $C_{29}H_{45}N_2O_8S$ 581.2897, found 581.2872.

Ethyl (2*R*,3*R*)-3-(3'-Dodecylphenyl)-2-azido-3-hydroxypropionate [(+)-22]. A mixture of 0.37 g (0.66 mmol) of nosylate **21** and 0.46 g (7.1 mmol) of NaN_3 in 12 mL of dry DMF was stirred vigorously under Ar at 55 °C until TLC analysis indicated that no nosylate was still present. After addition of 30 mL of H_2O, the product was extracted with Et_2O (3 × 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated, and the residue was purified by column chromatography (hexane/EtOAc 3:1) to give 0.20 g (75%) of 2-azido derivative **22** as a colorless oil: $[\alpha]^{25}_D$ +6.4° (*c* 1.6, $CHCl_3$); IR 3602, 2116, 1736 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, *J* = 7.0 Hz), 1.23 (m, 21H), 1.57 (m, 2H), 2.59 (t, 2H, *J* = 7.9 Hz), 2.82 (br s, 1H), 4.06 (d, 1H, *J* = 7.1 Hz), 4.22 (q, 2H, *J* = 7.1 Hz), 4.97 (d, 1H, *J* = 7.0 Hz), 7.16 (m, 3H), 7.26 (m, 1H); ^{13}C NMR δ 14.04, 14.11, 22.67, 29.30, 29.34, 29.49, 29.58, 29.62, 29.65, 31.45, 31.90, 35.92, 62.11, 66.69, 74.22, 123.84, 126.59, 128.51, 128.87, 138.81, 143.44, 168.97; HR-MS [DCI, MNH_4^+] *m/z* calcd for $C_{23}H_{41}O_3N_4$ 421.3179, found 421.3186.

2-Bromo-6-(1'-hydroxydodecyl)pyridine (24). To a solution of 1.0 g (4.2 mmol) of 2,6-dibromopyridine (**23**) in 10 mL of dry THF at -78 °C was added 1.9 mL (4.75 mmol) of *n*-BuLi (2.5 M solution in hexane) under Ar. After the mixture was stirred for 30 min, a solution of 0.80 g (4.3 mmol) of dodecanal in 8 mL of dry THF was added. The mixture was stirred at –78 °C for 1 h, then at rt for 1 h. Saturated aqueous $NaHCO_3$ solution (10 mL) was added, and vigorous stirring was maintained for 10 min.

The product was extracted with Et_2O (3 × 15 mL), the combined extracts were dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (hexane/EtOAc 7:3) to give 1.15 g (80%) of **24** as a white solid: mp 39.0-40.0 °C; IR 3613, 3460, 1584, 1554, 1437, 1161, 1125 cm^{-1}; ^1H NMR δ 0.82 (t, 3H, J = 7.0 Hz), 0.90-1.50 (m, 18H), 1.50-1.90 (m, 2H), 3.58 (br s, 1H), 4.63 (dd, 1H, J = 8.0, 4.5 Hz), 7.21 (d, 1H, J = 7.6 Hz), 7.29 (d, 1H, J = 4.8 Hz), 7.47 (t, 1H, J = 7.7 Hz); ^{13}C NMR δ 14.01, 22.57, 25.26, 29.24, 29.43, 29.48, 29.51, 29.54, 31.80, 38.24, 73.10, 118.96, 126.35, 138.83, 140.99, 164.75.

2-Bromo-6-(1'-bromododecyl)pyridine (25). To a solution of 0.34 g (1.0 mmol) of alcohol **24** and 0.31 g (1.2 mmol) of Ph_3P in 15 mL of dry CH_2Cl_2 was added 0.20 g (1.1 mmol) of NBS at 0 °C under Ar. The mixture was stirred at 0 °C for 1 h, then allowed to warm to rt and stirred for 1 h. The mixture was diluted with 30 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO. Concentration gave 0.38 g (93%) of **25** as a colorless oil. A small sample was purified by flash chromatography (hexane/EtOAc 9:1): ^1H NMR δ 0.85 (t, 3H, J = 7.0 Hz), 1.00-1.60 (m, 18H), 2.00-2.30 (m, 2H), 4.93 (dd, 1H, J = 8.2, 6.8 Hz), 7.38 (m, 2H), 7.52 (t, 1H, J = 7.7 Hz); ^{13}C NMR δ 14.09, 22.65, 27.85, 28.83, 29.30, 29.32, 29.46, 29.56, 31.87, 38.09, 54.16, 120.96, 127.30, 139.15, 141.16, 161.98.

2-Bromo-6-dodecylpyridine (26). To a solution of 1.12 g (2.77 mmol) of crude **25** in 10 mL of dry DMSO was added 0.63 g (16.6 mmol) of $NaBH_4$ at rt under Ar. After the mixture was stirred at rt overnight, the mixture was diluted with 50 mL of Et_2O and washed with brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (hexane/EtOAc 9:1) to give 0.81 g

(90%) of **26** as a colorless oil: ^{1}H NMR δ 0.83 (t, 3H, J = 7.0 Hz), 1.10-1.40 (m, 18H), 1.60-1.90 (m, 2H), 2.71 (t, 2H, J = 7.9 Hz), 7.05 (d, 1H, J = 7.4 Hz), 7.25 (d, 1H, J = 7.7 Hz), 7.39 (t, 1H, J = 7.7 Hz); ^{13}C NMR δ 14.05, 22.62, 29.25, 29.29, 29.37, 29.47, 29.56, 29.60, 29.73, 31.85, 37.97, 121.36, 125.08, 138.48, 141.36, 164.22.

***N-tert*-Butoxylcarbonyl (4*S*)-4-[1'-(6"-Dodecylpyridin-2"-yl)-hydroxymethyl]-2,2-dimethyl-1,3-oxazolidine (27, 28).** To a solution of 0.39 g (1.2 mmol) of **25** in 10 mL of dry THF was added 0.5 mL (1.25 mmol) of *n*-BuLi (a 2.5 M solution in hexane) dropwise at –78 °C under Ar. After the mixture was stirred for 1 h, a solution of 0.23 g (1.0 mmol) of aldehyde **5** in 5 mL of dry THF was added dropwise over a 5-min period. The mixture was stirred at –78 °C for 3 h. The reaction was quenched with aqueous saturated $NaHCO_3$ solution (5 mL). The organic phase was separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organic phases were washed twice with brine, dried ($MgSO_4$), and evaporated. Column chromatography (hexane/EtOAc 4:1) gave 0.26 g (55%) of diastereoisomers **27** and **28**, which have very similar R_f values (0.56, hexane/EtOAc 4:1). ^{1}H NMR (C_6D_6, 60 °C) δ 0.90 (t, 3H, J = 7.0 Hz), 1.10-1.93 (m, 37H), 2.68 and 2.69 (two sets of t, 2H, J = 7.0 Hz), 3.66 (dd, 0.83H, J = 9.7, 6.4 Hz), 3.73 (dd, 0.17H, J = 9.3, 6.7 Hz), 4.19-4.40 (m, 1.67H), 4.36 (m, 0.34H), 4.18-5.22 (m, 1H), 6.72 (m, 1H), 6.90-7.20 (m, 2H).

3-(6'-Dodecylpyridin-2'-yl)-2-aminopropane-1,3-diol (29). A solution of 48 mg (1.0 mmol) of **26** and **27** in 3 mL of 1 M HCl and 3 mL of THF was heated at 70 °C under Ar with stirring for 5 h. The reaction mixture was cooled to rt, and neutralized with 1 M NaOH (3 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Flash chromatography ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4) gave 28 mg (84%) of diastereomeric sphingosine analogue **29** as a white solid: mp 54.0-56.0 °C; R_f 0.52 ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4); ^1H NMR δ 0.84 (t, 3H, J = 7.0 Hz), 1.10-1.50 (m, 18H), 1.64 (t, 2H, J = 7.3 Hz), 2.71 (t, 2H, J = 7.9 Hz), 3.18 (m, 5H), 3.34 (dd, 1H, J = 11.2, 5.3 Hz), 3.58 (dd, 1H, J = 11.2, 6.2 Hz), 4.74 (d, 0.17H, J = 3.8 Hz), 4.78 (d, 0.83H, J = 4.8 Hz), 7.03 (d, 1H, J = 7.6 Hz), 7.16 (d, 0.17H, J = 7.7 Hz), 7.18 (d, 0.83H, J = 7.7 Hz), 7.58 (t, 1H, J = 7.7 Hz); ^{13}C NMR[26] δ 14.08, 22.65, 29.31, 29.43, 29.55, 29.61, 29.63, 31.88, 37.95, 57.20, (57.67), 63.42, (64.69), (73.49), 74.72, (117.77), 118.41, (121.53), 121.63, 137.16, (137.29), 158.77, (158.91), (161.17), 161.37.

(2*S*,3*S*)-3-(6'-Dodecylpyridin-2'-yl)-2-butanoylamidopropane-1,3-diol [(-)-3]. To a solution of 31 mg (0.92 mmol) of **29** in 3 mL of dry THF was added 35 mg (0.18 mmol) of *p*-nitrophenyl butyrate at rt. The reaction was stirred for 48 h and then concentrated under reduced pressure. TLC analysis ($CHCl_3$/MeOH 9:1) showed the formation of **(-)-3** (R_f 0.52) and its diastereoisomer (R_f 0.45). Purification by column chromatography (by gravity, $CHCl_3$/MeOH 9:1) afforded 24 mg (70%) of **(-)-3** as a white solid: mp 52.2-53.5 °C; $[\alpha]^{25}_D$ -100.5° (*c* 2.1, $CHCl_3$); IR 3425, 3305, 1644, 1507, 1096 cm^{-1}; ^1H NMR δ 0.74 (t, 3H, J = 7.4 Hz), 0.85 (t, 3H, J = 7.0 Hz), 0.90-1.40 (m, 18H), 1.40-1.60 (m, 2H), 1.60-1.80 (m, 2H), 1.90-2.20 (m, 2H), 2.60-2.90 (t, 2H, J = 7.9 Hz), 3.66 (dd, 1H, J = 11.8 Hz, 4.5 Hz), 4.00 (dd, 1H, J = 11.8, 2.2 Hz), 4.30 (m, 1H), 4.98 (s, 1H), 5.89 (br s, 1H), 6.46 (d, 1H, J = 6.3 Hz), 7.07 (d, 1H, J = 7.7 Hz), 7.46 (d, 1H, J = 7.8 Hz), 7.66 (t, 1H, J = 7.8 Hz); ^{13}C NMR δ 13.43, 14.08, 19.02, 22.65, 29.26, 29.31, 29.40, 29.54, 29.62, 29.72, 31.88, 37.51, 38.07, 57.33, 62.62, 77.25, 119.07, 121.78, 138.28, 150.78, 160.52, 174.94; HR-MS (FAB, MH$^+$) calcd for *m/z* $C_{24}H_{43}N_2O_3$ 407.3274, found 407.3269.

(2*S*,3*S*)-3-(6'-Dodecylpyridin-2'-yl)-2-*tert*-butyloxycarbonylaminopropane-1,3-diol [(-)-30]. Amberlyst 15 (0.15 g) was added to a solution of **27** and **28** (0.10 g, 0.21 mmol) in MeOH (5 mL). The mixture was stirred at rt for 48 h, filtered through Celite, and concentrated. The residue was purified by column chromatography (by gravity, $CHCl_3$/MeOH 9:1). Unreacted starting material was recovered, retreated with Amberlyst, and the product was purified as above. There was obtained 59 mg (65%) of diol **30** as a colorless oil: $[\alpha]^{25}_D$ −46.2° (*c* 3.4, $CHCl_3$); IR 3025, 1701, 1496 1454, 1167 cm^{-1}; ^{1}H NMR (C_6D_6) δ 0.75 (t, 3H, *J* = 6.9 Hz), 1.10-1.50 (m, 27H), 1.60 (m, 2H), 2.57 (t, 2H, *J* = 7.6 Hz), 3.47 (m, 1H), 3.80 (dd, 1H, *J* = 11.3, 4.4 Hz), 4.11 (br s, 1H), 4.59 (br s, 1H), 5.02 (s, 1H), 5.55 (m, 2H), 6.55 (d, 1H, *J* = 7.6 Hz), 7.07 (t, 1H, *J* = 7.7 Hz), 7.28 (d, 1H, *J* = 7.7 Hz); ^{13}C NMR (C_6D_6) δ 14.35, 23.09, 28.24, 29.57, 29.79, 29.83, 29.88, 29.99, 30.08, 30.11, 32.30, 38.00, 58.10, 62.71, 77.12, 79.41, 119.05, 121.43, 137.43, 157.36, 159.96, 160.93.

(4*S*,5*S*)-4-(6'-Dodecylpyridin-2'-yl)-5-*tert*-butyloxycarbonylamino-2,2-dimethyl-1,3-dioxane [(-)-32]. This compound was prepared in 66% yield by using the same procedure as described for (+)-**16**: $[\alpha]^{25}_D$ −25.0° (*c* 3.4, $CHCl_3$); IR 1713, 1214, 1161 cm^{-1}; ^{1}H NMR (C_6D_6, 60 °C) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.10-1.50 (m, 35H), 1.81 (m, 2H), 2.76 (t, 2H, *J* = 7.8 Hz), 3.71 (t, 1H, *J* = 10.2 Hz), 3.81 (br s, 1H), 4.40 (dd, 1H, *J* = 10.8, 4.8 Hz), 4.96 (d, 1H, $J_{4,5}$ = 9.6 Hz), 5.73 (s, 1H), 6.68 (d, 1H, *J* = 7.5 Hz), 7.18 (1H, overlap with C_6H_6), 7.35 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (C_6D_6) δ 14.34, 19.06, 23.09, 28.36, 29.40, 29.79, 29.87, 29.95, 30.05, 30.08, 30.12, 32.30, 38.46, 51.51, 64.09, 74.71, 78.69, 99.09, 118.05, 121.78, 137.26, 155.58, 159.48, 160.69.

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12. The ratio of **4**:**4a** was determined by 400-MHz ^1H NMR (C_6D_6 at 60 °C): C(1')-H: δ 5.10 (br s) for **4**, δ 4.92 (d, J = 7.8 Hz) for **4a**.

13. Herold[14] found that the reaction of 1-pentadecyllithium with aldehyde **5** at -78 °C produced high erythro stereoselectivity when HMPA was used as the co-solvent. HMPA competes effectively with the *N*-Boc group for coordination to the metal ion; a non-chelated Felkin-Ahn model can rationalize attack of the alkynyl anion at the less-hindered *re* face of aldehyde **5**, leading to D-erythro product.[8d] However, we noted only a slight effect on the diastereoselectivity when HMPA was added as a co-solvent (ratio of **4**:**4a**, 5.7:1.0).

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19. The enantiomeric excess (ee) was determined by ^{1}H NMR analysis of the corresponding bis-Mosher esters derived from diol (-)-**20** and its enantiomer (+)-**20**.

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22. The ratio of **3** to its threo diastereoisomer was ~5:1.

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25. The oxazolidine system undergoes a dynamic equilibrium at ambient temperature.

26. The values in parentheses represent the diastereoisomeric peaks.

Chapter 2

Synthesis of New Trans Double Bond Sphingolipid Analogues: $\Delta^{4,6}$ and Δ^{6} Ceramides

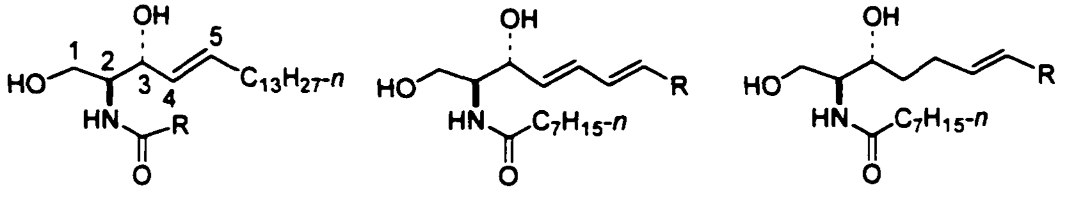
Abstract

Unsaturation was introduced at $\Delta^{4,6}$ and Δ^{4} of the sphingoid chain of naturally occurring ceramide **1** via a β-keto-sulfoxide (**12**) and -sulfone (**18**) derived from *N*-Boc L-serine methyl ester acetonide (**9**), affording two novel ceramide analogues, (2*S*,3*R*)-2-octanoylamidooctadeca-(4*E*,6*E*)-diene-1,3-diol (**2**) and (2*S*,3*R*)-2-octanoylamidooctadec-(6*E*)-ene-1,3-diol (**3**). After *C*-alkylation of **12** with (*E*)-1-bromo-2-tetradecene (**8**), a trans double bond was installed by elimination of PhS(O)H, providing conjugated dienone oxazolidine **13**. Reaction of **18** with **8**, followed by desulfonation (Al(Hg)), afforded keto-oxazolidine **20**, which bears a (*E*)-Δ^{6} double bond. The syntheses of analogues **2** and **3** from ketones **13** and **20**, respectively, were completed by the following sequence of reactions: diastereoselective reduction ($NaBH_4/CeCl_3$ or DIBAL-H), hydrolysis of the oxazolidine ring, liberation of the amino group, and installation of the *N*-amide group.

Introduction

Ceramide (*N*-acylsphingosine, **1**) is a long-chain aliphatic 2-amido-1,3-diol with a C(4),C(5)-trans double bond (Chart 1).[1] Considerable interest has been focused on its biochemistry and cell biology. Ceramide is a key intermediate in the biosynthesis of many other biologically important sphingolipids;[2] it has been implicated in many physiological events, including the regulation of cell growth and differentiation, inflammation, and programmed cell death (apoptosis).[3] Ceramide is also a regulator of many biochemical and cellular responses to stress, such as exposure to heat, radiation, oxidative conditions, and chemotherapeutic agents.[4]

Chart 1



1 **2** R = $C_{11}H_{23}$-n **3**

Ceramide as well as more complex sphingolipids are required for activation of membrane fusion of Semliki Forest virus (SFV) and other alphaviruses.[5] The C(4)-C(5) trans double bond in the sphingoid base of naturally occurring **1** may be crucial for ceramide's capacity to modulate various fundamental biological functions.[6] For example, it was shown that unnatural ceramide analogues having a cis C(4)-C(5) or a trans C(5)-C(6) double bond do not support fusion of SFV.[6] Similarly, the presence of the (*E*)-C(4)-C(5) double bond in the long-chain base of ceramide is important for the apoptotic response of **1**, since analogues that lack this double bond have reduced activity.[7,8] An understanding of the structural biology of ceramide may be aided by the synthesis of its homologues, which can be tested as both substrates for ceramide-utilizing enzymes and as substitutes for naturally occurring ceramide in cell signaling events and other biological activities.

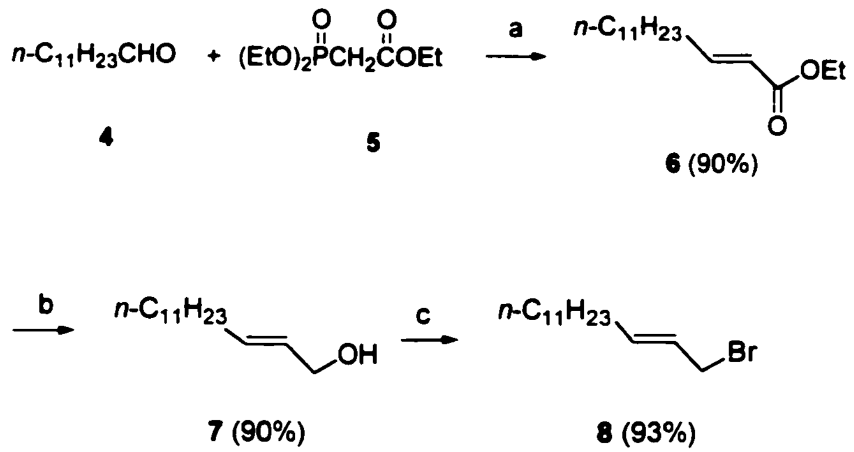
Synthetic routes to sphingosine and ceramide have been reviewed recently.[9] In an extension of our previous studies on the (*E*)-C(5)-C(6),[6] (*E*)-C(7)-C(8),[10] and (*E*)-C(15)-C(16)[10] double-bond analogues of ceramide, we report here a novel approach that allows the efficient syntheses of two new unnatural analogues of ceramide in which a diene [(*E*)-C(4)-C(5) and (*E*)-C(6)-C(7), compound **2**; see Chart 1] and an (*E*)-C(6)-C(7) double bond are incorporated (compound **3**). Diene **2** may have a higher reactivity than ceramide **1** in mitochondria[11] by undergoing facilitated oxidation at C(3) to give an α,β-unsaturated ketone and hydrogen peroxide as the initial oxidation products, which eventually lead to apoptotic cell death. Although 4,6-sphingadienes have not been reported to occur naturally, it is of interest to note that 4,8-sphingadiene is the principal sphingoid backbone of the glucosylceramides found in soybean and wheat.[12] Its function is not known.

Results and Discussion

Since organosulfur compounds play important roles in the formation of carbon-carbon bonds,[13] we decided to synthesize the novel analogues of ceramide **2** and **3** by use of the α-keto-sulfoxide and sulfone intermediates shown in the retrosynthetic plan (eq. 1). Both of these intermediates are derived from the commercially available protected L-serine building block **9**.

Synthesis of Allylic Bromide 8. As shown in Scheme 1, allylic bromide **8** was synthesized via α,β-unsaturated ester (*E*)-**6**. The latter was obtained in 90% yield (*E*/*Z* ratio, ~18:1) by Horner-Wadsworth-Emmons (HWE) reaction of dodecanal (**4**) with the phosphonoacetate **5**. Reduction of ester **6** with DIBAL-H afforded allylic alcohol **7** in 90% yield. Treatment of allylic alcohol **7** with NBS via Mitsunobu reaction[14] gave crude allylic bromide **8** in 93% yield.

Scheme 1. Synthesis of Allylic Bromide 8[a]

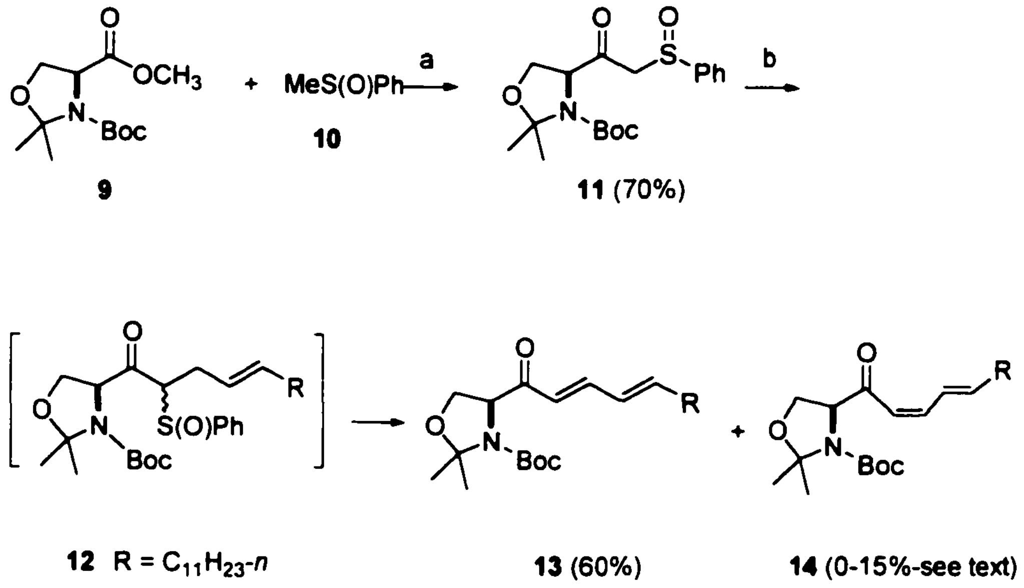


[a]Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$ (**5**), LiBr, Et_3N, rt; (b) DIBAL-H, THF, -78 °C-0 °C; (c) NBS, Ph_3P, CH_2Cl_2, rt.

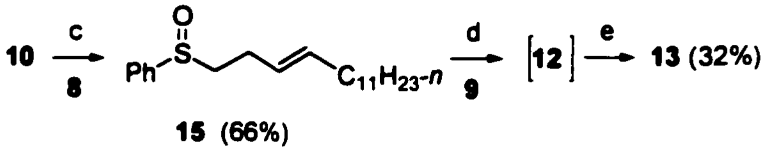
Synthesis of Dienone 13. The synthesis of dienone **13** started with the protected serine-derived methyl ester **9**, which is readily prepared from L-serine in three high-yielding steps.[15] Condensation of ester **9** with 2 equiv of the carbanion of methyl phenyl sulfoxide at -78 °C gave β-ketosulfoxide **11** in 70% yield (Scheme 2). Several methods were screened for alkylation of **11** with allylic bromide **8**. Cesium carbonate in DMF provided elimination product **13**, presumably via intermediate **12**. However, DBU in benzene was more convenient than Cs_2CO_3 in DMF, since no workup was required to obtain dienone **13**; the reaction mixture was simply passed through a pad of silica gel to remove the precipitate (DBU•HBr). Heating the filtrate in benzene for several hours afforded a mixture of two $\Delta^{4,6}$-dienones, (4*E*,6*E*)-**13** and (4*Z*,6*E*)-**14**, in a ratio of ~4:1. We were able to avoid the formation of **14** by carrying out the reaction at room temperature, even though the reaction was slow.[16] However, we noted that the optical rotation of **13** formed by using any of the above conditions was low, suggesting that racemization had occurred. The synthesis of chiral **13** was finally realized by using K_2CO_3[17] in DMF. This two-step reaction was very efficient at room temperature, providing diene **13** exclusively in 60% yield. These conditions not only avoided the difficult task of separating dienones **13** and **14**, which are formed together at elevated temperature, but also afforded **13** without racemization.

Scheme 2. Synthesis of Ketone 13[a]

Method A



Method B



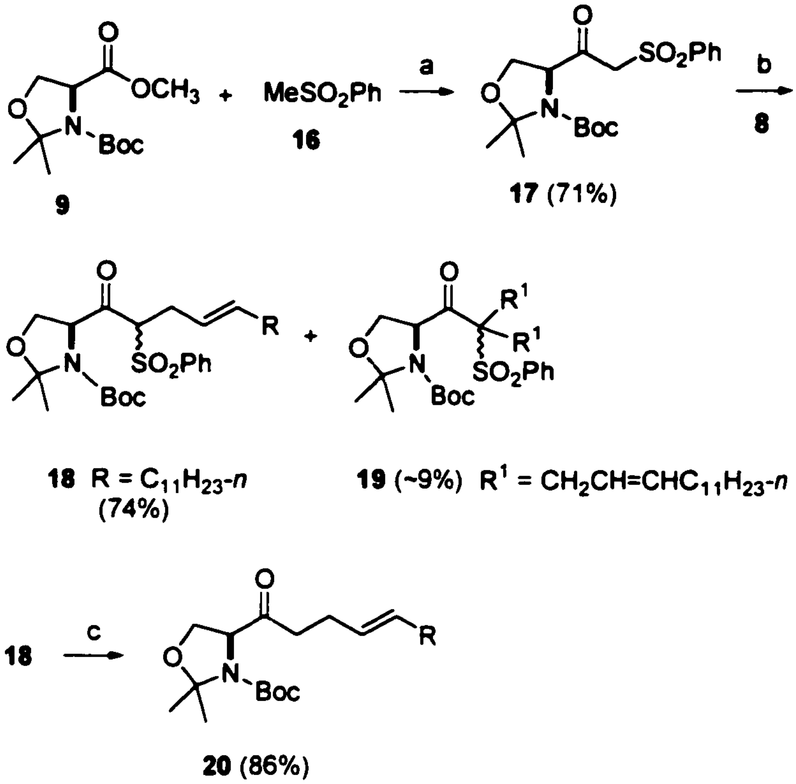
[a] Reagents and conditions: (a) LDA, THF, -78 °C-rt; (b) K_2CO_3, DMF, rt, 3 d; (c) LDA, THF, -78 °C - rt; (d) LDA, THF, -78 °C; (e) K_2CO_3, DMF, rt.

Method B (Scheme 2) shows a less desirable route to dienone **13**. Reaction of methyl phenyl sulfoxide (**10**) with allylic bromide **8** gave long-chain sulfoxide **15**. Treatment of sulfoxide **15** (2 equiv) with LDA, followed by reaction of the α-sulfinyl carbanion with ester **9** at -78 °C, gave the presumed β-ketosulfoxide intermediate **12**. After workup, the reaction mixture was dissolved in DMF and a small amount of K_2CO_3 was added, affording dienone **13** in ~32% overall yield. Thus Method A is a better choice.

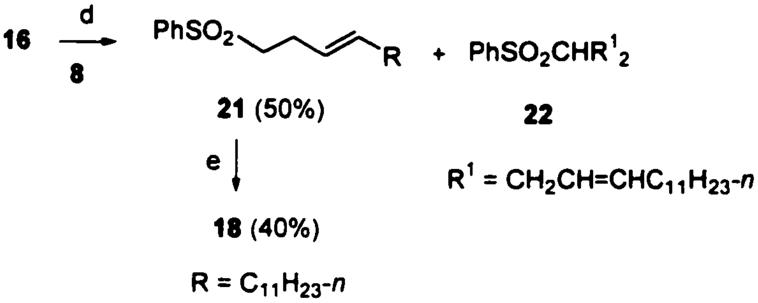
Synthesis of Ketone 20. Since α-ketosulfoxide **12** undergoes elimination even at room temperature it is not a suitable synthon for ketone **20**. Scheme 3 outlines the use of sulfone chemistry for the synthesis of **20**. Reaction of **9** with 2 equiv of the carbanion of methyl phenyl sulfone (**16**) at -78 °C gave β-ketosulfone **17** in 71% yield. Alkylation of **17** with allylic bromide **8** in the presence of a variety of bases was investigated. In Cs_2CO_3/DMF and DBU/DMF, dialkylation was the predominant reaction, giving **19** as the principal product. In DBU/benzene, monoalkylation of **17** was very efficient, giving **18**/**19** in a ratio of ~8:1. Sulfone intermediates **18** and **19** were readily separated by silica gel column chromatography. Desulfonylation of β-ketosulfone **18** with aluminum amalgam[18] afforded ketone **20** in 86% yield.

Scheme 3. Synthesis of Ketone 20[a]

Method A



Method B

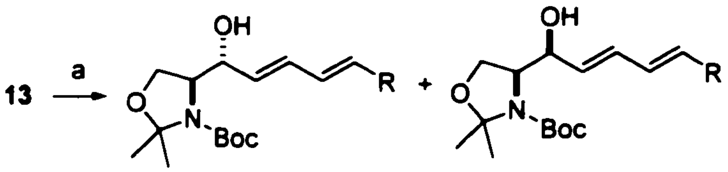


[a]Reagents and conditions: (a) *n*-BuLi, THF, -78 °C; (b) DBU, benzene, rt; (c) Al(Hg), THF/H_2O (20/1); (d) *n*-BuLi, THF, -78 °C (e) *n*-BuLi, THF, -78 °C.

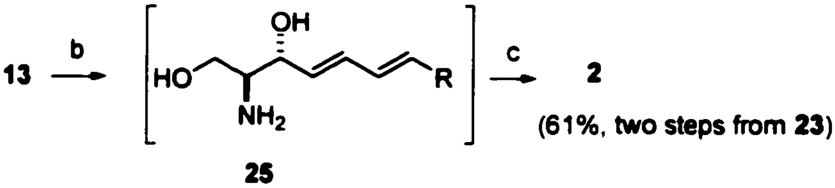
Another approach to sulfone **18** is shown in Scheme 3 (Method B). Methyl phenyl sulfone (**16**) was treated with *n*-butyllithium and reacted with allylic bromide **8** to give sulfone **21**. The yield of this reaction was low (50%) because of the formation of significant amounts of dialkylation product **22**. Sulfone **21** was treated with *n*-butyllithium and reacted with ester **9** to give sulfone **18** in 40% yield. Thus we chose Method A for its efficiency in the preparation of **18**.

Synthesis of Ceramide Analogues 2 and 3. Several reducing agents were screened for the attempted diastereoselective reduction of ketone **13** (Scheme 4).[19] A higher *erythro* selectivity was observed with $NaBH_4/CeCl_3$ in MeOH or DIBAL-H in THF, which gave *erythro*-**23**/*threo*-**24** in a ratio of 4-5 to 1. However, the undesired *threo* diastereomer was formed as the major product with the other reducing agents we used: L-selectride gave a ~1.3:1 ratio of *threo*/*erythro* isomers, and Red-Al gave **24**:**23** in 2:1 ratio). After diastereomers **23** and **24** were separated by column chromatography, acid hydrolysis of **23** (1 M HCl in THF, 70 °C) provided D-*erythro*-sphingosine **25**. Ceramide analogue **2** was obtained by *N*-acylation of sphingosine **25** with *p*-nitrophenyl caprylate.

Scheme 4. Synthesis of Ceramide Analogue 2[a]



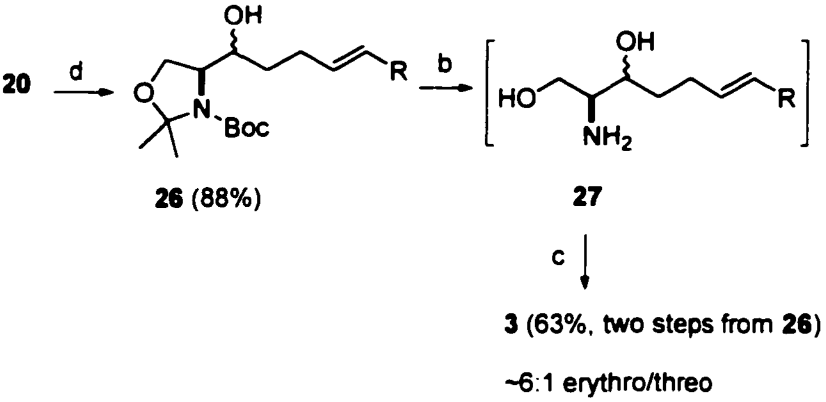
23 R = $C_{11}H_{23}$-*n* (72%) **24**



[a]Reagents and conditions: (a) $NaBH_4$, $CeCl_3$, MeOH or DIBAL-H, THF, -15 °C to 0 °C; (b) 1 M HCl, THF, 70 °C; (c) $p\text{-}O_2NC_6H_4CO_2C_7H_{15}\text{-}n$.

Reduction of **20** with $NaBH_4$ or DIBAL-H gave alcohol **26**, but unfortunately the two diastereoisomers could not be isolated at this step (Scheme 5). Acid hydrolysis of **26** (1 M HCl in THF, 70 °C) provided crude sphingosine **27**. *N*-Acylation of sphingosine **27** with *p*-nitrophenyl caprylate gave two diasteroisomers, which were separated by column chromatography. The erythro/threo ratio was about 6:1.

Scheme 5. Synthesis of Ceramide Analogue 3[a]



[a]Reagents and conditions: (a) (d) $NaBH_4$, MeOH, or DIBAL-H, THF, -15 °C; (b) 1 M HCl, THF, 70 °C; (c) $p\text{-}O_2NC_6H_4CO_2C_7H_{15}\text{-}n$.

In summary, a simple and convenient method has been established for the regiospecific introduction of unsaturation (both at a single site and as a conjugated diene) into the sphingoid backbone of ceramide by employing organosulfur chemistry.

Experimental Section

General Information. See Chapter 1 for the detailed information. $NaBH_4$, L-selectride, and *p*-nitrophenyl caprylate were purchased from Acros. Red-Al was purchased from Fluka. Ester **9** and diisopropylamine were purchased from Aldrich. Methyl phenyl sulfoxide (**10**) and methyl phenyl sulfone (**16**) were purchased from Alfa-Aesar.

(*E*)-Ethyl 2-Tetradecenoate (6). To a nitrogen-flushed solution of 21.0 g (245 mmol) of LiBr in 200 mL of dry THF was injected 11.7 mL (13.2 g, 59 mmol) of $(EtO)_2P(O)CH_2CO_2Et$ (**5**) at rt. After the solution was stirred at rt for 10 min, 13.6 mL (98 mmol) of Et_3N was injected, and stirring was continued for 10 min. A solution of dodecanal (**4**, 9.0 g, 49 mmol) in 20 mL of dry THF was injected. The reaction mixture was stirred vigorously at rt until the full consumption of the aldehyde **4** 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 11.2 g (90%) of ester **6** as a colorless oil: ^1H NMR δ 0.84 (t, 3H, J = 7.0 Hz), 1.10-1.50 (m, 21H), 2.17 (m, 2H), 4.15 (q, 2H, J = 7.2 Hz), 5.77 (dt, 1H, J = 15.6, 1.4 Hz), 6.92 (dt, 1H, J = 15.6, 7.0 Hz); ^{13}C NMR δ 14.1, 14.2, 22.6, 28.0, 29.1, 29.3, 29.4, 29.5, 29.58, 29.59, 31.9, 32.2, 60.0, 121.2, 149.4, 166.7.

(*E*)-2-Tetradecen-1-ol (7). To a solution of 5.0 g (20 mmol) of ester **6** in 150 mL of dry THF was added 40 mL of DIBAL-H (a 1.5 M solution in toluene, 60 mmol) at -78 °C under N_2. After 1 h, the solution was allowed to warm to 0 °C. The reaction was quenched by slow addition of 5 mL of MeOH followed by 20 mL of cold 5% aqueous potassium sodium tartrate solution. The product was extracted with EtOAc (3 × 30 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 4:1) gave 4.0 g (90%) of alcohol **7** as a colorless oil: ^1H NMR δ 0.84 (t, 3H, J = 6.7 Hz), 1.10-1.50 (m, 18H), 1.82 (s, 1H), 2.02 (m, 2H), 4.02 (d, 2H, J = 5.3 Hz), 5.61 (m, 2H); ^{13}C NMR δ 14.0, 22.6, 29.1, 29.2, 29.3, 29.47, 29.58, 29.60, 29.64, 31.9, 32.2, 63.6, 128.8, 133.3.

(*E*)-1-Bromo-2-tetradecene (8). To a solution of 2.12 g (10 mmol) of alcohol **7** and 2.75 g (1.05 mmol) of Ph_3P in 30 mL of dry CH_2Cl_2 was added 1.96 g (11 mmol) of NBS at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to rt and stirred for 1 h. The mixture was diluted with 60 mL of hexane and passed through a pad of silica gel with suction to remove the precipitate of Ph_3PO. The filtrate was concentrated, and the resulting residue was dissolved in 60 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO again. Concentration gave 2.56 g (93%) of bromide **8** as a colorless oil: ^1H NMR δ 0.86 (t, 3H, J = 6.5 Hz), 1.10-1.50 (m, 18H), 2.04 (m, 2H), 3.92 (d, 2H, J = 7.3 Hz), 5.69 (m, 2H); ^{13}C NMR δ 14.1, 22.7, 28.8, 29.1, 29.3, 29.4, 29.57, 29.62, 29.64, 31.9, 32.1, 33.7, 126.2, 136.8.

***N-tert*-Butoxycarbonyl (4*S*)-4-[(Phenylsulfinyl)acetyl]-2,2-dimethyl-1,3-oxazolidine (11).** To a solution of diisopropylamine (930 µL, 6.6 mmol) in 8 mL of THF was added 2.64 mL of *n*-butyllithium (a 2.5 M solution in hexane, 6.6 mmol) at -15 °C under N_2. After the mixture was stirred at this temperature for 30 min, a solution of methyl phenyl sulfoxide (**10**, 841 mg, 6.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred at -15 °C for 30 min, and then chilled to -78 °C. A solution of ester **9** (778 mg, 3.0 mmol) in 5 mL of THF was added dropwise. The solution was stirred at -78 °C for 2 h and allowed to warm to rt overnight. After saturated aqueous NH_4Cl (10 mL) solution was added, the product was extracted with EtOAc, washed with brine, and dried ($MgSO_4$). Purification by column chromatography (hexane/EtOAc 1:4, R_f 0.72) gave 771 mg (70%) of **11** as a yellow solid: mp 82.5-85.0 °C; IR 1710, 1385, 1170, 1090, 1055 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 1.35 (s, 9H), 1.40 (s, 3H), 1.67 (s, 3H), 3.75 (m, 4H), 4.26 and 4.40 (two sets of s, 1H), 7.10 (m, 3H), 7.49 (m, 2H). The ^{13}C NMR spectrum was very complex because several diastereoisomers are present. HR-MS (FAB, MH$^+$) calcd for *m/z* $C_{18}H_{26}NO_5S$ 368.1532, found 368.1516.

(3*E*)-Pentadecenyl Phenyl Sulfoxide (15). To a solution of diisopropylamine (930 µL, 6.6 mmol) in 8 mL of THF was added 2.64 mL of *n*-butyllithium (a 2.5 M solution in hexane, 6.6 mmol) at -15 °C under N_2. After the mixture was stirred at this temperature for 30 min, a solution of PhS(O)Me (**10**, 841 mg, 6.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred at -15 °C for 30 min. A solution of bromide **8** (1.65 g, 6.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred at -15 °C for 2 h and allowed to warm to rt overnight. Saturated aqueous NH_4Cl solution (10 mL) was added, and the product was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue by column chromatography (hexane/EtOAc 3:2, R_f 0.72) gave 1.32 g (66%) of **15**

as a colorless liquid: ^{1}H NMR δ 0.83 (t, 3H, *J* = 6.6 Hz), 1.10-1.40 (m, 18H), 1.92 (q, 2H, *J* = 6.5 Hz), 2.23 (dt, 1H, *J* = 21.5, 7.1 Hz), 2.41 (dt, 1H, *J* = 22.3, 7.5 Hz), 2.76 (q, 2H, *J* = 8.0 Hz), 5.30 (m, 1H), 5.49 (m, 1H), 7.45 (m, 3H), 7.58 (m, 2H); ^{13}C NMR δ 14.0, 22.6, 25.2, 29.0, 29.17, 29.24, 29.4, 29.5, 29.53, 29.56, 29.6, 31.8, 32.4, 56.9, 57.0, 123.9, 125.9, 129.1, 130.8, 133.5, 143.8.

N-*tert*-Butoxycarbonyl (4*S*)-4-[1'-Oxo-(2'*E*,4'*E*)-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine [(-)-13]. **Method A**: To a solution of β-ketosulfoxide **11** (370 mg, 1.0 mmol) in 4 mL of DMF was added K_2CO_3 (170 mg, 1.2 mmol) at rt. After the mixture was stirred at rt for 1 h under N_2, a solution of bromide **8** (275 mg, 1 mmol) in 2 mL of DMF was added. The mixture was stirred at rt for 3 days. Water (5 mL) was added, and the product was extracted with Et_2O (3 x 15 mL), washed with brine, and dried ($MgSO_4$). Concentration and purification by flash column chromatography (hexane/EtOAc 4:1, R_f 0.75) gave 261 mg (60%) of **13** as a white solid. **Method B**: To a solution of diisopropylamine (334 mg, 0.47 mL, 3.3 mmol) in 4 mL of THF was added 1.3 mL of *n*-butyllithium (a 2.5 M solution in hexane, 3.3 mmol) dropwise at -15 °C under nitrogen. After 30 min a solution of phenyl sulfoxide **15** (1.0 g, 3.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred for 30 min, then cooled to -78 °C. A solution of ester **9** (389 mg, 1.5 mmol) in 5 mL of THF was added dropwise. After the mixture was stirred at -78 °C for 3 h, the reaction was quenched by addition of saturated aqueous NH_4Cl solution (10 mL). The product was extracted with EtOAc, the organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The residue was dissolved in 5 mL of DMF containing a small amount of K_2CO_3, and the mixture was stirred at rt for 3 days. Purification by flash chromatography (hexane/EtOAc 3:2, R_f

0.72) gave 140 mg (32%) of **13** as a white solid: mp 48.5-50.0 °C; $[\alpha]^{25}_{D}$ -36.8° (*c* 2.5, CHCl$_3$); IR 1700, 1385 cm^{-1}; ^{1}H NMR (C$_6$D$_6$, 70 °C) δ 0.88 (t, 3H, *J* = 7.0 Hz), 1.10-1.30 (m, 18H), 1.39 (s, 9H), 1.55 (s, 3H), 1.82 (s, 3H), 1.92 (q, 2H, *J* = 6.9 Hz), 3.78-3.84 (m, 2H), 4.43 (br s, 1H), 5.81-5.89 (m, 1H), 5.95-6.01 (m, 1H), 6.23 (d, 1H, *J* = 15.3 Hz), 7.29 (dd, 1H, *J* = 15.3, 10.7 Hz); ^{13}C NMR (C$_6$D$_6$)^{20} δ 14.1, 20.1, 28.4, 29.0, 29.1, 29.4, 29.5, 29.7, 32.2, 33.2, 33.3, (64.7) 65.0, (66.0) 66.3, (79.9) 80.0, (94.4) 95.3, 123.3 (124.4), (128.2) 129.2, (144.0) 144.2, (145.8) 146.4, 151.7 (152.2), (195.5) 196.0; HR-MS (FAB, MNa^{+}) calcd for *m/z* C$_{26}$H$_{45}$NO$_4$Na 458.3247, found 458.3249.

N-*tert*-Butoxycarbonyl (4*S*)-4-[1'-Oxo-(2'*Z*,4'*E*)-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine [14]. ^{1}H NMR (C$_6$D$_6$, 70 °C) δ 0.89 (t, 3H, *J* = 6.2 Hz), 1.10-1.30 (m, 16H), 1.39 (s, 9H), 1.52 (s, 3H), 1.50-1.60 (m, 2H), 1.81 (s, 3H), 2.81 (m, 2H), 3.77 (m, 2H), 4.27 (br s, 1H), 5.13 (d, 1H, *J* = 10.8 Hz), 5.50 (d, 1H, *J* = 17.4 Hz), 6.13 (m, 2H).

N-*tert*-Butoxycarbonyl (4*S*)-4-[1'-Hydroxy-(2'*E*,4'*E*)-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine [(-)-23]. To a solution dienone **13** (325 mg, 0.75 mmol) in 15 mL of dry MeOH was added anhydrous CeCl$_3$ (61.5 mg, 0.25 mmol) at -15 °C. After the mixture was stirred for 10 min, NaBH$_4$ (32 mg, 0.85 mmol) was added. The temperature was gradually raised to 0 °C. After 2 h, water (20 mL) was added, the product was extracted with Et$_2$O (3 x 25 mL), washed with brine, and dried (MgSO$_4$). Concentration and purification by column chromatography (hexane/EtOAc 4:1, R$_f$ 0.45) gave 235 mg (72%) of **23** as a colorless oil: $[\alpha]^{25}_{D}$ -18.6° (*c* 1.5, CHCl$_3$); IR 1690, 1375 cm^{-1}; ^{1}H NMR (C$_6$D$_6$, 70 °C) δ 0.89 (t, 3H, *J* = 6.6 Hz), 1.28 (m, 18H), 1.38 (s, 9H), 1.43 (s, 3H), 1.61 (s, 3H), 2.02 (q, 2H, *J* = 6.8 Hz), 3.65 (m, 1H), 3.79 (br s, 1H), 3.94 (br s, 1H), 4.34 (m, 1H), 5.59-5.66 (m, 2H), 6.09 (m, 1H), 6.38 (m, 1H); ^{13}C NMR (C_6D_6, 70 °C) δ 12.5, 14.1, 19.0, 22.9, 24.4, 26.8, 28.1, 28.4, 29.5, 29.59, 29.68, 29.71, 29.88, 29.99, 30.04, 32.3, 32.9, 33.1, 62.8, 65.0, 73.6, 80.2, 94.6, 128.7, 130.5, 131.8, 134.8; HR-MS (FAB, MNa$^+$) calcd for *m/z* $C_{26}H_{47}NO_4Na$ 460.3403, found 460.3419.

(2*S*,3*R*)-(4*E*,6*E*)-2-Caprylamidooctadecadiene-1,3-diol [(-)-2]. A solution of 88 mg (0.2 mmol) of **23** in 4 mL of 1 M HCl and 4 mL of THF was heated at 70 °C with stirring for 10 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (4 mL). The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided crude sphingosine analogue **25** as a white solid, which was used in the next reaction without further purification. To a solution of **25** in 6 mL of dry THF was added 108 mg (0.40 mmol) of *p*-nitrophenyl caprylate at rt. The mixture was stirred for 48 h and concentrated. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 51 mg (61%, two steps) of $\Delta^{4,6}$-ceramide analogue **2** as a white solid: mp 69.0-71.0 °C; $[\alpha]^{25}_D$ -4.30° (*c* 2.2, $CHCl_3$); IR 1620, 1540, 1455 cm^{-1}; ^{1}H NMR δ 0.86 (t, 6H, *J* = 6.6 Hz), 1.10-1.40 (m, 26H), 1.60 (m, 2H), 2.05 (q, 2H, *J* = 7.1 Hz), 2.20 (t, 2H, *J* = 7.4 Hz), 3.69 (m, 1H), 3.90 (m, 2H), 4.37 (br s, 1H), 5.60 (dd, 1H, *J* = 15.3, 6.3 Hz), 5.73 (m, 1H), 6.00 (m, 1H), 6.27 (m, 2H); ^{13}C NMR δ 14.05, 14.10, 22.6, 22.7, 25.8, 29.0, 29.15, 29.21, 29.3, 29.49, 29.59, 29.62, 29.65, 31.7, 31.9, 32.7, 36.8, 54.5, 62.5, 74.5, 128.9, 132.7, 136.7, 174.0; HR-MS (FAB, MNa$^+$) calcd for *m/z* $C_{26}H_{49}NO_3Na$ 446.3610, found 446.3598.

***N*-*tert*-Butoxycarbonyl (4*S*)-4-[(Phenylsulfonyl)acetyl]-2,2-dimethyl-1,3-oxazolidine [(-)-17].** A solution of sulfone **16** (937 mg, 6.0 mmol) in 10 mL of THF was added 2.64 mL of *n*-butyllithium (a 2.5 M solution in hexane, 6.6 mmol) at -15 °C under N_2. The reaction mixture was stirred at -15 °C for 30 min, and then chilled to -78 °C. A solution of ester **9** (778 mg, 3.0 mmol) in 5 mL of THF was added dropwise. The reaction mixture was stirred at -78 °C for 2 h and allowed to warm to rt overnight. Saturated aqueous NH_4Cl solution (10 mL) was added, and the product was extracted with EtOAc, washed with brine, and dried ($MgSO_4$). Purification by column chromatography (hexane/EtOAc 1:1, R_f 0.72) gave 817 mg (71%) of **17** as a white solid: mp 105-106 °C; $[\alpha]^{25}_{D}$ -93.2° (*c* 2.2, $CHCl_3$); ^1H NMR (C_6D_6, 70 °C) δ 1.33 (s, 9H), 1.45 (s, 3H), 1.58 (s, 3H), 3.90 (br s, 2H), 3.99 (d, 1H, J = 14.0 Hz), 4.15 (br s, 1H), 4.53 (br s, 1H), 6.99 (m, 3H), 7.78 (m, 2H); HR-MS (FAB, MH^+) calcd for *m/z* $C_{18}H_{26}NO_6S$ 384.1481, found 384.1487.

(3*E*)-Pentadecenyl Phenyl Sulfone (21). To a solution of sulfone **16** (780 mg, 5.0 mmol) in 5 mL of THF was added 2.4 mL of *n*-butyllithium (a 2.5 M solution in hexane, 6.0 mmol) at -78 °C under N_2. The solution was stirred at -78 °C for 30 min. A solution of bromide **8** (140 mg, 5.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred at –78 °C for 2 h and allowed to warm to rt overnight. After saturated aqueous NH_4Cl solution (10 mL) was added, the product was extracted with EtOAc, washed with brine, and dried ($MgSO_4$). Purification by column chromatography (hexane/EtOAc 3:2, R_f 0.72) gave 175 mg (50%) of **21** as a colorless liquid: ^1H NMR δ 0.83 (t, 3H, J = 7.0 Hz), 1.10-1.40 (m, 18H), 1.87 (m, 2H), 2.35 (m, 2H), 3.08 (m, 2H), 5.22 (m, 1H), 5.41 (m, 1H), 7.51 (m, 2H), 7.61 (m, 1H), 7.86 (m, 2H); ^{13}C NMR δ 14.0, 22.6, 25.8, 29.1, 29.2, 29.3, 29.47, 29.53, 31.8, 32.3, 56.0, 124.8, 128.0, 129.1, 133.5, 139.0.

***N-tert*-Butoxycarbonyl (4*S*)-4-[1'-Oxo-2'-phenylsulfonyl-(4'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (18).** **Method A**: To a solution of β-ketosulfone **17** (383 mg, 1.0 mmol) in 10 mL of benzene was added DBU (153 mg, 1.0 mmol) at rt. After the mixture was stirred at rt for 1 h under N_2, a solution of bromide **8** (275 mg, 1 mmol) in 5.0 mL of benzene was added dropwise. The reaction mixture was stirred at rt for 3 h and passed through a pad of silica gel to remove the precipitate (DBU•HBr). The pad was washed with benzene. Purification by column chromatography (hexane/EtOAc 4:1, R_f 0.70) gave 428 mg (74%) of **18** as a colorless liquid. **Method B**: To a solution of phenyl sulfone **21** (766 mg, 2.0 mmol) in 5 mL of THF was added 0.8 mL of *n*-butyllithium (a 2.5 M solution in hexane, 2.0 mmol) dropwise at -15 °C under N_2. After 30 min the mixture was cooled to -78 °C, and a solution of ester **9** (259 mg, 1.0 mmol) in 5 mL of THF was added dropwise. The reaction mixture was stirred at -78 °C for 2 h, then allowed to warm to rt, with stirring for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution (10 mL). The product was extracted with EtOAc, the organic layer was washed with brine, dried ($MgSO_4$), and concentrated. Purification by column chromatography (hexane/EtOAc 4:1, R_f 0.70) gave 231 mg (40%) of **18** as a colorless liquid: IR 1732, 1698, 1390, 1360, 1315, 1175, 1145 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 0.96 (t, 3H, J = 7.0 Hz), 1.35 (m, 18H), 1.44 (s, 9H), 1.55 (s, 3H), 1.74 (s, 3H), 1.88 (m, 1H), 1.97 (m, 1H), 2.60-3.00 (m, 2H), 3.80-4.19 (m, 1H), 4.01-4.30 (m, 1.5H), 4.78-4.90 (m, 1H), 5.39 (br s, 1H), 5.46-5.49 (m, 1.5H), 7.05-7.17 (m, 3H), 7.23 (m, 1H), 8.04 (br s, 1H); HR-MS (FAB, MH$^+$) calcd for *m/z* $C_{32}H_{52}NO_6S$ 578.3515, found 578.3515.

***N-tert*-Butoxycarbonyl (4*S*)-4-[1'-Oxo-(4'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(-)-20].** To a solution of ketosulfone **18** (290 mg, 0.50 mmol) in 25 mL of

THF/H_2O 20/1 was added Al(Hg) (freshly prepared from aluminum foil; 135 mg, 5 mmol, 2% aqueous $HgCl_2$).[18] After the mixture was stirred at rt overnight, it was passed through a pad of silica gel with suction, which was washed with EtOAc. Concentration and purification by flash column chromatography (hexane/EtOAc 4:1, R_f 0.85) gave 186 mg (86%) of ketone **20** as a colorless oil: $[\alpha]^{25}_D$ -5.6° (*c* 2.8, $CHCl_3$); IR 1709, 1463, 1390, 1380, 1365, 1167 cm^{-1}; ^1H NMR (C_6D_6, 70 °C) δ 0.86 (t, 3H, *J* = 7.0 Hz), 1.29 (m, 18H), 1.38 (m, 9H), 1.47 (s, 3H), 1.75 (s, 3H), 1.96 (m, 2H), 2.30 (m, 2H), 2.39 (m, 2H), 3.69 (m, 2H), 4.16 (br s, 1H), 5.43 (m, 2H); ^{13}C NMR (C_6D_6, 70 °C) δ 14.1, 23.0, 26.7, 28.4, 29.6, 29.7, 29.9, 30.0, 30.1, 32.3, 32.9, 65.6, 65.7, 80.2, 129.1, 131.8, 206.2; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{26}H_{47}NO_4Na$ 460.3403, found 460.3393.

***N-tert*-Butoxycarbonyl (4*S*)-4-[1'-Hydroxy-(4'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (26).** To a solution of ketone **20** (65 mg, 0.15 mmol) in 4 mL of dry MeOH was added $NaBH_4$ (6.4 mg, 0.17 mmol) at -15 °C. The temperature was gradually raised to 0 °C. After 2 h, water (5 mL) was added, and the product was extracted with Et_2O (3 x 15 mL), washed with brine, dried ($MgSO_4$), and concentrated. Purification by column chromatography (hexane/EtOAc 4:1, R_f 0.45) gave 58 mg (88%) of **26** as a colorless oil: IR 1701, 1671, 1457, 1390, 1365 cm^{-1}; ^1H NMR (C_6D_6, 70 °C) δ 0.89 (t, 3H, *J* = 6.9 Hz), 1.22 (m, 20H), 1.30 (s, 9H), 1.47 (s, 3H), 1.63 (s, 3H), 2.00 (m, 2H), 2.20 (m, 1H), 2.34 (m, 1H), 3.61-3.69 (m, 2H), 3.84 (br s, 2H), 5.46-5.55 (m, 2H); ^{13}C NMR (C_6D_6, 70 °C) δ 14.1, 23.0, 27.2, 28.4, 29.1, 29.6, 29.7, 30.0, 30.1, 32.3, 33.0, 62.7, 65.1, 80.3, 94.4, 130.5, 131.2.

(2*S*,3*R*)-(6*E*)-2-Caprylamidooctadecadiene-1,3-diol [(-)-3]. A solution of 88 mg (0.2 mmol) of **26** in 4 mL of 1 M HCl and 4 mL of THF was heated at 70 °C with stirring for 10 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (4 mL). The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided crude sphingosine analogue **27** as a white solid, which was used in the next reaction without further purification. To a solution of **27** in 6 mL of dry THF was added 108 mg (0.40 mmol) of *p*-nitrophenyl caprylate at rt. The mixture was stirred for 48 h and then concentrated under reduced pressure. Purification by column chromatography (EtOAc) afforded 53 mg (63%, two steps) of Δ^6-ceramide analogue **3** as a low-melting white solid: $[\alpha]^{25}_D$ -2.39° (*c* 2.2, $CHCl_3$); IR 1631, 1542 cm^{-1}; ^1H NMR δ 0.86 (t, 6H, *J* = 7.1 Hz), 1.10-1.40 (m, 26H), 1.52 (m, 2H), 1.62 (m, 2H), 1.93 (m, 2H), 2.07 (m, 2H), 2.21 (t, 2H, *J* = 7.7 Hz), 2.57 (br s, 2H), 3.78 (m, 2H), 3.88 (m, 1H), 3.96 (m, 1H), 5.38 (m, 2H), 6.20 (d, 1H, *J* = 8.3 Hz); ^{13}C NMR δ 14.05, 14.10, 22.5, 22.7, 25.8, 28.8, 29.0, 29.2, 29.4, 29.52, 29.53, 29.6, 29.7, 31.4, 31.7, 31.9, 32.6, 34.0, 36.9, 53.2, 65.4, 72.6, 129.1, 131.8, 174.1; HR-MS (DCI, MH^+) calcd for *m/z* $C_{26}H_{52}NO_3$ 426.3947, found 426.3952.

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we observed that the elimination reaction proceeds even at room temperature, affording a conjugated dienone (e.g., **13**).

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20. The oxazolidine system undergoes a dynamic equilibrium at ambient temperature.

Chapter 3

Synthesis of D-*erythro*-Sphingosine-d_{27} and Sphingosine-d_{27} Phosphate

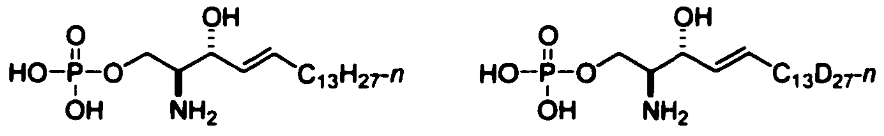
Abstract

Deuterium was introduced into the sphingoid backbone of ceramide by addition of lithium pentadecyne-d_{27} to Garner aldehyde in the presence of HMPA, giving a high erythro selectivity (erythro/threo 18/1). Red-Al reduction of propargylic alcohol **9** and acid hydrolysis provided sphingosine **11**. Sphingosine-d_{27}-1-phosphate **2** was obtained by treatment of **11** with ATP in the presence of sphingosine kinase.

Introduction

Sphingosine 1-phosphate is currently considered to be a predictor of coronary artery disease, the leading cause of death in many countries.[1] Although sphingosine 1-phosphate is a constituent of normal serum, its level in the serum is elevated under conditions associated with coronary disease (such as hypoxia).[1] Therefore, it is desirable to develop a convenient method for estimating the level of this lipid in serum. In order to use stable isotope dilution MS^2 for quantification of sphingosine 1-phosphate (**1**) in biological samples (same as plasma), we plan to use deuterium in the sphingoid backbone (**2**), thereby increasing the m/z of sphingosine 1-phosphate by 27 in the internal standard.

Chart 1

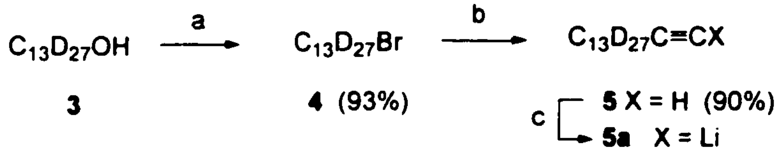


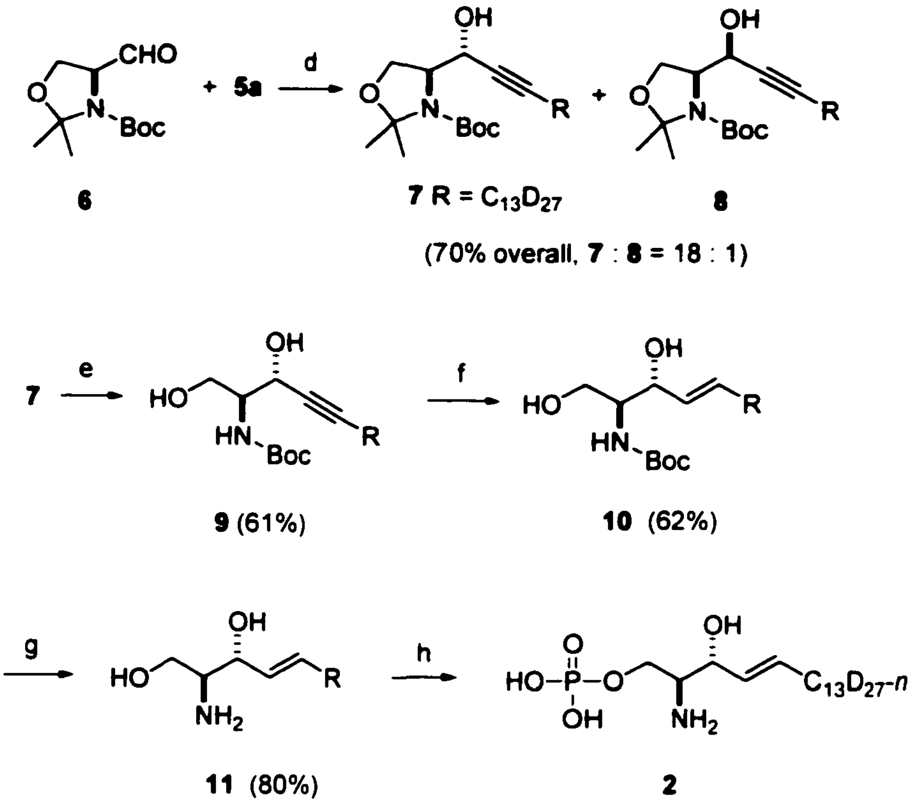
1 2

Results and Discussion

Scheme 1 shows the preparation of perdeuteriotridecyl bromide **4** from $C_{13}D_{27}OH$ (**3**) via Mitsunobu reaction[3] (93% yield). Reaction of **4** with lithium acetylenide-ethylenediamine complex gave alkyne **5** in 90% yield. Addition of lithium pentadecyne to Garner aldehyde[4] **6** produced either the erythro or the threo stereochemistry, depending on the reaction conditions used.[5] *threo*-Selectivity was predominant under chelation conditions ($ZnBr_2$ in Et_2O). Solvents such as HMPA, which competes effectively with the *N*-Boc group for coordination to the RCHO-Li species, result in high erythro selectivity (erythro/threo > 20/1). The acetonide was removed by acid hydrolysis (Amberlyst-15, MeOH). Red-Al reduction[6] of propargylic alcohol **9** afforded *N*-Boc-sphingosine-d_{27} **10**. Acid hydrolysis of **10** (1 M HCl in THF, 70 °C) provided sphingosine-d_{27} **11** in 80% yield. Treatment of **11** with ATP in the presence of sphingosine kinase provided sphingosine-d_{27} 1-phosphate **2** (this step was carried out by Dr. Dan Baker, University of Tennessee Health Sciences Center, Memphis).

Scheme 1. Synthesis of Sphingosine-d_{27} 11 and Sphingosine-d_{27} 1-phosphate 2[a]





[a]Reagents and Conditions: (a) NBS, Ph_3P, CH_2Cl_2, 0 °C-rt; (b) lithium acetylide ethylenediamine complex, DMSO, 0 °C-rt; (c) *n*-BuLi, THF, -23 °C; (d) *n*-BuLi, HMPA, THF, -23 °C; (e) Amberlyst 15, MeOH, rt; (f) Red-Al, Et_2O, 0 °C-rt; (g) 1 M HCl, THF, 70 °C; (h) sphingosine kinase, ATP (by Dr. Dan Baker).

Experimental Section

General Information. See Chapter 1 for the detailed information. THF and Et_2O were distilled from Na and benzophenone immediately before use. CH_2Cl_2 and DMSO were dried over CaH_2. HMPA was distilled from CaH_2. $C_{13}D_{27}OH$ (**3**) was purchased from Cambridge Isotope Laboratories. Lithium acetylide-ethylenediamine complex was purchased from Aldrich. Garner aldehyde **6** was prepared from *N*-Boc-L-serine methyl ester (purchased from Aldrich) as described previously.[4c]

1-Bromotridecane-d_{27} (4). To a solution of 0.5 g (2.2 mmol) of alcohol **3** and 0.64 g (2.4 mmol) of Ph_3P in 15 mL of dry CH_2Cl_2 was added 0.47 g (2.6 mmol) of NBS at 0 °C under argon atmosphere. The mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature and stirred for 1 h. The mixture was diluted with 30 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO. Purification by column chromatography (hexane) gave 0.59 g (93%) of **4** as a colorless oil.

1-Pentadecyne-d_{27} (5). A 25-mL, three-necked flask equipped with a pressure-equalizing dropping funnel, condenser, and stirrer was flushed with argon and charged with 0.40 g (4.0 mmol) of lithium acetylide-ethylenediamine complex (90% purity). Dry DMSO (5 mL) was added to form a solution-slurry. The solution was stirred and brought to 0 °C. A solution of compound **4** (0.59 g, 2.0 mmol) in 5 mL of dry DMSO was then added dropwise over 5 min. When the addition was completed, the reaction mixture was held at room temperature for 2 h. Water (2 mL) was added slowly, followed by 1 M HCl (5 mL). The mixture was extracted with three 15-mL portions of hexane. The extracts were combined and washed successively with 10 mL of water and 10 mL of saturated aqueous NaCl solution, dried ($MgSO_4$), and concentrated. Purification by column chromatography (hexane) gave 0.42 g (90%) of **5** as a colorless oil.

***tert*-Butyl (4*S*,1'*R*)-2,2-Dimethyl-4-(1'-hydroxy-2'-hexadecynyl)-3-oxazolidinecarboxylate-d_{27} (7).** To a solution of 0.40 g (1.7 mmol) of **5** in 10 mL of dry THF at −23 °C was added 0.72 mL (1.8 mmol) of *n*-BuLi (a 2.5 M solution in hexane) dropwise under argon atmosphere. After the mixture was stirred for 1 h, HMPA (0.60 mL, 3.4 mmol) was added. After 10 min, a solution of 0.39 g (1.7 mmol) of aldehyde **6**

in 5 mL of dry THF was added dropwise over a 5-min period. The mixture was stirred at −23 °C for 3 h. The reaction was quenched with aqueous saturated $NaHCO_3$ solution (5 mL). The organic phase was separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organic phase was washed twice with brine, dried ($MgSO_4$), and evaporated. Column chromatography (hexane/EtOAc 4:1) gave a 18:1 mixture of **7**/**8** (0.55 g, 70%). Pure **7** was obtained by chromatography (hexane/EtOAc 4:1) by gravity on multiple columns: ^1H NMR δ 1.20-1.80 (m, 15H), 3.77 (t, 1H, J = 8.6 Hz), 3.94 (s, 1H), 4.03 (s, 1H), 4.69 (s, 1H).

***tert*-Butyl (1*S*,2*R*)-*N*-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate-d_{27} (9).** A solution of 0.40 g (0.86 mmol) of **7** in 5 mL of MeOH was added 0.40 g of Amberlyst 15, and the heterogeneous mixture was stirred at room temperature for 48 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 0.22 g (61%) of **9** as a white solid: ^1H NMR δ 1.20-1.60 (s, 9H), 3.05 (br s, 1H), 3.70 (br s, 2H), 3.98 (br s, 1H), 4.06 (d, 1H, J = 9.9 Hz), 4.70 (s, 1H), 5.50 (d, 1H, J = 7.7 Hz).

***tert*-Butyl (3*E*,1*S*,2*R*)-*N*-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate-d_{27} (10).** A solution of 50 mg (0.12 mmol) of **9** in dry Et_2O (10 mL) was treated dropwise with 3.3 mL (1.1 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched by slow addition of 0.5 mL of MeOH at 0 °C. The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography ($CHCl_3$/MeOH 9:1) gave 31 mg (62%) of **10**: ^1H NMR δ 1.40-

1.60 (s, 9H), 2.69 (br s, 1H), 2.80 (br s, 2H), 3.59 (d, 1H, J = 4.2 Hz), 3.70 (d, 1H, J = 3.1 Hz), 3.80 (d, 1H), 4.21 (br s, 1H), 5.31 (d, 1H, J = 7.4 Hz), 5.43 (dd, 1H, J = 15.4, 5.9 Hz), 5.63 (d, 1H, J = 15.3 Hz).

D-*erythro*-Sphingosine-d_{27} (11). A solution of 20 mg (0.047 mmol) of **10** in 3 mL of 1 M HCl and 3 mL of THF was heated at 70 °C with stirring for 8 h under argon. The reaction mixture was cooled to room temperature, and neutralized with 1 M NaOH (3 mL). The product was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Flash chromatography ($CHCl_3$/MeOH/conc. NH_4OH 130:25:4) gave a white solid that was dissolved in a minimum volume of $CHCl_3$ and passed through a Cameo filter (Fisher Scientific) to remove dissolved silica gel. Finally, the product was lyophilized from benzene to give 12 mg (80%) of sphingosine-d_{27} **11** as a white powder: ^{1}H NMR δ 2.62 (br s, 1H), 3.48 (br s, 1H), 3.53 (s, 1H), 3.83 (s, 1H), 3.80 (d, 1H, J = 5.9 Hz), 5.32 (dd, 1H, J = 15.4, 5.9 Hz), 5.62 (d, 1H, J = 15.3 Hz).

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Chapter 4

Synthesis and Growth Inhibitory Activity of Chiral 5-Hydroxy-2-*N*-Acyl-3*E*-Sphingenines: Ceramides with an Unusual Sphingoid Backbone

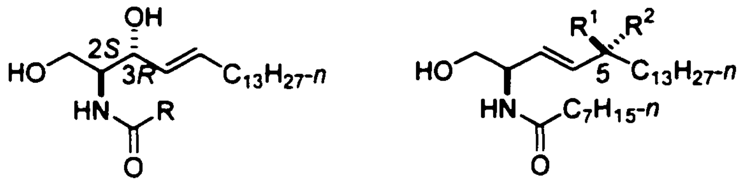
Abstract

The unusual sphingoid base 5-hydroxy-3-sphingenine was identified in the hydrolysate of brain sphingolipids more than 40 years ago. We present here the first synthesis of the 5*R* and 5*S* diastereoisomers of the *N*-acyl derivatives of 5-hydroxy-3-sphingenine, **2** and **3**, respectively, which represent regioisomers of 2*S*,3*R*-ceramide (**1**). The key steps include the synthesis of α,β-unsaturated ketone intermediates **4** and **5** from *N*-Cbz- and *N*-Boc-L-serine and diastereoselective reduction of the enones. The configuration at the new carbinol center was deduced by proton NMR analysis of (*R*)- and (*S*)-Mosher [methoxy(trifluoromethyl)phenylacetate] ester derivatives. Ceramide analogues **2** and **3** showed a markedly higher antiproliferative activity than **1** on MCF-7 cells.

Introduction

Sphingolipids are widely distributed in mammalian membranes, where they play a structural role and also participate in a plethora of cellular events. They all have, by definition, a "sphingoid base" backbone, the most common of which is (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol (C_{18}-sphingosine).[1] A variety of sphingosines exist that differ with respect to the lipid chain length and location of unsaturation, as well as the number of hydroxy groups. The sphingoid base 5-hydroxy-3*E*-sphingenine occurs naturally. It was isolated by TLC[2] and HPLC[3] from the acid hydrolysate of a human brain sphingolipid mixture; however, the configuration at C5 was not established. As part of our interest in analyzing structure-function relationships of ceramides (*N*-acylsphingosines) that differ with respect to the location of the double bond and hydroxy groups in the sphingoid base,[4] we report here the synthesis of ceramides **2** and **3** (see Chart 1). These diastereomers represent regioisomers of 2*S*,3*R*-ceramide (**1**),[5] which occupies the "hub" of sphingolipid metabolism and serves as a coordinator of eukaryotic stress responses and other biological activities.[6] In view of the capacity of **1** to regulate various biological functions, the availability of some of its analogues, such as **2** and **3**, would contribute to our understanding of the complex structural biology of ceramide. We report here that **2** and **3** are significantly more effective than **1** in inhibiting the growth of a breast tumor cell line, although the mechanism by which they exert their antiproliferative action is unclear.

Chart 1



1

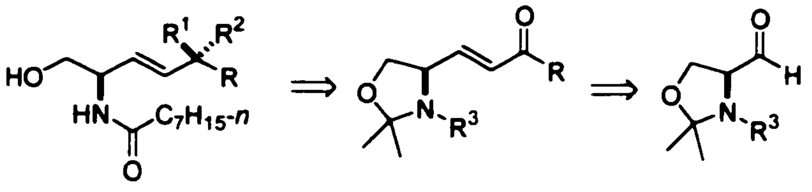
2 R^1 = H, R^2 = OH
3 R^1 = OH, R^2 = H

Results and Discussion

Retrosynthetic Analysis. Scheme 1 illustrates our strategy for the preparation of ceramide **2** and **3**. The stereochemistry of the C5-hydroxy group is generated by diastereoselective reduction of the key enone intermediates **4** and **5**. The stereochemistry of the *E*3-double bond results from the Horner-Wadsworth-Emmons (HWE) reaction of

L-serine-derived aldehydes **6** and **7** with ketophosphonate **14**. The configuration at C2 is derived from L-serine as the chiral precursor.

Scheme 1. Retrosynthetic Plan



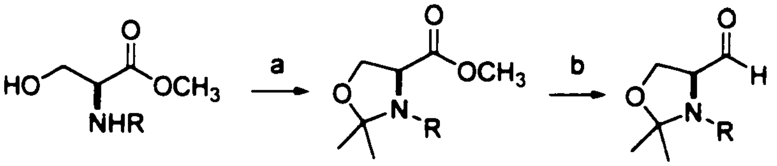
Ceramides 2 and 3
2 $R^1 = H$, $R^2 = OH$
3 $R^1 = OH$, $R^2 = H$
$R = C_{13}H_{27}$-*n*

Enones 4 and 5
4 R^3 = Cbz
5 R^3 = Boc

Aldehydes 6 and 7
6 R^3 = Cbz
7 R^3 = Boc

Synthesis of Aldehydes 6 and 7 (Scheme 2). Aldehyde **6** was prepared by oxazolidine formation of *N*-Cbz-L-serine **8** (2,2-dimethoxypropane, catalytic *p*-TsOH, benzene),[7] followed by reduction of acetonide **10** with DIBAL-H in toluene at –78 °C. Similarly, (*S*)-Garner aldehyde **7** was prepared from *N*-Boc-L-serine **9** via acetonide **11**.[8]

Scheme 2. Synthesis of Aldehydes 6 and 7[a]



8 R = Cbz
9 R = Boc

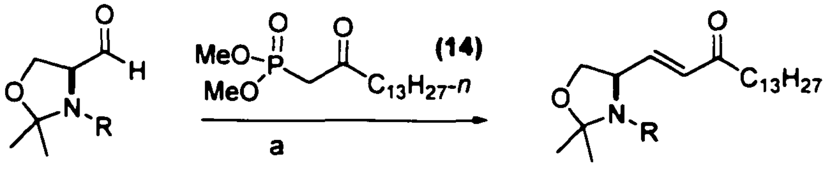
10 R = Cbz (88%)
11 R = Boc (87%)

6 R = Cbz (75%)
7 R = Boc (76%)

[a]Reagents and conditions: (a) $Me_2C(OMe)_2$, *p*-TsOH, C_6H_6, reflux; (b) DIBAL-H, toluene, -78 °C.

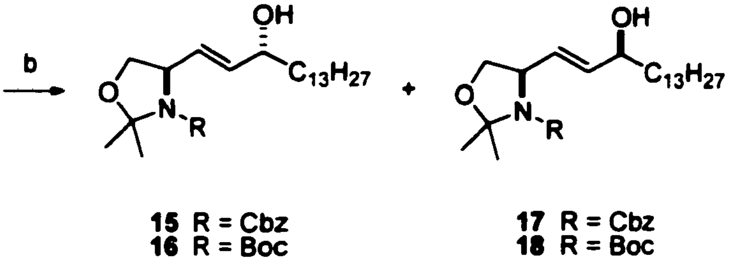
Installation of the Lipid Chain and Diastereoselective Reduction of Enones 4 and 5 (Scheme 3). Treatment of dimethyl methanephosphonate (**12**) with *n*-BuLi in THF at −78 °C and reaction with methyl tetradecanoate (**13**) provided β-ketophosphonate **14** in 95% yield. As shown in Scheme 3, HWE reaction of **14** with L-(*N*-Cbz)- and (*N*-Boc)-serinals **6** and **7** gave enones **4** and **5**, respectively, in high yield. Several reducing agents were screened for the attempted diastereoselective reduction of the ketone. Table 1 shows that reduction of *N*-Boc-protected enone **5** generally showed modestly higher selectivity with a variety of reducing agents than that of *N*-Cbz-protected enone **4**. The *tert*-butyl group appears to be more effective than the benzyl group with respect to blocking one face of the carbonyl group with all of the reducing agents shown in Table 1 except DIBAL-H. We found that reduction of **4** and **5** at 0 °C with $NaBH_4$ in the presence of $CeCl_3$ in methanol gave alcohols **15** and **16** in low stereoselectivity. Reduction with $LiAlH_4$ in the presence of a Chirald® (a chiral ligand)[9] provided diastereomers **15** and **16** as the major products in a ~2:1 and 5.2:1 ratio of isomers, respectively. Similar results were obtained with oxazaborolidine-catalyzed reduction.[10] Fortunately, high stereoselectivity was achieved by using the bulky L-Selectride in THF (1 h, 0 °C -room temperature), and high ratios of **15**/**17** (8:1) and **16**/**18** (18:1) were obtained. The two diastereoisomers were readily separated by column chromatography. Thus L-Selectride is the reducing agent of choice for the synthesis of ceramide **2** (via **16**), whereas $NaBH_4/CeCl_3$ is preferred for the preparation of ceramide **3** (via **18**).

Scheme 3. Diastereoselective Reduction of Enones 4 and 5[a]



6 R = Cbz
7 R = Boc

4 R = Cbz (91%)
5 R = Boc (90%)



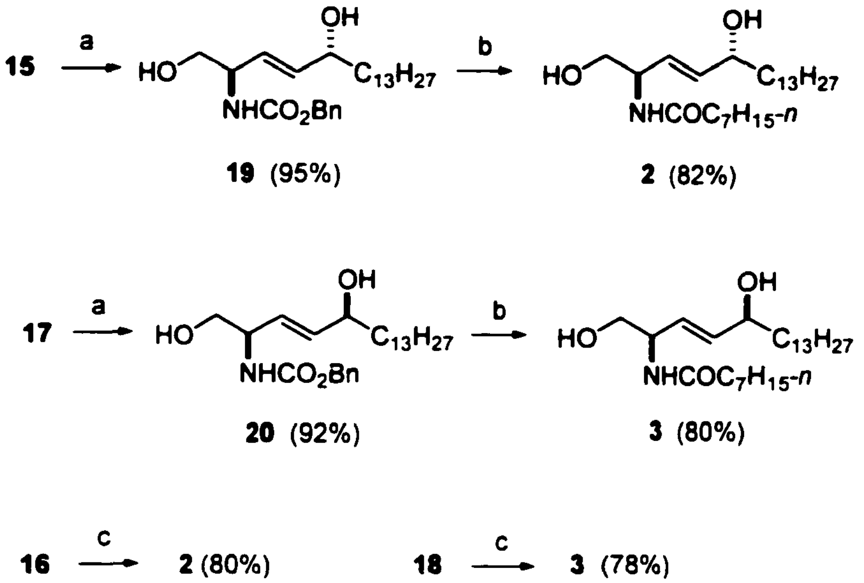
*a*Reagents and conditions: (a) Cs_2CO_3, 2-PrOH, rt; (b) see Table 1.

Table 1. Diastereoselective Reduction of Enones 4 and 5

reducing agent	reduction conditions	reduction of enone **4**		reduction of enone **5**	
		ratio of **15**:**17**	overall yield (%)	ratio of **16**:**18**	overall yield (%)
$NaBH_4$, $CeCl_3$	MeOH, 0 °C, 3 h	1.0:1.0	88	1.0:1.8	90
DIBAL-H	THF, 0 °C, 1 h	1.0:1.0	90	1.0:1.1	89
$LiAlH_4$, Chirald®	Et_2O, -78 °C, 6 h	2.0:1.0	86	5.2:1.0	87
$BH_3 \cdot Me_2S$, oxazaborolidine	THF, rt, 1 h	1.4:1.0	85	2.3:1.0	84
L-Selectride	THF, 0 °C-rt, 1 h	8.0:1.0	91	18.0:1.0	90

Deprotection and *N*-Acylation (Scheme 4). Acid hydrolysis of **15** (*p*-TsOH, MeOH) provided **19** in 95% yield; similarly, **20** was obtained from **17** in 92% yield. After the corresponding regioisomeric sphingosine analogues were obtained by removal of the Cbz group with lithium in liquid NH_3, *N*-acylation with *p*-nitrophenyl octanoate gave the diastereoisomeric ceramide analogues **2** and **3** in 82% and 80% yield, respectively. Ceramides **2** and **3** were also obtained in good yield from **16** and **18**, respectively, by removal of the *N*-Boc and isopropylidene protecting groups (1 M HCl, dioxane, 100 °C) and *N*-acylation with *p*-nitrophenyl octanoate.

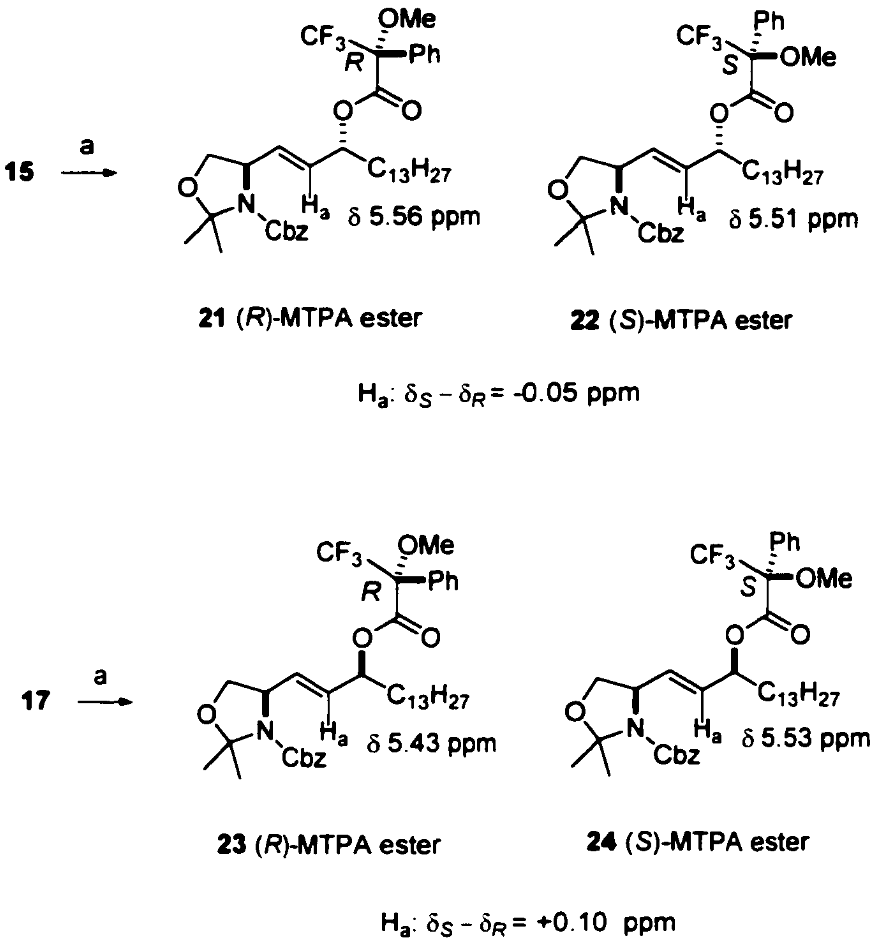
Scheme 4. Deprotection and *N*-Acylation[a]



[a] Reagents and conditions: (a) *p*-TsOH, MeOH, rt; (b) (i) Li, NH_3, -78 °C; (ii) *p*-$O_2NC_6H_4CO_2C_7H_{15}$-*n*, THF, rt; (c) (i) 1 M HCl, dioxane, 100 °C; (ii) *p*-$O_2NC_6H_4CO_2$-C_7H_{15}-*n*, THF, rt.

Configurational Assignment (Scheme 5). The assignment of the configuration at C-5 of **15** and **17** was made by ^1H NMR analysis[11] of the corresponding *S* and *R* Mosher esters, which were prepared by the reaction of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) chloride or (*R*)-MTPA chloride with **15** and **17** in the presence of DMAP. The difference between the chemical shift of the vinyl proton H_a in the (*R*)- and (*S*)-MTPA ester derivatives was used to determine the absolute configuration at C-5.[11,12] The upfield signal of H_a in the (*S*)-MTPA ester **22** (δ 5.51 ppm) compared to that in the (*R*)-MTPA ester **21** (δ 5.56 ppm) indicates that **15** has the *R* configuration ($\Delta\delta_{Ha} = \delta_S - \delta_R = -0.05$ ppm). Similarly, the *S* configuration was assigned to **17** by the downfield shift of H_a in **24** (δ 5.53 ppm) compared with **25** (δ 5.43 ppm) ($\Delta\delta_{Ha} = \delta_S - \delta_R = +0.10$ ppm).

Scheme 5. Configurational Assignment by ^{1}H NMR Analysis of the Diastereomeric (*S*)- and (*R*)- MTPA Esters[a]



21 (*R*)-MTPA ester

22 (*S*)-MTPA ester

H_a: $\delta_S - \delta_R = -0.05$ ppm

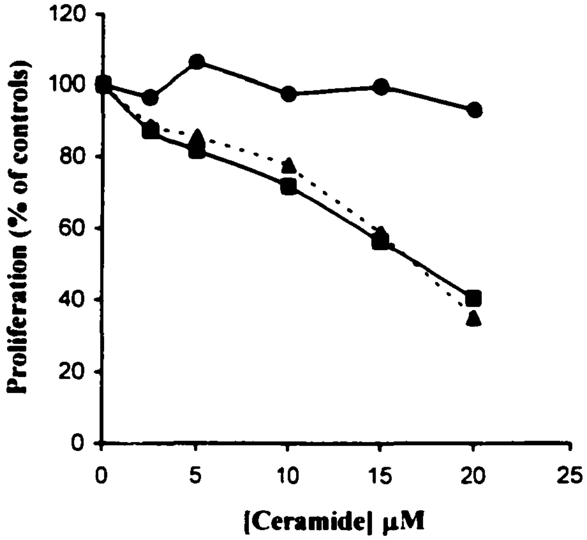
23 (*R*)-MTPA ester

24 (*S*)-MTPA ester

H_a: $\delta_S - \delta_R = +0.10$ ppm

[a] Reagents and conditions: (a) (*S*)-(+)- or (*R*)-(-)-MTPA chloride, CH_2Cl_2, DMAP, rt.

Figure 1. Effects of ceramides 1-3 on the proliferation of MCF-7 cells. Cells were grown in medium containing 5% serum and 1-3 (0-20 μM) for 48 h. The cell numbers were determined as described in the Experimental Section. (●), 1; (■), 2; (▲), 3.



Biological Evaluation of Compounds 2 and 3 (Figure 1). Synthetic ceramides with a short *N*-acyl chain (such as octyl) have been widely used for in vitro studies because they tend to be more cell permeable than the long-chain endogenous ceramides. To assess whether C8-ceramides having a 5-hydroxy-3*E*-sphingenine backbone show antiproliferative activity against epithelial tumor cells, exponentially growing MCF-7 cells were treated in Gilbert Arthur's laboratory (University of Manitoba) with varying concentrations of compounds **2** and **3** (0-20 μM) for 48 h. Figure 1 shows a comparison of the effects of **1**-**3** on MCF-7 cell growth. Surprisingly, we found that ceramides **2** and **3** possessed significantly higher antiproliferative activity than **1**, which is known to induce apoptosis in many cells.[13] The IC_{50} value (the drug concentration required to inhibit growth by 50%) for **2** and **3** was ~15 μM, indicating that the configuration at C-5 did not affect the activity, whereas the IC_{50} value of **1** was >>20 μM. Thus MCF-7 cells are significantly more resistant to C8-ceramides with the prevalent 3-hydroxy-4*E*-sphingenine backbone than with a 5-hydroxy-3*E*-sphingoid backbone. No information is available concerning the metabolism and intracellular localization of lipids containing the unusual 5-hydroxy-3*E*-sphingenine core. Further studies are planned to clarify the mechanisms by which ceramides with an altered alkenyl sphingoid chain such as **2** and **3** exert their antiproliferative action.

Conclusion

In summary, the first synthesis of 5*R* and 5*S* diastereoisomeric ceramides **2** and **3**, which have the naturally occurring but unusual 5-hydroxy-3*E*-sphingenine long-chain base and a *N*-octanoyl residue, was achieved in several steps from serinal derivatives **6** and **7**. A higher degree of diastereoselectivity was achieved in the L-Selectride reduction of *N*-Boc-enone **5** than *N*-Cbz-enone **4**. The in vitro antiproliferative activity of 3-alkenylceramides **2** and **3** in the breast tumor cell line MCF-7 was much higher than that of D-*erythro*-*N*-C8-ceramide **1**.

Experimental Section[14]

General Information. Chirald® (Darvon alcohol), L-Selectride, L-(*N*-Cbz)-serine methyl ester (**8**), L-(*N*-Boc)-serine methyl ester (**9**), and (*R*)-(-)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) chloride were used directly as obtained commercially.

***N*-Benzyloxycarbonyl 4(*S*)-(1'-Methoxycarbonyl)-2,2-dimethyl-1,3-oxazolidine [(-)-10].** To a solution of *N*-Cbz-L-serine methyl ester (**8**) (11.6 g, 0.046 mol) in benzene (160 mL) were added 2.2-dimethoxypropane (9.7 g, 0.092 mol) and *p*-TsOH·H_2O (0.122 g, 0.64 mmol) under N_2 at rt. The solution was heated under reflux for 40 min, then slowly distilled until a volume of 135 mL was collected over 2 h, when the reaction was judged to be complete by TLC. Saturated aqueous $NaHCO_3$ solution (40 mL) was added, and the mixture was extracted with Et_2O (2 x 100 mL). The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated to give the crude product as a colorless oil. Purification by flash chromatography gave 11.9 g (88% yield) of oxazolidine methyl ester **10** as a colorless liquid. The NMR data are in full accord with the literature data.[7a]

***N*-Butoxycarbonyl 4(*S*)-(1'-Methoxycarbonyl)-2,2-dimethyl-1,3-oxazolidine [(-)-11].** This compound was prepared from **9** in 87% yield by the procedure described above.[8] The NMR data are in full accord with the literature data.[8]

***N*-Benzyloxycarbonyl 4(*S*)-(1'-Formyl)-2,2-dimethyl-1,3-oxazolidine [(-)-6].** To a solution of oxazolidine ester **10** (3.8 g, 13.0 mmol) in dry toluene (25 mL) was slowly added 15.2 mL (22.8 mmol) of DIBAL-H (a 1.5 M solution in toluene) at –78 °C under N_2. The rate of addition was adjusted so as to keep the internal temperature below –65 °C. The reaction mixture was stirred at –78 °C for an additional 2 h, when TLC showed the reaction to be complete. The reaction was quenched by slowly adding 6 mL of cold (-78 °C) MeOH. The resulting mixture was slowly poured into 90 mL of ice-cold 1 M HCl and the mixture was extracted with EtOAc (3 × 80 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated to give the crude product as a pale yellow oil. Vacuum distillation gave oxazolidine aldehyde **6** (2.56 g, 75% yield) as a colorless liquid. The NMR data are in full accord with the literature data.[7a]

***N*-Butoxycarbonyl 4(*S*)-(1'-Formyl)-2,2-dimethyl-1,3-oxazolidine [(-)-7].** This compound was prepared from **11** in 76% yield by the procedure described above.[8] The NMR data are in full accord with the literature data.[8]

Dimethyl 2-Oxopentadecanephosphonate (14). To a solution of 1.5 g (12.0 mmol) of dimethyl methanephosphonate (**12**) in 30 mL of dry THF was added 4.8 mL (12.0 mmol) of *n*-BuLi (a 2.5 M solution in hexanes) at –78 °C under N_2. After the mixture was stirred for 30 min at –78 °C, a solution of 2.4 g (10.0 mmol) of methyl tetradecanoate (**13**) in 10 mL of THF was added dropwise with stirring. The mixture was kept at –78 °C for 1 h and then allowed to warm to 0 °C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution, extracted with $CHCl_3$ (3 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was recrystallized (EtOAc/hexane) to give 2.8 g (92%) of ketophosphonate **14** as a white solid: mp 40.2-41.5 °C; ^{1}H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.25 (m, 20H), 1.57 (t, 2H, J = 7.0 Hz), 2.61 (t, 2H, J = 7.3 Hz), 3.10 (d, 2H, J = 22.7 Hz), 3.76 (d, 2H, J = 10.8 Hz); ^{13}C NMR δ 14.1, 22.7, 23.4, 29.0, 29.4, 29.6, 29.7, 31.9, 40.6 (J = 128.3 Hz), 44.2, 53.0, 202.1; HR-MS [DEI, M^+] *m/z* calcd for $C_{17}H_{35}PO_4$ 334.2273, found 334.2271.

***N*-Benzyloxycarbonyl 4(*R*)-[3'-Oxo-(1'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(-)-4].** To a suspension of ketophosphonate **14** (3.70 g, 11.1 mmol) and Cs_2CO_3 (3.58 g, 11.0 mmol) in 40 mL of 2-propanol was added a solution of aldehyde **6** (2.63 g, 10.0 mmol) in 10 mL of 2-propanol at 0 °C. After stirring at rt overnight, the mixture was diluted with 200 mL of EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (hexane/EtOAc 4:1) to give 4.30 g (91%) of enone **4** as a colorless oil: $[\alpha]^{25}_D$ -35.6° (*c* 5.0. $CHCl_3$): IR 1702. 1631. 1467. 1408. 1349. 1256. 1094 cm^{-1}; ^1H NMR δ 0.88 (t. 3H. *J* = 6.6 Hz). 1.26 (s. 20H). 1.49-1.60 (m. 2H). 1.57 (s. 3H) 1.67 (s. 3H). 2.54 and 2.40 (two sets of t. 2H. *J* = 7.2 Hz). 3.83 (dd. 1H. *J* = 9.2. 2.2 Hz). 4.12 (dd. 1H. *J* = 9.2. 6.4 Hz). 4.45-4.55 and 4.55-4.65 (two sets of m. 1H). 5.00-5.16 (m. 2H), 6.06 (d. 0.68H. *J* = 15.7 Hz). 6.24 (d. 0.32H. *J* = 15.7 Hz). 6.68 and 6.64 (two sets of dd. 1H. *J* = 15.7. 7.0 Hz). 7.26-7.36 (m. 5H): ^{13}C NMR δ 14.1. 22.6 (23.9).[15] 24.4 (24.7). 26.3 (27.3). 28.9. 29.1. 29.2. 29.3. 29.36. 29.4. 29.56. 29.58 (29.60), 31.9 (33.8). 58.0 (58.6). 66.8. (67.4) 67.7. 76.69 (77.00). 77.3. (94.3) 94.9. 128.0. 128.4. 130.5. 136.1. (142.3) 142.7. 152.1 (152.5). 200.0: HR-MS [DCI/NH_3. MH^+] *m/z* calcd for $C_{29}H_{46}NO_4$ 472.3426. found 472.3421.

***N-tert*-Butoxycarbonyl 4(*R*)-[3'-Oxo-(1'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(-)-5].** This compound was prepared from aldehyde **7** in 90% yield by the same procedure as described for **4**: mp 38.0-39.0 °C: $[\alpha]^{25}_D$ –43.7° (*c* 1.0. $CHCl_3$): IR 1693. 1633. 1456. 1391. 1255. 1172. 1098 cm^{-1}: ^1H NMR (C_6D_6. 70 °C)[16] δ 0.88 (t. 3H. *J* = 6.7 Hz). 1.0-1.3 (m. 20H). 1.37 (s. 9H). 1.48 (s. 3H). 1.67 (m. 5H). 2.29 (t. 2H. *J* = 7.2 Hz). 3.43 (dd. 1H. *J* = 9.0. 2.7 Hz). 3.67 (dd. 1H. *J* = 9.0. 6.4 Hz). 4.15 (br s. 1H). 6.13 (d. 1H. *J* = 15.8 Hz). 6.60 (dd. 1H. *J* = 15.8. 7.0 Hz): ^{13}C NMR (C_6D_6) δ 14.3. 23.1. 23.7. 24.4. 25.8. 26.8. 27.6. 28.4. 29.6. 29.8. 29.9. 30.1. 32.3. 40.5. 58.5. (67.2) 67.5. 79.6 (79.9). (93.8) 94.6. 130.7. (142.7) 143.4. 151.7. 198.6: HR-MS [DCI/NH_3. MH^+] *m/z* calcd for $C_{26}H_{48}NO_4$ 438.3583. found 438.3588.

***N*-Benzyloxycarbonyl 4(*R*)-[3'-Hydroxy-(1'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (15, 17).** To a solution of CeCl$_3$ (0.81 g, 3.29 mmol) and NaBH$_4$ (0.12 g, 3.71 mmol) in 30 mL of MeOH was added a solution of enone **4** (1.18 g, 2.50 mmol) in 10 mL of MeOH at 0 °C. The mixture was stirred for 3 h at 0 °C, then 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 under reduced pressure. The residue was purified by chromatography (hexane/EtOAc 3:1) to give diastereoisomers **15** (0.53 g, 45%) and **17** (0.51 g, 43%) as colorless oils: **15**: $[\alpha]^{25}_{D}$ -9.8° (*c* 1.3, CHCl$_3$): IR 1698, 1467, 1410, 1350, 1253, 1095 cm^{-1}: ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz): 1.25 (s, 22H), 1.40-1.60 (m, 2H), 1.55 (s, 3H), 1.65 (s, 3H), 1.84 (br s, 2H), 3.75 (dd, 1H, *J* = 2.2, 8.9 Hz), 3.99 (br s, 1H), 4.05 (dd, 1H, *J* = 6.1, 8.9 Hz), 4.30-4.40 and 4.40-4.50 (two sets of m, 1H), 4.97-5.19 (m, 2H), 5.30-5.70 (m, 2H), 7.29-7.39 (m, 5H): ^{13}C NMR δ 14.1, 22.7 (23.6), 24.9 (25.4), 26.4, 27.27, (29.33) 29.5, 29.56, 29.59, 29.63, 29.65, 29.66 (30.9), 31.9, 37.0 (37.1), 58.5, (66.5) 66.9, 68.3 (68.6), (94.0) 94.4, 128.0, 128.1, (128.3) 128.5, 129.1, 135.3 (135.4), 136.4, 136.6, 152.4: HR-MS [DCI/NH$_3$, MH$^+$] *m/z* calcd for C$_{29}$H$_{48}$NO$_4$ 474.3583, found 474.3561. **17**: $[\alpha]^{25}_{D}$ -18.9° (*c* 2.0, CHCl$_3$): IR 1698, 1466, 1410, 1350, 1254, 968 cm^{-1}: ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.25 (s, 22H) 1.43-1.50 (m, 2H), 1.55 (s, 3H), 1.65 (s, 3H), 1.73 (br s, 1H), 3.77 (dd, 1H, *J* = 2.1, 8.9 Hz), 4.01 (br s, 1H), 4.06 (dd, 1H, *J* = 6.0, 8.9 Hz), 4.34-4.43 and 4.45-4.51 (two sets of m, 1H), 4.95-5.35 (m, 2H), 5.50-5.90 (m, 2H), 7.28-7.40 (m, 5H): ^{13}C NMR δ 14.1, 22.7 (23.6), 25.3 (26.4), 29.5, 29.58, 29.60, 29.63, 29.65, 29.7, 30.9, 31.9, 37.1 (37.2), 58.5 (59.1), 66.5 (66.9), (68.3) 68.6, 72.0, 94.4 (94.5), 127.95 (128.02), 128.4 (128.5), 129.0, 135.5 (135.7), (136.4)

136.5, 153.4; HR-MS [DCI/NH$_3$, MH$^+$] *m/z* calcd for $C_{29}H_{48}NO_4$ 474.3583, found 474.3583.

***N-tert*-Butoxycarbonyl 4(*R*)-[3'-Hydroxy-(1'*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (16, 18).** Compounds **16** and **18** was prepared in 90% overall yield by the same procedure as described for **15** and **17** (NaBH$_4$ reduction). Purification by chromatography (hexane/EtOAc 2:1) afforded diastereoisomers **16** (32%) and **18** (58%) as colorless oils: **16**: $[\alpha]^{25}_D$ −22.5° (*c* 1.0, CHCl$_3$); IR 1690, 1601, 1392, 1171, 1099 cm^{-1}; ^{1}H NMR δ (C_6D_6, 70 °C) 0.88 (t, 3H, *J* = 6.1 Hz), 1.20-1.80 (m, 39H), 3.54 (dd, 1H, *J* = 8.7, 2.4 Hz), 3.75 (dd, 1H, *J* = 8.7, 6.2 Hz), 3.98 (d, 1H, *J* = 5.2 Hz), 4.19 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR δ (C_6D_6) 14.3, 23.1, 23.8, 25.2, 26.0, 27.0, 27.5, 28.5, 29.8, 30.1, 32.3, 37.8, 38.0, 59.0 (59.3), 68.4, 72.0 (72.2), 79.2 (79.8), (93.6) 94.2, (129.0) 129.3, 135.7 (136.7), 152.0; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{26}H_{49}NO_4Na$ 462.3559, found 462.3579. **18**: $[\alpha]^{25}_D$ −40.0° (*c* 1.0, CHCl$_3$); IR 1689, 1602, 1392, 1253, 1171 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.80 (m, 39H), 3.54 (dd, 1H, *J* = 6.3, 2.3 Hz), 3.75 (dd, 1H, *J* = 8.7, 6.1 Hz), 4.00 (dd, 1H, *J* = 11.3, 5.8 Hz), 4.19 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR (C_6D_6) δ 14.3, 23.1, 23.8, 25.0, 26.0, 27.1, 27.7, 28.5, 29.8, 30.1, 30.2, 32.3, 37.7, 38.0, 59.0, (68.2) 68.5, 72.0 (72.2), 79.1, 79.1 (79.7), (93.6) 94.2, (128.6) 129.6, 135.8 (136.2), 152.0; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{26}H_{49}NO_4Na$ 462.3559, found 462.3566.

L-Selectride Reduction of Enone 5. To a solution of enone **5** (101 mg, 0.23 mmol) in 10 mL of dry THF was added 0.46 mL (0.46 mmol) of L-Selectride (lithium tri-*sec*-butylborohydride, a 1.0 M solution in THF) dropwise at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C, then allowed to warm to rt for another 0.5 h. The mixture was then 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 under reduced pressure. The residue was purified by chromatography (hexane/EtOAc 3:1) to give diastereoisomers **16** (86 mg, 85%) and **18** (4.8 mg, 4.7%) as colorless oils (the ratio of **16**/**18** was 18:1).

(2*R*,5*R*)-2-[(Benzyloxycarbonyl)amino]-(3*E*)-octadecen-1,5-diol [(+)-19]. A solution of oxazolidine **15** (0.45 g, 0.95 mmol) and *p*-TsOH·H_2O (0.18 g, 10 μmol) in 20 mL of MeOH was stirred overnight at rt. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH 25:1) to give 0.39 g (95%) of **19** as a white solid: mp 92.0-93.0 °C; $[\alpha]^{25}_D$ +8.6° (*c* 2.5, $CHCl_3$); IR 1720, 1601, 1503, 1467, 1232 cm^{-1}; ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.25 (s, 22H), 1.45-1.50 (m, 2H), 2.85 (br s, 2H), 3.60 (dd, 1H, *J* = 11.0, 4.0 Hz), 3.66 (dd, 1H, *J* = 11.0, 3.8 Hz), 4.06 (q, 1H, *J* = 6.1 Hz), 4.27 (br s, 1H), 5.09 (s, 2H), 5.42 (br s, 1H), 5.59 (dd, 1H, *J* = 15.7, 4.5 Hz), 5.66 (dd, 1H, *J* = 15.7, 5.8 Hz), 7.27-7.36 (m, 5H); ^{13}C NMR δ 14 1, 22.7, 25.4, 29.3, 29.5, 29.6, 29.7, 31.9, 37.1, 53.9, 64.9, 66.9, 72.3, 128.1, 128.2, 128.5, 135.2, 136.3, 158.0; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{26}H_{43}NO_4Na$ 456.3090, found 456.3089.

(2*R*,5*S*)-2-[(Benzyloxycarbonyl)amino]-(3*E*)-octadecen-1,5-diol [(-)-20]. This compound was prepared from **17** in 92% yield by the procedure described above: mp 93.5-95.0 °C; $[\alpha]^{25}_D$ −2.5° (*c* 2.5, $CHCl_3$); IR 1719, 1602, 1503, 1467, 1232 cm^{-1}; ^1H NMR ($CDCl_3$/MeOD) δ 0.88 (t, 3H, *J* = 6.5 Hz), 1.10-1.70 (m, 24H), 3.59 (d, 2H, *J* = 4.0 Hz), 4.02 (m, 1H), 4.20 (m, 1H), 5.09 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR ($CDCl_3$/MeOD) δ 14.2, 22.9, 25.7, 29.6, 29.8, 29.9, 32.1, 37.1, 54.5, 64.5, 67.0, 72.1, 127.8, 128.1, 128.3, 128.7, 135.7, 136.7, 157.0; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{26}H_{43}NO_4Na$ 456.3090, found 456.3090.

(2*R*,5*R*)-2-[Octanoylamido]-(3*E*)-octadecen-1,5-diol [(+)-2]. Method A: To the blue solution prepared by addition of 0.10 g (14.4 mmol) of Li metal to 20 mL of liquid NH_3 was added a solution of **19** (0.26 g, 0.60 mmol) in 10 mL of dry THF at -78 °C. After the mixture was stirred for 30 min, the reaction was quenched by addition of NH_4Cl (0.78 g, 14.6 mmol). After removal of NH_3 by a stream of N_2, the mixture was diluted with 100 mL of Et_2O and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in 20 mL of THF and *p*-nitrophenyl octanoate (0.16 g, 0.60 mmol) was added. After the mixture was stirred overnight, it was concentrated, and the product was purified by column chromatography ($CHCl_3$/MeOH 9:1) to give 0.21 g (82%) of **2** as a white solid. **Method B**: A solution of 44 mg (0.1 mmol) of **16** in 5 mL of 1 M HCl and 5 mL of dioxane was heated at 100 °C with stirring for 1 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (5 mL). The product was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided a crude sphingosine analogue as a white solid, which was dissolved in 6 mL of dry THF. After 54 mg (0.20 mmol) of *p*-nitrophenyl octanoate was added at rt, the reaction mixture was stirred for 24 h and concentrated. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 34 mg (80%, two steps) of ceramide **2** as a white solid: mp 69.0-70.0 °C; $[\alpha]^{25}_D$ +4.3° (*c* 1.0, $CHCl_3$); IR 1659, 1602, 1503, 1466, 1219 cm^{-1}; ^1H NMR δ 0.88 (t, 6H, J = 6.5 Hz), 1.30 (s, 32H), 1.48-1.52 (m, 2H), 1.62-1.65 (m, 2H), 2.31 (t, 2H, J = 7.5 Hz), 3.41 (br s, 2H), 3.64 (dd, 1H, J = 11.5, 4.3 Hz), 3.68 (dd, 1H, J =

11.5, 3.7 Hz), 4.08 (q, 1H, J = 6.0 Hz), 4.30-4.53 (m, 1H), 5.62 (dd, 1H, J = 15.8, 4.1 Hz), 5.67 (dd, 1H, J = 15.8, 4.9 Hz), 6.35 (d, 1H, J = 7.5 Hz); ^{13}C NMR δ 14.0, 14.1, 22.6, 22.7, 24.9, 25.5, 25.7, 25.8, 29.0, 29.1, 29.25, 29.3, 29.59, 29.61, 29.63, 29.65, 29.67, 31.7, 33.9, 36.8, 37.1, 52.6, 65.0, 72.3, 127.8, 135.4, 173.5; HR-MS [DCI/NH$_3$, MH$^+$] *m/z* calcd for $C_{26}H_{52}NO_3$ 426.3947, found 426.3938.

(2*R*,5*S*)-2-[Octanoylamido]-(3*E*)-octadecen-1,5-diol [(-)-3]. This compound was prepared from **18** and **20** by the procedure described above (Methods A and B): mp 78.5-79.5 °C; $[\alpha]^{25}_D$ −11.0° (*c* 1.0, CHCl$_3$); IR 1657, 1503, 1466, 1218 cm^{-1}; ^{1}H NMR δ 0.85 (t, 6H, J = 6.5 Hz), 1.10-1.70 (m, 34H), 2.19 (t, 2H, J = 7.4 Hz), 3.63 (d, 2H, J = 4.5 Hz), 4.06 (dd, 1H, J = 12.2, 5.9 Hz), 4.49 (m, 1H), 5.62 (m, 2H), 6.04 (d, 1H, J = 7.7 Hz); ^{13}C NMR δ 14.0, 22.6, 22.7, 25.5, 25.7, 29.0, 29.2, 29.3, 29.6, 29.7, 31.7, 31.9, 36.8, 37.1, 40.4, 52.6, 65.1, 72.1, 127.3, 135.7, 173.7; HR-MS [DCI/NH$_3$, MH$^+$] *m/z* calcd for $C_{26}H_{52}NO_3$ 426.3947, found 426.3955.

Cell Cultures. MCF-7 (breast cancer) cells, originally obtained from the American Type Culture Collection, were grown in Professor Arthur's laboratory to the exponential phase in medium supplemented with 5% fetal bovine serum and antibiotics, as described previously.[17] The cells were treated with compounds **1-3** (0-20 μM) for 48 h, and the increase in cell numbers after 48 h was determined[17] and expressed as a percentage of the controls, which received no drug. The results are the means of experiments made with quadruplicate wells. The standard deviations from the means were <10%. Stock solutions of the ceramides were made in EtOH. The final concentration of EtOH was <0.1%.

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5. Note that the priority sequence at C2 is reversed in compounds **2** and **3** compared with that in compound **1**.

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12. H_a is affected by the anisotropic magnetic field around the phenyl ring (see ref. 11). The chemical shifts reported here for H_a are for the central line of the double doublet.

13. For a recent review of apoptosis induced by **1**. see: Andrieu-Abadie. N.; Gouaze. V.; Salvayre. R.; Levade. T. *Free Radic. Biol. Med.* **2001**. *31*. 717-728.

14. General experimental methods have been described; see Chapter 1.

15. The ^{13}C-NMR chemical shifts in parentheses indicate the small peaks arising from the minor rotamers in the dynamic equilibrium of the oxazolidine system. which is slow at ambient temperature.

16. Proton NMR spectra of *N*-Boc-containing compounds were recorded at elevated temperature in order to facilitate the interconversion of the rotamers and thus simplify the spectra.

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Chapter 5

First Asymmetric Synthesis of 6-Hydroxy-4-Sphingenine-containing Ceramides. Use of Chiral Propargylic Alcohols to Prepare a Lipid Found in Human Skin

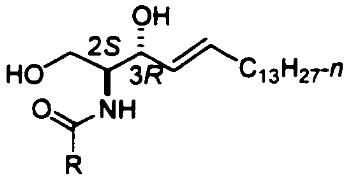
Abstract

6-Hydroxy-4*E*-sphingenine-containing ceramides were found recently in human skin. This chapter describes the first synthesis of the 6*S* and 6*R* diastereoisomers **2** and **3**, which represent analogues of (2*S*,3*R*)-ceramide (**1**) having two allylic hydroxyl groups. Chiral propargylic alcohols **8** and **11**, which were prepared by asymmetric dihydroxylation of α,β–unsaturated ester **13** and allylic chloride **22**, respectively, were employed as precursors of **2** and **3**. Nucleophilic addition of lithiated TBS-protected propargylic ethers **25** and **32** to L-serine-derived aldehyde **26**, respectively, afforded oxazolidine intermediates **27** and **33**. Acid-mediated deprotection of the oxazolidine, followed by *N*-acylation and Birch reduction completed the syntheses of **2** and **3**.

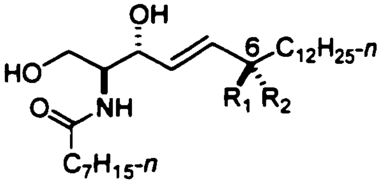
Introduction

Ceramides (*N*-acylsphingosines) were reported to occur in human epidermis in 1975,[1] and ceramide (**1**) has since been discovered to be a potent lipid mediator of many cellular functions, including proliferation, differentiation, and apoptosis.[2] The role of ceramides in skin function, particularly in the regulation of the water content of the epidermis,[3] has led to the development of synthetic "pseudoceramides" as ceramide replacements in the cosmetic industry.[4] The sphingolipids in stratum corneum are structurally heterogeneous. A substantial proportion of skin ceramides are hydroxylated at various positions of the sphingoid base or at the α and ω positions of the *N*-linked fatty acyl chain.[5] A novel sphingoid base, (6*R*/6*S*)-6-hydroxy-4*E*-sphingenine, with predominately eighteen and twenty carbon atoms, was identified in the ceramide fraction of human stratum corneum; ~25% of these ceramides bear ω-hydroxy fatty acids[6a-c] and ~9% have nonhydroxylated fatty acids.[6d] We report here the first asymmetric synthesis of these diastereomeric ceramides of human stratum corneum. The amide chain of skin ceramides often contains as many as thirty carbons;[5] however, for ease of handling, we synthesized model ceramides **2** and **3** with a *N*-octanoyl chain (see Chart 1). The synthetic route to **2** and **3** described here can be applied to the preparation of other 6-hydroxy-substituted ceramides bearing longer *N*-acyl chains, thereby affording homogeneous stratum corneum sphingolipids for biophysical investigations.

Chart 1



1



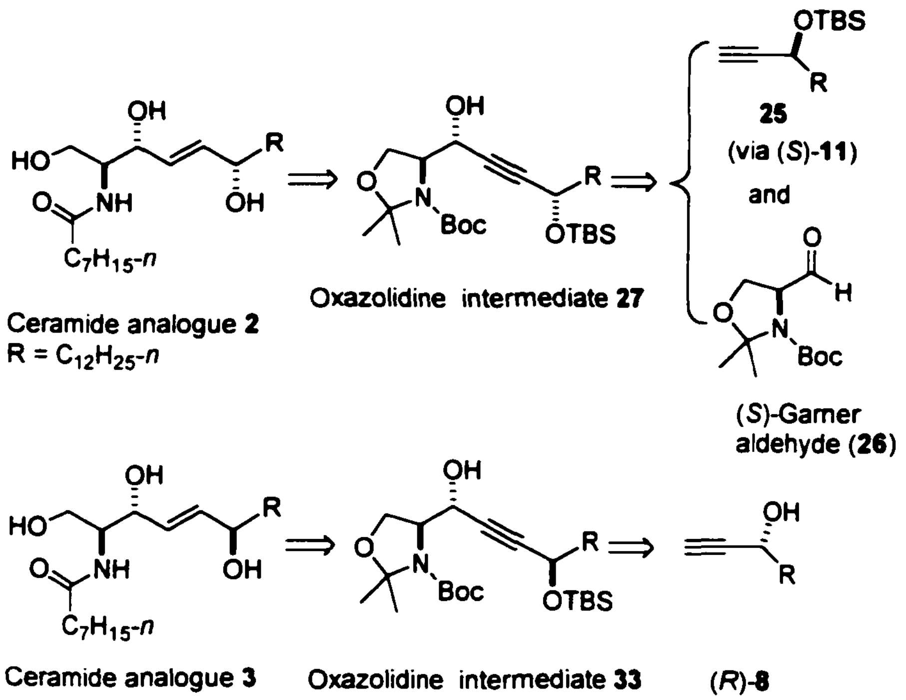
2 R_1 = H, R_2 = OH

3 R_1 = OH, R_2 = H

Results and Discussion

Synthetic Plan. As outlined in Scheme 1, our strategy for the preparation of ceramide **2** involves the key oxazolidine intermediate **27**, which is obtained by the addition of the acetylide ion derived from propargylic alcohol **11** to *N*-Boc-*N,O*-isopropylidene-L-serinal ((*S*)-Garner aldehyde, **26**).[7] Similarly, diastereomer **3** arises via intermediate **33**, which is obtained by the reaction of the acetylide ion derived from **8** with aldehyde **26**. Thus, the success of this synthetic plan depends on the ability to prepare long-chain propargylic alcohols (*R*)-**8** and (*S*)-**11** in high enantiomeric purity (see below).

Scheme 1. Retrosynthetic Plan

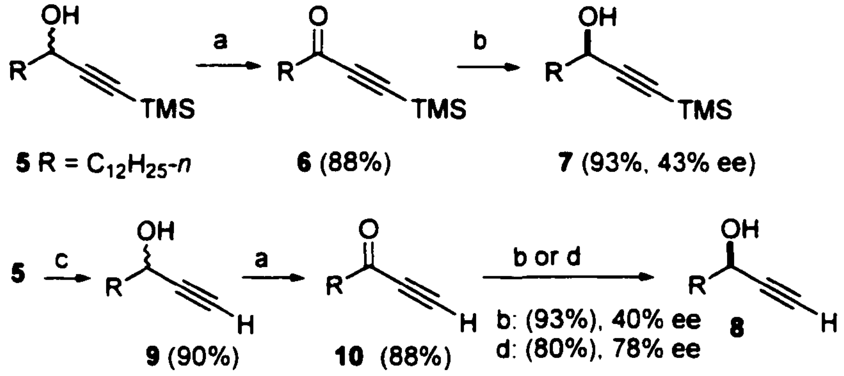


Synthesis of Chiral Propargylic Alcohols. Chiral propargylic alcohols are important synthons. They have been prepared by many methods, e.g., addition of an organometallic compound to an acetylenic aldehyde in the presence of chiral ligands.[8] enantioselective aldol addition to an α,β-ynal,[9] asymmetric reduction of an α,β-ynone,[10] resolution via enzymatic techniques,[11] Sharpless asymmetric epoxidation of an allylic alcohol,[12] and asymmetric dihydroxylation (AD) of an allylic chloride.[13]

Attempted Enantioselective Reduction of Ynones 6 and 10 (Scheme 2). We first examined whether the enantioenriched propargylic alcohols **8** and **11** could be prepared by asymmetric reduction of α,β-ynones **6** and **10** (Scheme 2). Treatment of trimethylsilylacetylene with *n*-BuLi in THF at −78 °C followed by reaction with tridecanal **4** provided alcohol **5** in 92% yield. PCC oxidation of **5** afforded α,β-ynone **6** in 88% yield. Asymmetric reduction of ynone **6** with $LiAlH_4$ in diethyl ether in the presence of Darvon alcohol (Chirald®)[10,14] gave alcohol **7** but the resulting ee[15] was low (43%). As shown in Scheme 2, analogous treatment of ynone **10**, obtained by removal of the TBS group of **5** and PCC oxidation of the resulting alcohol **9**, also provided **8** in poor enantiomeric purity (40%). Similarly, reduction of ynone **10** with *B*-(3-pinanyl)-9-borabicyclo[3.3.1]nonane ((*R*)-Alpine-Borane)[16] gave moderate enantioselectivity (78% ee).

Therefore, we examined alternative routes for the preparation of chiral propargylic alcohols **8** and **11**. As outlined in Schemes 3 and 4, the routes we chose involve the AD reaction with an α,β-unsaturated ester[17] or an allylic chloride,[13] and conversion of the resultant diol to a 4-(chloromethyl)-1,3-dioxolane, followed by a double elimination reaction.

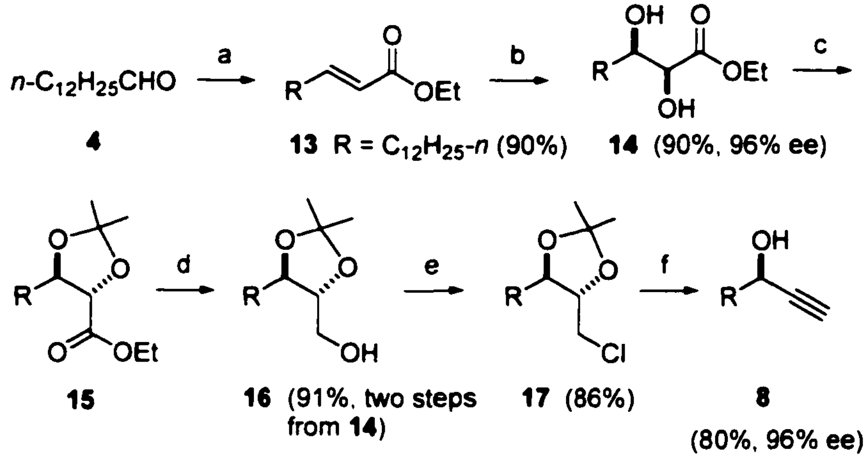
Scheme 2. Attempted Preparation of Alcohol 8 via Asymmetric Reduction[a]



[a]Reagents and conditions: (a) PCC, CH_2Cl_2, rt; (b) $LiAlH_4$, Chirald®, Et_2O, -78 °C; (c) K_2CO_3, MeOH, rt; (d) (*R*)-Alpine-Borane, 0 °C-rt.

Synthesis of 8 via AD Reaction of α,β-Unsaturated Ester 13. On base-mediated double elimination, chloromethyl-1,3-dioxolanes (+)-**17** (Scheme 3) and (-)-**24** (Scheme 4) afforded alcohols **8** and **11**, respectively. α,β-Unsaturated ester **13** was prepared by Horner-Wadsworth-Emmons (HWE) reaction of tridecanal **4** with diisopropyl ester phosphonate **12** (Scheme 3).[18] Reaction of **13** with AD-mix-β provided diol ester **14** in 90% yield and 96% ee. This result is consistent with previous reports of very high ee values attained in AD reactions of α,β-unsaturated esters.[17b] Treatment of diol **14** with 2,2-dimethoxypropane in the presence of *p*-TsOH gave acetonide ester **15**, which on reduction with DIBAL-H gave alcohol **16**. During the acetonide formation, a trace of the methyl ester analogue of **15** was formed by transesterification. However, subsequent reduction provided the same alcohol **16**. The latter was converted to chloride **17** in 88% yield by Mitsunobu reaction.[19] Propargylic alcohol (*R*)-**8** was obtained in good yield and high ee[20] by treatment of **17** with excess *n*-BuLi and HMPA in THF at low temperature.

Scheme 3. Synthesis of Alcohol (*R*)-8[a]

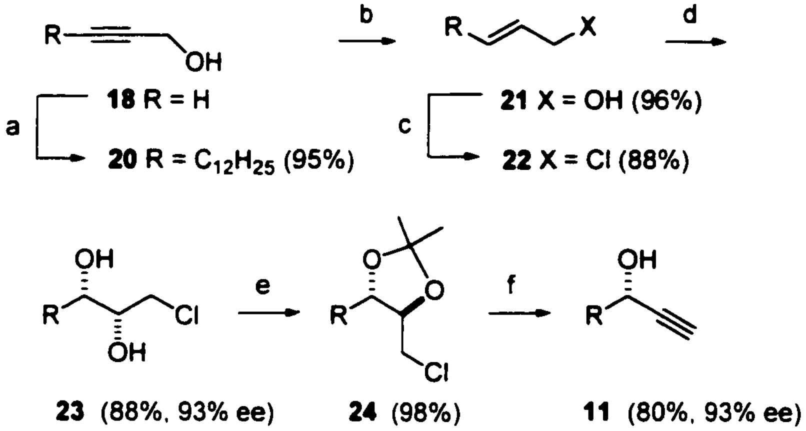


[a] Reagents and conditions: (a) (i-PrO)$_2$P(O)CH$_2$CO$_2$Et (**12**), Et$_3$N, LiBr, THF, rt; (b) AD-mix-β, MeSO$_2$NH$_2$, t-BuOH/H$_2$O 1/1, rt; (c) Me$_2$C(OMe)$_2$, p-TsOH, CH$_2$Cl$_2$, rt;

(d) DIBAL-H, THF, 0 °C; (e) NCS, Ph_3P, CH_2Cl_2, 0 °C–rt; (f) *n*-BuLi, HMPA, THF, -42 °C-rt.

Synthesis of 11 via Allylic Chloride 22 (Scheme 4). Takano et al. prepared short-chain propargylic alcohols from allylic chlorides.[13] We applied this method to the preparation of long-chain alcohol **11**. Thus, alkylation of propargyl alcohol (**18**) with dodecyl bromide (**19**) in THF[22a] gave propargylic alcohol **20** in 95% yield (Scheme 4). The latter was converted to (*E*)-allylic alcohol **21** in 96% yield by $LiAlH_4$ reduction,[23] then to chloride **22** in 88% yield by Mitsunobu reaction of **21**. Reaction of **22** with AD-mix-α[23] provided diol **23**[24] in 88% yield and 93% ee. In order to minimize epoxide formation, the AD reaction was carried out under "buffered" conditions (with 3 equiv of $NaHCO_3$).[25] Treatment of diol **23** with 2,2-dimethoxypropane in the presence of *p*-TsOH gave acetonide **24**, which was treated with excess *n*-BuLi in the presence of HMPA to provide alcohol (*S*)-**11** in 80% yield (93% ee).

Scheme 4. Synthesis of Alcohol (*S*)-11[a]

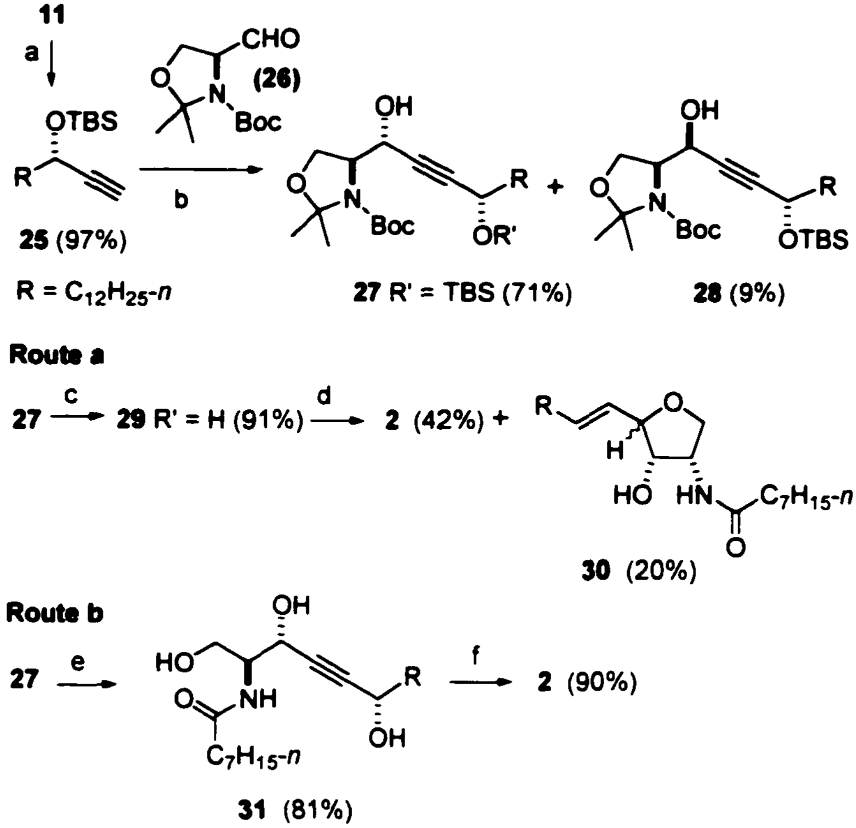


[a]Reagents and conditions: (a) Li, NH_3, $Fe(NO_3)_3$, $C_{12}H_{25}Br$ (**19**), THF, -78 °C; (b) $LiAlH_4$, THF, reflux; (c) NCS, Ph_3P, CH_2Cl_2, 0 °C–rt; (d) AD-mix-α, $NaHCO_3$,

$MeSO_2NH_2$, *t*-BuOH/H_2O 1/1, 0 °C; (e) $Me_2C(OMe)_2$, *p*-TsOH, CH_2Cl_2, rt; (f) *n*-BuLi, HMPA, THF, -42 °C-rt.

Synthesis of Ceramide Analogue 2 (Scheme 5). Coupling of protected propargylic alcohols **8** and **11** with (*S*)-aldehyde **26**[7,26] led to ceramide analogues **2** and **3**. The C-2 and C-6 configurations in **2** and **3** are derived from the stereogenic centers in serinaldehyde **26** and the propargylic alcohols, respectively. The new stereocenter at C-3 is generated by the asymmetric addition of the lithium acetylide of **8** or **11** to the Boc-protected aldehyde. As shown in Scheme 5, protection of the hydroxy group of **11** (TBSCl, Im) gave ether **25**, which was lithiated (*n*-BuLi, THF, -78 °C) and reacted with aldehyde **26** to give an 8:1 mixture of *erythro*-**27** and *threo*-**28** in 80% overall yield.[27] The diastereoselectivity was improved significantly (ratio > 20:1) by the addition of 2 equiv of HMPA.[28] After diastereomers **27** and **28** were separated by chromatography, the TBS group of **27** was removed by treatment with *n*-Bu_4NF in THF to give diol **29**, and Birch reduction was used to convert the triple bond to a trans double bond. Acid hydrolysis (1 M HCl in dioxane, 100 °C) followed by *N*-acylation with *p*-nitrophenyl octanoate provided target ceramide analogue **2** (Route a). Unexpectedly, we found the yield of **2** to be only 42%; about 20% of byproduct **30** was also formed. A much better result (Route b) was obtained when we changed the sequence of these reactions: acid hydrolysis of **27** followed by *N*-acylation provided 4-alkynylceramide analogue **31**. In the last step, Birch reduction of **31** (Li, $EtNH_2$, THF, -78 °C) gave ceramide analogue **2** in 90% yield.

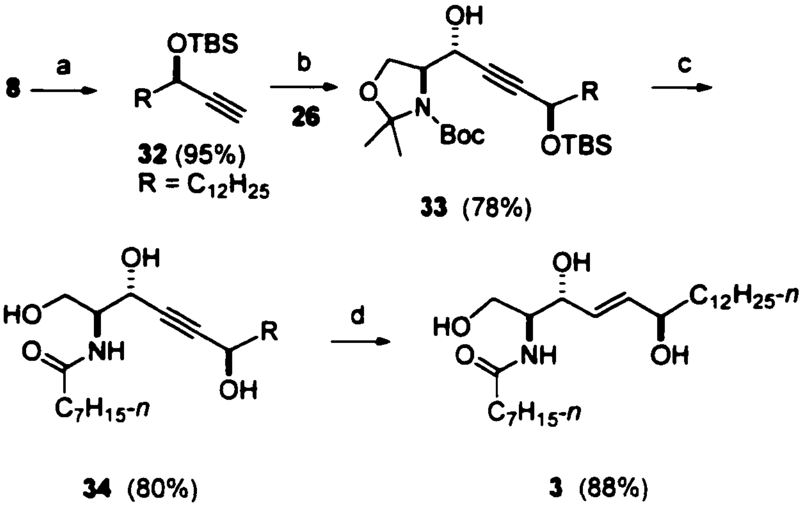
Scheme 5. Synthesis of Ceramide Analogue 2[a]



[a]Reagents and conditions: (a) TBSCl, imidazole, DMF, rt; (b) *n*-BuLi, THF, -78 °C-rt; (c) *n*-Bu_4NF, THF, rt; (d) (i) Li, $EtNH_2$, -78 °C, (ii) 1 M HCl, dioxane, 100 °C, (iii) *p*-$O_2NC_6H_4CO_2C_7H_{15}$-*n*, THF, rt; (e) (i) 1 M HCl, dioxane, 100 °C, (ii) *p*-$O_2NC_6H_4CO_2$-C_7H_{15}-*n*, THF, rt; (f) Li, $EtNH_2$, -78 °C.

Synthesis of Ceramide Analogue 3 (Scheme 6). Protection of the hydroxy group of **8** as the TBS ether followed by lithiation (*n*-BuLi, THF, -78 °C) and reaction with aldehyde **26** in the presence of HMPA afforded **33** in 78% yield (Scheme 6). Acid hydrolysis (1 M HCl in dioxane, 100 °C) of **33** followed by *N*-acylation with *p*-nitrophenyl octanoate afforded 4-alkynylceramide analogue **34**, which on Birch reduction provided ceramide analogue **3** in 88% yield.

Scheme 6. Synthesis of Ceramide Analogue 3



[a]Reagents and conditions: (a) TBSCl, imidazole, DMF, rt; (b) *n*-BuLi, HMPA, THF, -78 °C-rt; (c) (i) 1 M HCl, dioxane, 100 °C, (ii) *p*-$O_2NC_6H_4CO_2C_7H_{15}$-*n*, THF, rt; (d) Li, $EtNH_2$, -78 °C.

In summary, we have reported the first synthesis of naturally occurring sphingolipids with the 6(*R*/*S*)-hydroxysphing-4*E*-enine backbone, a novel long-chain base bearing allylic hydroxy groups at C-3 and C-6. The key precursors were the propargylic alcohols **8** and **11**, which were prepared by AD reactions with α,β-unsaturated ester **13** and allylic chloride **22**, respectively, followed by acetonide formation and elimination. The target ceramide analogues **2** and **3** were obtained with high stereoselectivity and good yields.

Experimental Section

General Information. See the previous report for general experimental details.[29] (*S*)-Garner aldehyde **26** was prepared from *N*-Boc-L-serine methyl ester as described previously.[26b,c] Mosher esters were prepared as described previously.[15] ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. The solvent was $CDCl_3$ unless otherwise noted. IR spectra were recorded in chloroform. The mobile phase used in electrospray mass spectrometry contained ~50 μM NH_4OAc and 0.1% HOAc.

***rac*-1-(Trimethylsilyl)pentadecyn-3-ol (5).** To a solution of 0.43 mL (3.0 mmol) of (trimethylsilyl)acetylene in 15 mL of dry THF was added 1.3 mL (3.2 mmol) of *n*-BuLi (2.5 M solution in hexanes) at −78 °C under N_2. After 30 min, a solution of 0.48 g (2.8 mmol) of tridecanal (**4**) in 10 mL of THF was added dropwise. After the mixture was stirred for 2 h at −78 °C and for 1 h at rt, the reaction was quenched by the addition of aqueous saturated NH_4Cl solution. The product was extracted with EtOAc (3 × 30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Pentadecynyl alcohol **5** (0.77 g, 92%) was obtained by chromatography on silica gel (hexane/EtOAc 9:1); IR 3597, 2169 cm^{-1}; ^1H NMR δ 0.14 (br s, 9H), 0.84 (t, 3H, J = 6.7 Hz), 1.10-1.50 (m, 20H), 1.64 (m, 2H), 2.12 (br s, 1H), 4.31 (t, 1H, J = 6.6 Hz); ^{13}C NMR δ -0.2, 14.1, 22.6, 22.7, 25.1, 27.8, 29.2, 29.3, 29.5, 29.61, 29.64, 31.9, 37.1, 37.6, 62.8, 89.1, 107.1; HR-MS [DCI/NH_3, MNH_4^+] *m/z* calcd for $C_{18}H_{14}NOSi$ 314.1879, found 314.1879.

1-(Trimethylsilyl)pentadecyn-3-one (6). To a solution of 0.30 g (1.0 mmol) of **5** in 10 mL of CH_2Cl_2 was added 0.43 g (2.0 mmol) of PCC. The mixture was stirred at rt for 5 h, then filtered through a pad of silica gel, which was washed with CH_2Cl_2. The filtrate was concentrated to give a residue that was purified by flash chromatography (hexane/EtOAc 9:1), affording 0.26 g (88%) of ynone **6**; IR 2172, 1665, 1256 cm^{-1}; ^1H NMR δ 0.16 (s, 9H), 0.82 (t, 3H, J = 6.6 Hz), 1.10-1.60 (m, 18H), 1.57 (m, 2H), 2.46 (t, 1H, J = 7.4 Hz); ^{13}C NMR δ -0.9, 14.0, 22.2, 22.6, 22.7, 23.7, 23.8, 28.8, 29.1, 29.2, 29.3, 29.4, 29.51, 29.52, 31.8, 42.3, 42.6, 45.1, 97.1, 102.0, 187.6; MS [ESI, 295.2 (M^+), 312.2 (MNH_4^+), 317.1 (MNa^+)].

1-(Trimethylsilyl)pentadecyn-3-ol (7). To a solution of Chirald® (1.70 g, 6.0 mmol) in 10 mL of dry Et_2O was added dropwise 2.8 mL (2.8 mmol) of $LiAlH_4$ (a 1.0 M solution in Et_2O) at 0 °C. After stirring for 5 min, the mixture was chilled to -78 °C and a solution of ketone **6** (590 mg, 2.0 mmol) in 10 mL of Et_2O was added over a 15-min period. The mixture was stirred at -78 °C for 6 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 column chromatography (hexane/EtOAc 4:1) gave 275 mg (93%) of **7** as a colorless oil: IR 3597, 2158 cm^{-1}; MS [ESI, 314.2 (MNH_4^+), 319.2 (MNa^+)].

***rac*-1-Pentadecyn-3-ol (9).** A mixture of 0.60 g (2.0 mmol) of TMS-acetylide **5** and 0.62 g (4.6 mmol) of K_2CO_3 in 4 mL of MeOH was stirred at rt for 4 h. After the solvent was removed, the residue was dissolved in 30 mL of Et_2O and washed with water and dried ($MgSO_4$). Concentration and purification of the residue by column chromatography (hexane/EtOAc 9:1) gave 0.40 g (90%) of **9** as a white solid: mp 44.0-45.0 °C; IR 3597, 3302, 1457 cm^{-1}; ^1H NMR δ 0.84 (t, 3H, J = 6.6 Hz), 1.20-1.60 (m, 20H), 1.68 (m, 2H), 2.31 (br s, 1H), 2.42 (d, 1H, J = 3.7 Hz), 4.32 (dt, 1H, J = 6.7, 2.1 Hz); ^{13}C NMR δ 14.1, 22.6, 25.1, 29.3, 29.5, 29.62, 29.69, 29.7, 32.0, 37.6, 62.2, 72.7, 85.1; HR-MS [DCI/NH_3, MNH_4^+] m/z calcd for $C_{15}H_{32}NO$ 242.2484, found 242.2477.

1-Pentadecyn-3-one (10). This compound was prepared in 88% yield by using the same procedure as described for **6**: IR 2093, 1681 cm^{-1}; ^1H NMR δ 0.82 (t, 3H, J =

6.6 Hz), 1.10-1.60 (m, 18H), 1.61 (m, 2H), 2.51 (t, 1H, $J = 7.4$ Hz), 3.17 (s, 1H); ^{13}C NMR δ 14.0, 22.6, 23.7, 28.8, 29.2, 29.3, 29.48, 29.53, 29.54, 31.8, 45.3, 78.2, 81.4, 187.3; HR-MS [DCI/NH_3, MNH_4^+] m/z calcd for $C_{15}H_{30}NO$ 240.2327, found 240.2333.

Ethyl (2*E*)-Pentadecenoate (13). To a nitrogen-flushed solution of 10.5 g (122 mmol) of LiBr in 100 mL of dry THF was injected 7.2 mL (7.6 g, 30 mmol) of $(i\text{-}PrO)_2P(O)CH_2CO_2Et$ (**12**) at rt. After the solution was stirred at rt for 10 min, 6.8 mL (49 mmol) of Et_3N was added, and stirring was continued for 10 min. A solution of 4.3 g (25 mmol) of tridecanal (**4**) in 10 mL of dry THF was added. The reaction mixture was stirred vigorously at rt until the full consumption of tridecanal 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 6.0 g (90%) of ester **13** as a colorless oil. The NMR data are in full accord with the literature data.[18]

Ethyl (2*S*,3*R*)-2,3-Dihydroxypentadecanoate [(+)-14]. To a solution of 14.0 g of AD-mix-β in 200 mL of *t*-BuOH/H_2O 1/1 that had been stirred vigorously at room temperature for 30 min was added 950 mg (10.0 mmol) of $MeSO_2NH_2$. Stirring was continued for 10 min at rt. Then 2.7 g (10.0 mmol) of α,β-unsaturated ester **13** was added. The reaction mixture was stirred vigorously until the disappearance of ester **13** was noted (TLC). Sodium sulfite (15.0 g, 14.6 mmol) was added to quench the reaction, and stirring was continued for another 30 min. The product was extracted with EtOAc (3 × 80 mL), and the combined extracts were dried (Na_2SO_4) and concentrated to give a yellow residue. Purification of the residue by column chromatography (hexane/EtOAc

2:1) gave 2.7 g (90%) of diol ester **14** as a white solid; mp 69.0-70.0 °C; $[\alpha]^{25}{}_D$ +8.3° (*c* 1.0. $CHCl_3$); IR 3629. 3019, 2398. 1518, 1425, 1212 cm^{-1}; ^1H NMR δ 0.88 (t, 3H, *J* = 6.5 Hz). 1.10-1.70 (m. 25H). 3.06 (d, 1H. *J* = 8.4 Hz). 3.86 (m. 2H), 4.06 (dd, 1H. *J* = 6.5, 2.3 Hz), 4.24 (q, 2H, *J* = 7.1 Hz); ^{13}C NMR δ 14.15. 14.17. 22.8, 25.9, 29.5, 29.70. 29.72, 29.74. 29.76. 29.8. 32.0. 33.6. 61.8. 72.8. 73.6, 173.8; MS [ESI. 320.2 (MNH_4^+). 325.2 (MNa^+)].

(4*S*,5*R*)-5-Dodecyl-4-ethoxycarbonyl-2,2-dimethyl-1,3-dioxolane (15). To a solution of 2.4 g (8.0 mmol) of diol ester **14** in 60 mL of dry CH_2Cl_2 was added 1.67 g (16.0 mmol) of 2,2-dimethoxypropane and 50 mg (0.26 mmol) of *p*-TsOH at room temperature. The mixture was stirred at rt for 2 h and then passed through a pad of silica gel in a sintered glass funnel. The pad was washed with 50 mL of CH_2Cl_2. Concentration gave ester **15** as a colorless oil. which was used without further purification in the subsequent reaction.

(4*R*,5*R*)-5-Dodecyl-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane [(+)-16]. To a solution of ester **15** obtained above in 15 mL of dry THF was added dropwise 10.7 mL (16.0 mmol) of DIBAL-H (a 1.5 M solution in toluene) at 0 °C under N_2. The reaction mixture was stirred for 2 h at 0 °C until TLC analysis showed the reaction to be complete. The reaction was quenched by slow addition of 2 mL of MeOH followed by 15 mL of cold 5% aqueous potassium sodium tartrate solution. The product was extracted with EtOAc (3 × 40 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 4:1) gave 2.18 g (91%) of **16** as a colorless oil: $[\alpha]^{25}{}_D$ +18.8° (*c* 1.8. $CHCl_3$): IR 3597. 1463. 1382. 1103 cm^{-1}: ^1H NMR δ 0.88 (t. 3H. *J* = 6.6

Hz), 1.10-1.70 (m, 28H), 2.90 (t, 1H, $J = 6.0$ Hz), 3.59 (m, 1H), 3.72 (m, 2H), 3.84 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 26.0, 27.1, 27.4, 29.4, 29.58, 29.63, 29.70, 29.73, 29.8, 32.0, 33.2, 62.2, 77.1, 81.8, 108.6; HR-MS [DCI, MH$^+$] m/z calcd for $C_{18}H_{37}O_3$ 301.2743, found 301.2751.

(4*S*,5*R*)-4-Chloromethyl-5-dodecyl-2,2-dimethyl-1,3-dioxolane [(+)-17]. To a solution of 1.74 g (5.8 mmol) of alcohol **16** and 1.83 g (6.9 mmol) of Ph_3P in 30 mL of dry CH_2Cl_2 was added 930 mg (6.9 mmol) of NCS at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to rt and stirred for 2 h. The mixture was diluted with 100 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO. Concentration and purification of the residue by column chromatography (hexane/EtOAc 9:1) gave 1.59 g (86%) of **17** as a colorless oil: $[\alpha]^{25}_D$ +14.2° (c 0.9, $CHCl_3$); IR 1464, 1376, 1218, 1098 cm^{-1}; ^{1}H NMR δ 0.88 (t, 3H, $J =$ 6.6 Hz), 1.20-1.70 (m, 28H), 3.58 (d, 2H, $J = 4.9$ Hz), 3.88 (m, 2H); ^{13}C NMR δ 14.1, 22.7, 26.0, 27.0, 27.5, 29.4, 29.5, 29.6, 29.7, 32.0, 33.5, 44.4, 79.4, 80.3, 109.1; HR-MS [DCI, MH$^+$] m/z calcd for $C_{18}H_{36}O_2Cl$ 319.2404, found 319.2407.

(3*R*)-1-Pentadecyn-3-ol [(+)-8]. To a stirred solution of 4.5 mL (26.1 mmol) of HMPA in 20 mL of dry THF was added 10.4 mL (26.1 mmol) of *n*-BuLi (a 2.5 M solution in hexane) at –42 °C under N_2. After 10 min, a solution of 1.19 g (3.73 mmol) of chloride **17** 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 0.5 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 column chromatography (hexane/EtOAc 7:1) gave 668 mg (80%) of **8** as a white solid; mp 40.0-41.0 °C; $[\alpha]^{25}_D$ +2.60° (*c* 1.0, $CHCl_3$); IR 3597, 3302, 1458 cm^{-1}; ^1H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.20-1.60 (m, 20H), 1.72 (m, 2H), 2.41 (br s, 1H), 2.44 (d, 1H, J = 3.7 Hz), 4.36 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 25.1, 29.3, 29.5, 29.62, 29.69, 29.7, 32.0, 37.6, 62.3, 72.8, 85.1; HR-MS [DCI/NH_3, MNH_4^+] *m/z* calcd for $C_{15}H_{32}NO$ 242.2484, found 242.2489.

2-Pentadecyn-1-ol (20). This compound was prepared in 95% yield by a slight modification of the procedure of van Aar et al.[22a] we used 3 equiv of propargyl alcohol (**18**) rather than 1.5 equiv; mp 44.2-45.2 °C (lit.[22a] mp 38-41 °C, lit.[22b] mp 43-45 °C). The NMR data are in full accord with the literature data.[22a,b]

(2*E*)-Pentadecen-1-ol (21). This compound, which was a low-melting white solid, was prepared in 96% yield as described previously, and the NMR data are in full accord with the literature data.[23]

(2*E*)-1-Chloropentadecene (22). To a solution of 5.0 g (22.1 mmol) of alcohol **21** and 7.0 g (26.7 mmol) of Ph_3P in 50 mL of dry CH_2Cl_2 was added 3.3 g (24.4 mmol) of NCS at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to rt and stirred for 2 h. The mixture was diluted with 150 mL of hexane and passed through a pad of silica gel with suction to remove the precipitate of Ph_3PO. The filtrate was concentrated, and the resulting residue was dissolved in 100 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO again. Concentration gave 4.79 g (88%) of allylic chloride **22** as a colorless oil. This compound was used in the next step without further purification.

(2*R*,3*S*)-1-Chloropentadecane-2,3-diol [(-)-23]. After a solution of 14.0 g of AD-mix-α[23,30] in 200 mL of *t*-BuOH/H_2O 1/1 was stirred vigorously at rt for 30 min, 2.52 g (30.0 mmol) of $NaHCO_3$ was added. After 15 min, 950 mg (10.0 mmol) of $MeSO_2NH_2$ was added, and stirring was continued for 10 min at rt. The reaction mixture was chilled to 0 °C, and 2.47 g (10.0 mmol) of allylic chloride **22** was added. The reaction mixture was stirred vigorously for 8 h. Sodium sulfite (15.0 g, 14.6 mmol) was added to quench the reaction, and stirring was continued for another 30 min. The product was extracted with EtOAc (3 × 80 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a yellow solid residue, which was purified by column chromatography (hexane/EtOAc 2:1), giving 2.45 g (88%) of diol **23** as a white solid: mp 64.5-65.5 °C (lit.[23] mp 92-93 °C); $[\alpha]^{25}_D$ –9.6° (*c* 1.25, $CHCl_3$); $[\alpha]^{25}_D$ –8.9° (*c* 1.0, MeOH) (lit.[23] $[\alpha]^{25}_D$ –7.0° (*c* 1.0, MeOH)).[24] The NMR data are in full accord with the literature data.[23]

(4*R*,5*S*)-4-Chloromethyl-5-dodecyl-2,2-dimethyl-1,3-dioxolane [(-)-24]. This compound was prepared in 98% yield by using the same procedure as described for **15**; $[\alpha]^{25}_D$ –10.93° (*c* 2.1, $CHCl_3$). The IR and ^1H- and ^{13}C-NMR spectra were essentially identical to those of (+)-**17**. HR-MS [DCI, MH^+] *m/z* calcd for $C_{18}H_{36}O_2Cl$ 319.2404, found 319.2400.

(3*S*)-1-Pentadecyn-3-ol [(-)-11]. This compound was prepared in 80% yield by using the same procedure as described for **8**; mp 40.0-41.0 °C; $[\alpha]^{25}_D$ –2.59° (*c* 1.0, $CHCl_3$). The IR and ^1H- and ^{13}C-NMR spectra were essentially identical to those of (+)-**8**. HR-MS [DCI, MH^+] *m/z* calcd for $C_{15}H_{29}O$ 225.2218, found 225.2211.

(3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-pentadecyne [(-)-25]. To a solution of 0.45 g (2.0 mmol) of **11** and 0.29 g (4.2 mmol) of imidazole in 4 mL of DMF under N_2 was added 0.33 g (2.2 mmol) of *tert*-butylchlorodimethylsilane. After the solution was stirred at rt overnight, it was diluted with water (5 mL) and the product was extracted with Et_2O (3 x 20 mL). The combined extracts were washed with brine and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by chromatography (hexane) to give 0.66 g (97%) of **25** as a colorless oil; $[\alpha]^{25}_D$ –26.9° (*c* 1.7, $CHCl_3$); IR 1714, 1463, 1256, 1093, 837 cm^{-1}; ^1H NMR δ 0.10 (s, 3H), 0.13 (s, 3H), 0.89 (m, 12H), 1.20-1.40 (m, 20H), 1.65 (m, 2H), 2.34 (d, 1H, J = 2.1 Hz), 4.32 (dt, 1H, J = 6.5, 2.1 Hz); ^{13}C NMR δ -5.1, -4.5, 14.1, 18.2, 22.7, 25.2, 25.8, 29.3, 29.4, 29.61, 29.64, 29.71, 29.74, 32.0, 38.6, 62.8, 71.8, 85.8; HR-MS [DCI, MH^+] *m/z* calcd for $C_{21}H_{43}SiO$ 339.3083, found 339.3086.

N-*tert*-Butoxycarbonyl (4S,1'R,4'S)-4-[4'-(*tert*-Butyldimethylsilyloxy)-1'-hydroxy-2'-hexadecynyl]-2,2-dimethyl-1,3-oxazolidine [(-)-27]. To a solution of 1.01 g (3.0 mmol) of alkyne **25** in 15 mL of dry THF was slowly added 1.3 mL (3.2 mmol) of *n*-BuLi (2.5 M solution in hexanes) at –78 °C under N_2. The mixture was stirred for 30 min at –78 °C, and a solution of 0.69 g (3.0 mmol) of Garner aldehyde **26** in 10 mL of THF was added dropwise with stirring. The mixture was kept at –78 °C for 2 h and then allowed to warm to rt for 2 h. The reaction was quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (3 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The major diastereomer **27** was isolated by chromatography on silica gel (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ –41.1° (*c* 2.9, $CHCl_3$); IR 3334, 3012, 1688, 1380, 1256, 1152 cm^{-1}; ^1H NMR (C_6D_6, 70 °C)[31] δ 0.13 (s, 3H), 0.19 (s, 3H), 0.88 (t, 3H, J = 6.1 Hz), 0.96 (s, 9H), 1.20-1.80 (m, 37H), 3.79 (t, 1H, J = 7.5 Hz), 4.00 (br s, 2H), 4.41 (t, 1H, J = 6.1 Hz), 4.70 (br s, 1H); ^{13}C NMR (C_6D_6)[32] δ -5.2, -4.9, 14.3, 18.1, 18.4, 18.5, 18.7, 23.1, 23.3, 25.7, 25.9, 26.0, 26.3, 28.3, 29.5, 29.7, 29.8, 30.00, 30.05, 30.07, 30.1, 32.3, 38.2, 39.2. (62.0) 63.3. 64.2 (64.6). 67.1, (79.9) 81.7, 83.0 (83.5), 87.6 (88.1), 101.0. (151.7) 154.3: HR-MS [FAB. MH$^+$] *m/z* calcd for $C_{32}H_{62}NO_5Si$ 568.4397. found 568.4366. **Minor diastereomer 28**: IR 3597, 3008. 1692, 1398, 1256. 1163. 1092 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 0.14 (s. 3H). 0.20 (s. 3H). 0.88 (t. 3H. J = 6.1 Hz). 0.99 (s. 9H). 1.20-1.80 (m. 37H). 3.26 (br s. 1H). 3.49 (t. 1H. J = 6.4 Hz), 3.83 (dd. 1H. J = 9.5, 6.5 Hz), 4.13 (m. 2H). 4.46 (m. 1H). 4.76 (d. 1H. J = 6.4 Hz): HR-MS [FAB. MNa$^+$] *m/z* calcd for $C_{32}H_{61}NO_5SiNa$ 590.4217. found 590.4236.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*,4'*S*)-4-[1',4'-Dihydroxy-2'-hexadecynyl]-2,2-dimethyl-1,3-oxazolidine [(-)-29].** To a solution of 0.47 g (0.82 mmol) of **27** in 5.0 mL of THF was added 1.64 mL (1.64 mmol) of a 1.0 M solution of *n*-Bu_4NF in THF at rt under N_2. After the mixture was stirred for 1 h. the reaction was quenched with 5 mL of water. The product was extracted with EtOAc (3 × 20 mL). dried (Na_2SO_4). and concentrated under reduced pressure. The residue was purified by chromatography (EtOAc/hexane 2:3) to give 0.34 g (91%) of **29** as a colorless oil: $[\alpha]^{25}_{D}$ −41.1° (*c* 1.9. $CHCl_3$); IR 1666. 1467. 1394. 1368. 1245. 1167. 1082 cm^{-1}; ^{1}H NMR (C_6D_6. 70 °C) δ 0.89 (t. 3H. J = 6.6 Hz). 0.96 (s. 9H). 1.20-1.80 (m. 37H). 2.22 (d. 1H. J = 4.6 Hz). 3.78 (dd. 1H. J = 9.0. 7.0 Hz). 3.96 (br s. 1H). 4.05 (br s. 1H). 4.31 (dd. 1H. J = 11.1. 5.2 Hz). 4.69 (br s. 1H); ^{13}C NMR (C_6D_6) δ 14.3. 14.8. 22.9. 23.0. 23.1. 23.3. 23.5. 25.3. 25.8. 26.1. 26.7. 28.3. 29.8. 30.09. 30.1. 30.2. 32.1. 32.3. 34.5. 38.2. (62.0) 62.3. 62.9. 63.7. (64.8) 65.2. (79.9) 80.9. 83.6. 87.9 (88.4). 94.9 (95.4). (151.9) 154.2: HR-MS [FAB. MH$^+$] *m/z* calcd for $C_{26}H_{48}NO_5$ 454.3532. found 454.3517.

(2*S*,3*R*,6*S*)-2-Octanoylamido-4-octadecyn-1,3,6-triol [(-)-31]. A solution of 113 mg (0.20 mmol) of **28** in 5 mL of 1 M HCl and 5 mL of dioxane was heated at 100

°C with stirring for 1 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (5 mL). The product was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided a crude sphingosine analogue as a white solid, which was dissolved in 6 mL of dry THF. After 108 mg (0.40 mmol) of *p*-nitrophenyl octanoate was added at rt, the reaction mixture was stirred for 24 h and concentrated. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 71 mg (81%, two steps) of 4-alkynylceramide analogue **31** as a white solid: mp 95.2-96.2 °C; $[\alpha]^{25}_D$ −13.6° (*c* 1.1, $CHCl_3$/MeOH 4:1); IR 1659, 1509, 1052 cm^{-1}; ^1H NMR ($CDCl_3$/MeOD) δ 0.88 (t, 6H, *J* = 6.5 Hz), 1.20-1.70 (m, 32H), 2.24 (t, 2H, *J* = 7.5 Hz), 3.67 (dd, 1H, *J* = 11.5, 4.7 Hz), 3.90 (dd, 1H, *J* = 11.5, 4.6 Hz), 4.06 (d, 1H, *J* = 4.7 Hz), 4.30 (m, 1H), 4.53 (dd, 1H, *J* = 4.8, 1.3 Hz); ^{13}C NMR ($CDCl_3$/MeOD) δ 14.1, 22.7, 22.8, 25.4, 25.8, 29.1, 29.3, 29.5, 29.7, 31.8, 32.0, 36.66, 36.7, 37.6, 55.2, 61.5, 61.8, 62.8, 82.3, 87.8, 175.3; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{26}H_{49}NO_4Na$ 462.3559, found 462.3552.

(2*S*,3*R*,6*S*)-2-Octanoylamido-(4*E*)-octadecen-1,3,6-triol [(+)-2]. To the blue solution prepared by addition of 50 mg (7.2 mmol) of lithium metal to 5 mL of liquid $EtNH_2$ was added a solution of **31** (44 mg, 0.10 mmol) in 5 mL of dry THF at -78 °C. After the mixture was stirred for 1 h, the reaction was quenched by addition of saturated aqueous NH_4Cl solution (5 mL). After removal of $EtNH_2$ by a stream of N_2, the mixture was diluted with 50 mL of $CHCl_3$ and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. Purification by column chromatography ($CHCl_3$/MeOH 9:1) gave 40 mg (90%) of **2** as a white solid: mp 119.5-121.0 °C; $[\alpha]^{25}_D$ +5.1° (*c* 1.1, $CHCl_3$/MeOH 4:1); IR 1659, 1509, 1465, 1222 cm^{-1}; ^1H NMR ($CDCl_3$/MeOD) δ 0.88 (t, 6H, *J* = 6.4 Hz), 1.20-1.70 (m, 32H), 2.21 (t, 2H, *J* = 7.4 Hz), 3.63 (dd, 1H, *J* = 10.9, 3.4 Hz), 3.84 (m, 2H), 4.06 (q, 1H, *J* = 6.1 Hz), 4.20 (t, 1H, *J* = 5.3 Hz), 5.65 (dd, 1H, *J* = 15.5, 5.8 Hz), 5.73 (dd, 1H, *J* = 16.7, 6.0 Hz); ^{13}C NMR ($CDCl_3$/MeOD) δ 14.1, 22.7, 22.8, 25.6, 25.9, 29.1, 29.4, 29.5, 29.7, 29.8, 31.8, 32.0, 36.7, 37.1, 54.7, 61.6, 71.9, 72.6, 129.5, 135.6, 174.9; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{26}H_{51}NO_4Na$ 464.3716, found 464.3707.

(3*S*,4*S*)-2-[(1'*E*)-1'-Tetradecenyl]-3-hydroxy-4-octanoylamidotetrahydrofuran [(+)-30]. mp 142.0-143.0 °C; $[\alpha]^{25}_D$ +12.9° (*c* 0.9, $CDCl_3$/MeOD 4:1): IR 1665, 1510, 1467, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$/MeOD) δ 0.88 (t, 6H, *J* = 6.4 Hz), 1.20-1.70 (m, 30H), 2.08 (q, 2H, *J* = 6.9 Hz), 2.21 (t, 2H, *J* = 7.4 Hz), 3.62 (t, 1H, *J* = 8.2 Hz), 3.79 (br s, 3H), 4.06 (m, 2H), 4.29 (q, 1H, *J* = 3.0 Hz), 4.53 (m, 1H), 5.60 (dd, 1H, *J* = 15.4, 7.4 Hz), 5.83 (dt, 1H, *J* = 15.4, 8.5 Hz); ^{13}C NMR ($CDCl_3$/MeOD) δ 13.56, 13.6, 22.2, 22.3, 25.4, 28.6, 28.7, 28.8, 28.9, 29.2, 29.27, 29.3, 31.3, 31.6, 32.2, 36.0, 52.4, 69.4, 71.8, 83.1, 124.0, 136.4, 174.3; HR-MS [DCI, MH$^+$-H_2O] *m/z* calcd for $C_{26}H_{48}NO_2$ 406.3685, found 406.3669; MS [ESI, MH$^+$] *m/z* calcd for $C_{26}H_{50}NO_3$ 424.3, found 424.3.

(3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-pentadecyne [(+)-32]. This compound was prepared in 95% yield by using the same procedure as described for **25**: $[\alpha]^{25}_D$ +20.7° (*c* 0.7, $CHCl_3$): IR 1708, 1467, 1256, 1093, 837 cm^{-1}; ^{1}H NMR δ 0.10 (s, 3H), 0.13 (s, 3H), 0.89 (m, 12H), 1.20-1.40 (m, 20H), 1.65 (m, 2H), 2.34 (d, 1H, *J* = 2.1 Hz), 4.32 (m, 1H); ^{13}C NMR δ -5.1, -4.5, 14.1, 18.2, 22.7, 25.2, 25.8, 29.3, 29.4, 29.61, 29.64, 29.71, 29.74, 32.0, 38.6, 62.8, 71.8, 85.8; MS [ESI, 356.3 (MNH$_4^+$)].

***N*-*tert*-Butoxycarbonyl (4*S*,1'*R*,4'*R*)-4-[4'-(*tert*-Butyldimethylsilyloxy)-1'-hydroxy-2'-hexadecynyl]-2,2-dimethyl-1,3-oxazolidine [(-)-33].** To a solution of 1.01

g (3.0 mmol) of alkyne **32** in 15 mL of dry THF was slowly added 1.3 mL (3.2 mmol) of *n*-BuLi (a 2.5 *M* solution in hexanes) at −78 °C under N_2. After the mixture was stirred for 30 min at −78 °C, HMPA (1.05 mL, 6.0 mmol) was added, followed by a solution of 0.69 g (3.0 mmol) of Garner aldehyde **26** in 10 mL of dry THF. The mixture was kept at −78 °C for 2 h, and then allowed to warm to rt for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (3 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. *N*-Boc-1,3-oxazolidine **33** was isolated by chromatography on silica gel (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ −4.3° (*c* 1.8, $CDCl_3$); IR 1696, 1375, 1010 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 0.13 (s, 3H), 0.18 (s, 3H), 0.88 (t, 3H, *J* = 6.1 Hz), 0.96 (s, 9H), 1.20-1.80 (m, 37H), 3.79 (t, 1H, *J* = 7.1 Hz), 4.00 (br s, 2H), 4.41 (t, 1H, *J* = 5.0 Hz), 4.70 (br s, 1H); ^{13}C NMR (C_6D_6) δ -4.8, -3.9, 14.4, 18.4, 23.1, 23.4, 24.0, 25.5, 25.7, 26.1, 26.3, 28.2, 28.4, 29.7, 29.8, 30.0, 30.1, 37.0, (63.1) 63.4, 64.2 (64.6), (65.0) 65.2, (79.8) 80.8, 83.6 (83.7), 87.5 (88.0), 95.0, (151.7) 154.3; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{32}H_{61}NO_5SiNa$ 590.4217, found 590.4231.

(2*S*,3*R*,6*R*)-2-Octanoylamido-4-octadecyn-1,3,6-triol [(-)-34]. This compound was prepared in 80% yield by using the same procedure as described for **31**; mp 82.5-83.5 °C; $[\alpha]^{25}_D$ −1.56° (*c* 1.8, $CDCl_3$); IR 3427, 1657, 1512, 1463, 1053 cm^{-1}; ^{1}H NMR δ 0.85 (t, 6H, *J* = 6.4 Hz), 1.20-1.70 (m, 32H), 2.22 (t, 2H, *J* = 7.5 Hz), 3.71 (dd, 1H, *J* = 10.9, 3.4 Hz), 4.04 (m, 2H), 4.32 (t, 1H, *J* = 6.5 Hz), 4.59 (d, 1H, *J* = 3.6 Hz), 6.65 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR δ 14.06, 14.1, 22.6, 22.7, 25.4, 25.7, 29.1, 29.3, 29.4, 29.67, 29.72, 31.7, 32.0, 36.7, 37.7, 54.9, 61.8, 62.0, 63.2, 82.5, 88.1, 174.9; HR-MS [DCI, MH$^+$] *m/z* calcd for $C_{26}H_{50}NO_4$ 440.3740, found 440.3742.

(2*S*,3*R*,6*R*)-2-Octanoylamido-(4*E*)-octadecen-1,3,6-triol [(-)-3]. This compound was prepared in 88% yield by using the same procedure as described for **2**; mp 92.0-93.0 °C; $[\alpha]^{25}_{D}$ −10.0° (*c* 1.0, $CHCl_3$); IR 1658, 1510, 1467, 1224 cm^{-1}; ^1H NMR δ 0.88 (t, 6H, *J* = 6.4 Hz), 1.20-1.70 (m, 32H), 2.23 (t, 2H, *J* = 7.5 Hz), 3.70 (dd, 1H, *J* = 10.9, 3.4 Hz), 3.94 (m, 2H), 4.13 (q, 1H, *J* = 6.1 Hz), 4.37 (t, 1H, *J* = 4.1 Hz), 5.79 (dd, 1H, *J* = 15.5, 5.8 Hz), 5.81 (dd, 1H, *J* = 16.7, 6.0 Hz), 6.38 (d, 1H, *J* = 7.4 Hz); ^{13}C NMR δ 14.1, 22.6, 22.7, 25.5, 25.7, 29.0, 29.2, 29.4, 29.6, 29.7, 31.7, 31.9, 36.8, 37.3, 54.4, 62.1, 71.9, 73.8, 129.4, 135.6, 174.2; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{26}H_{51}NO_4Na$ 464.3716, found 464.3700.

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24. Zhang et al.[23] assigned the 2*S*,3*S* configuration to compound **23**; however, it should be 2*R*,3*S*. Moreover, because of the discrepancy between the mp and [α] with reference 23, we prepared the enantiomer of **23**, 2*S*,3*R*-1-chloropentadecane-2,3-diol, by the reaction of **22** with AD-mix-β, and found no change in mp (64.5-65.5 °C) and the same magnitude of specific rotation but opposite sign ($[\alpha]^{25}_{D}$ +8.9° (*c* 1.0, MeOH)).

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27. The ratio of **27**:**28** was estimated by high-temperature ^{1}H-NMR spectroscopy.

28. For examples of high *anti/syn* ratios in the addition of alkynyllithium reagents to **26** in the presence of HMPA, see: (a) Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362.

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31. The 1,3-oxazolidine-carbamate system exists as a pair of rotamers, the interconversion of which is enhanced at high temperature; therefore proton NMR spectra were recorded at 70 °C.

32. Some of the signals in the ambient temperature ^{13}C NMR spectra appear as a pair of singlets (denoted in parentheses).

Chapter 6

Preparation of Propargylic Alcohols from α,β-Unsaturated Esters. Control of C-3 Stereochemistry via Asymmetric Dihydroxylation

Abstract

A series of chiral propargylic alcohols with high enantiomeric excess was prepared by asymmetric dihydroxylation of α.β-unsaturated esters and elimination via 4-chloromethyl-1.3-dioxalane intermediates.

Introduction

Chiral propargylic alcohols are widely used synthons for many natural products.[1] Among the methods for preparing chiral propargylic alcohols are addition of organometallic compounds to α.β-acetylenic aldehydes in the presence of chiral ligands,[2] enantioselective aldol additions to α.β-ynals,[3] asymmetric reduction of α.β-ynones,[4] enzymatic resolution,[5] base-mediated double elimination of substrates (1-chloro-2.3-epoxy[6] and 4-chloromethyl-1.3-dioxolane[7]), elimination of chiral vinyl sulfoxides,[8] alkynylation of aldehydes catalyzed by a chiral ligand,[9] and reductive cleavage of α.β-alkynyl acetals.[10] Since it is well known that the Sharpless asymmetric dihydroxylation (AD) reaction is a very efficient method for building chirality from nonchiral olefinic substrates, and since α.β-unsaturated esters are excellent substrates for the AD reaction (giving high yield and very high ee),[11] we have developed a new method for the preparation 4-chloromethyl-1.3-dioxolane intermediates from α.β-unsaturated esters. These dioxolane intermediates are easily converted to chiral propargylic alcohols via double elimination. We demonstrate here the utility of this method by preparing a series of representative propargylic alcohols **1a-e** in high chiral purity.

Chart 1

1a $R = n\text{-}C_{15}H_{31}$

1b R = Ph

1c $R = n\text{-}C_{12}H_{25}$

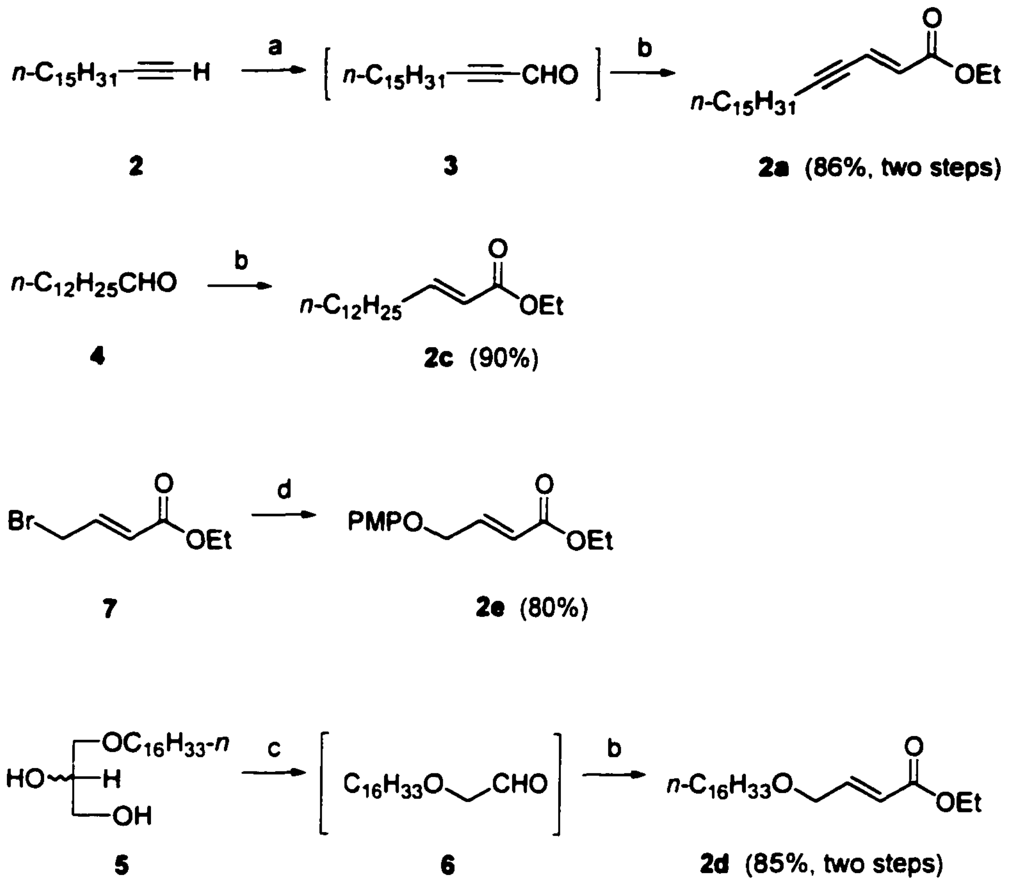
1d $R = n\text{-}C_{16}H_{33}OCH_2$

1e $R = PMPOCH_2$

Results and Discussion

Synthesis of α,β-Unsaturated Esters 2a (Scheme 1). The Horner-Wadsworth-Emmons (HWE) reaction[12] of aldehydes with a phosphonate reagent provides α,β-unsaturated esters. For example, reaction of heptadecyne with *n*-BuLi followed by formylation with 1-formylpiperidine provided ynal intermediate **3**, which was converted to ester **2a** in 86% yield (two steps from **2**). With triethyl phosphonoacetate, the *E*/*Z* ratio of **2a** was about 16:1, but when diisopropyl(ethoxycarbonylmethyl)phosphonate was used in the HWE reaction, the *E*/*Z* ratio was greatly improved,[13] and *E*-**2a** was obtained nearly exclusively. HWE reaction of tridecanal (**4**) with diisopropyl(ethoxycarbonylmethyl)-phosphonate gave ester **2c** in 90% yield. Treatment of diol **5** with $NaIO_4$ provided aldehyde **6**, which was converted to ester **2d** in 85% yield (two steps from **12**). Coupling of *p*-methoxyphenol with allylic bromide **7** provided ester **2e** in 80% yield.[14]

Scheme 1. Synthesis of α,β-Unsaturated Esters 2a, 2c-e[a]

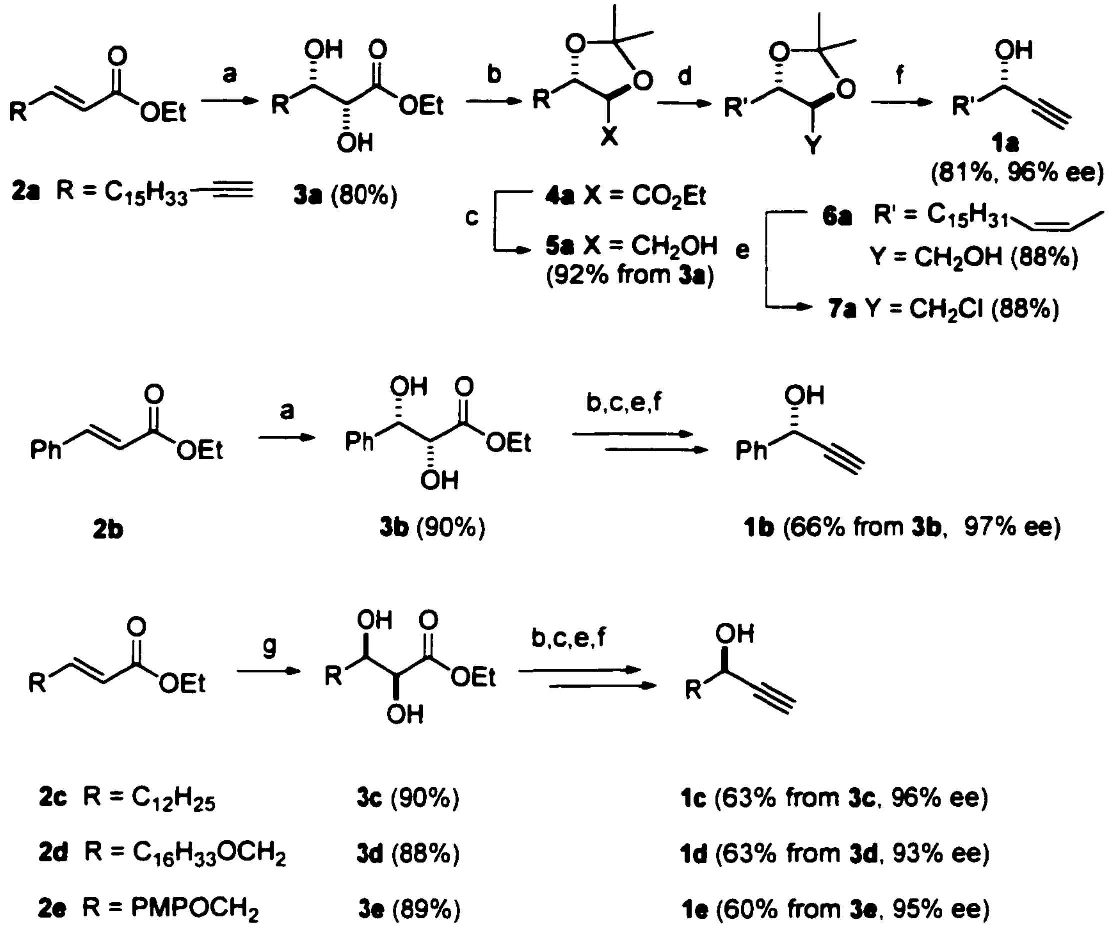


[a]Reagents and conditions: (a) 1-formylpiperidine, *n*-BuLi, THF, -15 - 0 °C; (b) (*i*-PrO)$_2$P(O)CH$_2$CO$_2$Et, Et$_3$N, LiBr, THF, rt; (c) NaIO$_4$, CH$_2$Cl$_2$, rt; (d) *p*-methoxyphenol, K$_2$CO$_3$, Me$_2$CO, 18-crown-6, rt.

Synthesis of Chiral Propargylic Alcohols 2a-e (Scheme 2). Compound **1a** is the *cis* double bond analogue of eicos-(4*E*)-en-1-yn-3-ol. The *E* stereoisomer, which has been isolated from a marine sponge and has also been synthesized,[15] exhibits immunosuppressive and antitumor activities.[16] Analogue **1a**, which may be useful for structure-function relationships, is readily available in geometrically pure form by the reaction sequence illustrated in Scheme 2. The AD reaction of α,β-unsaturated ester **2a** using AD-mix-α gave diol **3a** in low yield (30-40%). However, on supplementation of the reaction mixture with additional osmium reagent ($K_2OsO_4 \cdot 2H_2O$) and chiral ligand $(DHQ)_2$-PHAL, the reaction proceeded well, giving diol **3a** in 70-80% yield. After conversion of diol **3a** to acetonide ester **4a**, reduction with DIBAL-H provided alcohol **5a** in high yield (86%, two steps from **3a**). Lindlar-catalyzed hydrogenation of **5a** in MeOH provided *cis* compound **6a** in 88% yield. Treatment of alcohol **6a** with NCS via Mitsunobu reaction[17] gave chloride **7a** in 88% yield. Chiral propargylic alcohol **1a** was obtained by treatment of **7a** with *n*-BuLi in the presence of HMPA[7] (80% yield, 96 % ee).[18]

Synthesis of Chiral Propargylic Alcohols 2b-e (Scheme 2). We recently used propargylic alcohol **1c** as a building block for the synthesis of 6-hydroxy-4*E*-sphingosine.[19] As shown in Scheme 2, application of the method used to prepare compound **2a** afforded **2b-e** with high ee. The AD reaction of α,β-unsaturated esters **2b-e** gave diol esters **3b-e**, which were treated with 2,2-dimethoxypropane in the presence of *p*-TsOH to obtain acetonides **4b-e**. DIBAL-H reduction gave alcohols **5b-e**, which were converted to chlorides **6b-e** by Mitsunobu reaction. Chiral propargylic alcohols **1b-e** were obtained by treatment of chlorides **6b-e** with *n*-BuLi in the presence of HMPA. As expected, all alcohols **1b-e** have high ee.

Scheme 2. Synthesis of Chiral Propargylic Alcohol 2[a]



[a] Reagents and conditions: (a) AD-mix-α (for **3a**, more $K_2OsO_4\cdot 2H_2O$ and $(DHQ)_2$-PHAL were added).); (b) $Me_2C(OMe)_2$, *p*-TsOH, CH_2Cl_2, rt; (c) DIBAL-H, THF, 0 °C-rt; (d) H_2, Pd-$CaCO_3$ (cat.), MeOH, rt, (e) NCS, Ph_3P, CH_2Cl_2, 0 °C – rt; (f) *n*-BuLi, HMPA, THF, -42 °C - rt; (g) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH/H_2O 1/1, rt.

Experimental Section

General Information. See Chapter 1 for the detailed information. 2,2-Dimethoxypropane, NCS, and *n*-BuLi were purchased from Acros. DIBAL-H, HMPA, and lithium acetylide-ethylenediamine complex were purchased from Aldrich.

1-Heptadecyne (2). To a mixture of 3.45 g (34.5 mmol) of lithium acetylide-ethylenediamine complex[20] (90% purity) in 25 mL of DMSO was added a solution of 5.03 g (17.3 mmol) of pentadodecyl bromide in 10 mL of dry DMSO at 0 °C. When the addition was completed, the reaction mixture was held at rt for 5 h. Water (20 mL) was added slowly, followed by 1 M HCl (20 mL), and the mixture was extracted with hexane (3 x 30 mL). The combined extracts were washed successively with 30 mL of water and 30 mL of saturated aqueous NaCl solution, dried (Na_2SO_4), and concentrated. Purification by chromatography (hexane) gave 3.30 g (90%) of **2** as low melting white solid: ^{1}H NMR δ 0.85 (t, 3H, J = 6.6 Hz), 1.10-1.50 (m, 26H), 1.84 (t, 1H, J = 2.7 Hz), 2.12 (m, 2H); ^{13}C NMR δ 14.0, 18.4, 22.7, 28.6, 28.8, 29.2, 29.4, 29.6, 29.7, 29.8, 32.0, 68.0, 84.3.

Ethyl (2*E*)-Eicos-2-en-4-ynoate (2a). Eicos-2-ynal (**3**) was prepared by formylation of 1-heptadecyne (**2**) as follows. To a solution of 1-heptadecyne **2** (3.03 g, 12.9 mmol) in 100 mL of dry THF was added 5.7 mL (14.3 mmol) of *n*-butyllithium (a 2.5 M solution in hexane) at -15 °C. The mixture was stirred at -15 °C for 1 h, and then 1.67 g (14.8 mmol) of 1-formylpiperidine was added. The reaction mixture was stirred at -15 °C for 2 h and rt for 2 h. The reaction was quenched by the addition of 50 mL of 10% aqueous $NaHSO_4$ solution. The product was extracted with EtOAc (3 × 50 mL), and the organic layer was washed with 10% aqueous $NaHSO_4$ solution (3 × 30 mL), dried (Na_2SO_4), and concentrated. Ynal **3** was obtained as a pale yellow liquid, which was used without further purification in the subsequent HWE reaction. To a nitrogen-flushed solution of 5.58 g (64.3 mmol) of LiBr in 100 mL of dry THF was injected 3.89 g (15.4 mmol) of $(i\text{-PrO})_2P(O)CH_2CO_2Et$ at rt. After the solution was stirred at rt for 10 min, 2.7

mL (25.7 mmol) of Et_3N was injected, and stirring was continued for another 15 min. The solution of ynal **3** in 20 mL of dry THF was injected. The reaction mixture was stirred vigorously at rt until the full consumption of **3** 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 200 mL of hexane-EtOAc (10:1). Concentration and column chromatography (hexane/EtOAc 40/1) gave **2a** (86% yield from **2**) as a white solid; mp 31.0-31.5 °C; ^{1}H NMR δ 0.81 (t, 3H, J = 6.7 Hz), 1.10-1.60 (m, 29H), 2.28 (m, 2H), 4.12 (q, 2H, J = 7.1 Hz), 6.04 (d, 1H, J = 15.8 Hz), 6.67 (dt, 1H J = 15.8, 2.2 Hz); ^{13}C NMR δ 14.0, 19.6, 22.5, 22.6, 25.1, 28.3, 28.8, 28.9, 29.0, 29.3, 29.4, 29.5, 29.58, 29.61, 29.62, 31.5, 31.8, 34.5, 60.3, 77.8, 100.5, 125.9, 129.1, 165.8; HR-MS [DCI, MH$^+$] *m/z* calcd for $C_{22}H_{39}O_2$ 335.2950, found 335.2937.

Ethyl (2*R*,3*S*)-2,3-Dihydroxyeicos-4-ynoate [(+)-3a]. A solution of 14.0 g of AD-mix-α, 180 mg of $(DHQ)_2$-PHAL, 55 mg (0.15 mmol) of $K_2OsO_4 \cdot 2H_2O$, and 950 mg (10.0 mmol) of $MeSO_2NH_2$ in 300 mL of *t*-BuOH/H_2O 1/1 was stirred vigorously at rt for 30 min. Then 3.12 g (9.3 mmol) of unsaturated ester **2a** was added. The reaction mixture was stirred vigorously until the disappearance of ester **2a** was noted. Sodium sulfite (15.0 g, 14.6 mmol) was added to quench the reaction. Stirring was continued for another 30 min. The product was extracted with EtOAc (3 × 100 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a yellow solid residue, which was purified by chromatography (hexane/EtOAc 2:1), giving 2.74 g (80%) of diol **3a** as a white solid: mp 61.2-62.0 °C; $[\alpha]^{25}_D$ +1.6° (*c* 5.0, $CHCl_3$); ^{1}H NMR δ 0.83 (t, 3H, J = 6.6 Hz), 1.10-1.60 (m, 29H), 2.20 (m, 2H), 3.09 (br s, 2H), 4.26 (m, 3H), 4.60 (q, 1H J = 1.9 Hz); ^{13}C NMR δ 14.0, 18.6, 22.6, 28.4, 28.8, 29.1, 29.3, 29.4, 29.6, 31.8, 62.2, 64.0, 73.9, 77.2, 87.2, 171.8; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{22}H_{40}O_4Na$ 391.2824, found 391.2841.

(4*R*,5*S*)-4-Ethoxycarbonyl-5-(1'-heptadecynyl)-2,2-dimethyl-1,3-dioxolane (4a). To a solution of diol **3a** (1.18 g, 3.2 mmol) in 25 mL of dry CH_2Cl_2 was added 0.67 g (6.4 mmol) of 2,2-dimethoxypropane at rt. After *p*-TsOH (50 mg) was added, the mixture was stirred at room temperature for 2 h. The reaction mixture was passed through a pad of silica gel in a sintered glass funnel. The pad was washed with 50 mL of CH_2Cl_2. Concentration gave a colorless oil which was used without further purification in the subsequent reaction.

(4*R*,5*S*)-4-Hydroxymethyl-5-(1'-heptadecynyl)-2,2-dimethyl-1,3-dioxolane [(-)-5a]. To a solution of ester **4a** obtained above in dry THF (15 mL) was added, dropwise, 4.3 mL (6.4 mmol) of DIBAL-H (a 1.5 M solution in toluene) at 0 °C under N_2. The reaction mixture was stirred for 2 h at 0 °C until TLC analysis showed the reaction to be complete. The reaction was quenched by slow addition of 2.0 mL of MeOH followed by 15.0 mL of cold 5% aqueous potassium sodium tartrate solution. The product was extracted with EtOAc (3 × 30 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 4:1) gave 1.05 g (90%) of **5a** as a colorless oil: $[\alpha]^{25}_D$ −12.7° (*c* 1.3, $CHCl_3$); ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.10-1.60 (m, 32H), 2.20 (m, 2H), 2.74 (br s, 1H), 3.65 (dd, 1H, *J* = 12.2, 3.6 Hz), 3.86 (dd, 1H, *J* = 12.2, 2.8 Hz), 4.06 (m, 1H), 4.54 (d, 1H, *J* = 7.8 Hz); ^{13}C NMR δ 14.2, 18.8, 22.7, 26.4, 26.8, 28.5, 28.9, 29.2, 29.4, 29.6, 29.7, 32.0, 60.9, 66.9, 76.5, 82.4, 87.7, 110.0; HR-MS [DCI/NH$_3$, MH$^+$] *m/z* calcd for $C_{23}H_{43}O_3$ 367.3212, found 367.3203.

(*Z*) (4*R*,5*S*)-4-Hydroxymethyl-5-(1'-heptadecenyl)-2,2-dimethyl-1,3-dioxolane [(-)-6a]. To a solution of 290 mg (0.79 mmol) of **5a** in 15 mL of dry MeOH was added 22 mg of Lindlar catalyst (Pd-$CaCO_3$). The apparatus was evacuated, then flushed with hydrogen from a H_2-filled balloon. The reaction mixture was stirred at rt for 1 h. The solvent was removed, and the residue was purified by chromatography (hexane/EtOAc 7:3), providing 255 mg (88%) of the desired product **6a** as a colorless oil: $[\alpha]^{25}_D$ −7.85° (*c* 1.3, $CHCl_3$); ^1H NMR δ 0.86 (t, 3H, *J* = 6.6 Hz), 1.10-1.40 (m, 26H), 2.06 (s, 6H), 2.13 (m, 2H), 2.30 (dd, 1H, *J* = 7.6, 4.8 Hz), 3.57 (m, 1H), 3.74 (m, 1H), 3.82 (m, 1H), 4.70 (t, 1H, *J* = 8.7 Hz), 5.37 (t, 1H, *J* = 9.1 Hz), 5.70 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 27.0, 27.2, 27.8, 29.2, 29.4, 29.5, 29.6, 29.70, 29.73, 32.0, 60.6, 72.6, 81.5, 109.0, 125.9, 136.8; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{23}H_{44}O_3Na$ 391.3188, found 391.3175.

(*Z*)-(4*R*,5*S*)-4-Chloromethyl-5-(1'-heptadecenyl)-2,2-dimethyl-1,3-dioxolane [(-)-7a]. To a solution of 763 mg (2.09 mmol) of alcohol **6a** and 656 mg (2.5 mmol) of Ph_3P in 30 mL of dry CH_2Cl_2 was added 307 mg (2.3 mmol) of NCS at 0 °C under N_2. The mixture was stirred at 0 °C for 1 h, then allowed to warm to rt and stirred for 1 h. The mixture was diluted with 100 mL of hexane and passed through a pad of silica gel to remove the precipitate of Ph_3PO. Concentration and purification of the residue by column chromatography (hexane/EtOAc 9:1) gave 691 mg (86%) of **7a** as a colorless oil; $[\alpha]^{25}_D$ −5.73° (*c* 3.0, $CHCl_3$); ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.10-1.40 (m, 26H), 1.46 (two sets of s, 6H), 2.11 (m, 2H), 3.56 (dd, 1H, *J* = 11.9, 4.6 Hz), 3.69 (m, 1H), 3.88 (m, 1H), 4.70 (t, 1H, *J* = 8.8 Hz), 5.38 (t, 1H, *J* = 9.2 Hz), 5.73 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 26.6, 26.9, 27.0, 27.3, 27.5, 27.9, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 43.1, 64.4, 80.1, 109.5, 125.4, 137.2; HR-MS [FAB, MH$^+$] *m/z* calcd for $C_{23}H_{44}O_2Cl$ 387.3030, found 387.3030.

(3*S*)-Eicos-4-(*Z*)-en-1-yn-3-ol [(+)-1a]. To a stirred solution of 0.47 mL (2.7 mmol) of HMPA in 4 mL of dry THF was injected 1.0 mL (2.5 mmoL) of *n*-BuLi (a 2.5 M solution in hexane) at –42 °C under N_2. After 10 min, a solution of chloride **7a** (155 mg, 0.42 mmol) in 4 mL of THF was added dropwise within 3 min. After 0.5 h, the reaction was quenched with saturated aqueous NH_4Cl solution (10 mL). 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 chromatography (hexane/EtOAc 7:1) gave 97 mg (80%) of **1a** as a white solid: mp 32.5-33.0 °C, $[\alpha]^{25}_D$ +76.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.10-1.60 (m, 26H), 2.03 (s, 1H), 2.11 (m, 2H), 2.49 (d, 1H *J* = 2.2 Hz), 5.14 (m, 1H), 5.56 (m, 2H); ^{13}C NMR δ 14.1, 22.7, 27.6, 29.2, 29.3, 29.4, 29.5, 29.6, 27.7, 32.0, 58.0, 84.1, 128.7, 134.1; HR-MS [DEI, M$^+$] *m/z* calcd for $C_{20}H_{36}O$ 292.2766, found 292.2761.

Ethyl (2*R*,3*S*)-2,3-Dihydroxy-3-phenylpropionate [(+)-3b]. $[\alpha]^{25}_D$ +7.9° (*c* 1.0, MeOH); ^{1}H NMR δ 1.17 (t, 3H, *J* = 7.2 Hz), 3.54 (d, 1H, *J* = 6.6 Hz), 3.69 (d, 1H, *J* = 6.5 Hz), 4.13 (m, 2H), 4.24 (dd, 1H, *J* = 6.4, 3.6 Hz), 4.90 (dd, 1H, *J* = 6.4, 4.6 Hz), 7.28 (m, 5H); ^{13}C NMR δ 13.8, 61.7, 74.5, 74.9, 126.2, 127.7, 128.1, 139.8, 172.6.

(4*S*,5*S*)-2,2-Dimethyl-5-phenyl-4-hydroxymethyl-1,3-dioxolane [(+)-5b]. $[\alpha]^{25}_D$ +31.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.51 (s, 3H), 1.56 (s, 3H), 2.73 (br s, 1H), 3.60 (m, 1H), 3.86 (m, 2H), 4.86 (d, 1H, *J* = 8.6 Hz), 7.34 (m, 5H); ^{13}C NMR δ 26.9, 60.2, 78.6, 83.6, 109.1, 126.4, 128.1, 128.5, 137.5; MS [ESI, MNH$_4^+$] 226.1.

(4*R*,5*S*)-4-Chloromethyl-2,2-dimethyl-5-phenyl-1,3-dioxolane (6b). ^{1}H NMR δ 1.53 (s, 3H), 1.57 (s, 3H), 3.58 (dd, 1H, *J* = 12.1, 4.8 Hz), 3.70 (dd, 1H, *J* = 12.0, 3.6 Hz), 4.00 (m, 1H), 4.88 (d, 1H, *J* = 8.2 Hz), 7.34 (m, 5H); ^{13}C NMR δ 27.0, 27.2, 42.9, 80.4, 82.3, 109.7, 126.6, 128.55, 128.60, 128.7, 137.1.

(1*R*)-1-Phenyl-2-propyn-1-ol [(-)-1b]. $[\alpha]^{25}_D$ −28.0° (*c* 1.0, $CHCl_3$) (lit.[8] $[\alpha]^{21}_D$ −26.7° (*c* 1.5, $CHCl_3$); ^{1}H NMR δ 2.61 (d, 1H, *J* = 5.8 Hz), 2.64 (d, 1H, *J* = 2.2 Hz), 5.43 (d, 1H, *J* = 3.7 Hz), 7.33 (m, 3H), 7.53 (m, 2H); ^{13}C NMR δ 64.3, 74.8, 83.5, 126.6, 128.5, 128.6, 140.0; MS [ESI, MNH_4^+] 150.1.

Ethyl (2*S*,3*R*)-2,3-Dihydroxypentadecanoate [(+)-3c]. mp 69.0-70.0 °C; $[\alpha]^{25}_D$ +8.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.5 Hz), 1.10-1.70 (m, 25H), 3.06 (d, 1H, *J* = 8.4 Hz), 3.86 (m, 2H), 4.06 (dd, 1H, *J* = 6.5, 2.3 Hz), 4.24 (q, 2H, *J* = 7.1 Hz); ^{13}C NMR δ 14.15, 14.17, 22.8, 25.9, 29.5, 29.70, 29.72, 29.74, 29.76, 29.80, 32.0, 33.6, 61.8, 72.8, 73.6, 173.8.

(4*R*,5*R*)-5-Dodecyl-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane [(+)-5c]. $[\alpha]^{25}_D$ +18.8° (*c* 1.8, $CHCl_3$); ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.10-1.70 (m, 28H), 2.90 (t, 1H, *J* = 6.0 Hz), 3.59 (m, 1H), 3.72 (m, 2H), 3.84 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 26.0, 27.1, 27.4, 29.4, 29.58, 29.63, 29.70, 29.73, 29.8, 32.0, 33.2, 62.2, 77.1, 81.8, 108.6; HR-MS [DCI, MH$^+$] *m/z* calcd for $C_{18}H_{37}O_3$ 301.2743, found 301.2751.

(4*S*,5*R*)-4-Chloromethyl-5-dodecyl-2,2-dimethyl-1,3-dioxolane [(+)-6c]. $[\alpha]^{25}_D$ +14.2° (*c* 0.9, $CHCl_3$); ^{1}H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.70 (m, 28H), 3.58 (d, 2H, *J* = 4.9 Hz), 3.88 (m, 2H); ^{13}C NMR δ 14.1, 22.7, 26.0, 27.0, 27.5, 29.4, 29.5, 29.6, 29.7, 32.0, 33.5, 44.4, 79.4, 80.3, 109.1; HR-MS [DCI, MH$^+$] *m/z* calcd for $C_{18}H_{36}O_2Cl$ 319.2404, found 319.2407.

(3*R*)-1-Pentadecyn-3-ol [(+)-1c]. mp 40.0-41.0 °C; $[\alpha]^{25}_D$ +2.60° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.60 (m, 20H), 1.72 (m, 2H), 2.41 (br s, 1H), 2.44 (d, 1H, *J* = 3.7 Hz), 4.36 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 25.1, 29.3, 29.5, 29.62, 29.69, 29.7, 32.0, 37.6, 62.3, 72.8, 85.1; HR-MS [DCI, MH^+] *m/z* calcd for $C_{15}H_{29}O$ 225.2218, found 225.2211.

Ethyl 4-Hexadecyloxy-(2*E*)-butenoate (2d). ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.60 (m, 29H), 1.57 (m, 2H), 3.45 (t, 2H, *J* = 6.6 Hz), 4.10 (q, 2H, *J* = 2.0 Hz), 4.19 (m, 2H), 6.07 (dt, *J* = 15.7, 2.0 Hz), 6.96 (dt, *J* = 15.7, 4.2 Hz); ^{13}C NMR δ 14.1, 22.8, 26.2, 29.5, 29.58, 29.69, 29.71, 29.76, 29.79, 32.0, 60.3, 69.4, 71.3, 121.1, 144.7, 166.3; MS [ESI, MNH_4^+] 372.3.

Ethyl (2*S*,3*R*)-2,3-Dihydroxy-4-(hexadecyloxy)butyrate [(+)-3d]. mp 62.2-63.0 °C; $[\alpha]^{25}_D$ +1.2° (*c* 5.0, $CHCl_3$); ^1H NMR δ 0.85 (t, 3H, *J* = 6.6 Hz), 1.20-1.40 (m, 29H), 1.54 (m, 2H), 1.63 (br s, 1H), 2.47 (d, 1H, *J* = 7.2 Hz), 3.16 (d, 1H, *J* = 5.6 Hz), 3.46 (t, 2H, *J* = 6.7 Hz), 3.56 (m, 2H), 4.09 (m, 1H), 4.23 (m, 3H); ^{13}C NMR δ 14.1, 22.7, 26.0, 26.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 60.3, 70.8, 71.0, 71.3, 71.8, 173.1; MS [ESI, MNH_4^+] 406.3.

(4*S*,5*R*)-5-(Hexadecyloxy)methyl-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane [(-)-5d]. $[\alpha]^{25}_D$ –6.5° (*c* 2.0, $CHCl_3$); ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.40 (m, 26H), 1.42 (s, 6H), 1.57 (m, 2H), 2.59 (br s, 1H), 3.49 (m, 3H), 3.65 (dd, 1H, *J* = 9.7, 4.8 Hz), 3.73 (m, 2H), 3.92 (m, 1H), 3.98 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 26.0, 26.9, 27.0, 29.4, 29.52, 29.59, 29.63, 29.7, 32.0, 62.6, 71.2, 72.1, 77.4, 80.0, 109.3; MS [ESI, MNH_4^+] 404.3.

(4*S*,5*S*)-4-Chloromethyl-5-(hexadecyloxy)methyl-2,2-dimethyl-1,3-dioxolane [(+)-6d]. $[\alpha]^{25}_{D}$ +1.9° (*c* 1.6, $CHCl_3$); ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.40 (m, 26H), 1.48 (s, 6H), 1.57 (m, 2H), 3.49 (m, 2H), 3.65 (m, 4H), 4.05 (m, 2H); ^{13}C NMR δ 14.1, 22.7, 26.0, 27.0, 27.1, 29.4, 29.56, 29.62, 29.69, 29.7, 31.9, 44.6, 71.3, 72.0, 77.9, 78.3, 110.0; MS [ESI, MNH_4^+] 422.2.

(3*S*)-1-Hexadecyloxybut-3-yn-2-ol [(+)-1d]. mp 46.0-47.0; $[\alpha]^{25}_{D}$ +6.96° (*c* 2.3, $CHCl_3$); ^1H NMR δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20-1.40 (m, 26H), 1.57 (m, 2H), 2.44 (d, 1H, *J* = 2.2 Hz), 2.57 (d, 1H, *J* = 4.8 Hz), 3.52 (m, 3H), 3.60 (m, 1H), 4.53 (m, 1H); ^{13}C NMR δ 14.1, 22.7, 26.0, 29.3, 29.45, 29.53, 29.60, 29.62, 29.69, 29.7, 31.9, 61.4, 71.8, 73.6, 74.0, 81.7; HR-MS [DCI, MNH_4^+] *m/z* calcd for $C_{20}H_{42}NO_2$ 328.3216, found 328.3216.

Ethyl (2*S*,3*R*)-2,3-Dihydroxy-4-(4'-methoxyphenoxy)butyrate [(+)-3e]. $[\alpha]^{25}_{D}$ +10.3° (*c* 3.2, $CHCl_3$); ^1H NMR δ 1.31 (t, 3H, *J* = 7.2 Hz), 2.73 (d, 1H, *J* = 7.8 Hz), 3.34 (d, 1H, *J* = 5.8 Hz), 4.04 (s, 3H), 4.05 (m, 2H), 4.32 (m, 4H), 6.83 (m, 4H); ^{13}C NMR δ 14.2, 55.7, 62.3, 69.0, 70.7, 70.8, 114.7, 115.6, 152.5, 154.2, 173.1; MS [ESI, MNH_4^+] 288.1.

(4*S*,5*R*)-4-Chloromethyl-5-(4'-methoxyphenoxy)methyl-2,2-dimethyl-1,3-dioxolane [(+)-6e]. $[\alpha]^{25}_{D}$ +11.54° (*c* 1.6, $CHCl_3$); ^1H NMR δ 1.47 (s, 6H), 3.71 (d, 2H, *J* = 5.0 Hz), 3.75 (s, 3H), 4.09 (m, 2H), 4.23 (m, 2H), 6.83 (m, 4H); ^{13}C NMR δ 27.1, 44.5, 55.7, 69.2, 77.5, 78.1, 110.4, 114.6, 115.5, 152.5, 154.2; MS [ESI, MNH_4^+] 304.1.

(2*R*)-1-(4'-Methoxyphenoxy)-but-3-yn-2-ol [(+)-1e]. mp 50.0-51.0 °C (lit.[5b] mp 52-53 °C); $[\alpha]^{25}_{D}$ +28.6° (*c* 1.0, $CHCl_3$); ^1H NMR δ 2.52 (d, 1H, *J* = 2.2 Hz), 2.82 (d, 1H, J = 5.3 Hz), 3.75 (s, 3H), 4.09 (m, 2H), 4.73 (m, 1H), 6.83 (m, 4H); ^{13}C NMR δ 55.7, 61.2, 72.3, 74.3, 81.2, 114.7, 115.9, 152.3, 154.4; MS [ESI, MNH_4^+] 210.1.

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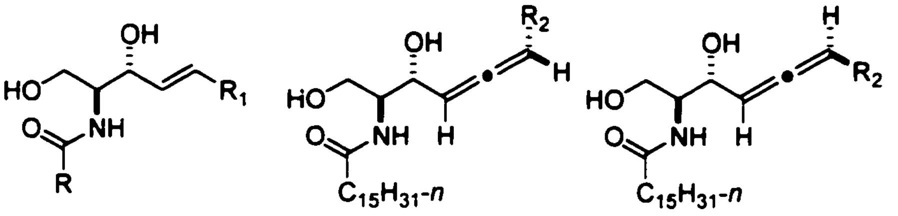
Chapter 7

Synthesis of New Ceramide Analogues with Allene in the Sphingoid Backbone

Abstract

New ceramide analogues **2** and **3** with an allene group in the sphingoid backbone were synthesized. The synthesis of the key intermediates **12** and **18** involved the $LiAlH_4$ reduction of oxazolidine intermediates **11** and **17** via a S_N2' reaction. Hydrolysis of the oxazolidine, liberation of the amino group, and *N*-acylation provided new ceramide analogues **2** and **3**.

Chart 1



1 $R_1 = C_{13}H_{27}$-*n* **2** $R_2 = C_{12}H_{25}$-*n* **3**

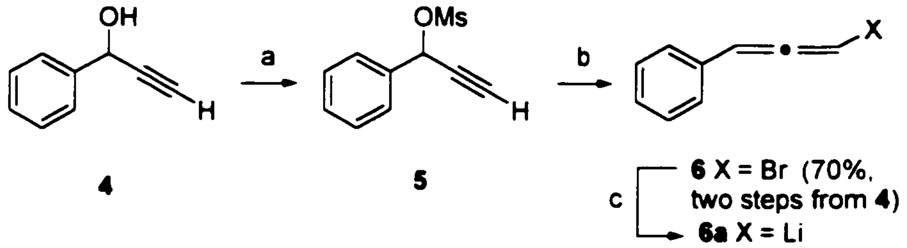
Introduction

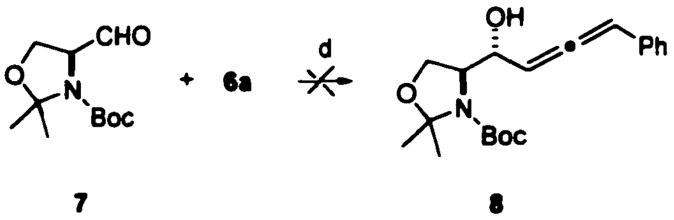
The C(4)-C(5) trans double bond in the sphingoid base of naturally occurring ceramide **1** may be crucial for the ceramide's capacity to modulate various fundamental biological functions (including membrane fusion of Semliki Forest virus and induction of apoptosis). We prepared new ceramide analogues in which the C(4)-C(5) double bond of the long-chain base was replaced by an allene (Chart 1, compounds **2** and **3**). These analogues will allow us to test whether replacing the C(4)-C(5)-double bond with an allene group can support apoptosis or the fusion of SFV with target membranes.

Results and Discussion

Attempted Synthesis of Allene 8 (Scheme 1). There are several methods to prepare allenic alcohols: reaction of propargylic cyclic carbonates and sulfites with organometallic reagents;[1] addition of allenyl trichlorosilanes or allenylstannanes to aldehydes;[2] reaction of allenic zirconium[3] or zinc[4] species to aldehydes; base-mediated reduction of dibromocyclopropylcarbinols;[5] addition of hydride ion to propargylic alcohols;[6] copper-catalyzed addition of Grignard reagents to alkynyl epoxides;[7] and hydrostannation and deoxystannylation of propargylic alcohols.[8] We first tried to prepare model compound **8** from allenic bromide **6** via nucleophilic addition of a lithiated allene[9] to L-serine-derived aldehyde **7** (Garner aldehyde). Treatment of phenyl propargyl alcohol (**4**) with *n*-BuLi and reaction with mesyl chloride provided mesylate **5**. Allenic bromide **6** was obtained in 70% yield by the S_N2' reaction of $LiCuBr_2$ with mesylate **5**.[10] However, the expected allenic alcohol **8** was not formed when bromide **6** was treated with *n*-BuLi and reacted with Garner aldehyde **7**.[11]

Scheme 1. Attempted Synthesis of Allene 8[a]



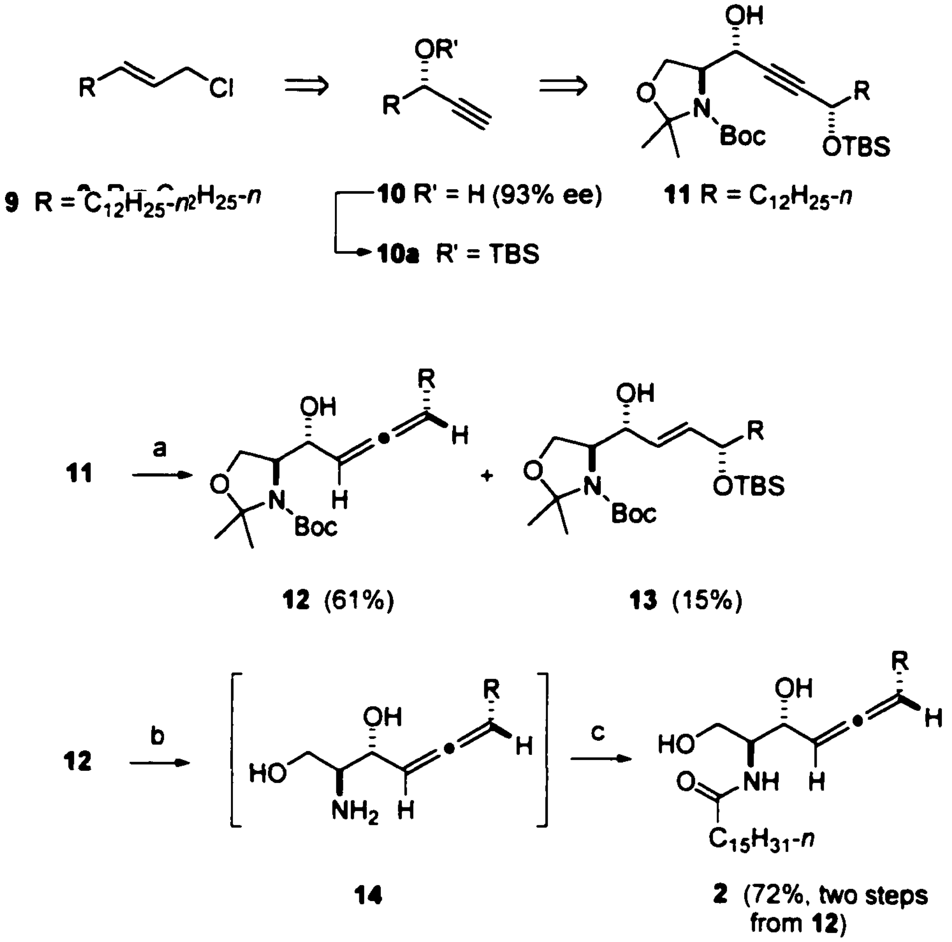


[a]Reagents and conditions: (a) LiBr, *n*-BuLi, MsCl, THF, -78 °C-rt; (b) CuBr, LiBr, THF, rt; (c) *n*-BuLi, THF, -78 °C; (d) THF, -78 °C-rt.

Scheme 2. Synthesis of Ceramide Analogue 2 (Scheme 2). The addition of a hydride to propargylic alcohols (S_N2' reaction)[6,12] to install the allene group is a widely used method for the allenic alcohol synthesis. Many leaving groups have been used, including methoxy,[13] tetrahydropyranyloxy,[14] silyloxy,[6b] quaternary ammonium salts,[15] and nitro groups.[16] $LiAlH_4$ is the most commonly used hydride provider.[6,12-16] We first chose TBS group as the leaving group.[17] As shown in Scheme 2, chiral propargylic alcohol **10** (93% ee) was prepared via Sharpless asymmetric dihydroxylation of allylic chloride **9**.[18] The reaction of TBS-protected chiral propargylic alcohol **10a** with Garner aldehyde **7** provided intermediate **11**.[18] $LiAlH_4$ reduction of propargylic alcohol **11** gave a mixture of allenic alcohol **12** and allylic alcohol **13**, the yields of which depended on the reaction conditions. First, the choice of reaction solvent was very important. When the solvent was THF, the yields of both **12** and **13** were low and some unidentified byproducts were formed. When diethyl ether was used, only **12** and **13** were obtained. The ratio of **12** to **13** depended on the reaction temperature and time. If the temperature was very low (<40 °C), the reaction with excess of $LiAlH_4$ was not completed and some of the starting material **11** could be recovered. When the reaction was carried out at –15

°C for 3 h, then raised to room temperature for 0.5 h, allenic alcohol **12** was obtained as the major product. Conversely, when the reaction was carried out at –15 °C for 0.5 h, then allowed to warm to room temperature for 2 h, allylic alcohol **13** was formed as the major product. The amount of $LiAlH_4$ was also very important. It was found that 1.5-2.0 equiv. of $LiAlH_4$ was suitable to form the allenic alcohol, and 2.5-3.0 equiv. of $LiAlH_4$ was suitable to form the allylic alcohol. Hydrolysis of the oxazolidine (100 °C, 1 M HCl, dioxane) of **12** provided sphingosine analogue **14**. Ceramide analogue **2** was obtained by *N*-acylation of **14** with *p*-nitrophenyl palmitate.

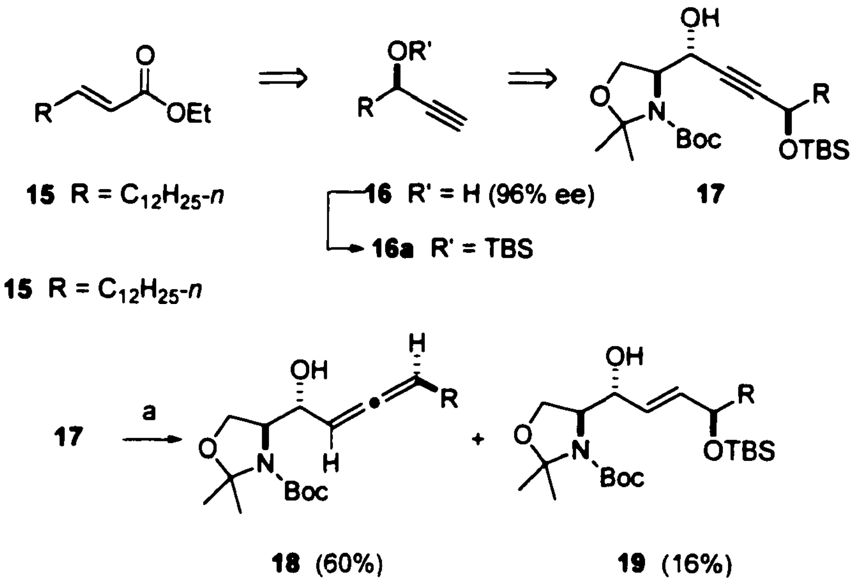
Scheme 2. Synthesis of Ceramide Analogue 2[a]

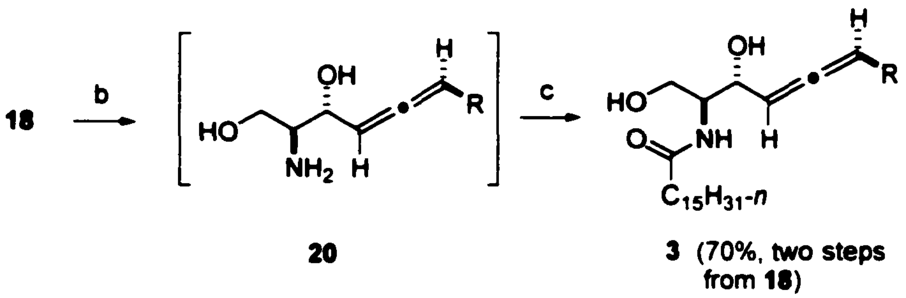


[a]Reagents and conditions: (a) $LiAlH_4$, Et_2O, -15 °C (3 h)-rt (0.5 h); (b) 1 M HCl, dioxane, 100 °C; (c) p-$O_2NC_6H_4CO_2C_{15}H_{31}$-$n$, THF, rt.

Synthesis of New Ceramide Analogue 3. Chiral propargylic alcohol **16** (96% ee) was prepared from Sharpless asymmetric dihydroxylation of α,β-unsaturated ester **15**.[17] The reaction of TBS-protected chiral propargylic alcohol **16a** with Garner aldehyde **7** afforded intermediate **17**.[17] As shown in Scheme 3, $LiAlH_4$ reduction of **17** provided allenic alcohol **18** and allylic alcohol **19**. As discussed before, the ratio of **18** and **19** depended on the reaction conditions. When Et_2O was the solvent and the reaction was carried out at low temperature (−15 °C) for 3 h, then raised to room temperature for 0.5 h, allenic alcohol **18** was formed as the major product. Ceramide analogue **3** was obtained by acid hydrolysis of the oxazolidine (100 °C, 1 M HCl, dioxane) of **18** and *N*-acylation with *p*-nitrophenyl palmitate.

Scheme 3. Synthesis of Ceramide Analogue 3[a]





[a]Reagents and conditions: (a) $LiAlH_4$, Et_2O, -15 °C (3 h)-rt (0.5 h); (b) 1 M HCl, dioxane, 100 °C; (c) p-$O_2NC_6H_4CO_2C_{15}H_{31}$-$n$, THF, rt.

Experimental Section

General Information. See the Chapter 1 for general experimental details. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. The solvent was $CDCl_3$ unless otherwise noted.

1-Bromo-3-phenyl 1,2-propadiene (6). To a solution of 132 mg (1.0 mmol) of propargyl alcohol **4** and 86 mg (1.0 mmol) of dry LiBr in 20 mL of dry THF was added 0.4 mL of n-BuLi (1.0 mmol, a 2.5 M solution of in hexane) at −78 °C under N_2. After stirring for 30 min, methanesulfonyl chloride (115 mg, 1.0 mmol) was added and stirring was continued for 30 min. A solution of 1.5 mmol of $LiCuBr_2$ (130 mg (1.5 mmol) of LiBr + 214 mg (1.5 mmol) of CuBr) in 15 mL of THF was added at −78 °C. After stirring for 1 h at −78 °C, the reaction mixture was allowed to warm to rt for 5 h. The reaction was quenched by the slow addition of saturated aqueous NH_4Cl solution, filtered, extracted with Et_2O (3 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was chromatographed on silica gel (hexane) to afford 137 mg (70%) of **6** as a colorless liquid: the IR spectrum is the same as that in the literature;[7] ^{1}H NMR δ 6.20 (s, 1H), 6.25 (s, 1H), 7.25 (m, 5H) (lit.[7] 6.25 (m, 2H), 7.25 (m, 5H)); ^{13}C NMR δ 74.8, 102.6, 127.9, 128.6, 128.8, 131.7, 202.9.

***N-tert*-Butoxycarbonyl (4*S*,3'*R*)-4-[1'-Hydroxy-(2',3')-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine [(-)-12].** To a solution of 0.57 g (1.0 mmol) of alcohol **11** in 10 mL of dry Et$_2$O was added 2.0 mL of 1.0 M LiAlH$_4$ in Et$_2$O (2.0 mmol) at –15 °C under N$_2$. The solution was stirred for 3 h at this temperature and allowed to warm to rt for 0.5 h. The reaction was quenched by the slow addition of cold water. The mixture was extracted with EtOAc (3 × 20 mL), dried over MgSO$_4$, and concentrated under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc 9:1-4:1) to afford 267 mg (61%) of **12**: $[\alpha]^{25}_{D}$ –67.6° (*c* 1.5, CHCl$_3$); ^{1}H NMR (C$_6$D$_6$, 70 °C) 0.87 (t, 3H, *J* = 5.2 Hz), 1.10-1.70 (m, 35H), 1.65 (s, 3H), 1.97 (m, 2H), 3.72 (dd, 1H, *J* = 9.0, 6.6 Hz), 3.91 (br s, 1H), 3.97(br s, 1H), 4.46 (m, 1H), 5.18 (m, 1H), 5.27 (m, 1H); ^{13}C NMR (C$_6$D$_6$) δ 14.3, 23.1, 23.2, 25.0, 25.9, 26.0, 26.7, 28.3, 29.1, 29.5, 29.6, 29.8, 30.1, 32.3, (61.8) 63.1, 65.0, (71.3) 71.8, (79.5) 80.4, (92.8) 93.3, (93.4) 94.5, (152.0) 154.1, (203.5) 204.0; HR-MS [FAB, MH^{+}] *m/z* calcd for C$_{26}$H$_{48}$NO$_4$ 438.3583, found 438.3568.

***N-tert*-Butoxycarbonyl (4*S*,1'*R*,4'*S*)-4-[4'-(*tert*-Butyldimethylsilyloxy)-1'-hydroxy-2'-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(-)-13].** To a solution of 0.57 g (1.0 mmol) of alcohol **11** in 10 mL of dry Et$_2$O was slowly added 3.0 mL of 1.0 M LiAlH$_4$ in Et$_2$O (3.0 mmol) at –15 °C under N$_2$. The solution was stirred for 0.5 h at this temperature and allowed to warm to rt for 2 h. The reaction was quenched by the slow addition of cold water. The mixture was extracted with EtOAc (3 × 20 mL), dried over MgSO$_4$, and concentrated under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc 9:1-4:1) to afford 235 mg (50%) of **13**: $[\alpha]^{25}_{D}$ −18.7° (*c* 1.5, $CDCl_3$); ^{1}H NMR (C_6D_6, 70 °C) δ 0.11 (s, 6H), 0.89 (t, 3H, *J* = 7.1 Hz), 0.99 (s, 9H), 1.20-1.80 (m, 37H), 3.69 (t, 1H, *J* = 7.0 Hz), 3.81 (br s, 1H), 3.95 (br s, 1H), 4.19 (dd, 1H, *J* = 12.7, 5.7 Hz), 4.43 (br s, 1H), 5.71 (dd, 1H, *J* = 15.4, 5.1 Hz), 5.92 (dd, 1H, *J* = 14.8, 4.5 Hz); ^{13}C NMR (C_6D_6) δ -4.8, -3.9, 14.3, 18.2, 23.1, 24.6, 25.7, 25.9, 26.0, 26.1, 26.6, 28.3, 29.7, 30.1, 38.9, 62.9, 65.1, 73.2, 73.8, 77.7, 80.5, 94.6, 135.5, 154.4; HR-MS [FAB, MNa^+] *m/z* calcd for $C_{32}H_{63}NO_5SiNa$ 592.4373, found 592.4400.

(2*S*,3*R*,5*R*)-(4,5)-2-Palmitoylamidooctadecadiene-1,3-diol [(-)-2]. A solution of 88 mg (0.2 mmol) of **12** in 4 mL of 1 M HCl and 4 mL of dioxane was heated at 100 °C with stirring for 1 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (4 mL). The product was extracted with $CHCl_3$ (3 × 20 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided crude sphingosine analogue **14** as a white solid, which was used in the next reaction without further purification. To a solution of **14** in 6 mL of dry THF was added 108 mg (0.40 mmol) of *p*-nitrophenyl palmitate at rt. The mixture was stirred for 24 h and concentrated. Purification by flash chromatography ($CHCl_3$/MeOH 9:1) afforded 60 mg (72%, two steps) of ceramide analogue **2** as a white solid: mp 96.0-97.0 °C; $[\alpha]^{25}_{D}$ −30.5° (*c* 1.1, $CHCl_3$/MeOH 9:1); ^{1}H NMR δ 0.74 (t, 6H, *J* = 5.9 Hz), 0.9-1.30 (m, 44H), 1.47 (m, 2H), 1.87 (m, 2H), 2.09 (t, 2H, *J* = 7.6 Hz), 3.54 (dd, 1H, *J* = 11.3, 3.8 Hz), 3.74 (m, 1H), 4.08 (br s, 1H), 5.05 (br s, 1H), 5.11 (t, 1H, *J* = 6.4 Hz); ^{13}C NMR δ 13.7, 22.4, 25.6, 28.5, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 31.7, 36.4, 54.8, 61.3, 70.8, 91.8, 93.5, 174.6, 203.2. HR-MS [FAB, MH$^+$] *m/z* calcd for $C_{34}H_{66}NO_3$ 536.5043, found 536.5061. (See Fig. 1, page 129.)

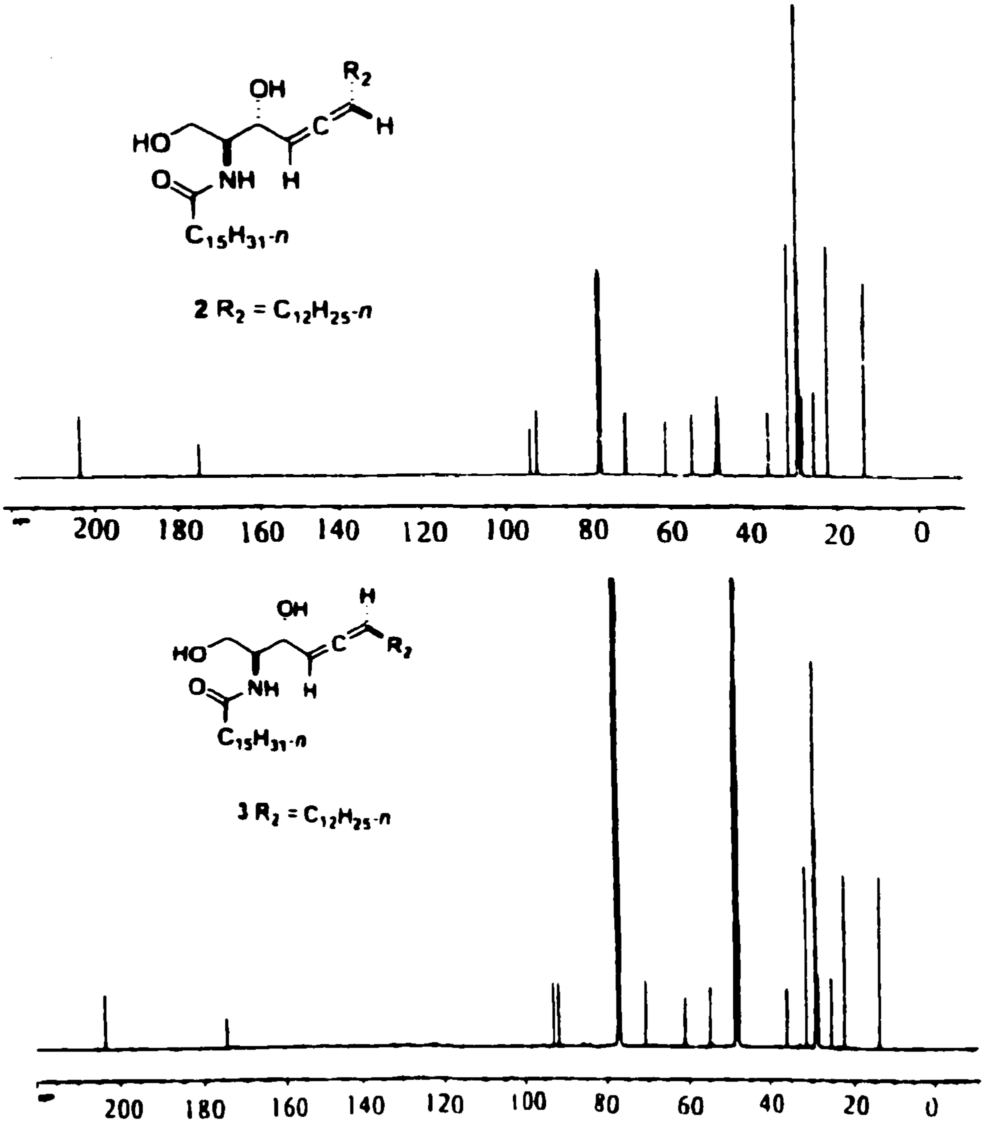
***N*-tert-Butoxycarbonyl (4*S*,3'*S*)-4-[1'-Hydroxy-(2',3')-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine [(+)-18].** This compound was prepared in 60% yield by using the same procedure as described for **12**; $[\alpha]^{25}_D$ +0.67° (*c* 2.0, $CHCl_3$); ^{1}H NMR (C_6D_6, 70 °C) 0.88 (t, 3H, *J* = 6.9 Hz), 1.10-1.70 (m, 35H), 1.66 (s, 3H), 1.98 (m, 2H), 3.72 (dd, 1H, *J* = 9.0, 6.6 Hz), 3.90 (br s, 1H), 3.98 (br s, 1H), 4.40 (m, 1H), 5.18 (m, 1H), 5.27 (m, 1H); ^{13}C NMR (C_6D_6) δ 14.2, 23.0, 23.1, 25.0, 26.7, 27.0, 28.3, 29.3, 29.6, 29.8, 29.9, 30.1, 32.0, (61.8) 63.0, 65.2, (71.8) 72.3, (79.5) 80.4, (92.7) 93.2, (93.6) 94.5, (152.0) 154.1, (203.7) 204.1; HR-MS [FAB, MH$^+$] *m/z* calcd for $C_{26}H_{48}NO_4$ 438.3583, found 438.3576.

***N*-tert-Butoxycarbonyl (4*S*,1'*R*,4'*R*)-4-[4'-(*tert*-Butyldimethylsilyloxy)-1'-hydroxy-2'-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(-)-19].** This compound was prepared in 51% yield by using the same procedure as described for **13**: $[\alpha]^{25}_D$ −15.7° (*c* 1.5, $CHCl_3$); ^{1}H NMR (C_6D_6, 70 °C) δ 0.11 (s, 6H), 0.89 (t, 3H, *J* = 6.6 Hz), 1.00 (s, 9H), 1.20-1.80 (m, 37H), 3.69 (t, 1H, *J* = 7.0 Hz), 3.80 (br s, 1H), 3.98 (br s, 1H), 4.19 (dd, 1H, *J* = 13.2, 5.7 Hz), 4.43 (br s, 1H), 5.71 (dd, 1H, *J* = 15.5, 5.5 Hz), 5.88 (dd, 1H, *J* = 15.5, 5.9 Hz); ^{13}C NMR (C_6D_6) δ -4.6, -3.9, 14.3, 18.5, 23.1, 24.6, 25.7, 26.0, 26.2, 26.6, 28.3, 29.8, 30.1, 32.3, 38.8, 62.6, 64.9, 73.2, 73.6, 80.5, 94.6, 135.9, 154.4; HR-MS [FAB, MNa$^+$] *m/z* calcd for $C_{32}H_{63}NO_5SiNa$ 592.4373, found 592.4352.

(2*S*,3*R*,5*S*)-(4,5)-2-Palmitoylamidooctadecadiene-1,3-diol [(+)-3]. This compound was prepared by using the same procedure as described for **2**: mp 107.0-108.0 °C; $[\alpha]^{25}_D$ +16.3° (*c* 1.0, $CHCl_3$/MeOH 9:1); ^{1}H NMR δ 0.63 (t, 6H, *J* = 6.6 Hz), 0.9-1.30

(m, 44H), 1.38 (m, 2H), 1.76 (m, 2H), 1.98 (t, 2H, J = 7.5 Hz), 3.44 (dd, 1H, J = 11.3, 3.8 Hz), 3.60 (dd, 1H, J = 11.3, 4.9 Hz), 3.65 (m, 1H), 3.94 (t, 1H, J = 5.3 Hz), 4.92 (m, 1H), 5.02 (dd, 1H, J = 6.6, 1.6 Hz); ^{13}C NMR δ 13.5, 22.2, 25.4, 28.4, 28.8, 28.9, 29.0, 29.1, 29.2, 31.5, 36.1, 54.8, 61.0, 70.6, 91.5, 92.8, 174.6, 203.3; HR-MS [FAB, MH$^+$] *m/z* calcd for $C_{34}H_{66}NO_3$ 536.5043, found 536.5042. (See Fig. 1 below.)

Figure 1. ^{13}C NMR Spectra of Ceramides **2** and **3**



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